Clinical Case Report

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The first case report of a large deletion of the *BRCA1* gene in Croatia

A case report

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Abstract

Rationale: Breast cancer is one of the most common cancers in women, and it is the leading cause of cancer related deaths in Croatia. *BRCA1* and *BRCA2* gene mutations are the most common cause of hereditary breast cancer.

Patient concerns: In this report we describe a Croatian patient with no apparent family history of cancer, who developed breast cancer first at 29, and again at 33.

Diagnosis: Due to the early development of first breast cancer and triple negative status of the second, the attending physician suspected a hereditary aspect.

Interventions: Patient was sent to *BRCA1* genetic testing. Subsequently, her mother and sister were sent to check for the mutation found in the patient.

Outcomes: *BRCA1* exons 4-6 deletion was determined and sequencing confirmed the deletion as NG_005905.2: g.107648_117905del10257. Mother and sister were not affected, but since there were no available family members on the fathers' side, it was not possible to determine if this was a case of *de novo* mutation. Until now, only in three reports with the similar mutation the exact mutation borders were determined. The mutation in this case was not the same as previously reported and was more than twice in size.

Lessons: All large deletions should be described at the nucleotide level, so that in cases with missing family data it would be possible to deduce if the mutation is already known. If the mutation is already known, it is probably not a *de novo* event, since it is unlikely that the breakpoints would be exactly the same more than once.

Abbreviations: HGVS = Human Genome Variation Society, HRM = high-resolution melting, MLPA = multiplex ligation-dependent probe amplification, QMPSF = quantitative multiplex polymerase chain reaction of short fluorescent.

Keywords: *BRCA1*, breast cancer, genetic testing, large deletion

1. Introduction

Breast cancer is the most common cancer in women after non-melanoma skin cancer, and it is the leading cause of cancer-related deaths in Croatia. About 5% to 10% of all breast cancer cases are hereditary, and heterozygous germline *BRCA1* and *BRCA2* mutations are responsible for the majority of hereditary breast and/or ovarian cancers. In the most cases, the mutations are small nucleotide alterations leading to premature stop of translation. Large rearrangements of *BRCA1* and less often, *BRCA2* have been described in recent years.[1]

In the Laboratory for Hereditary Cancer at the Rudjer Boskovic Institute about 240 patients have been analyzed for both small mutations and large rearrangements of *BRCA* genes and this is the first large deletion found.

2. Case presentation

The patient, a 36-year-old woman, developed Her-2 positive breast cancer at the age of 29 in her left breast, and triple negative breast cancer at the age of 33 in her right breast. The initial interview indicated that there was no history of cancer in the family, but testing for *BRCA1* gene mutation was indicated due to the early age of onset and her triple negative status (Table 1). The patient signed her informed consent for BRCA testing and the study was conducted according to the Declaration of Helsinki

Table 1

Milestones in the patient medical history and genetic testing.

Patient's age	Life event
29	Her-2 positive breast cancer diagnosed
33	Triple negative breast cancer diagnosed
33	*BRCA1* testing recommended
34	*BRCA1* mutation detected

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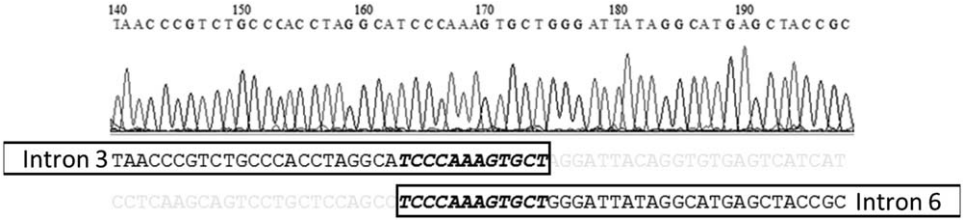

Figure 1. Deletion breakpoints. Breakpoint analysis of the deletion with partial sequence upstream and downstream of the deletion point. The stretch of 12 bases with identical sequence are shown in italic and bold.

