Prevalence of BRCA1 Mutations in Familial and Sporadic Greek Ovarian Cancer Cases

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Abstract

Germline mutations in the BRCA1 and BRCA2 genes contribute to approximately 18% of hereditary ovarian cancers conferring an estimated lifetime risk from 15% to 50%. A variable incidence of mutations has been reported for these genes in ovarian cancer cases from different populations. In Greece, six mutations in BRCA1 account for 63% of all mutations detected in both BRCA1 and BRCA2 genes. This study aimed to determine the prevalence of BRCA1 mutations in a Greek cohort of 106 familial ovarian cancer patients that had strong family history or metachronous breast cancer and 592 sporadic ovarian cancer cases. All 698 patients were screened for the six recurrent Greek mutations (including founder mutations c.5266dupC, p.G1738R and the three large deletions of exon 20, exons 23–24 and exon 24). In familial cases, the BRCA1 gene was consequently screened for exons 5, 11, 12, 20, 21, 22, 23, 24. A deleterious BRCA1 mutation was found in 43/106 (40.6%) of familial cancer cases and in 27/592 (4.6%) of sporadic cases. The variant of unknown clinical significance has p.V1833M was identified in 9/698 patients (1.3%). The majority of BRCA1 carriers (71.2%) presented a high-grade serous phenotype. Identifying a mutation in the BRCA1 gene among breast and/or ovarian cancer families is important, as it enables carriers to take preventive measures. All ovarian cancer patients with a serous phenotype should be considered for genetic testing. Further studies are warranted to determine the prevalence of mutations in the rest of the BRCA1 gene, in the BRCA2 gene, and other novel predisposing genes for breast and ovarian cancer.

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Introduction

Ovarian cancer is one of the highest mortality rated malignancies, an aspect mainly attributed to the advanced stage at diagnosis. Although new predisposing genes have been identified lately, the important players to ovarian cancer susceptibility are still the known BRCA1 and BRCA2 genes. Carriers of inherited mutations in BRCA1 and BRCA2 genes face a lifetime risk of ovarian cancer of 35–60% (average age of diagnosis 50 years) and 12–25% (average age of diagnosis 60 years) respectively, and also an elevated risk of fallopian tube and peritoneal carcinomas [1–3].

BRCA1 and BRCA2-associated ovarian malignancies have a distinct clinical phenotype, the majority of which being high-grade serous, advanced stage carcinomas [4], while generally are being associated with overall longer survival [5,6]. Moreover, the BRCA1/2 mutation status of an ovarian cancer patient can be an important aspect in regards to the decision of chemotherapy; BRCA1/2 carriers show increased sensitivity to platinum-based therapy [6,7], as well as to poly-ADP-ribose polymerase inhibitors [8].

Hereditary ovarian cancer can also occur in the context of Lynch syndrome, which is caused by inherited germline mutations within the MMR genes. The cumulative risk of ovarian cancer in MMR mutation carriers is estimated to be 10%, while histologically these tumours are of the endometrioid subtype in most cases [9].

Hereditary ovarian cancer is probably underestimated, since recent studies highlight new susceptibility genes (RAD51C, RAD51D, PALB2) that might predispose for ovarian cancer, but their exact prevalence is still under investigation [10–13]. Up-to-date sequencing technologies that provide the opportunity to test massively multiple targeted genes have been already applied in
ovarian cancer genetics. The most interesting is the test based on BROCA chip, a highly sensitive panel of 21 tumor suppressor genes, which tested 360 ovarian cancer cases and successfully identified deleterious mutations in 12 known ovarian susceptibility genes, with the substantial proportion represented by BRCA1 and BRCA2 mutations [13].

The prevalence of BRCA1 and BRCA2 mutations in ovarian cancer patients varies amongst populations; a quite thorough population study in North America demonstrates a 13–15% frequency of germline BRCA1/2 mutations in sporadic ovarian cases [14,15]. The prevalence of mutations can rise up to 30–40% in populations such as Ashkenazi Jews, where a number of founder mutations are apparent [16].

Although the Greek population is characterized by genetic heterogeneity, our extensive 15-year research on hereditary breast/ovarian cancer has highlighted the existence of 6 recurrent mutations including four founder (c.5266dupC, p.G1738R, BRCA1 and BRCA2 mutations in sporadic ovarian cases [17–21]. This bipolar project focuses on the prevalence of BRCA1 mutations among 106 familial and 592 sporadic Greek ovarian cancer cases with the simultaneous correlation of clinicopathological tumour features.

