

***BRCA1/BRCA2* variants of uncertain significance in clinical practice: A case report**

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Abstract. The influence of *BRCA1/2* variants of uncertain significance (VUSs) on the cancer risk and their association with the response to treatment is uncertain. The aim of the present study was to evaluate the role of *BRCA* VUS in patients with breast cancer. A total of two cases of breast cancer patients with the *BRCA* VUS were described. The complete coding sequence of *BRCA1/2* genes was analyzed from the genomic DNA material by next generation sequencing on the Ion Torrent platform. The presence of c.3454G>A (p.Asp1152Asn) VUS in the *BRCA1* gene was reported in a 64-year-old woman with invasive breast carcinoma. The characteristics of the breast tumors were the following: moderately differentiated-intermediate grade (NG-2 G-2), HER2 (+), estrogen receptor (ER) (+++), progesterone receptor (PR) (+++), luminal A subtype and pT2 N1a Mx. The second detected VUS was the c.2374T>C (p.Tyr792His) variant in the *BRCA2* gene. This variant was reported in a 33-year-old woman who was diagnosed with right breast cancer (cT2N1M0). The invasive breast carcinoma was characterized as follows: NG-2 G-2, ER (+++), PR (+++), Ki-67 10%, HER2 (+++) and luminal B subtype. The data demonstrated that patients with VUSs should be managed based on their family history of cancer and clinicopathological characteristics. The clinical significance of the VUS in *BRCA1/2* may change over time and reclassification of the variant to 'pathogenic' or 'benign' should be undertaken. Patients with VUS should be followed up regularly.

Introduction

Pathogenic mutations in the *BRCA1/BRCA2* genes increase the risk of developing breast and ovarian cancer or other types of cancer (prostate cancer, pancreatic cancer and melanoma). Women with *BRCA1* mutations exhibit a 45-85% lifetime risk of developing breast cancer. Other genes associated with the risk of developing breast cancer are *ATM*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *STK11*, and *TP53* (1-7). The risk of developing ovarian cancer by 70 years of age is 40-50% for *BRCA1* mutation carriers and 10-20% for *BRCA2* mutation carriers (8,9). Other suppressor genes and oncogenes associated with hereditary ovarian cancers, include the mismatch repair (MMR) genes in Lynch syndrome, the tumor suppressor gene, *TP53*, in the Li-Fraumeni syndrome, genes involved in the double-strand breaks repair system, such as *CHEK2*, *RAD51*, *BRIPI*, and *PALB2* (10).

The development and popularization of NGS technique led to the detection of new genetic variations, including VUS (variant of uncertain significance) (11). It is variant whose clinical significance to the function or medical condition is not known. In previous studies conducted on high-risk families, variants of uncertain significance exhibited a frequency up to 15% for the *BRCA* genes. It included the following mutations: missense variations, small in-frame deletions, variants in regulatory sequences and exonic or intronic variants that may affect pre-mRNA splicing (12). The International Agency on Cancer Research of the World Health Organization has proposed a simple five-tier system for the clinical management of *BRCA1/BRCA2* VUSs that is not widely known to clinicians. Class 1 and 2 variants are managed as neutral variants, whereas class 4 and 5 as pathogenic variants. Insufficient evidence has been reported regarding class 3 variants and therefore these are not included in the selection process (13). The American College of Medical Genetics and Genomics recommends the use of specific standard terminology as follows: 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign' and 'benign'. These terms are used to describe variants identified in genes (14). Variants of uncertain significance have also been identified in other genes such as: *TP53* (15), *ATM* (16) or *PALB2* (17). There are only few studies, which describe histopathologic characteristics of VUS (18).

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Assessing the pathogenicity of VUS in *BRCA1* and *BRCA2* poses a serious challenge. Functional assays provide an important tool for classification. *BRCA1/2* promote the maintenance of genome stability via homologous recombination. Thus, related assays are particularly relevant to cancer risk. Currently, there is need for high-throughput assays with sufficient sensitivity to characterize the large number of identified variants.

The influence of *BRCA1/2* VUSs on the cancer risk and their association with the response to treatment is uncertain. The aim of the present study was to characterized breast cancer patients according to clinical and histopathological factors.

