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Genetic Predisposition to Male Breast Cancer: A Case Series

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Abstract. *Background/Aim: Male breast cancer (MBC) is a very rare disorder affecting approximately 1 in 833 men. Genetic predisposition is one of the most important risk factors of MBC with BRCA2 being the most commonly mutated gene in males diagnosed with breast cancer. However, a large part of MBC heritability is still unexplained. This study sought to add to the data already available on the genetics of MBC. Materials and Methods: Our study initially involved comprehensive analysis of BRCA1 and BRCA2, followed by analysis of 43 genes implicated in cancer predisposition in a series of 100 Greek patients diagnosed with MBC between 1995-2015. Results: Pathogenic variants were identified in 13 patients, with BRCA2 being the most commonly affected gene, followed by BRCA1, RAD50, RAD51B, and MSH3. Conclusion: In agreement with previous reports, BRCA2 is the most important genetic factor of MBC predisposition, while the remaining known cancer predisposition genes are each very rarely involved, rendering conclusions as to their cumulative effect difficult to draw.*

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Breast cancer is the most common cancer type in women worldwide with an estimated lifetime risk of 1 in 8. In contrast, male breast cancer (MBC) is 70-100 times less common with an estimated lifetime incidence of 1 in 833, making it a very rare disease (1). As a result, diagnosis

usually occurs at more advanced stages and has therefore, therefore, a worse prognosis (2). Furthermore, research on MBC is very limited and management is extrapolated from our knowledge on one of the best-studied cancer types, *i.e.*, female breast cancer (FBC).

Well-documented risk factors of MBC include age, race, and radiation exposure (2). Nevertheless, genetic predisposition seems to be one of the most important risk factors of MBC as shown by the fact that 15-20% of patients report a family history of breast or ovarian cancer. The most frequently mutated gene in MBC patients is *BRCA2* although mutations in *BRCA1, CHEK2, ATM* and *PALB2* have also been frequently described (2-5). However, despite advances in technology available for genetic screening, much of the MBC heritability is still unexplained and more research is necessary in order to uncover the genes responsible for hereditary MBC.

In this study, we aimed to delineate the genetic contribution to MBC in a series of Greek patients with a diagnosis of male breast cancer as the sole criterion for inclusion in the study. When the study started, the only genes generally believed to be involved in MBC susceptibility were *BRCA1* and more importantly *BRCA2*. With widespread use of next-generation sequencing (NGS) and multigene panel analysis we decided to expand the study to involve a custom designed panel of 43 genes known or suspected to be involved in genetic predisposition to cancer, the ultimate goal being to be able to target breast cancer screening to men with MBC predisposition, providing diagnosis at earlier and therefore more manageable stages.

Materials and Methods

Study group. The study was coordinated and approved by the Male Breast Cancer Working Group of the Hellenic Society of Medical Oncology. Blood samples of 100 men diagnosed with breast cancer between 1995-2015 were collected by the referring physicians at various Greek Departments of Medical Oncology. The sole criterion for inclusion in the study was a histologically confirmed diagnosis of MBC. All participants signed informed consent agreeing to donate their genetic material for molecular analysis of genes related to hereditary breast cancer and to the anonymous use of the results of the analysis for research purposes and scientific publications.

BRCA1 and BRCA2 analysis. Analysis of the *BRCA1* and *BRCA2* genes was carried out in two Molecular Diagnostic Laboratories in Athens, Greece. Twenty-nine of the samples were analyzed by Sanger Sequencing (SS) using fluorescently labeled ddNTPs. Electrophoresis of sequencing products was performed on an ABI 3500 genetic analyzer. The remaining 71 samples were analyzed by NGS using the CE IVD BRCA MASTR Dx kit (MULTIPLICOM NV) according to the manufacturer's instructions (6).

Analysis for large genomic rearrangements was carried by multiplex ligation-dependent probe amplification (MLPA) according to the manufacturer's instructions (7), in samples negative for single nucleotide variants (SNVs) and small indels of the *BRCA1* and *BRCA2* genes.

