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Prevalence and founder effect of the *BRCA1* p.(Val1833Met) variant in the Greek population, with further evidence for pathogenicity and risk modification

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Abstract

Purpose: Multiple lines of evidence have suggested a likely causative role in breast/ovarian cancer (BrCa/OvCa) predisposition for the *BRCA1* p.(Val1833Met) variant, predominantly found among Greek patients. Our aim was to study the variant's prevalence and founder effect on the Greek population, while providing additional data for its pathogenicity.

Methods: We genotyped 3531 BrCa/OvCa patients using Sanger and next generation sequencing, as well as 1558 healthy, age-matched females with real-time PCR. Carriers underwent haplotype analysis to determine a founder effect. A co-segregation analysis was applied to estimate the likelihood ratio for pathogenicity.

Results: In total, 27 BrCa/OvCa patients (0.77%; 27/3531) were found to carry the p.(Val1833Met) variant. No carriers were identified in the control group diagnosis. A common shared haplotype, spanning 2.76 Mb on chromosome 17 was demonstrated among carriers, establishing the founder effect. *BRCA1*, p.(Val1833Met) is possibly a disease-associated variant, supported by a likelihood ratio of 1.88, while a correlation to ovarian cancer is suspected.

Conclusions: Altogether, *BRCA1*, p.(Val1833Met) variant is a Greek founder and is very likely to predispose for BrCa/OvCa. Therefore, such carriers should be counselled accordingly, with clinical recommendations supporting surveillance and risk-reduction strategies, while providing the option for targeted therapeutic interventions.

Keywords BRCA1, Missense variant, Breast cancer, Ovarian cancer, Familial cancer, Founder effect.

Introduction

The diverse mutational spectrum in the Greek population among hereditary breast and/or ovarian cancer patients was recently described in detail, concerning predominantly *BRCA1*, followed by *BRCA2* pathogenic variants. Interestingly, about 75% of all *BRCA1* pathogenic variants are located at the 3′ end of the gene, attributed mainly to strong founder effects, while *BRCA2* pathogenic variants are scattered along the gene sequence. Although the Greek population is characterized by genetic heterogeneity, Greek founder pathogenic variants account for approximately 68.5% of all *BRCA1* pathogenic variants detected, suggesting a rea-

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sonable first-step screening of breast and/or ovarian cancer patients for such variants [1]. *BRCA1* p.Gly1738Arg, a missense variant whose pathogenicity and founder effect were fully characterized in a previous study, is among them [2].

Nevertheless, many variants of unknown clinical significance (VUS) in the *BRCA1* and *BRCA2* genes await definite classification. These variants could explain a substantial percentage of hereditary cancer cases, thereby improving clinical management of cancer patients and their families. Although most truncating variations in these genes are proven to be cancer-predisposing, many missense variants lie in the grey zone and are difficult to classify [3,4]. Missense pathogenic variants have been shown, among other effects, to affect the binding site hampering the interactions with phosphorylated protein targets including the DNA helicase BACH1 and the CtBP-interacting protein CtIP [5].

BRCA1, c.5497G>*A*, p.(Val1833Met) (NM_007294.3/NP_009225.1/ClinGen:CA00366) missense variant, which is located in the last exon of the gene within the second BRCT domain, is such a variant, which attracted our attention mainly due to the number of Greek families found to carry it [6]. This variant (rs80357268) is classified as likely pathogenic with no conflicting interpretations, while being absent in population databases (ExAC, 1000 Genomes, ESP) (https://www.ncbi.nlm.nih.gov/clinvar/variation/55598/). *In silico* models predict that this variant is probably damaging to protein structure and function. Moreover, an *in silico* prior probability of pathogenicity of 0.03 has been attributed to p.(Val1833Met) variant [7,8].

