Next-Generation Sequencing Reveals a Nonsense Mutation (p.Arg364Ter) in *MRE11A* Gene in an Indian Patient with Familial Breast Cancer

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Established Facts

- The majority of families with hereditary breast and ovarian cancer (HBOC) harbor mutations in *BRCA1* and *BRCA2* genes.

Novel Insights

- Next-generation multigene panel testing is a better approach for molecular diagnosis of HBOC families.
- This case provides novel insight into the role of the *MRE11A* gene in causing breast cancer susceptibility in HBOC families.

Keywords

Breast cancer · *MRE11* · Next-generation sequencing

Summary

Background: The MRN complex consisting of *MRE11A*-*RAD50*-*NBS1* proteins is involved in the repair of double-strand breaks, and mutations in genes coding for the MRN complex have been identified in families with breast and ovarian cancer. **Case Report:** In a *BRCA*-negative family with positive history of breast and endometrial cancer, next-generation sequencing-based panel testing identified a mutation in the *MRE11A* gene (NM_005590 c.1090C>T: p.Arg364Ter). This mutation results in a shorter mutated protein lacking 2 DNA binding domains (the GAR domain and the *RAD50* binding site), abolishing the function of protein. **Conclusion:** This case provides insight into the role of the *MRE11A* gene in causing breast cancer susceptibility in families, and supports the use of multigene panel testing in cases with hereditary predisposition to breast cancer.

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Introduction

Approximately 5–10% of all breast and ovarian cancers are hereditary. Germline mutations in the *BRCA1* and *BRCA2* genes are observed in about 25% of the cases of hereditary breast and ovarian cancer (HBOC) worldwide [1]. In Indian patients, a compilation of 5 studies of patients with positive family history of breast and ovarian cancer (294 probands) in whom both *BRCA1* and 2 genes were analyzed, mutations were detected in either of the genes in 63 probands (21.4%) – a figure very similar to the studies in western countries [2–6]. Mutations in other genes such as *TP53*, *PTEN*, *ATM* and *STK11* were found in less than 5% of HBOC cases [7]. Several recent experimental and clinical reports [8–11] support the role of genes of the MRN complex in susceptibility to HBOC. Next-generation sequencing (NGS) technologies have helped understand the role of MRN genes in determining breast cancer susceptibility as it allows screening of multiple genes in a cost-effective manner. We present a female breast cancer patient (50-year-old at presentation) with a positive family history of cancer who underwent NGS-based gene panel testing, and in whom a pathogenic mutation in 1 of the intermediate penetrance genes, *MRE11A*, was identified.

Case Report

The index patient was a 60-year-old female (supplementary fig. 1, www.karger.com/?DOI=457786; III-5) of Indian Asian origin. At the age of 50 years, she was diagnosed with breast carcinoma. 1 of the proband's sisters was diagnosed with breast cancer at 60 years (III-1). The other sister (58 years old; III-3) and the younger brother (55 years old; III-7) are both healthy. The proband's mother (II-1) died at the age of 77 of endometrial cancer. A maternal aunt (79 years, II-5) died of breast cancer. Based on the family history of 3 cases of breast cancer, HBOC was diagnosed. Sanger sequencing and MLPA (MRC Holland) for *BRCA* genes were performed using genomic DNA extracted from blood leukocytes [12]. No mutation was identified in the *BRCA* genes. NGS-based panel testing for 21 known breast cancer susceptibility genes (list of genes available on request) on a high-throughput platform was done at the BGI Clinical Laboratories (Shenzhen, China). A pathogenic heterozygous mutation c.1090C>T (p.Arg364Ter) in the *MRE11A* gene was detected [13, 14]. This mutation was confirmed in the index patient by Sanger sequencing (supplementary fig. A; www.karger.com/?DOI=457786). A 308-bp DNA fragment spanning exon 10 was amplified and cut with *Rsa*I restriction enzyme (New England BioLabs) at 37°C for 4 h. The wild-type sequence was cut into 3 fragments of 158, 86 and 64 bp. The mutation eliminates 1 of the restriction site and *Rsa*I enzyme cuts the mutated amplicon into 2 fragments of 222 and 86 bp. Samples from family members of the proband and 100 normal controls were subjected to restriction fragment length polymorphism analysis (supplementary fig. B www.karger.com/?DOI=457786), which showed an absence of mutation in the 100 normal controls.

Clinical Management of Mutation Carriers

Two sisters (1 affected, III-1; 1 unaffected, III-3) of the index patient tested positive and 1 niece (IV-3) tested negative for the mutation. No recommended guidelines are yet available for carriers of a mutation in the *MRE11* gene. Regular self and clinical breast cancer examination and screening with annual magnetic resonance imaging and mammography was advised. Although there is no reported evidence of benefits of prophylactic bilateral salpingo-oophorectomy in *MRE11* gene mutation carriers, the proband's sister (III-3) made an informed decision to undergo prophylactic oophorectomy.