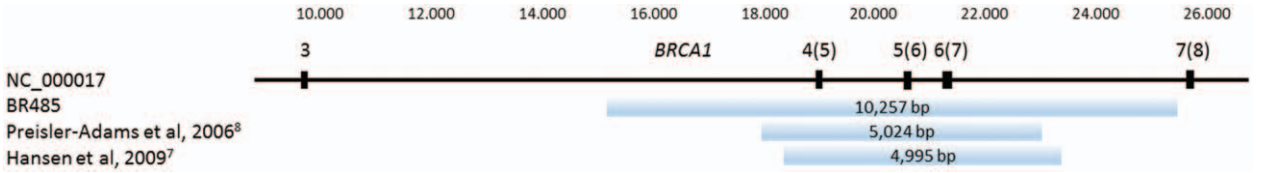

Figure 2. Size and position of other similar mutations. Comparison of the 2 previously determined mutations (Germany and Denmark) with the deletion in this case (BR485).[6,8] Exon positions are noted. Traditional exon numbers are in brackets.

Principles. The mutation analysis was conducted with HRM (high-resolution melting) for small mutations and QMPSF (quantitative multiplex polymerase chain reaction of short fluorescent) for larger rearrangements.[2] Deletion of exons 4 to 6 (traditionally named exons 5–7) in *BRCA1* was found with QMPSF and confirmed with MLPA (multiplex ligation-dependent probe amplification; name of the kit used for the experiment 'SALSA MLPA P002 BRCA1 probemix; MRC-Holland').

After the mutation was found, the consulting physician conducted an additional interview with following family history: There was no cancer history in maternal side (and testing the patients' mother and sister did not show the mutation), but on the fathers side there was a history of cancer. The father died from heart attack at the age of 47 and did not have cancer at the time of death. The patient's paternal grandfather died from lymphoma at the age of 64. Two grandfather's sisters developed uterine cancer and one of them died from it at the age of 43. Two grandfather's sisters developed colon cancer. Two daughters from one of the grandfather's sisters died from cancer, one had lung cancer and the other uterine cancer. No family member from the father's side was available for testing.

To determine the deletion breakpoints, several pairs of primers were developed to bridge the gap. Long range PCR using the Expand Long Range dNTPack (Roche) as recommended by the manufacturer was performed. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The bands were purified with QIAquick Gel Extraction Kit (QIAGEN) and sequenced on ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Long-range PCR resulted in the fragment of 356 bp. Sequencing determined a 10,257 bp deletion with HGVS nomenclature NC_000017.11:g.43100079_43110335del10257, NG_005905.2:g.107648_117905del, NM_007294.3:c.135–3803_442–199del, NP_009225.1:p.(Lys45Asnfs*16) (Fig. 1).

3. Discussion

De novo *BRCA1* mutations are very rare, and have been reported only few times.[3] Since it is unlikely that the same breakpoints would occur independently, if this mutation had the same breakpoints as previously reported, it is not new. The literature survey showed this deletion (almost always named exon 5–7 deletion) to be reported 6 times previously. In 3 cases the deletion size was not determined (Italy,[4] Slovenia,[5] and Myriad Genetic Laboratories[1]); in 2 cases the deletion was 4995 bp (Spain[6] and Denmark[7]), and in 1 case it was 5024 bp (Germany[8]).

This deletion did not correspond to the mutations found in Germany and Denmark/Spain, which were much smaller (Fig. 2), so the authors from Italy and Slovenia were contacted. The Slovenian DNA was not available, but the mutation from Italy is not the same, since the primers for the long-range PCR gave no product.

4. Conclusion

Large rearrangements in *BRCA* genes are not a common occurrence in Croatian population; this is the first one in more than 10 years of testing. Identification of this large deletion of *BRCA1* gene presents a valuable result that shows the need of doing the large rearrangement analysis even in populations where it is not usually seen.

It also demonstrates the problem of lack of the complete family history in determination if the mutation is de novo. It would be helpful if all large rearrangements were described at the nucleotide level, so the origin of these mutations can be more easily resolved.

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