Regarding physicochemical status, the valine to methionine substitution has been shown to reduce the thermodynamic stability of the BRCT domain comparable to a partly folded intermediate, disturbing the structural integrity of the molecule [9, 10], while its binding affinity to both BACH1 and CtIP phosphopeptides is preserved [11]. In particular, it has been demonstrated that p.(Val1833Met) variant disrupts the hydrophobic core of the C-terminal BRCT repeat, resulting in small but significant reduction in stability of protein folding [12,13]. In addition, structure-based analysis of various *BRCA1* variants in the BRCT-C domain showed that Val1833 residue is solvent inaccessible. It is located in the β4 strand of a four-stranded parallel β-sheet. Since valine is more favored in β-strands than methionine and methionine is larger than valine, a possible disruption of the tight packing in the domain's hydrophobic core of the BRCT-C domain is caused [12,14]. In an *E. coli* biophysical assay, the Val1833Met mutant presented severe impact on BRCA1 thermostability, giving rise to inclusion body formation [15]. Val1833Met destabilizing effect was also demonstrated in a yeast small colony phenotype assay, highlighting its functional and structural defects [16]. In a recent study, where pathogenicity of multiple *BRCA1* VUS was assessed via functional assays, p.(Val1833Met) was classified as a variant with intermediate activity in Class4 (likely pathogenic) [17]. Recently, p.(Val1833Met) was presented as an HR-defective variant with a subsequent increased cancer risk [18].

The aim of the present study was to investigate the prevalence of p.(Val1833Met) variant among Greek breast and/or ovarian cancer patients and explore its possible founder effect for the Greek population, while providing additional evidence for disease causality.

Patients and methods

Study group

For the conduction of the present study, three distinct patient groups were included. In detail, 2503 breast cancer patients, 918 ovarian cancer patients and 110 patients diagnosed with both breast and ovarian cancer were enrolled *ad hoc* from several hospitals in Greece between 2010 and 2018, all fulfilling National Comprehensive Cancer Network (NCCN) selection guidelines for genetic testing [19]. Mean age at diagnosis was 46.7 ± 11.0 years (range: 19–84 years) and 53.6 ± 11.6 years (range: 17–87 years) for breast cancer and for ovarian cancer diagnosis, respectively. In addition, 1558 Greek cancer-free, age-matched women were tested, constituting the control group. The study was approved by the Bioethics Committee of NCSR 'Demokritos' (240/EHΔ/11.3, updated on February 14, 2014). All individuals signed informed consent prior to genetic testing.

Genotyping

Total genomic DNA was isolated from peripheral blood lymphocytes using the salt extraction protocol proposed by Miller et al. [20]. For patients enrolled from 2010 to 2014, the *BRCA1* and *BRCA2* genes were analyzed by Sanger sequencing using the v3.1 BigDye Terminator Cycle Sequencing kit on an ABI 3130xl Genetic Analyzer (ThermoFisher Scientific, Carlsbad, CA, USA). The procedure has been described thoroughly in a previous study [6].

Patients enrolled from 2015 to 2018 were screened through massively parallel sequencing using the Illumina Trusight Cancer Panel sequenced on a MiSeq analyzer (Illumina, San Diego, USA), as previously described [21]. The control group genotyping was carried out by a real-time PCR protocol, using the SYBR green dye-based detection assay. KAPA SYBR FAST qPCR Master Mix Universal kit (KapaBiosystems, Wilmington, MA, USA) was used on a CFX96 Touch Real-Time PCR Detection System (BioRad, California, USA). Each reaction included a primer pair used to amplify the 106 bp product and one fluorescent probe, labelled with HEX. A fast-2-step cycling protocol was employed and for each group of assays, both negative and mutant control DNA samples were included. Probe and primer sequences provided by IDT (Integrated DNA Technologies, Leuven, Belgium) are available upon request.

Haplotype analysis

Haplotype analysis was conducted in 42 variant carriers from 27 families, 14 family relatives that were non-carriers and 40 cancer-free, age-matched Greek females. Four *BRCA1* extragenic polymorphic microsatellite markers (D17S951, D17S800, D17S250, and D17S1861) and one intragenic marker (D17S855) spanning a region of approximately 5.9 Mb on chromosome 17 were used. The markers D17S855, D17S800, and D17S250 were labeled with 6-FAM fluorescent dye, having size ranges of 141–155 bp, 168–178 bp, and 147–169 bp, respectively, marker D17S1861 was labeled with

6-HEX fluorescent dye, having a size range of 92–112 bp, while marker D17S951 was labeled with 6-TAMRA fluorescent dye, having a size range of 170–188 bp. The fluorescently labeled PCR products were electrophoresed on an ABI 3130XL Genetic Analyzer standardized with ROX-500 (ThermoFisher Scientific, Warrington, UK) and analyzed using the GeneScan 3.1 software (ThermoFisher Scientific, Warrington, UK).

