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ORIGINAL ARTICLE

Reinterpretation of *BRCA1* and *BRCA2* variants of uncertain significance in patients with hereditary breast/ovarian cancer using the ACMG/AMP 2015 guidelines

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Abstract

Background Although *BRCA1* or *BRCA2* (*BRCA1/2*) genetic testing plays an important role in determining treatment modalities in patients with hereditary breast and ovarian cancer, sequence variants with unknown clinical significance or variant of uncertain significance (VUS) have limited use in medical decision-making. With vast quantities of gene-related data being updated, the clinical significance of VUS may change over time. We reinterpreted the sequence variant previously reported as *BRCA1/2* VUS results in patients with breast or ovarian cancer and assessed whether the clinical significance of VUS was changed.

Methods We retrospectively reviewed medical records of 423 breast or ovarian cancer patients who underwent *BRCA1/2* genetic testing from 2010 to 2017. The VUSs in *BRCA1/2* were reanalyzed using the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines (ACMG/AMP 2015 guidelines) and the VUS was reclassified into five categories: "pathogenic", "likely pathogenic", "VUS", "likely benign", and "benign".

Results A total of 75 patients (48 sequence types of VUS) were identified as carrying either one or more VUS in *BRCA1/2*. Among the 75 patients, two patients (2.7%) were reclassified as "likely pathogenic", 30 patients (40.0%) were reclassified as either "benign" or "likely benign", and the remaining 43 patients (57.3%) were still classified as VUS category.

Conclusions Since the clinical significance of VUS in *BRCA1/2* may vary from time to time, reinterpretation of the VUS results could contribute to clinical decision-making.

Keywords Variant of uncertain significance · *BRCA* · Breast cancer · Reclassification · Genetic testing

Introduction

In hereditary cancers, genetic testing plays an important role in predicting cancer risk and making clinical decisions for disease treatment and prevention [1]. The genetic test for *BRCA1* or *BRCA2* (*BRCA1/2*) genes, which are associated with hereditary breast and ovarian cancer syndrome, is widely used in clinical practice [2, 3]. Breast or ovarian cancer patients who harbor disease-associated *BRCA1/2* germline mutations are administered prophylactic treatments, such as risk-reducing agents, risk-reducing bilateral mastectomy, and risk-reducing salpingo-oophorectomy (RRSO), to prevent cancer recurrence [3, 4]. In addition, targeted therapy such as poly (ADP-ribose) polymerase inhibitors (PARPi) may also be used for certain patients with *BRCA1/2* gene mutations [5, 6].

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Genetic testing can reveal germline mutation variants that may be pathogenic, benign or uncertain clinical significance. The recently introduced 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines (ACMG/AMP 2015 guidelines) provide a detailed framework for the interpretation of a sequence variant using a scoring rule based on various types of information, including computational, functional, and segregation data [7]. These guidelines classify a sequence variant into five categories: "pathogenic", "likely pathogenic", "uncertain significance", "likely benign", and "benign". The guidelines recommend reporting variants with ambiguous clinical significance as a variant of uncertain significance (VUS) [7].

Previous studies have shown that the VUS frequency of *BRCA1/2* genes differs depending on the patient's ethnicity or study population [8, 9]. Approximately 5–10% of patients that harbor *BRCA1/2* germline mutations are reported to carry VUS in world's population, but the VUS proportion in Asians is slightly higher than other group, at approximately 10–20% [8–11]. Test results that identify VUS cannot support clinicians in making treatment decisions, and also the ambiguous results can increase anxiety in patients and their families [12, 13]. One strategy to manage patients with identified VUS is to periodically reanalyze the variant using more recent and updated data. As the genetic data expand over time, there is always a possibility of a given variant ultimately being reclassified under another category.

In this study, we aim to reanalyze the VUS in *BRCA1/2* genes in patients with breast or ovarian cancer using ACMG/AMP 2015 guidelines with updated information to assess the possibility of reclassifying VUS to a more specific category to aid clinical decision-making.

Materials and methods

Study design

We retrospectively reviewed genetic testing results from 423 patients with breast or ovarian cancer at Ewha Womans University Mokdong Hospital (Seoul, Korea) between 2010 and 2017. All patients were simultaneously examined for both *BRCA1* and *BRCA2* germline mutations.