Multigene analysis. Multigene analysis was carried out on DNA samples from patients negative for pathogenic (PVs) or likely pathogenic variants (LPVs hence forward all described as PVs) in *BRCA1* and *BRCA2*. This was done by a solution-based capture approach using a custom target enrichment panel covering 43 genes (Table I) involved in hereditary predisposition to cancer (KAPA HyperExplore MAX 3Mb T1, Roche, Basel, Switzerland). Library preparation was carried out in MGISP-960 high-throughput automated sample preparation system (MGI) and sequencing was carried out using the DNBSEQ-G50 sequencing platform (MGI). All targeted regions were sequenced to a minimum depth of 20×. Unless otherwise stated (Table I), this assay targets all coding regions of the indicated transcripts and 20 base pairs of flanking intronic sequences. Reads were aligned to the reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. Variants were named according to Human Genome Variation Society nomenclature guidelines. Clinical interpretation of all identified variants was performed using an in-house local knowledge-base and a proprietary bioinformatics pipeline based on the standards and guidelines for the interpretation of sequence variants recommended by the American College of Medical Genetics and Genomics (ACMG Laboratory Quality Assurance Committee) and the Association for Molecular Pathology (AMP) (8). All clinically significant observations were confirmed by Sanger Sequencing analysis (primers available upon request) on SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The presence of copy number variations (CNVs) was investigated using the commercial computational algorithm SeqPilot Version 4.4 Build 505 (JSI Medical Systems, Ettenheim, Germany). In addition, the computational algorithm panelcn.MOPS (9) was also used in the analysis of *BRCA1* and *BRCA2*. The presence of CNVs was verified by MLPA (MRC Holland, Amsterdam, the Netherlands) (7).

Results

Pathogenic and likely pathogenic variants. Initial analysis was limited to the *BRCA1* and *BRCA2* genes. This revealed eight PVs/LPVs in nine patients (9%) (Figure 1). Two of the PVs were located in the *BRCA1* gene while the remaining 6, including a large genomic rearrangement involving exons 12 and 13 of the gene, were located in *BRCA2*. A single *BRCA2* PV, c.7879A>T, p.(Ile2627Phe) was identified in two presumably unrelated patients. Finally, variant c.6942delA, p.(Ile2315*), in *BRCA2*, identified in a single patient, was absent from mutation and population databases, but in accordance with the ACMG guidelines (8) was classified as likely pathogenic based on its predicted effect on the BRCA2 protein (Table II).

Enough good quality DNA, for further analysis by NGS, was available for 65 of the 91 patients negative for *BRCA1* and *BRCA2* PVs. This analysis revealed a further 4 PVs (Figure 1). Two of these variants were located in *RAD50* and one each in *MSH3* and *RAD51B* (Table II). All four variants have been previously described in mutation databases and/or in the international literature.

Table I. *Genes analyzed*.

Gene	Reference sequence	CNV analysis	Comments
APC	NM_000038		
ATM	NM_000051		
AXIN2	NM_004655		
BARD1	NM_000465		
BMPR1A	NM_004329		
BRCA1	NM_007294	✔	
BRCA2	NM_000059	✔	
BRIP1	NM_032043		
CDH1	NM_004360		
CDK4	NM_000075		
CDKN2A	NM_000077, NM_058195		p14ARF and p16INKa
CHEK2	NM_007194	✔	
EPCAM	NM_002354	✔ (Exons 8 and 9 only)	
FANCA	NM_000135		
FANCL	NM_001114636		
FANCM	NM_020937		
HOXB13	NM_006361		p(Gly84Glu only)
MEN1	NM_000244		
MLH1	NM_000249	✔	
MRE11	NM_005591		
MSH2	NM_000251	✔	
MSH3	NM_002439		
MSH6	NM_000179	✔	
MUTYH	NM_001128425	✔	
NBN	NM_002485		
NF1	NM_000267		
NTHL1	NM_002528		
PALB2	NM_024675	✔	
PMS2	NM_000535		
POLD1	NM_001256849		Exons 8-13
POLE	NM_006231		Exons 1-14
PPP2R2A	NM_002717		
PTEN	NM_000314		
RAD50	NM_005732	✔ (Exons 1, 2, 4, 10, 14, 21, 23 and 25 only)	
RAD51B	NM_133509		
RAD51C	NM_058216	✔	
RAD51D	NM_002878	✔	
RET	NM_020975		
SMAD4	NM_005359		
SMARCA4	NM_001128849		
STK11	NM_000455		
TP53	NM_000546	✔	
VHL	NM_000551		

CNV: Copy number variation.

Variants of uncertain significance (VUS). In the initial analysis of *BRCA1/2* genes, 7 patients carried variants of uncertain significance (VUS), and in 2 of these cases, the variant was later reclassified as likely benign (Figure 1). Among the 65 DNA samples that were subjected to multigene analysis, 60 VUS were identified (Figure 2). In two patients, the VUS were inherited in addition to PVs, suggesting that they are unlikely to be causative of the disease. In the majority of VUS (46.7%), the uncertainty of the clinical significance was due to the lack of additional evidence for a likely benign classification. In a further 36.7%, the available data were conflicting. In 6.7% of cases, there was no or limited information available to be used in the classification of the variants and only 10% (6 variants) were predicted to have a damaging effect on the encoded protein. These 6 variants were located in *CHEK2, MRE11, MSH2, PMS2, RAD51B,* and *TP53*. The evidence was, however, not enough for a definitive classification and were, therefore, reported as VUS.