Estimation of the p.(Val1833Met) variant age

The age of p.(Val1833Met) variant was determined using the DMLE2.2 software (http://www.dmle.org). The program uses the Markov Chain Monte Carlo method which generally allows Bayesian inference of the variant age based on the observed linkage disequilibrium at studied genetic markers [22]. The population growth rate was 0.135 and was based on demographic data, assuming a time interval of 25 years per generation.

Co-segregation analysis

In order to further assess the pathogenicity of the p.(Val1833Met) variant, we used the publicly available in http://www.msbi.nl/cosegregation algorithm, which requires information only about gender, genotype, present age and/or age of onset for breast and/or ovarian cancer, through of which the calculation of the likelihood ratios for or against causality is feasible [23].

Statistical analysis

All the emerged data underwent statistical analysis using the chi-square test at a significance level of 0.05.

Results

Prevalence of p.(Val1833Met) variant among Greek breast and/or ovarian cancer patients

In total, 27 breast and/or ovarian cancer patients were found to carry the p.(Val1833Met) variant, providing an overall prevalence of 0.77% (27/3531). Among breast and ovarian cancer patients, the variant prevalence was 0.44% (11/2503) and 1.53% (14/918), respectively, while a total of 1.82% (2/110) of patients diagnosed with both breast and ovarian cancer were carriers.

Direct comparison of said prevalence revealed a statistically significant difference between breast and ovarian cancer patients' groups ($p = 0.0009$, 95% CI [0.4, 2.1]), indicating that p.(Val1833Met) variant is more correlated to ovarian cancer diagnosis. A similar correlation was also demonstrated upon comparison of prevalence in breast/ovarian and breast cancer patients' groups ($p = 0.0442$, 95% CI [0.0, 5.9]), enhancing p.(Val1833Met) association to ovarian cancer. In the control group, consisting of 1558 Greek cancer-free, age-matched females, the variant was not detected.

Characteristics of *BRCA1* p.(Val1833Met) carriers

Among carriers, 40.7% (11/27) were diagnosed with breast cancer with a mean age of diagnosis at 41.6 ± 10.6 years (range: 27–66 years), while 51.9% (14/27) had ovarian cancer with a mean age of diagnosis at 57.2 ± 9.7 years (range: 45–74 years). Interestingly, 7.4% (2/27) of the carriers were diagnosed with both breast and ovarian cancer, with a metachronous age of onset for ovarian cancer. Although not statistically significant, carriers were diagnosed with breast cancer at a younger age, when compared to the patient's cohort ($p = 0.1250$, 95% CI [−1.4, 11.6]). On the contrary, age at ovarian cancer diagnosis was slightly higher, when compared to the cohort of total, yet not constituting a statistically significant observation ($p = 0.6541$, 95% CI [−7.5, 4.7]).

Of all 27 carriers, information on family history were available for the 26, of which 19 reported family history of breast and/or ovarian cancer. Direct comparison revealed statistically significant association for family history ($p = 0.0360$, 95% CI [3.9, 70.6]). Approximately half of the carriers (47.4%; 9/19) with reported family history described only ovarian cancer cases in their family, whereas 26.3% (5/19) of them had only breast cancer cases to report.

Segregation analysis for the *BRCA1* p.(Val1833Met) variant

Segregation analysis was conducted in 8 families of p.(Val1833Met) carriers, in order to investigate a possible correlation with pathogenicity. Detailed pedigrees of the families, members of which carry the p.(Val1833Met) variant are summarized in Fig. 1. Segregation is demonstrated in pedigrees 1e, 1f, 1 g and 1 h. Of these, the most illustrative pedigree for the p.(Val1833Met) segregation analysis is Fig. 1f, since both the proband (II:5) and her two daughters (III:4 and III:5), who were diagnosed with ovarian and breast cancer respectively, carry the variant, while the other daughter (III:2), cancer-free at the age of 49 years, is not a p.(Val1833Met) carrier. Moreover, in Fig. 1e, the proband (III:9), diagnosed with breast cancer at the age of 49 years, was found to have inherited the variant from her father (II:9), cancer-free at the age of 83 years but with a sister affected by ovarian cancer at age 67. This is a typical pattern of paternal inheritance for breast cancer susceptibility alleles.