Case report

The present case report describes two cases of breast cancer patients with *BRCA* VUSs. All diagnosis and treatment took place in National Research Institute of Oncology, Gliwice Branch. Genetic counseling was performed. During the genetic consultation, a family history was collected, the pedigree was prepared and the medical records were reviewed. The patients provided written informed consent regarding the use of their biological material for clinical research (all the samples were collected by routine laboratory analyses). The complete coding sequence of the *BRCA1* and *BRCA2* genes was analyzed on the genomic DNA material using next generation sequencing on the Ion Torrent platform. The libraries were prepared using the Oncomine *BRCA* Assay Chef Ready Kit, according to the manufacturer's instructions (ThermoFisher Scientific). Sequencing was performed on the Ion S5 sequencer (ThermoFisher Scientific) using the Ion 530 Chip Kit and 510&520&530 Kit-Chef. The raw data generated during sequencing was processed using Ion Reporter v.5.6 software (ThermoFisher Scientific). The wANNOVAR program (www.wannovar.usc.edu) was used to annotate the detected variants from Ion Reporter. The reference sequences were the following: NM_007294.3 (*BRCA1*) and NM_000059.3 (*BRCA2*). The complete coding sequence of the *BRCA1* and *BRCA2* genes was performed together with adjacent intron sequences. The interpretation of the variants was carried out in accordance with the following databases: NCBI ClinVar, dbSNP, 1000 Genomes Project, BIC, LOVD, VarSome in the current version on the day of the examination. Classification of variants was done according to guidelines of American College of Medical Genetics (ACMG) (14). The presence of these variants in the *BRCA1* and *BRCA2* genes was confirmed using the Sanger method.

A 63-year-old woman was admitted to the Genetic Outpatient Clinic with the diagnosis of bilateral breast cancer. At the age of 60, she was also diagnosed with endometrial cancer (T2N0M0). The patient was eligible for surgical treatment. Histopathological examination indicated adenocarcinoma *papillare serosum* (pT2N0Mx). Following surgery, she received chemotherapy, radiotherapy and brachytherapy (standard therapy). Genetic counseling was performed. There was no cancer in family history. Genetic pedigree Fig. 1.

Two years later (at the age of 62), the patient was diagnosed with bilateral breast cancer as determined by mammography. One tumor was located centrally in the right breast and

exhibited the following dimensions: 2x1.5 cm. The second tumor was situated in the upper outer quadrant of the left breast and exhibited the following dimensions: 9x8x5 mm. A thick-needle biopsy was performed. Histopathological examination indicated ductal invasive carcinoma with neuroendocrine differentiation. The biochemical and clinical characteristics of the tumors were the following: NG2, G2, ER (+++), PR (+++), HER2 (+), Ki-67 10%, luminal A subtype in the right breast and ductal invasive carcinoma G2, ER (+++), PR (+++), HER2 (+), Ki-67 5% and luminal A subtype in the left breast. Breast-conserving therapy with sentinel lymph node biopsy and IORT was performed in the left breast. Postoperative histopathological examination indicated the following tumor characteristics: pT2 N1a Mx, invasive breast carcinoma with neuroendocrine differentiation NG-2 G-2, HER2 (+), ER (+++), PR (+++) and luminal A subtype. Metastases were demonstrated in one lymph node. The patient was eligible for adjuvant radiotherapy and hormone therapy based on aromatase inhibitors. In addition, right-sided mastectomy with right arm SNB was performed.

Genetic analysis was conducted. The presence of the c.3454G>A (p.Asp1152Asn) mutation in the *BRCA1* gene, which is a VUS has been previously reported. The detected mutation causes a change of the amino acid aspartic acid to the amino acid asparagine at position 1,152 of the amino acid sequence of the *BRCA1* protein. This variant is found in the ClinVar and *BRCA* Share databases, where it has the status of a VUS. In order to determine the pathogenicity of this variant, *in silico* analyses were performed using the SIFT, Mutation Taster and Align GVGT algorithms. These algorithms indicate that the c.3454G>A (p.Asp1152Asn) variant in the *BRCA1* gene is probably not pathogenic. Mutations in the *BRCA2* gene were not detected. Moreover, the absence of mutations in the *CHEK2* (c.1100delC and mutation c.470T>C) (GenBank NM_145862.2.), the *PALB2* (c.508_509delAG and c.172_175delTTGT) and the *TP53* (exons 2-11) genes were observed. The presence of the c.3454G>A (p.Asp1152Asn) variant in the *BRCA1* gene was confirmed using the Sanger method. Genetic tests were also offered to the patient's relatives (brother and mother). They have not yet decided to examination. The woman has no children.