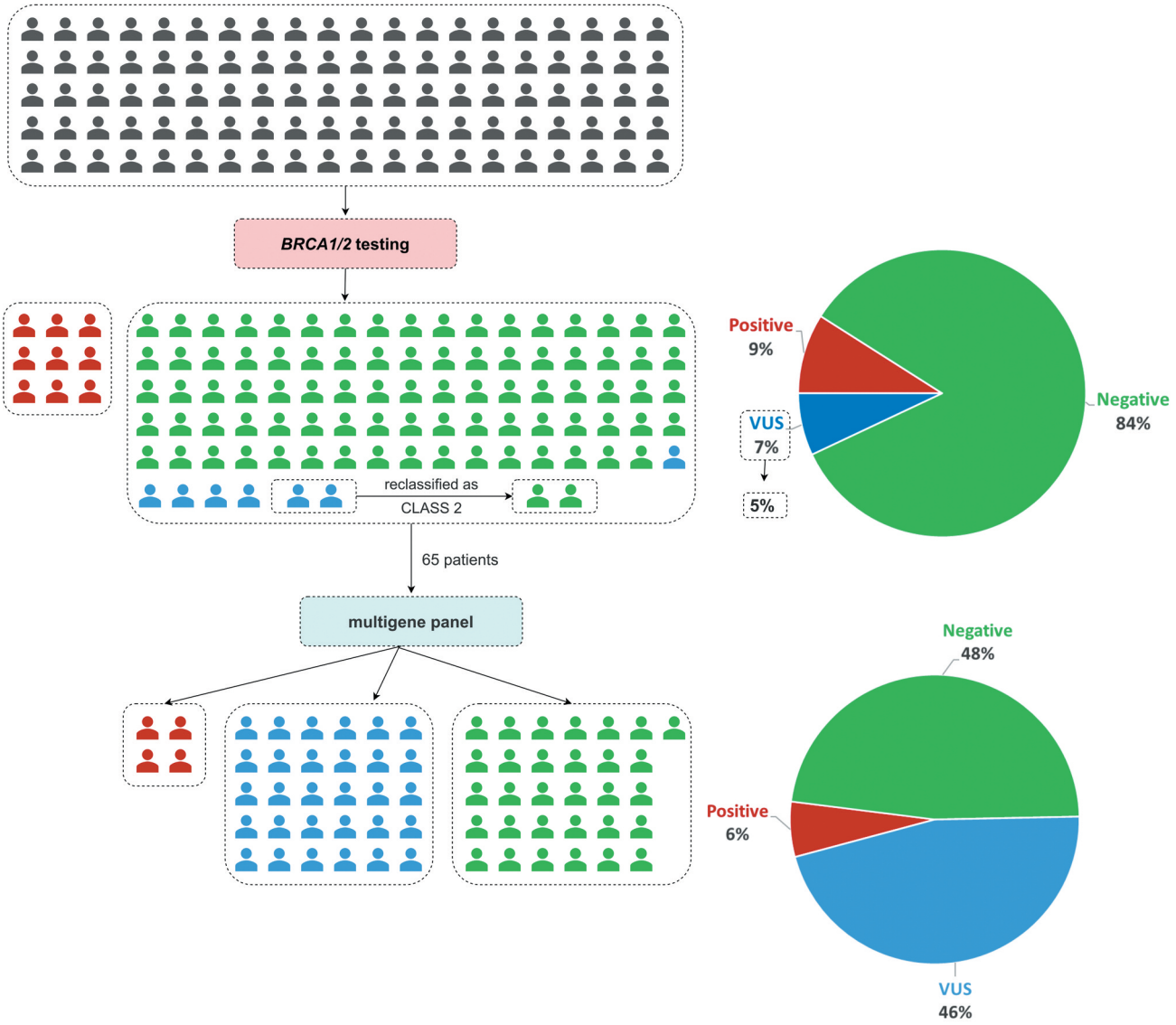

Figure 1. *The schematic representation of the study workflow and genetic testing results using the BRCA1/2 and multigene panel approach. Positive results refer to the individuals where a pathogenic/likely pathogenic variant was identified.*

The majority of VUS were detected in genes that have relatively recently been associated with cancer predisposition, such as *FANCA* and *FANCM,* and have therefore been less extensively analyzed. In contrast, no VUS were detected in well studied genes, such as *BRCA1* and *BRCA2*.