In Fig. 1h, the proband (II:8) and her sister (II:2), both diagnosed with ovarian cancer at the ages of 49 and 68 years, respectively, harbor the p.(Val1833Met) variant. The two daughters (III:8 and III:9) of the proband were found to carry the variant, yet are unaffected at the ages of 43 and 36 years, respectively. On the other hand, the variant was detected in one of her sister's daughters (III:3), diagnosed with breast cancer at the age of 41 years, while the other unaffected 48 years old daughter (III:2) does not carry the variant. Surprisingly, in Fig. 1b, the proband (IV:2), diagnosed with breast cancer at the age of 33 years and found to carry the p.(Val1833Met) variant, has a maternal grandmother (II:4) with a breast cancer diagnosis at the age of 58 years who was tested negative for this variant. This implies that the proband probably inherited the variant from her father; from the paternal side, a majority of males is observed, constituting a limiting factor for assessing further the pathogenicity of this variant within this family.

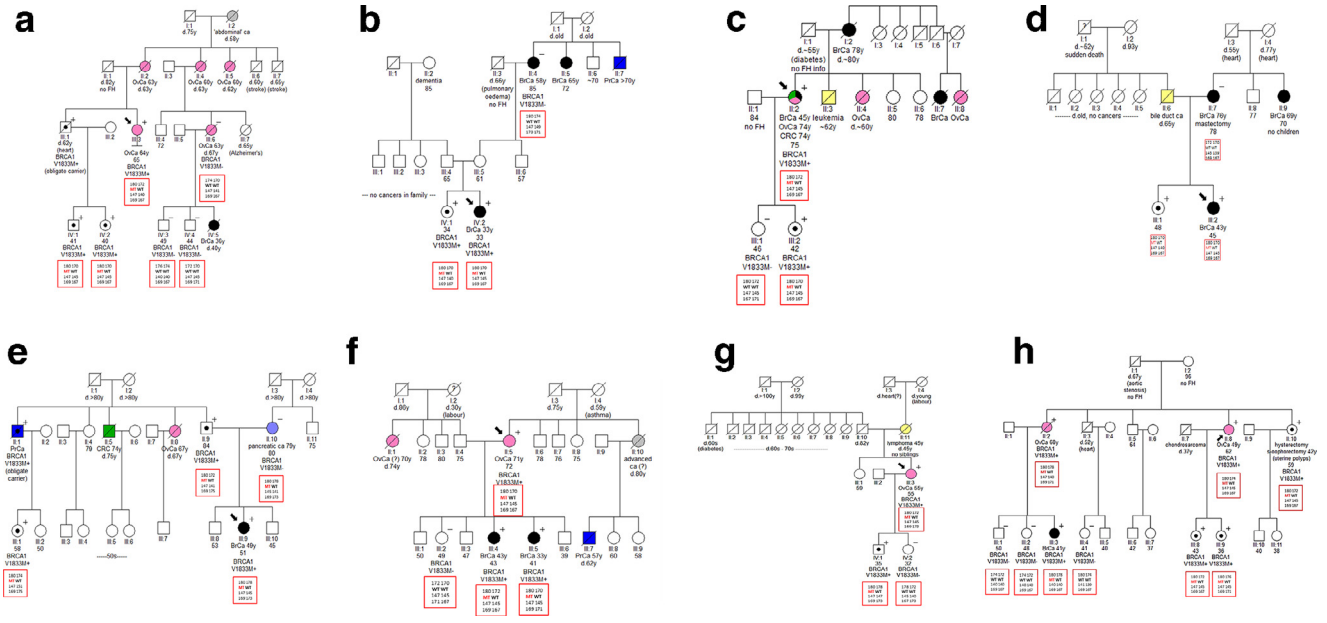

Fig. 1 Pedigrees of eight families (a-h) members of which carry the *BRCA1*, p.(Val1833Met) variant, who undergone segregation and haplotype analysis. The arrow depicts the proband and the bullets correspond to the cancer-free carriers. The plus sign indicates variant carriers, while minus sign indicates non-carriers. Breast (BrCa) and ovarian cancer (OvCa) are colored in black and pink, respectively, whereas colorectal cancer (CRC) and prostate cancer (PrCa) are colored in green and blue, respectively. Haplotype analysis is reported in red frame. *Abbreviations used: MT: Mutant, WT: Wild-Type. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)*

An interesting observation is extracted from segregation analysis in Fig. 1a. The proband (III:3), carrying the p.(Val1833Met) variant, was diagnosed with high-grade serous ovarian cancer at the age of 64 years. Her nephew (IV:1) and niece (IV:4) were found to carry this variant, although being cancer-free at the age of 41 and 40 years, respectively. Her maternal cousin (III:7), who was independently tested and was an ovarian cancer patient at the age of 63 years, was found to carry another known pathogenic variant in *BRCA1*, without being accompanied by the p.(Val1833Met) variant. This lack of coexistence with another deleterious variant reinforces the pathogenic effect of this variant.