The second patient was 33-year-old woman, who was admitted to the Genetic Outpatient Clinic with the diagnosis of right breast cancer (cT2N1M0) and with a previous family history of cancer (two of her relatives were diagnosed with cancer-one with lung cancer and the second with adrenal gland cancer) (genetic pedigree Fig. 2). At initial diagnosis, PET testing was carried out and the data indicated the presence of a 3 cm tumor in the right breast with involvement of the right armpit lymph nodes. A coarse-needle biopsy was performed. Histopathological examination demonstrated the presence of invasive breast carcinoma, which exhibited the following characteristics: NG-2 G-2, ER (+++), PR (+++), Ki-67 10%, HER2 (+++) and luminal B subtype. Metastases were reported in the lymph nodes of the right armpit. The patient was eligible for neoadjuvant chemotherapy (IV cycles of AC regimen and 12 cycles of paclitaxel monotherapy) and immunotherapy (pertuzumab and trastuzumab). In addition, the patient was eligible for surgical treatment following neoadjuvant therapy. A radical amputation of the right breast with

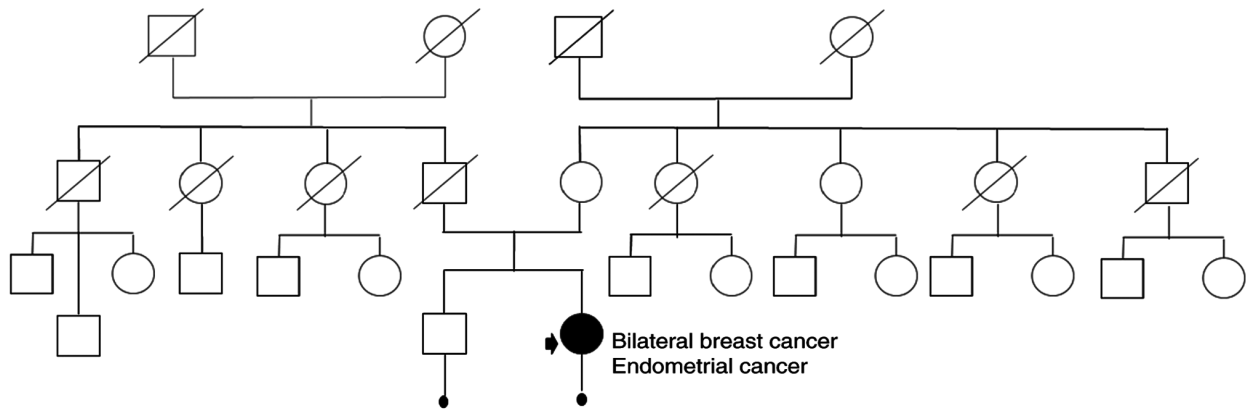


Figure 1. Genetic pedigree of first patient. White square, male without cancer disease; white circle, female without cancer disease; black circle, female with cancer disease.