Materials and Methods

Patient Study Group

The study group consisted of patients with epithelial ovarian cancer that were recruited from various hospitals around Greece in collaboration with the Hellenic Cooperative Oncology Group (HeCOG) between 2000 and 2012. Corresponding demographic, clinicopathological data had been registered for the majority of recruited patients in the frame of clinical service in HeCOG-affiliated hospitals. Samples from 698 patients in total, selected on the basis of a diagnosis of ovarian carcinoma, were analyzed for mutations in BRCA1. The 698 patients were categorized as: (a) Familial cases: 106 ovarian cancer patients with at least one first degree relative presented with either breast and/or ovarian cancer or cancer patients presented with breast and ovarian cancer. 53/106 (50%) patients had both breast and ovarian cancer, 29 of which had family history and 24 with no family history. The mean age of diagnosis was 49 years, ranging from 24 to 72 years old, and (b) sporadic cases: 592 ovarian cancer cases were included based on their diagnosis of ovarian cancer only and had no reported family history. The mean age of diagnosis was 59.1 years, ranging from 17 to 82 years old.

All patients had signed an informed consent form permitting the use of their biologic material for research purposes and a research nurse explained the purpose of the study to them; the possibility of increased risk of ovarian cancer (and possibly other cancers) in family members were explained in detail. Patients donated 10 ml of blood for genetic analysis as well as completed a questionnaire containing details on other cancers in family members. In cases where a mutation was identified, diagnosis and family history was confirmed and segregation analysis was performed. Furthermore, family relatives of mutation carriers were informed in specialized counselling sessions and if consented to genetic analysis, they were tested for the specific mutation. Histology report, where available, was provided by the originating hospital, from which information regarding tumour type, grading and staging was extracted. The study was approved by the Bioethics Committees of the IRB of Papageorgiou Hospital of Thessaloniki and NCSR “Demokritos”.

Mutation Analysis

Total genomic DNA was isolated from peripheral blood lymphocytes following the salt extraction procedure. DNA quantitation was assessed by UV absorbance using a Nanodrop™ ND-1000 spectrophotometer (Thermo Fisher Scientific, MA, USA). BRCA1 (NM_007294.3) mutation screening was based on a population-specific hierarchical protocol described previously (18), aiming to reduce time and cost involved. Two different experimental protocols were designed: For sporadic cases, screening was performed only for the six most common BRCA1 mutations identified in the Greek population. Exon 20 of the BRCA1 gene (encompassing the founder mutations: p.G1738R & c.5266dupC and the recurrent p.R1751X mutation) was analyzed by direct sequencing, while the three Greek founder genomic rearrangements involving exons 20, 23 and 24 were assessed by three individual diagnostic PCRs [20] (Pertesi et al. in preparation). Additionally, in familial cases full sequencing of exons 5, 11, 12, 20, 21, 22, 23, 24 (which cover 70% of the gene’s coding sequence) was performed. These regions contain all BRCA1 mutations previously identified in the Greek population. All PCR amplifications were performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA). PCR product purification was performed using a vacuum driven ultrafiltration purification system, where PCR samples are transferred to a filter plate with a membrane resin which retains the PCR products free from non-incorporated nucleotides and primers (Macherey-Nagel, Düren, Germany). Sequencing reactions were performed using the v.3.1 BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and PCR products were electrophoresed on the ABI Prism® 3130xl Genetic Analyzer. Sequences obtained were aligned, using Sequencher® PC software (Gene Codes, USA), with reference sequences from Genbank (NG_005905.2) and examined for the presence of mutations. Upon mutation identification, an independent blood sample was drawn from the patient and the mutation was confirmed by bi-directional sequencing. In addition statistical analysis was performed using the chi-square test.

Real-Time PCR (Allele Discrimination)

Allele discrimination of the c.5497G>A (p.V1833M) variant in the sporadic cohort was performed by TaqMan® assay using a Mx3000PTM Real-Time PCR System (Stratagene, USA) and analyzed using the allelic discrimination endpoint analysis with data collected at the end of the PCR process. Each reaction included a primer pair used to amplify the 106 bp product and two fluorescent probes, labelled with two spectrally distinct dyes, namely FAM (G) and HEX (A), allowing genotyping of the two possible variants at the single-nucleotide polymorphism (SNP) site in a target template sequence. The KAPA PROBE FAST Universal qPCR Master mix kit (KapaBiosystems) was used, containing all components except primers and probes. ROX reference dye was additionally added in each reaction mix. PCR amplification was performed in a 20 μl reaction using 30 ng genomic DNA, and a fast-2-step cycling protocol with the following conditions: enzyme activation at 95°C for 3 min, and 40 cycles of denaturation at 95°C for 3 sec and annealing/extension at 60°C for 15 sec. Post-run analysis determined either a normal homozygote (samples having both alleles normal) or heterozygote (samples having normal allele as well as the BRCA1 allele variant c.5497G>A). The Ct value of an allele-specific probe highlights the presence of the examined SNP. Heterozygotes were confirmed by direct DNA sequencing. Threshold fluorescence (used to determine Ct values) was adjusted by baseline-
corrected normalized fluorescence (dRn). Probe and primer sequences used are available from the authors upon request.