Briefly, genomic DNA isolated from peripheral blood leukocytes was analyzed by direct DNA sequencing or next generating sequencing for *BRCA1* (MIM 604370) and *BRCA2* (MIM 612555). The amplicons of whole exons and exon flanking regions, both 25 bp upstream and downstream, were analyzed. All sequencing results were aligned and analyzed to the human genome assembly GRCh37 (hg19). Each sequence variant was described according to the Human Genome Variation Society recommendations. We used the transcript sequences of NM_007294.3 for *BRCA1* and NM_000059.3 for *BRCA2*, respectively.

The author's institution has reported *BRCA1/2* test results according to the ACMG/AMP 2015 guidelines since July 2016. Prior to July 2016 ($n=254$), the clinical significance of sequence variants was determined through the relevant documents, literatures or public database at the time of reporting the results, and the results were categorized into three representative groups; "pathogenic (deleterious)", "VUS" and "benign" [14]. Since July 2016 ($n=169$), the clinical significance of sequence variants has been interpreted based on the ACMG/AMP 2015 guidelines, and the results were reported in five categories as recommended; "pathogenic", "likely pathogenic", "VUS", "likely benign", or "benign" [7].

We classified the study subjects into three groups: *BRCA1/2* pathogenic/likely pathogenic variant carriers; neutral (benign or likely benign variant or wild-type); or VUS carrier of *BRCA1* or *BRCA2* or both (Fig. 1). A total of 81 patients (81/423, 19.1%) were classified as carrying

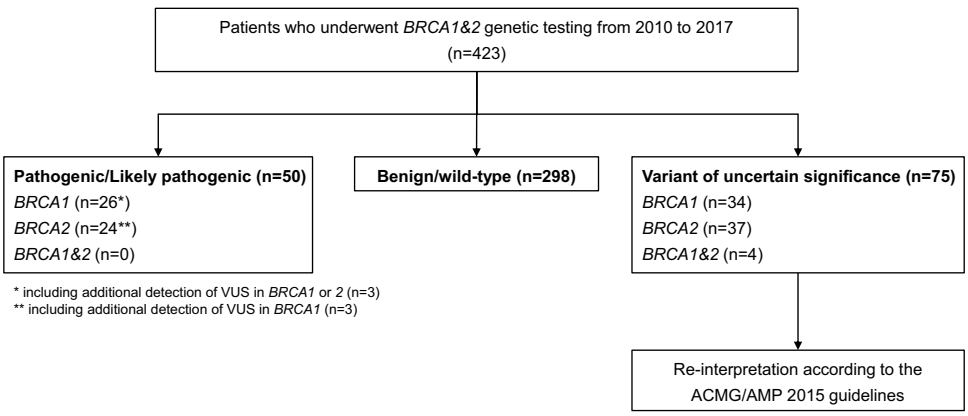

Fig. 1 Schematic diagram of the study design and patient selection

either one or more VUS in either *BRCA1* or *BRCA2*. Six of the patients carrying VUS also harbored accompanying pathogenic mutations and thus were excluded because their clinical decision-making would likely be influenced by the known deleterious mutations. Finally we enrolled 75 patients (breast cancer, $n=69$; ovarian cancer, $n=9$) with VUS variants. Among 75 patients, 59 cases were enrolled from January 2010 to June 2016 (59/254) and 16 cases were from July 2016 to December 2017 (16/169). We reinterpreted the sequence variants according to the ACMG/AMP 2015 guidelines as described below [7]. This study was approved by the Institutional Review Board of Ewha Womans University Mokdong Hospital (approval number 2018-09-010).

Reinterpretation of VUS according to the ACMG/AMP 2015 guidelines

Briefly, the description for each of the criterion is as follows. For pathogenic supporting (PP) evidence 5 or benign supporting (BP) evidence 6, which used reputable source recently reports variant as pathogenic or benign, respectively, we analyzed the VUS using the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/). The results from the database analysis were described as "pathogenic", "likely pathogenic", "uncertain significance", "conflicting", "likely benign" and "benign". Among these results, "pathogenic" and "likely pathogenic" were considered evidence of PP5, and "likely benign" and "benign" were regarded as BP6.