Discussion

In this study, we aimed to delineate the genetic contribution to cancer in a series of 100 Greek MBC patients, included in the study solely because of their MBC diagnosis. Initial analysis was restricted to the *BRCA1* and *BRCA2* genes. The results of this analysis compare favorably with those of similar studies (3-4, 10, 11). A PV or LPV in *BRCA1* or *BRCA2* was identified in 9% of the patients. As expected, *BRCA2* was the most frequently mutated gene, accounting for 46% of all the PVs detected in this series of patients.

Thus, we investigated whether the inclusion of additional genes could increase the number of PVs identified in our cohort, which could better explain the cancer phenotype in these patients. The application of a multigene NGS panel revealed the presence of 4 additional PVs in *RAD50* (2 cases), *RAD51B* (1 case), and *MSH3* (1 case). In contrast to previous reports, no PVs or LPVs were identified in *ATM* (5, 10), *CHEK2* (4) or *PALB2* (5, 11).

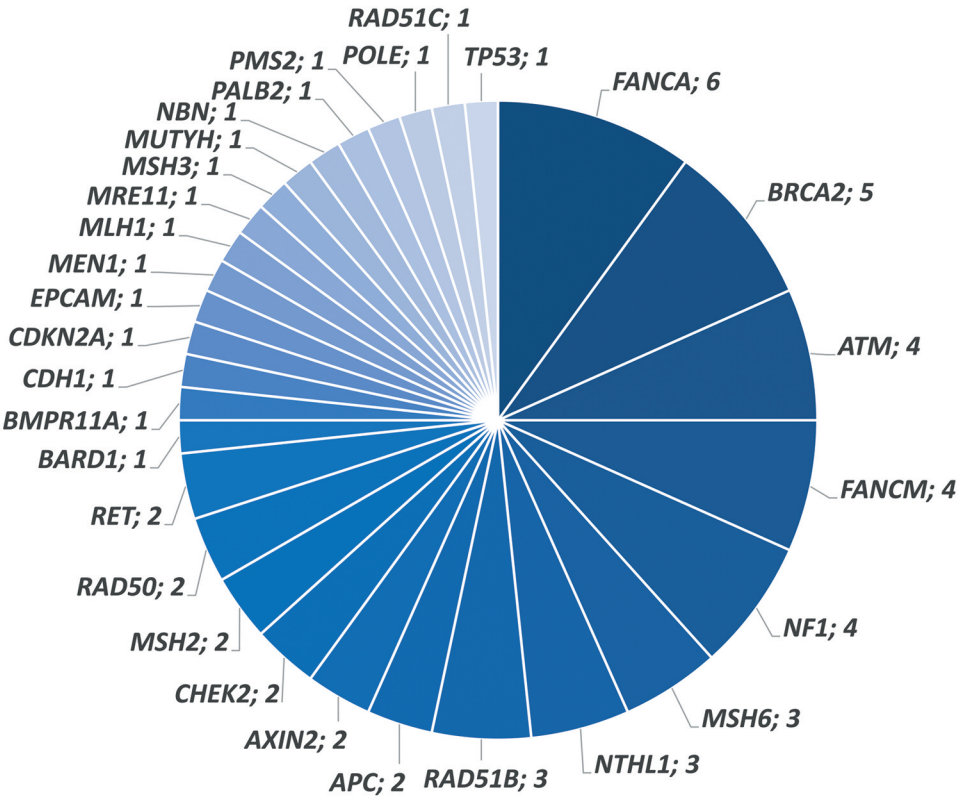

Figure 2. *The distribution of the 60 variants of uncertain significance (VUS) in each gene that were identified through the multigene panel approach.*