Results

106 familial and 592 sporadic ovarian cancer cases were analyzed to determine the frequency of the six most common mutations of the BRCA1 gene in the Greek population, namely c.5266dupC, p.Gly1738Arg, p.Arg1751X, deletion of exon 20 (c.5256_5277+3179del3200), deletion of exon 24 (c.5468-285_5592+4019del1429 InsCAG) and deletion of both exons 23 and 24 (g.169527_180579del11052). Considering the mutation prevalence of only the above six recurrent mutations, there is a substantial difference between familial (25.5%, 27/106) and sporadic (4.4%, 26/592) (p-value <0.0001). Familial cases were further screened for mutations in all BRCA1 exons where a mutation was previously identified in the Greek population (exons 5, 11, 12, 20, 21, 22, 23, 24), increasing mutation prevalence in this group to 40.6% (43/106). All mutations found, together with carriers’ age of onset and histology, are shown in Table 1 for familial cases and in Table 2 for sporadic cases. Interestingly, an extremely rare variant of unknown clinical significance (VUS), BRCA1 p.V1833M (rs80357268), was found in 9 patients (1.3%) out of the 698 in total. Prevalence in the sporadic group, screened by real-time PCR, was 0.85% (5/592), while 3.7% (4/106) in the familial group, screened by direct sequencing of exon 24 (Table 3).

Interestingly, all familial patients that carried this specific variant had a family history of breast and/or ovarian cancer. Segregation analysis done in one of the families tested positive showed that the p.V1833M variant co-segregated with the disease, suggesting a possible deleterious effect (Fig. 1). A broad cross-validation, based on biochemical and cell-based transcriptional assay, of BRCT missense variants highlighted the severe folding defect and the compromised effect on transcription of the p.V1833M variant [22]. Due to the scarcity of this variant no other segregation data were found in literature, however all available in vitro and in silico evaluation data agree upon its classification as deleterious [23–25]. In addition, no allele frequency was observed in dbSNP. In the present study, carriers of this variant are not included in our results as positive for a mutation, as we feel more solid clinical and functional data are needed to confirm its pathogenicity.

The age of onset distribution in BRCA1 carriers compared to total familial cases, as well as for BRCA1 carriers compared to the total sporadic cases is shown in Fig. 2. Mean age of onset in BRCA1 carriers in the familial cohort was 48.5 years, whereas in the total group was 49 years, with a range of 24–72 years. In the sporadic group, mean age of onset was 54.2 years for carriers and 59.1 years in the total group, with a range of 17–82 years. Among the 106 familial patients tested, 53 have developed both breast and ovarian cancer (50%). Of the 29 patients who developed both breast and ovarian cancer and had a family history 14/29 (48.3%) carried a BRCA1 mutation, whereas from those that had no clear family history 11/24 (45.8%) carried a BRCA1 mutation. However, there is no significant difference between the two groups (p-value 0.8593). Also, the majority of the familial (72.6%) and sporadic (64.6%) cohorts comprised of the serous type of ovarian carcinomas. Correlation between carriers and the total groups of both familial and sporadic cases showed that in accordance with other studies most of the BRCA1 carriers were of the serous type and there is no statistically significant difference between the two groups (p-value 0.1022). Serous histology presented in 78.8% of the familial BRCA1 carriers (Table 4) and in 61.5% of the BRCA1 carriers in the sporadic cohort (Table 5).

Discussion

In this study we have established the prevalence of BRCA1 mutations in a group of 698 Greek ovarian cancer patients, 106 of which were familial cases and 592 apparently sporadic. We identified 70 pathogenic mutations in all (10%), 43 in the familial cohort (40.6%) and 27 in the sporadic cohort (4.6%). To our knowledge, this is the first such study in the Greek population. A number of similar studies in other populations have been published, giving prevalence for BRCA1 mutations in ovarian cancer patients from 8 to 11%. Variation could be due to a number of reasons, mainly experimental design and populations with different degrees of genetic heterogeneity. A Canadian study of 1342 ovarian cancer cases revealed a combined mutation frequency of 13.3% of both BRCA1 and BRCA2, 8% of which was BRCA1 [14]. In another study 11.5% of all ovarian cancer cases in Colombia were attributable to a single BRCA1 founder mutation, while 15.6% of the total cohort was positive for mutations in either BRCA1 or BRCA2 [26]. 5.8% of a population-based series of ovarian cancer cases in Denmark were also found to be positive for BRCA1/2 mutations [27]. Of 209 women in the Tampa Bay area with invasive ovarian carcinoma, 15.3% had mutations in BRCA1 or BRCA2, 9.5% of which was due to BRCA1 mutations [15]. Similarly, in a recent Australian study, 8.8% (88/1001) of ovarian cancer patients tested had a BRCA1 mutation [28]. In a Polish study, BRCA1 or BRCA2 germline mutations were found in 13.9% of consecutive ovarian cancer patients, 11% of which was attributable to BRCA1 [29]. In a Swedish study, 13/161 (8%) of the patients were found to carry a BRCA1 or BRCA2 mutation, with 12/13 cases being BRCA1-positive [30].