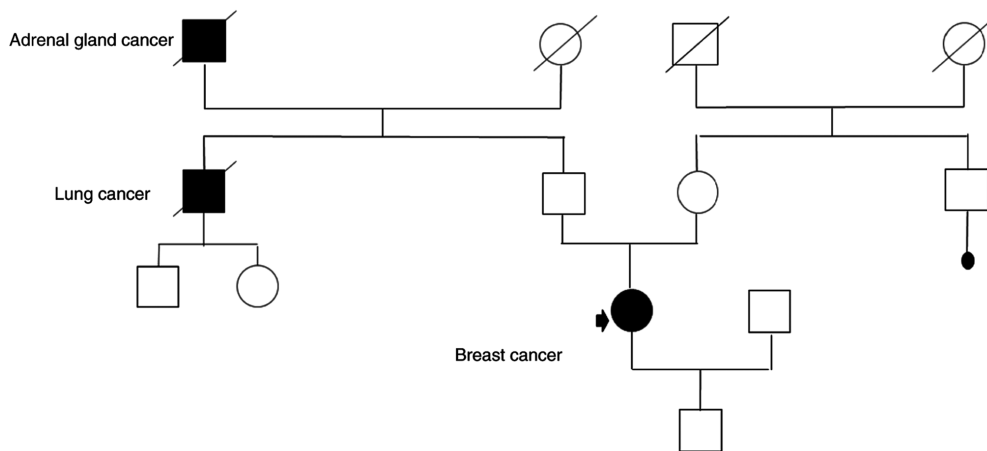


Figure 2. Genetic pedigree of second patient. Black square, male with cancer disease; black circle, female with cancer disease; white square, male without cancer disease; white circle, female without cancer disease.

the SNB of the lymph nodes was performed. Postoperative histopathological examination demonstrated a tumor with the presence of angioinvasion, without neuroinvasion. The exact clinical and biochemical characteristics were as follows: pT1c N0 M0, NST NG-2 G-2, Ki-67 10%, ER (+++), PR (+++), and HER2 overexpression.

Genetic analysis has shown the presence of the c.2374T>C (p.Tyr792His) variant in the *BRCA2* gene, which is a VUS. The detected variant caused a change of tyrosine to histidine at position 792 of the amino acid sequence of the *BRCA2* protein. This variant is found in the ClinVar and BRCA Share databases and it is characterized as a VUS. Genetic variants of this type may exert a diverse impact on protein function. The presence of the c.2374T>C (p.Tyr792His) mutation in the *BRCA2* gene was confirmed using the Sanger method. The analysis did not detect mutations in the *BRCA1* gene. Moreover, the data indicated the absence of mutations in the *PALB2* (c.508_509delAG and c.172_175delTTGT) gene. The *CHEK2* gene (c.1100delC and mutation c.470T>C) (GenBank NM_145862.2) was also examined. The c.470T>C mutation in *CHEK2* gene has been found. Genetic tests were also offered to the patient's first-degree relatives. However, they have not yet decided to examination. The patient's only son is underage (he is 5 year old now). The comparison of both

patients according to clinicopathological and molecular factors is shown in Tables I and II.

Discussion

In the present study, VUSs were detected in two breast cancer patients with family history of cancer. In the first case, the presence of the c.3454G>A (p.Asp1152Asn) variant was reported in the *BRCA1* gene. The detected variant causes a change of aspartic acid to asparagine at position 1,152 of the amino acid sequence of the *BRCA1* protein. In the second case, the presence of the c.2374T>C (p.Tyr792His) variant was detected in the *BRCA2* gene. The reported variant causes a change of tyrosine to histidine at position 792 of the amino acid sequence of the *BRCA2* protein.

According to the previous studies VUSs should be interpreted as non-informative and should not influence cancer management. In that cases clinical management should be based on personal and family history of cancer. Cancer prevention and screening strategies should be individualized (19). The determination of VUS pathogenicity should be based on multifactorial prediction models including the co-segregation test for family members with known cancers, periodic re-classification of VUSs, and other genetic tests, in addition to the

Table I. Clinicopathological characteristics of patients.

Factors	First patient with breast cancer	Second patient with breast cancer
Patient age, years	63	33
Second cancer	Endometrial cancer	No second cancer
Stage of disease	T2N1M0	T1N0M0
Histological grade	G2	G2
ER	Positive	Positive
PR	Positive	Positive
HER2-positive	Lack of overexpresion	Overexpression
Ki67, %	10	10
Breast cancer subtype	Luminal A subtype	Luminal B HER-positive subtype
History of cancer in family	No history of cancer	Lung cancer, adrenal gland cancer

G, grade; ER, estrogen receptor; PR, progesterone receptor.

Table II. Results of molecular examination.

Genes	First patient with breast cancer	Second patient with breast cancer
<i>BRCA1</i> NGS	c.3454G>A (p.Asp1152Asn)	Not detected
<i>BRCA2</i> NGS	Not detected	c.2374T>C (p.Tyr792His)
<i>CHEK2</i>	Not detected	c.470T>C mutation
<i>PALB2</i>	Not detected	Not detected
<i>TP53</i>	Not detected	Not examined

NGS, Next Generation Sequencing; CHEK2, checkpoint kinase 2; PALB2, partner and localizer of *BRCA2*.

detection of the *BRCA* gene mutations (20). NCCN guidelines recommend patients with VUS to be included in the variant reclassification programs (21).