Histopathological characteristics of p.(Val1833Met) variant carriers

Information regarding histopathology data was available for 11 out of all 13 variant carriers with breast cancer. It is noteworthy that 76.9% (10/13) were diagnosed before the age of 50 years. The predominant breast cancer type was ductal invasive (90.9%; 10/11), while one lobular breast cancer was reported. The majority of breast cancers were high grade (90%; 9/10), while 6 out of the 11 available histologies were triple negative (54.6%; 6/11) and 5 out of 10 breast tumors had lymph node involvement at diagnosis.

Among the 16 ovarian cancer cases, 13 cases had available histology reports. The majority involved serous carcinomas (76.9%; 10/13), following endometrioid carcinomas (15.3%; 2/13), while a single case was diagnosed with a mixed type (1/13). All 10 ovarian carcinomas with available information were high grade. Histopathology and family history data

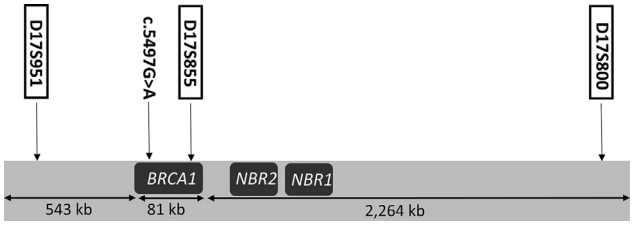

Fig. 2 Genomic map showing the three microsatellite markers (D17S951, D17S855 and D17S800) that defined the haplotype with the indicated distances, spanning a 2.76 Mb region on chromosome 17, surrounding *BRCA1* gene.

for all the carriers are summarized in Table 1. It should be noted, that 9 out of the 27 Greek families reported herein, have been initially identified in one of our previous studies [6] and more specifically these involve Families #123, 301, 460, 791, 1319, 1320, 1321, 1322 and 1323, which are listed in Table 1.

Haplotype analysis

Haplotype analysis conducted on 42 carriers, including probands and family relatives from 27 families, revealed the commonly shared haplotype '180–147–169' along a region of 2.76 Mb on chromosome 17, indicating a common ancestral origin for the *BRCA1* p.(Val1833Met) variant. The resulted haplotype was defined by two extragenic markers (D17S951 and D17S800) and one intragenic marker (D17S855). Fig. 2 depicts the particular locus extending from marker D17S951

Table 1 Summary of the histopathological characteristics and family history status of p.(Val1833Met) carriers diagnosed with breast cancer ($n=13$) and ovarian cancer ($n=16$).

Family ID	Age dx BrCa (years)	Age dx OvCa (years)	Histology	TNBC	High-grade	FH
123		59	Endometrioid		Yes	Yes
301		45	NA		NA	Yes
460		63	Serous		NA	Yes
636	66		Ductal	Yes	Yes	Yes
649	51		Ductal	No	NA	Yes
791		49	Serous		Yes	Yes
1294	33		Ductal	Yes	Yes	Yes
1319		62	Mixed		Yes	No
1320		48	NA		NA	No
1321		48	Serous		Yes	No
1322		70	NA		NA	No
1323		69	Serous		NA	No
1434	45	74	Ductal/Endometrioid	NA	Yes	Yes
1439	43		Lobular	No	No	Yes
1460	27		NA	No	NA	Yes
1967	49		Ductal	Yes	Yes	Yes
2107	52	59	Ductal/serous	No	Yes	Yes
2164	38		Ductal	Yes	Yes	Yes
2233		71	Serous		Yes	Yes
2250		48	Serous		Yes	Yes
369D		64	Serous		Yes	Yes
2390	39		NA	NA	NA	Yes
2425		55	Serous		Yes	No
2596	38		Ductal	Yes	Yes	Yes
4699	45		Ductal	Yes	Yes	No
5037		73	Serous		Yes	NA
3367	29		Ductal	No	Yes	Yes

NA: not available; TNBC: triple-negative breast cancer; dx: diagnosis; BrCa: Breast Cancer; OvCa: Ovarian Cancer, FH: Family history.

upstream of *BRCA1*, up to marker D17S800, located downstream of *BRCA1*.