Finally, two pioneer studies using next-generation sequencing techniques [13,31] reported an 18% prevalence in BRCA1 & BRCA2 (11.3% for BRCA1) mutations in ovarian cancer patients, while the Cancer Genome Atlas (TCGA) Research Network reported 14% (8.5% for BRCA1) [32].

The frequency of 10% for BRCA1 mutations found in the present study in all patients screened, regardless of family history, is consistent with previous observations in other populations. Given the fact that not the entire BRCA1 gene was screened, this percentage is probably an underestimate of the true frequency in ovarian cancer patients in our population. We estimate that the true frequency could be up to 20% higher, as in 85% of our cohort (592/698 patients) screening included only the Greek founder or recurrent mutations, representing 73% of all BRCA1 mutations observed in this population [17–21]. Based on this figure, we support the recommendation of at least BRCA1 testing for all ovarian cancer patients, regardless of family history and age of diagnosis.

The notably high prevalence of BRCA1 mutations in our familial patient group (40.6%) places patients in this category (personal history of ovarian cancer and at least one family member with breast and/or ovarian cancer, including personal history of breast cancer) in a high-risk setting, making screening for BRCA1 mutations mandatory. Previous studies are consistent with this observation and recommendation, reporting BRCA1 mutation prevalence in hereditary ovarian cancer patients from 24–66% [33]. In accordance with other studies, the age of disease onset was not included in our criteria on selecting familial ovarian cancer patients. Walsh et al. [13] have shown that >35% of carriers of a predisposing allele for ovarian cancer are over 60 years of age at diagnosis.

This study also showed that the most common phenotype of BRCA1-associated ovarian carcinomas is high-grade serous histology and advanced stage disease. Other histological types were also
Table 1. BRCA1 pathogenic mutations identified in familial cases.