The variant *BRCA1* c.3454G>A results in the p.Asp1152Asn (D1152N) mutation which causes a change of an Aspartic Acid to an Asparagine (GAC>AAC) residue in the polypeptide chain of the protein. Based on alternative nomenclature, this variant would be defined as *BRCA1* 3573G>A. This variant has been previously identified in patients with cancer or subjects with family history of cancer (family history of breast and/or ovarian cancer). It has also been identified in a pediatric patient with leukemia (22-26). *BRCA1* Asp1152Asn was not observed at a significant allele frequency in large population cohorts (27). *BRCA1* Asp1152Asn is not located in a known functional domain. The aspartic acid at codon 1152 is moderately conserved, and computational analyses (SIFT, PolyPhen-2) predict that this variant is tolerated. *In vitro* functional analyses demonstrate activity similar to wildtype in a homology-directed repair assay (28). One individual reported in BRCA Share database also carried a likely pathogenic variant in *BRCA1* c.4485_4675del/p.Ser1496GlyfsX14, further supporting benign nature of this variant. Based on current data, *BRCA1* Asp1152Asn is a VUS as reported by multiple laboratories in ClinVar (Variation ID: 54890).

Currently, limited information exists with regard to the c.2374T>C (p.Tyr792His) mutation of the *BRCA2* gene, suggesting that it has uncertain classification and consequently

uncertain clinical significance. This variant causes a change of tyrosine to histidine at position 792 of the amino acid sequence of the *BRCA2* protein. The tyrosine and histidine are amino acids with similar properties. This amino acid position is not well conserved in vertebrate species. In addition, this alteration is predicted to be tolerated by *in silico* analysis (SIFT, Mutation Taster). There is no functional studies concerning this variant. In the present study, the c.2374T>C (p.Tyr792His) mutation in the *BRCA2* gene was detected in women with stage IIB cancer according to the TNM classification and luminal B HER2 positive breast cancer subtype. The other characteristics of the patient were a diagnosis at a younger age (33 years) and family history of cancer (one relative with lung cancer and the second relative with adrenal gland cancer). A diagnosis at a younger age and cancer family history are suggestive of the pathological nature of c.2374T>C (p.Tyr792His) variant in the *BRCA2* gene. The absence of detected mutations was noted in the *BRCA1* gene and *PALB2* gene. However, the c.2374T>C (p.Tyr792His) mutation in the *BRCA2* gene coexisted with c.470T>C mutation in *CHEK2* gene. The meta-analysis demonstrates that the *CHEK2* I157T (also known as c.470T>C) variant increases cancer risk, especially breast and colorectal cancer in Caucasian (28).

Borg *et al* (23) examined a young woman with contralateral breast cancer (CBC, n=705) and with unilateral breast cancer (UBC, n=1,398) according to the presence of mutations and sequence variants of unknown clinical significance (VUS).

The majority of VUSs were not associated with an increased risk of CBC and represented neutral alleles of no or little significance in the etiology of breast cancer (23). In the present study, one patient had unilateral breast cancer (UBC) and the second patient exhibited contralateral breast cancer (CBC). Both cases presented stage IIB disease according to the TNM classification (AJCC system, effective since January 2018).

In some studies *BRCA1* mutation carriers were characterized by ER-negative/PR-negative steroid receptor status of breast cancer (29,30). A Meta-Analysis conducted by Chen *et al* (31) has suggested that TNBC was more common among the breast cancer patients with *BRCA1* mutation than among *BRCA2* mutation carriers or non-carriers. Authors have not found information about clinicopathological characteristics of patients with *BRCA1* c.3454G>A variant. There is also no studies concerning c.2374T>C (p.Tyr792His) variant in the *BRCA2* gene. In our study, the c.2374T>C (p.Tyr792His) mutation in the *BRCA2* gene was associated with luminal B HER2 positive breast cancer subtype. The variant *BRCA1* c.3454G>A was characterized by ductal invasive carcinoma histologic type G2, ER (+++), PR (+++), HER2 (+), Ki-67 5% and luminal A subtype.