Discussion

A previously reported, pathogenic, heterozygous mutation c.1090C>T (p.Arg364Ter) in the *MRE11A* gene was detected in this case [13, 14]. The *MRE11A* protein is a component of the MRN (*MRE11A*/*RAD50*/*NBS1*) complex, which is part of the *BRCA1*-associated genome surveillance complex (BASC). In response to DNA double-strand breaks (DSB), the MRN complex serves as the sensor of DNA breakage, and activates the key signal transducer of the DNA damage response machinery [15, 16]. The wild-type 70-kDa *MRE11A* protein is highly conserved across species. The C to T change at position 1,090 of the gene results in the termination of the *MRE11A* protein after 363 amino acids. The truncated protein lacks 2 DNA-binding domains, the GAR domain and the *RAD50* binding site, and a few amino acids from the C-terminal region of capping site (supplementary fig. 2, www.karger.com/?DOI=457786). The mutated protein is not able to interact with *RAD50* and form the MRN core complex. Single- and double-stranded (ds) DNA endonuclease, and a 3'-5' dsDNA exonuclease activity of the phosphoesterase domain of *MRE11A* protein is also integral to the DNA DSB repair mechanism [17]. The absence of DSB repair results in an accumulation of genomic instability, leading to increased sus-

ceptibility to cancer. Potentially pathogenic germline mutations have been reported in MRN complex genes in a number of studies in families with an hereditary predisposition to breast and ovarian cancer [10, 11, 18–21]. Heikkinen et al. [21] reported a mutation in the *MRE11* gene (p.R305W) in a family with ovarian and cervical cancer in a study of 151 HBOC families. Absence of mutation in 1,000 controls confirmed its role in increasing susceptibility to breast cancer. Another study described mutations in 3 out of 708 consecutive breast cancer patients. A deletion causing a splice site defect in the *MRE11A* gene was found in a patient with serous ovarian carcinoma in a study of 273 women with ovarian carcinoma [18]. Although these reports provided evidence that mutations in *MRE11* increase breast cancer risk, the tumorigenic contribution of MRN genes in breast cancer formation needs to be evaluated.

Hsu et al. [9] reported that the association of cancer risk with high-risk MRN genotypes is stronger in women who are more susceptible to estrogen exposure, i.e. those older than > 26 years of age at first full-term pregnancy (FTP) than those with fewer FTPs and no history of breast feeding. The suggested mechanism is that the estrogen metabolites bind to DNA and initiate DSB formation, and cells with defective MRN-mediated DSB repair are more susceptible to cancer formation. In the present case, the proband's mother and her sister had their first FTP before the age of 26 years and had more FTPs compare to affected daughters (supplementary fig. 1, www.karger.com/?DOI=457786). The proband and her sister developed breast cancer at 50 and 60 years of age, respectively, while their mother and her sister developed endometrial cancer (77 years) and breast cancer (79 years), respectively, at a later age. This observation supports the hypothesis given by Hsu et al. [9] that the breast cancer risk due to high-risk MRN genotypes can be modified by reproductive factors.

The proband's mother died at 77 years of endometrial cancer. This case and other studies highlight the association of mutations in the *MRE11A* gene with endometrial cancer [22–24]. Koppensteiner et al. [23] demonstrated that endometrial cancer associated with *MRE11* mutation has higher sensitivity to poly (ADP-ribose) polymerase (PARP) inhibitor therapy. PARP inhibitors are effective against tumors with deficient DNA repair mechanisms, and recent reports provide evidence that *MRE11* mutations can serve as a predictive biomarker for the efficacy of PARP inhibitors.

In conclusion, the management of subjects with mutations in genes other than *BRCA* is a challenge in the clinic, as no recommended guidelines are yet available for such individuals. The level of associated cancer risk needs to be determined in larger number of patients with adequate follow-up to define appropriate management guidelines. The identification of mutations in non-*BRCA* susceptibility genes will provide a better understanding of genetic heterogeneity in high-risk families.

Supplementary Figures

Supplementary fig. 1. Pedigree chart of the family.

Supplementary fig. 2. Domain organization of wild-type and mutant (*MRE11A*_p.Arg364Ter) *MRE11A* proteins.

Supplementary fig. A. Electropherogram of the mutated sequence. The c.1090C>T alteration in the *MRE11A* gene results in p.Arg364Ter amino acid change.

Supplementary fig. B. Ethidium bromide stained agarose gel showing RFLP analysis. Lane M (Molecular marker); Lanes 1-4: family members (lane 1 (III-1), lane 2 (III-2) and lane 3 (III-3) are heterozygous for c.1090C>T mutation while lane 4 (IV-1) is unaffected); Lanes 5-10 (normal controls); Lane 11 (undigested PCR product).

To access the supplementary figures please refer to www.karger.com/?DOI=457786.

Disclosure Statement

All authors declare that there were no conflicts of interest concerning this work.

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