In addition, 14 relatives that were non-carriers were also haplotyped, where different haplotypes were revealed (Tables A.1a, and A.1b, Supplementary Material). The detailed haplotypes among carriers and non-carriers are also illustrated in Fig. 1. Moreover, 40 cancer-free, age-matched Greek individuals were haplotyped giving the population allele frequencies for this genomic region (Table A.2, Supplementary Material). Accordingly, the founder effect of the p.(Val1833Met) variant in the Greek population is demonstrated.

Age estimation of the p.(Val1833Met) variant

The p.(Val1833Met) variant is estimated to have originated between 45 and 79 generations ago, including a time period from 1125 to 1975 years ($r=0.135$). Accordingly, the age of the variant is determined approximately at 58 generations, corresponding to 1450 years.

Evaluation of pathogenicity through co-segregation analysis

For each one of the 8 families that undergone segregation analysis for the p.(Val1833Met) variant a likelihood ratio (LR) against or in favor of pathogenicity was calculated, ranging from 0.07 to 4.74. Combining the LRs of each family, an overall likelihood ratio of 1.88 was determined. Taking into consideration that a variant with an overall likelihood ratio above 1 is assessed as pathogenic and one with an overall likelihood ratio below 1 is regarded neutral, the p.(Val1833Met) variant is considered pathogenic.

Discussion

The present study reveals that the *BRCA1*, p.(Val1833Met) variant, though extremely rare in public databases [7,8], is recurrent among Greek breast and/or ovarian cancer patients, with a possible stronger correlation to ovarian cancer diagnosis, thus constituting a disease-associated variant. According to our data, p.(Val1833Met) variant carriers are presented with histopathological characteristics that are associated to *BRCA1*-associated diagnoses, i.e. triple-negative breast cancer and high-grade serous ovarian cancer, while reporting strong family history of breast/ovarian cancer.

BRCA1, p.(Val1833Met) is a Greek founder variant which originated approximately 1450 years ago. This finding enriches the distinct mutational spectrum of Greek population, which, as previously reported, consists of at least five founder *BRCA1* pathogenic variants, four of which are large genomic rearrangements [1,24,25].

We also provided further evidence in favor of pathogenic classification of the variant. *BRCA1*, p.(Val1833Met) variant co-segregated with breast and ovarian cancer, did not co-exist with any other *BRCA1* pathogenic variant and was absent in cancer-free individuals. On top of that, the estimated overall likelihood ratio of 1.88 enhances p.(Val1833Met) causality. Although such ratio is not very outspoken, which could be attributed to family structure of the segregated families, the available family members being genotyped and the fact that missense variants do not profoundly impair protein function like truncating ones [26,27], a considerable key feature is added in the multifactorial approach of p.(Val1833Met) pathogenicity.

Rare *BRCA1* and *BRCA2* missense variants being regarded as VUS, whose reclassification involve integrating evidence of pathogenicity from different sources, leading to updated recommendations for surveillance and therapy, is a continuous challenge [28]. ENIGMA consortium (Evidence-based Network for the Interpretation of Germline Mutant Alleles) plays a crucial role in such variants' classification by collecting all available information on variants and associated phenotypes [29]. Through expert groups, interesting observations have been illustrated. Such an example is the *BRCA1*, p.(Arg1699Gln) variant, which has been characterized as an intermediate risk variant by ENIGMA, with cumulative risks of breast and ovarian cancer diagnosed being calculated as 20% and 6%, respectively [30]. This was the first such case revealing a new category of variant and disease association. In another similar study evaluating a missense variant, and more specifically, *BRCA1*, p.(Gly1770Val), through multifactorial analysis the pathogenicity of said missense variant was demonstrated [31].

Considering the data altogether, it seems reasonable to consider incorporation of p.(Val1833Met) variant in first-line screening for Greek breast and/or ovarian cancer patients, especially for those with ovarian cancer diagnosis. The identification of certain founder pathogenic variants in Greek population could improve focused screening and prevention strategies by testing at first all breast and/or ovarian cancer patients of whether they harbor any of the confirmed founder pathogenic variants or not. Thereby, cost- and time-effective genetic services could be provided, supporting the best possible clinical management of Greek patients.

At the same time, cancer-free p.(Val1833Met) variant carriers should be offered genetic counseling and subsequent clinical surveillance based on established guidelines [32], while breast and ovarian cancer patients that are variant carriers should be considered as good candidates for targeted therapies, like PARP inhibitors.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest related to this work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.06.006.

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