For PP3 or BP4 belonging to supporting evidence criteria based on multiple lines of computational evidence, we used the following five in silico missense prediction algorithms and one splice site prediction algorithm: MutationTaster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), PROVEN (http://provean.jcvi.org/index.php), SIFT (http://sift.jcvi.org/), Align-GVGD (http://agvgd.hci.utah.edu/agvgd_input.php) and Human Splicing Finder (http://umd.be/HSF3/). Agreement among at least five algorithms was considered evidence of either PP3 or BP4. However, if the in silico algorithms could not be applied according to the variant type, such as for variants in intronic sites, deletion variants, and synonymous (silent) variants, then agreement among all available algorithms was required. For evidence of BP7, the Human Splicing Finder was also used; evidence of BP7 was established when the results showed that a synonymous variant had no impact on the splice consensus sequence or creation of a new splice site.

To provide evidence associated with population data, such as pathogenic moderate (PM) evidence 2 and benign strong (BS) evidence 1, we used the Exome Aggregation Consortium database (ExAC, http://exac.broadinstitute.org/). PM2 was considered when the prevalence of the variant was absent from the controls. BS1 was considered when the allele frequency was greater than 1% and less than 5% in ExAC for all the population of East Asian.

We reviewed previously published literature for analyzing the variants for pathogenic strong (PS) evidence 3 or BS3, which is evidence of the presence of well-established in vitro or in vivo functional studies supporting damaging effects or suggesting no impact on the genes or their products [15–25]. The variants were interpreted independently by two investigators (MKS and TDJ). If the initial results were inconsistent, the results were discussed until a consensus was obtained.

Results

Reclassification of the patients carrying VUS

Among the 423 patients, VUS without *BRCA1/2* pathogenic variants were observed in 75 patients (Fig. 1). Of the 75 subjects, 22 types of VUS were identified in *BRCA1* (Table 1) and 26 types of VUS were identified in *BRCA2* (Table 2). These 48 types of VUS included 30 nonsynonymous, 7 synonymous, 3 in-frame deletion, and 8 intronic variants (Tables 1, 2).

Among the 75 *BRCA1/2* VUS carriers, 32 (42.7%) were reclassified: two patients were reclassified with "likely pathogenic" variants, eight patients were reclassified with "benign" variants, and 22 patients were reclassified with "likely benign" variants (Fig. 2). Among the 32 patients, the medical records of 30 patients were collected from 2010 to June 2016 and 2 of them were after July 2016. Forty-three patients (43/75, 57.3%) remained classified as carrying VUS after reinterpretation.

At the time of this study, 8 sequence types of the 22 VUS of *BRCA1* were reclassified as follows: two as "likely pathogenic", one as "benign", and five as "likely benign" (Fig. 3a). The remaining 14 VUS in *BRCA1* were still classified as "VUS". Similarly, of the 26 VUS of *BRCA2*, two were classified as "benign" and five as "likely benign" (Fig. 3b). The remaining 19 VUS of *BRCA2* were still classified as "VUS".

Two VUS cases reclassified as "likely pathogenic"

In this study, two cases which previously classified as "VUS" was reclassified as "likely pathogenic". One patient with a history of bilateral breast cancer. The variant was a missense mutation, NM_007294.3(BRCA1):c.5339T>C (p.Leu1780Pro), leading to a single amino acid substitution. The other patient with a history of breast cancer was found to carry a sequence variant, NM_007294.3(BRCA1):c.5017_5019delCAC (p.His1673del), creating an in-frame deletion in exon 16.

Table 1 Reinterpretation of variants of unknown significance in *BRCA1* according to the ACMG/AMP 2015 guidelines