The limitation of this study is small number of patients. We presented two patients carrying two VUSs. A larger group of breast cancer patients with VUSs is required to verify the current findings and additional data derived from literature studies are necessary to redefine the clinical significance of VUSs (change to benign or pathological variant). We do not have possibility to check *ATM* or *PTEN* genes. There was no mutation in *TP53* gene in first patient. The second women hasn't come yet to continue genetic diagnosis.

In conclusion, the c.3454G>A mutation in the *BRCA1* gene was characterized by NG-2 G-2, HER2 (+), ER (+++), PR (+++), luminal A subtype and endometrial cancer in history. However, the c.2374T>C (p.Tyr792His) mutation carriers in the *BRCA2* gene had breast cancer with NG-2 G-2, ER (+++), PR (+++), Ki-67 10%, HER2 (+++), luminal B subtype, cancer in family history, younger age at diagnosis and c.470T>C mutation in *CHEK2* gene. Patients with VUSs should be managed based on their family history of cancer and clinicopathological characteristics. The clinical significance of VUSs in *BRCA1/2* may change over time and may be reclassified as 'pathogenic' or 'benign' variants. It is advisable to examine the presence of VUSs in healthy and sick family members in order to analyze the co-existence of the variant with breast and/or ovarian cancer. Patients with VUS should be followed up regularly with use of individualized screening and prevention strategies.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JH was responsible for study design, preparation of the manuscript and final approval of the version to be published. WP, MM, JPP, AZ, AFK and MOW were responsible for genetic analysis and gave final approval of the version to be published. AFK and MOW confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The patients provided written informed consent regarding the use of their biological material for clinical research.

Patient consent for publication

Written informed consent was obtained from the patients for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Prokopcova J, Kleibl Z, Banwell CM and Pohlreich P: The role of ATM in breast cancer development. *Breast Cancer Res Treat* 104: 121-128, 2007.
2. Corso G, Montagna G, Figueiredo J, La Vecchia C, Fumagalli Romario U, Fernandes MS, Seixas S, Roviello F, Trovato C, Guerini-Rocco E, *et al*: Hereditary gastric and breast cancer syndromes related to CDH1 germline mutation: A multidisciplinary clinical review. *Cancers (Basel)* 12: 1598, 2020.
3. Cybulski C, Wokołorczyk D, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Masojć B, Deebniak T, Górski B, Blecharz P, *et al*: Risk of breast cancer in women with a CHEK2 mutation with and without a family history of breast cancer. *J Clin Oncol* 29: 3747-3752, 2011.
4. Evans MK and Longo DL: PALB2 mutations and breast-cancer risk. *N Engl J Med* 371: 566-568, 2014.
5. Tan MH, Mester JL, Ngoew J, Rybicki LA, Orloff MS and Eng CH: Lifetime cancer risks in individuals with germline PTEN mutations. *Clin Cancer Res* 18: 400-407, 2012.
6. Lim W, Olschwang S, Keller JJ, Westerman AM, Menko FH, Boardman LA, Scott RJ, Trimbath J, Giardiello FM, Gruber SB, *et al*: Relative frequency and morphology of cancers in STK11 mutation carriers. *Gastroenterology* 126: 1788-1794, 2004.
7. Schon K and Tischkowitz M: Clinical implications of germline mutations in breast cancer: TP53. *Breast Cancer Res Treat* 167: 417-423, 2018.
8. Practice bulletin No. 182 summary: Hereditary breast and ovarian cancer syndrome. *Obstet Gynecol* 130: 657-659, 2017.
9. King MC, Marks JH and Mandell JB: New York Breast Cancer Study Group: Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* 302: 643-646, 2003.
10. Toss A, Tomasello Ch, Razzaboni E, Contu G, Grandi G, Cagnacci A, Schilder RJ and Cortesi L: Hereditary ovarian cancer: Not only BRCA 1 and 2 genes. *Biomed Res Int* 2015: 341723, 2015.
11. Cheon JY, Moersky J and Cook-Deegan R: Variants of uncertain significance in BRCA: A harbinger of ethical and policy issues to come? *Genome Med* 6: 121, 2014.
12. Radice P, De Summa S, Caleca L and Tommasi S: Unclassified variants in BRCA genes: Guidelines for interpretation. *Ann Oncol* 22 (Suppl 1): i18-i23, 2011.
13. Lindor NM, Goldgar DE, Tavtigian SV, Plon SE and Couch FJ: BRCA1/2 sequence variants of uncertain significance: A primer for providers to assist in discussions and in medical management. *Oncologist* 18: 518-524, 2013.