| Patient | Exon | Mutation (cDNA) | Mutation (protein) | Age of onset | Type | Grade | Stage | Other primary cancers in proband (Age) | Family history (including proband) |
|---------|------|-----------------|--------------------|--------------|------|-------|-------|--------------------------------------|----------------------------------|
| 406     | 5    | c.181T>G        | p.Cys61Gly         | 39           | serous papillary adenocarcinoma | III   | lc    | _                                   | 1 BrCa <40y & 1 OvCa            |
| 1126    | 6    | c.181T>G        | p.Cys61Gly         | 52           | N.A. | N.A.  | N.A.  | _                                   | 3 BrCa (1 BrCa <40y), 1 OvCa    |
| 98      | 11   | c.1504_1508delTTAAA | p.Leu502fs         | 44           | serous papillary cystadenocarcinoma | N.A.  | IV    | _                                   | 1 BrCa <30y & 1 OvCa            |
| 99      | 11   | c.2980delT      | p.Leu203X          | 46           | papillary serous                  | II–III| N.A.  | BrCa 26y & bilateral BrCa 40y       | 4 BrCa <40y & 2 OvCa            |
| 483     | 12   | c.3775_3776delTC | p.Ser1125fs        | 38           | N.A. | N.A.  | N.A.  | _                                   | 1 BrCa <30y +1 OvCa             |
| 1181    | 11   | c.3750_3754delTG | p.Leu1218fs        | 48           | serous papillary adenocarcinoma   | N.A.  | N.A.  | BrCa 48y                            | 1 BrCa+OvCa                     |
| 230     | 11   | c.3607C>T       | p.Arg1203X         | 46           | papillary serous                  | II–III| N.A.  | BrCa 26y & bilateral BrCa 40y       | 4 BrCa <40y & 2 OvCa            |
| 1152    | 11   | c.1505T>G       | p.Leu502x          | 51           | bilateral serous                  | III   | N.A.  | _                                   | 2 OvCa & 2 BrCa                 |
| 1181    | 11   | c.1210insCT     | p.Glu404fs         | 50           | N.A. | N.A.  | BrCa 39y | 1 BrCa <40y & 2 OvCa          |
| 483     | 12   | c.4168_4169delTG | p.Ser1380fs        | 57           | N.A. | N.A.  | N.A.  | _                                   | 1 BrCa <35y & 2 OvCa            |
| 1077    | 19   | c.5161A>C       | p.Gln1721Pro*      | 35           | bilateral serous                  | III   | N.A.  | _                                   | 2 OvCa                          |
| 272     | 20   | c.5194_5277+3638del174 | p.His1732_Lys1759del | 52           | serous cystadenocarcinoma        | II–III| N.A.  | BrCa 40y                            | 1 BrCa+OvCa                     |
| 398     | 20   | c.5212G>A       | p.Gly1738Arg       | 57           | adenocarcinoma                    | N.A.  | IV    | _                                   | 2 OvCa                          |
| 798     | 20   | c.5212G>A       | p.Gly1738Arg       | 41           | adenocarcinoma                    | N.A.  | N.A.  | BrCa 39y                            | 1 BrCa+OvCa                     |
| 287     | 20   | c.5212G>A       | p.Gly1738Arg       | 32           | N.A. | N.A.  | N.A.  | _                                   | 1 BrCa <50y & 2 OvCa            |
| 99      | 20   | c.5212G>A       | p.Gly1738Arg       | 68           | serous cystadenocarcinoma         | III   | N.A.  | BrCa 72y                            | 1 BrCa+OvCa                     |
| 439     | 20   | c.5251C>T       | p.Arg1751X         | 48           | N.A. | N.A.  | N.A.  | _                                   | 6 BrCa (1 BrCa+OvCa) & 2 OvCa   |
| 290     | 20   | c.5251C>T       | p.Arg1751X         | 61           | serous adenocarcinoma             | N.A.  | III  | BrCa 66y                            | 1 BrCa+OvCa                     |
| 288     | 20   | c.5251C>T       | p.Arg1751X         | 40           | serous papillary cystadenocarcinoma | III  | IV   | BrCa 30y                            | 3 BrCa <50y (1 BrCa+OvCa <40y) |
| 189     | 20   | c.5251C>T       | p.Arg1751X         | 42           | serous                          | III   | N.A.  | BrCa 41y                            | 2 BrCa <40y (1 BrCa+OvCa)       |
| 1024    | 20   | c.5256_5277+3179del3200 | p.Arg1753fs     | 48           | serous papillary cystadenocarcinoma | III   | N.A.  | _                                   | 3 BrCa (1 BrCa <50y) & 1 OvCa (1 BrCa+OvCa) |
| 428     | 20   | c.5256_5277+3179del3200 | p.Arg1753fs     | 56           | N.A. | N.A.  | BrCa 58y | 1 BrCa+OvCa & 1 BrCa          |
| 698     | 20   | c.5256_5277+3179del3200 | p.Arg1753fs     | 48           | N.A. | N.A.  | BrCa 58y & 42y | 4 BrCa (3 BrCa <45y) & 2 OvCa |
| 296     | 20   | c.5266dupC      | p.Gln1756fs        | 56           | serous cystadenocarcinoma         | II    | IV   | BrCa 48y                            | 1 BrCa+OvCa                     |
| 280     | 20   | c.5266dupC      | p.Gln1756fs        | 45           | N.A. | N.A.  | N.A.  | _                                   | 3 OvCa                          |
## Table 1. Cont.