Genomic location[a]	Nucleotide change (NM_007294.3)	Amino acid change	Number of cases (%)	rs number	Allele frequency from ExAC		Functional assay [Ref.]	Initial report[b]	Reinterpretation[c]	
					All	East Asians			Type of evidence	Reclassification
Chr17: 41258531	c.154C>T	p.Leu52Phe	2 (4.9%)	rs80357084	0.00013	0.00175	–	VUS	–	VUS
Chr17: 41251844	c.495G>A	p.Leu165=	2 (4.9%)	rs745321499	–	–	–	VUS	PM2, PP3, BP6	VUS
Chr17: 41251778	c.547+14delG	–	2 (4.9%)	rs273902771	0.00012	–	No deleterious [15]	VUS	BP4,BS3	Likely benign
Chr17: 41251762	c.547+30A>G	–	1 (2.4%)	–	–	–	–	VUS	PM2, BP4	VUS
Chr17: 41246297	c.1251T>C	p.Asn417=	1 (2.4%)	rs80357197	–	–	–	VUS	BP4, BP6, BP7, PM2	Likely benign
Chr17: 41245803	c.1745C>T	p.Thr582Met	1 (2.4%)	rs786202386	–	–	–	VUS	PM2, PP3	VUS
Chr17: 41245377	c.2171C>T	p.Pro724Leu	1 (2.4%)	–	–	–	–	VUS	PM2	VUS
Chr17: 41244982	c.2566T>C	p.Tyr856His	4 (9.7%)	rs80356892	0.00152	0.02080	No deleterious [16–18]	VUS	BS1, BS3, BP6	Benign
Chr17: 41244100	c.3448C>T	p.Pro1150Ser	2 (4.9%)	rs80357272	0.00009	0.00127	–	VUS	PP3	VUS
Chr17: 41234431	c.4347A>G	p.Thr1449=	2 (4.9%)	rs80356840	0.00007	0.00092	–	VUS	BP6	VUS
Chr17: 41228567	c.4422T>C	p.Ala1474=	4 (9.7%)	rs756281673	0.00002	–	–	VUS	PP3, BP6	VUS
Chr17: 41228530	c.4459A>G	p.Lys1487Glu	1 (2.4%)	–	–	–	–	VUS	PM2	VUS
Chr17: 41228491	c.4484+14A>G	–	8 (19.5%)	rs80358022	0.00079	0.00972	–	VUS	BS1, BP6	Likely benign
Chr17: 41226426	c.4597G>C	p.Asp1533His	1 (2.4%)	–	–	–	–	VUS	PM2	VUS
Chr17: 41223119	c.4812A>G	p.Gln1604=	1 (2.4%)	rs28897693	0.00129	0.00046	–	VUS	BP4, BP6, BP7	Likely benign
Chr17: 41223048	c.4883T>C	p.Met1628Thr	1 (2.4%)	rs4986854	0.00152	0.00636	No deleterious [17–19]	VUS	BS3, BP6	Likely benign
Chr17: 41222980	c.4951T>C	p.Ser1651Pro	1 (2.4%)	rs879254042	–	–	Not clear [17]	VUS	PM2, PP3	VUS
Chr17: 41219680–41219682	c.5017_5019delCAC	p.His1673del	1 (2.4%)	rs80358343	–	–	–	VUS	PS4, PM2, PM4	Likely pathogenic
Chr17: 41203106	c.5306A>G	p.Tyr1769Cys	2 (4.9%)	rs397509257	0.00008	0.00000	–	VUS	–	VUS
Chr17: 41201205	c.5339T>C	p.Leu1780Pro	1 (2.4%)	rs80357474	–	–	Deleterious [20, 21]	VUS	PS3, PS4, PM2, PP3	Likely pathogenic
Chr17: 41201172	c.5372T>A	p.Val1791Glu	1 (2.4%)		–	–	–	VUS	PM2	VUS
Chr17: 41199753	c.5407-23C>T	–	1 (2.4%)	–	–	–	–	VUS	PM2, BP4	VUS

BP benign supporting evidence, *BS* benign strong evidence, *PM* pathogenic moderate evidence, *PP* pathogenic supporting evidence, *PS* pathogenic strong evidence, *VUS* variant of uncertain significance

[a]GRCh37 reference (hg19) was used for the chromosome locus

[b]Initial report at author's lab

[c]Reinterpretation according to ACMG/AMP 2015 guidelines

Table 2 Reinterpretation of variants of unknown significance in *BRCA2* according to the ACMG/AMP 2015 guidelines