Table II. *Variants detected in our study.*

Gene	HGVS cDNA	Consequence	IARC class category	dbSNP	ClinVar Variation ID
BRCA1	c.4327C>T	p.(Arg1443*)	CLASS 5	rs41293455	17675
BRCA1	c.5266dupC	p.(Gln1756Profs*74)	CLASS 5	rs80357906	17677
BRCA2	c.5645C>A	p.(Ser1882*)	CLASS 5	rs80358785	37984
BRCA2	c.6275_6276delTT	p.(Leu2092Pfs)	CLASS 5	rs11571658	9318
BRCA2	c.(6841+1_6842-1)_(7007+1_7008-1)del	-	CLASS 5	-	216030
BRCA2	c.6942delA	p.(Ile2315*)	CLASS 4	-	-
BRCA2	c.7879A>T	p.(Ile2627Phe)	CLASS 5	rs80359014	52430
BRCA2	c.7879A>T	p.(Ile2627Phe)	CLASS 5	rs80359014	52430
BRCA2	c.9501+1G>A	Splicing	CLASS 4	rs397508058	52853
MSH3	c.703C>T	p.(Gln235*)	CLASS 4	rs371356175	837189
RAD50	c.326_329del	p.(Thr109Asnfs*20)	CLASS 5	rs587780155	128017
RAD50	c.2922+1G>A	Splicing	CLASS 4	rs1581004749	822272
RAD51B	c.84G>A	p.(Gln28=)	CLASS 4	rs764896402	221910

HGVS: Human Genome Variation Society; IARC: International Agency for Research on Cancer; dbSNP: single nucleotide polymorphism database.

RAD50 and *RAD51B* encode for proteins involved in the homologous recombination (HR) repair pathway of which BRCA1 and BRCA2 are major components. RAD50 is a protein in the MRN complex, part of the mechanism responsible for detecting and early processing of double-strand breaks (12). RAD51B is one of the human paralogs of RAD51 recombinase. RAD51 recombinase binds to single stranded DNA at the break site to initiate double strand repair (13). PVs in the *RAD50* gene

have been previously described in various multigene panel analyses of FBC (12, 14, 15) and MBC (5, 16). In contrast, *RAD51B* has been less frequently included in multigene panel analysis in the international literature but implications of its involvement in breast cancer susceptibility have recently emerged (17). To date, the penetrance and clinical significance of mutations in these genes in breast cancer in general and more specifically in MBC are unknown. According to the latest version of the NCCN guidelines, the evidence regarding *RAD50* is currently insufficient to allow for specific management recommendations for carriers of PVs, whereas *RAD51B* is not even mentioned (18). Regardless of the current evidence as to the penetrance of PVs in these two genes, the proteins encoded by them are known to interact with *BRCA1* and *BRCA2* in the HR pathway. Involvement of additional HR pathway genes to MBC predisposition would be encouraging considering that mutations in genes involved in this pathway are predictive of response to PARP inhibitors (PARPi) (19, 20).

Finally, a PV in *MSH3* was identified in a single individual. The MSH3 protein is involved in the mismatch repair mechanism (MMR) but has not been implicated in Lynch Syndrome, the hereditary cancer predisposition syndrome caused by PVs in genes belonging to this pathway. Instead, biallelic mutations in *MSH3* have recently been described in patients diagnosed with adenomatous polyposis syndromes (21). PVs in *MSH3* have also been described in three families of a Chinese cohort with breast cancer; the disease, however, did not segregate with the mutation in two of the cases (22). The association of MBC with the PV in this case is not clear. Segregation analysis of the mutation in this patient's family would help delineate its effect on the phenotype. Unfortunately, DNA from the patient's family was not available.

A final observation in this series of patients was the relatively high frequency of VUS identified with the multigene panel (48%) compared to the analysis of *BRCA*1/2 (5%). The majority were identified in genes that have only recently been implicated in hereditary cancer predisposition and are therefore not extensively analyzed in large population series. However, international guidelines suggest that these variants should not be used to alter the medical management of patients. However, it is anticipated that as a large amount of data is generated by the increasing number of centers offering multigene panel testing and by the increasing number of genes included in these panels, the proportion of patients receiving a report of VUS will gradually decrease.

A potential limitation of this study is the quality and quantity of the clinical and family history information collected for these patients. This lack of information prohibits segregation analysis of the variants identified in genes that are not traditionally associated with male breast cancer predisposition.

In conclusion, in an effort to add to the international data available on the heritability of MBC, we analyzed *BRCA1* and *BRCA2*, followed by a panel of 41 additional cancer predisposition cancer genes in a series of Greek MBC patients. The results are in agreement with previous reports highlighting *BRCA2* as the most important gene responsible for MBC predisposition. However, extensive research in a larger number of patients is needed in order to further delineate the effect and extend of hereditary predisposition to MBC.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

AA designed and drafted the manuscript. KA, GP, CB, and PP carried out the DNA extraction, sequencing, and contributed to the analysis and interpretation of the variant data. GNT performed the bio-informatics analysis. ES, EK, DT, NK, EB, FZ, AK, AK, IK, IB, GL, GF, VM, SX, and HL provided the materials, demographic data, and family history. EP, LF, GN, and VG conceived of the study and participated in its design and coordination. All Authors read and approved the final manuscript.

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