| Patient | Exon | Mutation (cDNA) | Mutation (protein) | Age of onset | Type | Grade | Stage | Other primary cancers in proband (Age) | Family history (including proband) |
|---------|------|-----------------|--------------------|--------------|------|-------|-------|--------------------------------------|----------------------------------|
| 1186    | 20   | c.5266dupC      | p.Gln1756fs        | 49           | serous papillary adenocarcinoma | III  | III  | _                                   | 1 BrCa < 30y & 3 OvCa            |
| 520     | 20   | c.5266dupC      | p.Gln1756fs        | 35           | bilateral endometrioid carcinoma | N.A. | III  | _                                   | 1 bil OvCa                       |
| 344     | 20   | c.5266dupC      | p.Gln1756fs        | 50           | serous cyst-adenocarcinoma      | III  | N.A. | BrCa (72y)                          | 2 BrCa (1 BrCa < 40y) & 1 OvCa   |
| 365x    | 20   | c.5266dupC      | p.Gln1756fs        | 54           | clear cell                      | III  | N.A. | _                                   | 1 BrCa < 35y & 2 OvCa            |
| 155     | 23   | c.5467G>A       | p.1803_1822del20   | 50           | serous papillary cystadenocarcinoma | I    | III  | BrCa (40y)                          | 2 BrCa (1 BrCa + OvCa)           |
| 159     | 23   | c.5434C>G       | p.Pro1812Ala**     | 61           | N.A.                            | N.A. | N.A. | BrCa                                | 1 BrCa + OvCa                    |
| 284     | 24   | c.5468-285_5592+4019del4429_insCACAG | p.0          | 52           | endometrioid carcinoma          | N.A. | N.A. | BrCa (53y)                          | 2 BrCa (1 BrCa + OvCa)           |
| 1174    | 24   | c.5468-285_5592+4019del4429_insCACAG | p.0          | 53           | serous papillary cystadenocarcinoma | II   | N.A. | BrCa (61y)                          | 1 BrCa + OvCa, 5 BrCa            |
| 332     | 24   | c.5468-285_5592+4019del4429_insCACAG | p.0          | 31           | bilateral serous cystadenocarcinoma | N.A. | N.A. | BrCa (28y)                          | 1 BrCa + OvCa                    |
| 440     | 24   | c.5468-285_5592+4019del4429_insCACAG | p.0          | 48           | undifferentiated carcinoma       | N.A. | N.A. | BrCa (69y)                          | 1 BrCa + OvCa                    |
| 469     | 24   | c.5468-285_5592+4019del4429_insCACAG | p.0          | 51           | serous surface papillary         | III  | IV   | _                                   | 1 BrCa & 1 OvCa                  |
| 416     | 23,24| g.169527-180579del11052 | p.Gly1803_Tyr1863del11052 | 39           | serous papillary adenocarcinoma  | N.A. | N.A. | BrCa (37y)                          | 1 BrCa + OvCa                    |
| 1036    | 23,24| g.169527-180579del11052 | p.Gly1803_Tyr1863del11052 | 51           | serous                          | III  | N.A. | BrCa (51y)                          | 3 BrCa (1 BrCa < 50y) & 2 OvCa (1 BrCa + OvCa) |
| 661     | 23,24| g.169527-180579del11052 | p.Gly1803_Tyr1863del11052 | 67           | N.A.                            | N.A. | N.A. | BrCa (47y & 60y)                    | 5 BrCa & 2 OvCa                  |
| 1247    | 23,24| g.169527-180579del11052 | p.Gly1803_Tyr1863del11052 | 36           | serous papillary adenocarcinoma  | III  | III  | _                                   | 3 OvCa (3 OvCa < 35y)            |

*Unclassified variant.
**Splicing variant causing exon 23 skipping [47].
N.A. Non Applicable.
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### Table 2. BRCA1 pathogenic mutations identified in sporadic cases.

| Patient | Exon | Mutation       | Age of onset | Type                        | Grade | Stage |
|---------|------|----------------|--------------|-----------------------------|-------|-------|
| 944     | 5    | p.C61G         | 59           | serous papillary cystadenocarcinoma | III   | N.A.  |
| 399     | 20   | p.G1738R       | 61           | endometrioid                | III   | N.A.  |
| 915     | 20   | p.G1738R       | 67           | serous papillary cystadenocarcinoma | II    | IV    |
| 1129    | 20   | p.G1738R       | 51           | adenocarcinoma              | III   | N.A.  |
| 1079    | 20   | p.G1738R       | 53           | serous papillary            | III   | N.A.  |
| 952     | 20   | p.R1751X       | 55           | clear cell mesonephroid     | III   | IV    |
| 737     | 20   | p.R1751X       | 69           | serous papillary cystadenocarcinoma | N.A.  | IV    |
| 1205    | 20   | c.5266dupC     | 56           | papillary cystadenocarcinoma | III   | N.A.  |
| 899     | 20   | c.5266dupC     | 51           | endometrioid                | III   | IV    |
| 1078    | 20   | c.5266dupC     | 63           | endometrioid                | III   | IV    |
| 709x    | 20   | c.5266dupC     | 65           | N.A.                        | N.A.  | N.A.  |
| 1080    | 20   | c.5266dupC     | 58           | serous                      | III   | N.A.  |
| 946     | 20   | c.5266dupC     | 57           | endometrioid adenocarcinoma | III   | IV    |
| 951     | 20   | c.5266dupC     | 39           | serous papillary cystadenocarcinoma | III   | IIIc  |
| 521     | 20   | c.5266dupC     | 40           | serous cystadenocarcinoma   | N.A.  | IV    |
| 954     | 20   | c.5266dupC     | 45           | serous papillary cystadenocarcinoma | III   | IIIb  |
| 955     | 20   | c.5266dupC     | 55           | serous papillary cystadenocarcinoma | I     | IIIc  |
| 900     | 20   | c.5256,5277+3179del1200 | 65 | serous papillary cystadenocarcinoma | III   | IV    |
| 1121    | 24   | c.5468-285_5592+4019del4429_insCACAG | 46 | endometrioid               | III   | N.A.  |
| 1130    | 24   | c.5468-285_5592+4019del4429_insCACAG | 49 | papillary cystadenocarcinoma | III   | N.A.  |
| 893     | 24   | c.5468-285_5592+4019del4429_insCACAG | 50 | papillary cystadenocarcinoma | III   | IV    |
| 896     | 24   | c.5468-285_5592+4019del4429_insCACAG | 59 | serous papillary cystadenocarcinoma | III   | IIa   |
| 1055    | 23,24 | g.169527_180579del11052 | 68 | adenocarcinoma          | N.A.  | N.A.  |
| 1056    | 23,24 | g.169527_180579del11052 | 39 | mucinous cystadenocarcinoma | II    | IIIc  |
| 1057    | 23,24 | g.169527_180579del11052 | 49 | papillary cystadenocarcinoma | III   | IIIc  |
| 1058    | 23,24 | g.169527_180579del11052 | 59 | serous                  | N.A.  | N.A.  |
| 1214    | 23,24 | g.169527_180579del11052 | 34 | undifferentiated carcinoma | N.A.  | N.A.  |