14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, *et al*: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet Med* 17: 405-424, 2015.
15. Bittar CM, Vieira IA, Sabato CS, Andreis TF, Alemar B, Artigalás O, Galvão HCR, Macedo GS, Palmero EI and Ashton-Prolla P: TP53 variants of uncertain significance: Increasing challenges in variant interpretation and genetic counseling. *Fam Cancer* 18: 451-456, 2019.
16. Han FF, Guo CL and Liu LH: The effect of CHEK2 variant I157T on cancer susceptibility: Evidence from a meta-analysis. *DNA Cell Biol* 32: 329-335, 2013.
17. Boonen RACM, Vreeswijk MPG and van Attikum H: Functional characterization of PALB2 variants of uncertain significance: Toward cancer risk and therapy response prediction. *Front Mol Biosci* 7: 169, 2020.
18. Abdulrahman AA, Heintzelman RC, Corbman M and Garcia FU: Invasive breast carcinomas with ATM gene variants of uncertain significance share distinct histopathologic features. *Breast J* 24: 291-297, 2018.
19. Chern JY, Lee SS, Frey MK, Lee J and Blank SV: The influence of BRCA variants of unknown significance on cancer risk management decision-making. *J Gynecol Oncol* 30: e60, 2019.
20. Choi MC: Clinical significance of variants of unknown significances in BRCA genes. *J Gynecol Oncol* 30: e80, 2019.
21. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Genetic/familial high-risk assessment: Breast and ovarian. Version 3.2019, 2019.
22. Hansen NT, Brunak S and Altman RB: Generating genome-scale candidate gene lists for pharmacogenomics. *Clin Pharmacol Ther* 86: 183-189, 2009.
23. Borg Å, Haile RW, Malone KE, Capanu M, Diep A, Törngren T, Teraoka S, Begg CB, Thomas DC, Concannon P, *et al*: Characterization of BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance in unilateral and bilateral breast cancer: The WECARE study. *Hum Mutat* 31: E1200-E1240, 2010.
24. Stegel V, Krajc M, Zgajnar J, Teugels E, De Grève J, Hočevár M and Novaković S: The occurrence of germline BRCA1 and BRCA2 sequence alterations in Slovenian population. *BMC Med Genet* 12: 9, 2011.
25. Kluska A, Balabas A, Paziewska A, Kulecka M, Nowakowska D, Mikula M and Ostrowski J: New recurrent BRCA1/2 mutations in Polish patients with familial breast/ovarian cancer detected by next generation sequencing. *BMC Med Genomics* 8: 19, 2015.
26. Zhang J, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, Hedges D, Ma X, Zhou X, Yergeau DA, *et al*: Germline mutations in predisposition genes in pediatric cancer. *N Engl J Med* 373: 2336-2346, 2015.
27. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, *et al*: Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536: 285-291, 2016.
28. Lu Ch, Xie M, Wendl MC, Wang J, McLellan MD, Leiserson MD, Huang KL, Wyczalkowski MA, Jayasinghe R, Banerjee T, *et al*: Patterns and functional implications of rare germline variants across 12 cancer types. *Nat Commun* 6: 10086, 2015.
29. Byrski T, Gronwald J, Huzarski T, Grzybowska E, Budryk M, Stawicka M, Mierzwa T, Szwiec M, Wiśniowski R, Siolek M, *et al*: Response to neo-adjuvant chemotherapy in women with BRCA1-positive breast cancers. *Breast Cancer Res Treat* 108: 289-296, 2008.
30. Kirk R: Surgical oncology: Cancer risk reduction in BRCA mutation carriers. *Nat Rev Clin Oncol* 7: 609, 2010.
31. Chen H, Wu J, Zhang Z, Tang Y, Li X, Liu S, Cao S and Li X: Association between BRCA status and triple-negative breast cancer: A meta-analysis. *Front Pharmacol* 9: 909, 2018.