### Table 3. Showing the characteristics of patients carrying the BRCA1 p.V1833M variant.

| Patient | Exon | Mutation       | Age of onset | Type                        | Grade | Stage |
|---------|------|----------------|--------------|-----------------------------|-------|-------|
| 426     | 24   | p.V1833M       | 62           | N.A.                        | N.A.  | N.A.  |
| 2043    | 24   | p.V1833M       | 48           | serous papillary            | III   | N.A.  |
| 2118    | 24   | p.V1833M       | 48           | serous papillary cyst-adenocarcinoma | III   | N.A.  |
| 3249    | 24   | p.V1833M       | 71           | Endometrioid                | III   | N.A.  |
| 3275    | 24   | p.V1833M       | 69           | Serous                      | II    | N.A.  |
| 123     | 24   | p.V1833M       | 59           | Endometrioid                | III   | 2 OvCa|
| 301     | 24   | p.V1833M       | 45           | N.A.                        | N.A.  | 2 OvCa & 1 BrCa <50y |
| 460     | 24   | p.V1833M       | 63           | serous papillary cyst-adenocarcinoma | N.A.  | IV    |
| 791     | 24   | p.V1833M       | 49           | serous adenocarcinoma       | III   | 2 OvCa|

Table 2. BRCA1 pathogenic mutations identified in sporadic cases.

Table 3. Showing the characteristics of patients carrying the BRCA1 p.V1833M variant.
observed in *BRCA1* carriers, the second most common being the endometrioid type, but there were also clear cell, adenocarcinomas, undifferentiated carcinoma and other types (Tables 4, 5). Recent histopathological studies on early stage lesions of ovarian cancer in *BRCA1* or *BRCA2* carriers have suggested that the primary origin of high-grade serous ovarian cancer is the fallopian tube, implicating both genes as key players in the fallopian differentiation [34–39]. In our study, 61.5% of the *BRCA1* carriers of the sporadic cases and 78.8% of the familial cases had a serous histology. Further studies on the histology of ovarian cancer subtypes and the molecular events that control the initiation and progression of serous cancers are warranted by a number of research groups.

Nowadays, genetic testing is offered primarily to patients with early onset breast/ovarian cancer (<45 years) or when a strong family history is present, and it primarily involves analysis of *BRCA1* and *BRCA2* genes. Clinical intervention strategies are offered to carriers that will dramatically reduce ovarian cancer risk [33]. The most effective preventive measure today for ovarian and fallopian tube cancer, is salpingo-oophorectomy by the age of 40 years and after completion of child bearing. Unfortunately, ovarian cancer surveillance has not yet been proven effective [40].

In our study, 48.3% of familial patients with breast/ovarian cancer that carry a *BRCA1* mutation have a family history. Furthermore, 45.8% of breast/ovarian cancer patients with inherited *BRCA1* and *BRCA2* mutations do not have a clear family history due to the small family structure, predominance of males in the family and/or paternal inheritance, adoption or non-biological father. *BRCA1* and *BRCA2* mutation carriers in these families are at the same risk level for breast and ovarian cancer as women from high-incidence families. At present, women from such families rarely use genetic services. There is no significant difference between the percentage of *BRCA1* mutation carriers in patients with breast/ovarian cancer and those without a family history. This further supports what is highlighted by all recent mutation prevalence studies, including the present one, that all women with ovarian, fallopian tube, or peritoneal carcinoma should undergo comprehensive genetic testing, regardless of age or family history. However, the screening strategy being employed until now, testing one gene at a time is costly, laborious and time-consuming. Analysing first the most commonly mutated regions in each population, provides a population-specific, cost-effective way for testing hereditary breast/ovarian cancer patients for mutations in predisposing genes. This study provides a fast protocol, for testing all ovarian cancer patients in Greece for mutations in the *BRCA1* gene. Massively parallel sequencing is now being used in order to sequence many genes simultaneously at low cost.

It has now emerged that more ovarian cancer patients carry cancer-predisposing mutations and in more genes than previously appreciated. Recent studies using next-generation sequencing technologies have identified more than 12 low-to-medium penetrance novel susceptibility genes that might predispose for ovarian cancer, but their exact prevalence is still under investigation [13]. Thus, inherited breast/ovarian cancer is genetically highly heterogeneous with respect to both genetic loci and alleles involved.

Furthermore, despite mutations in cancer predisposing genes there are also a number of other molecular aberrations that cause ovarian cancer. It is critical to identify these aberrations as they will lead to the development of novel therapeutic strategies. The Cancer Genome Atlas project has analysed 489 high-grade serous ovarian adenocarcinomas for genomic and epigenomic alterations [32] and identified three microRNA subtypes, four promoter methylation subtypes, four ovarian cancer transcriptional subtypes, and a transcriptional signature associated with survival duration. In contrast to other histological types of ovarian cancer, 96% of high-grade serous (HGS)-OvCa samples had TP53 mutations leading to FOXM1 overexpression and 22% of tumours
Figure 2. Age of onset distribution in familial and sporadic cases. A) Age of onset distribution among the total of 106 familial patients with ovarian cancer and 41 patients with a BRCA1 mutation. B) Age of onset distribution among 492/592 of sporadic patients and 27/27 patients with a BRCA1 mutation.

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Table 4. Tumour histology in familial ovarian cancer cases compared to that of BRCA1 carriers in each group. Histological type was known for 33/43 BRCA1 carriers and 62/106 in total.

| Tumour histology | BRCA1 carriers | Total No. of cases |
|------------------|----------------|--------------------|
| Serous           | 26 (78.8%)     | 45 (72.6%)         |
| Mucinous         | 0              | 0                  |
| Clear cell       | 1 (3%)         | 1 (1.6%)           |
| Endometrioid     | 2 (6.9%)       | 8 (13.8%)          |
| Undifferentiated | 1 (3.45%)      | 1 (1.72%)          |
| Adenocarcinoma   | 3 (10.3%)      | 6 (10.3%)          |
| Mixed            | 0              | 0                  |
| other            | 0              | 1 (1.72%)          |

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Table 5. Tumour histology in sporadic ovarian cancer cases compared to that of BRCA1 carriers in each group. Histological type was known for 26/27 BRCA1 carriers and 450/592 in total.

| Tumour histology | BRCA1 carriers | Total No. of Cases |
|------------------|----------------|--------------------|
| Serous           | 16 (61.5%)     | 291 (64.6%)        |
| Mucinous         | 1 (3.8%)       | 15 (3.3%)          |
| Clear cell       | 1 (3.8%)       | 24 (5.3%)          |
| Endometrioid     | 5 (19.2%)      | 44 (9.7%)          |
| Undifferentiated | 1 (3.8%)       | 7 (1.5%)           |
| Adenocarcinoma   | 2 (7.7%)       | 21 (4.6%)          |
| Mixed            | 0              | 7 (1.5%)           |
| other            | 0              | 41 (9.1%)          |

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had germline or somatic mutations in BRCA1 and BRCA2. This study also showed that BRCA1 and BRCA2 mutations had a positive impact on survival while BRCA1 epigenetically silenced cases have poorer outcomes.

Identifying individuals with mutations in breast/ovarian cancer susceptibility genes is clinically important as BRCA1/2 carriers have been shown to have increased sensitivity to the novel poly-ADP-ribose (PARP) inhibitors [41,42]. It is hoped that this novel treatment will benefit both familial and sporadic ovarian or breast tumours that lack BRCA1/2 expression. In addition, BRCA1/2 carriers are also more sensitive to platinum-based chemotherapy [43,44]. Several studies have also demonstrated improved survival outcomes in carriers versus non-carriers [5,45,46].

With the advance and speed of mutation detection strategies nowadays, it is anticipated that more ovarian cancer cases will be avoided as hereditary cancers will be identified before disease onset and preventive measures would be employed.

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Author Contributions

Conceived and designed the experiments: AVS IK DY FF. Performed the experiments: AVS FF MP MT GEV. Analyzed the data: AVS IK DY FF. Contributed reagents/materials/analysis tools: AB MAD ET DP GC GK CP TM GP GA VK GF OT. Wrote the paper: AVS FF IK DY.

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