Genomic location[a]	Nucleotide change (NM_000059.3)	Amino acid change	Number of cases (%)	rs number	Allele frequency from ExAC		Functional assay [Ref.]	Initial report[b]	Reinterpretation[c]	
					All	East Asians			Type of evidence	Reclassification
Chr13: 32890650	c.53G>A	p.Arg18His	1 (2.4%)	rs80358762	0.00004	0.000469	No deleterious [22]	VUS	BS3, BP4, BP6	Likely benign
Chr13: 32893198	c.68-16T>A	–	1 (2.4%)	rs397507882	0.0000184	0.000000	–	VUS	BP6	VUS
Chr13: 32893344	c.198A>G	p.Gln66=	1 (2.4%)	rs28897700	0.00107	0.007028	–	VUS	BP6, BP7	Likely benign
Chr13: 32903560	c.632-20C>T	–	1 (2.4%)	–	–	–	–	VUS	PM2, BP4	VUS
Chr13: 32906388	c.794-21T>C	–	1 (2.4%)	–	–	–	–	VUS	PM2	VUS
Chr13: 32906579	c.964A>C	p.Lys322Gln	3 (7.1%)	rs11571640	0.00006	0.000812	–	VUS	–	VUS
Chr13: 32907235	c.1620G>A	p.Leu540=	1 (2.4%)	–	–	–	–	VUS	PM2, BP4, BP7	VUS
Chr13: 32907359	c.1744A>C	p.Thr582Pro	6 (14.3%)	rs80358457	0.00022	0.003134	No deleterious [22]	VUS	BS3, BP6	Likely benign
Chr13: 32912061	c.3569G>A	p.Arg1190Gln	1 (2.4%)	rs80358605	0.00006	0.000000	–	VUS	–	VUS
Chr13: 32913027	c.4535G>C	p.Arg1512Pro	1 (2.4%)	rs80358685	–	–	–	VUS	PM2, BP4	VUS
Chr13: 32913346	c.4854T>A	p.Asp1618Glu	1 (2.4%)	rs80358708	0.00001	0.000116	–	VUS	–	VUS
Chr13: 32913998–32914000	c.5506_5508delAAT	p.Asn1836del	1 (2.4%)	–	–	–	–	VUS	PM2, PM4	VUS
Chr13: 32914082	c.5590G>A	p.Asp1864Asn	1 (2.4%)	rs587781536	0.00002	–	–	VUS	–	VUS
Chr13: 32914277	c.5785A>G	p.Ile1929Val	1 (2.4%)	rs79538375	0.00096	0.009827	No deleterious [22]	VUS	BS1, BS3, BP4, BP6	Benign
Chr13: 32914461	c.5969A>C	p.Asp1990Ala	1 (2.4%)	rs148618542	0.00004	0.000578	–	VUS	PP3	VUS
Chr13: 32914521	c.6029T>G	p.Val2010Gly	1 (2.4%)	rs80358839	0.00003	0.000462	–	VUS	–	VUS
Chr13: 32914531	c.6039A>G	p.Lys2013=	1 (2.4%)	–	–	–	–	VUS	PM2	VUS
Chr13: 32914817	c.6325G>A	p.Val2109Ile	2 (4.8%)	rs79456940	0.00028	0.003835	–	VUS	BP4	VUS

Table 2 (continued)

Genomic location[a]	Nucleotide change (NM_000059.3)	Amino acid change	Number of cases (%)	rs number	Allele frequency from ExAC		Functional assay [Ref.]	Initial report[b]	Reinterpretation[c]	
					All	East Asians			Type of evidence	Reclassification
Chr13: 32915309–32915311	c.6817_6819delAGA	p.Arg2273del	1 (2.4%)	–	–	–	–	VUS	PM2, PM4, PP3	VUS
Chr13: 32929297	c.7307A>T	p.Asn2436Ile	1 (2.4%)	rs80358955	0.00001	0.000000	No deleterious [22]	VUS	BS3, BP6	Likely benign
Chr13: 32930651	c.7522G>A	p.Gly2508Ser	3 (7.1%)	rs80358978	0.00011	0.001505	–	VUS	PP3	VUS
Chr13: 32931952	c.7691C>G	p.Thr2564Ser	1 (2.4%)	rs431825355	0.00003	0.000463	–	VUS	–	VUS
Chr13: 32937526	c.8187G>T	p.Lys2729Asn	5 (11.9%)	rs80359065	0.00082	0.009711	No deleterious [22–25]	VUS	BS1, BS3, BP6	Benign
Chr13: 32937554	c.8215G>C	p.Val2739Leu	1 (2.4%)	rs80359069	0.000008238	0.000116	–	VUS	BP4	VUS
Chr13: 32954247	c.9221T>C	p.Leu3074Pro	1 (2.4%)	–	–	–	–	VUS	PM2	VUS
Chr13: 32972280	c.9649-19G>A	–	3 (7.1%)	rs11571830	0.00135	0.017260	–	VUS	BS1, BP4, BP6	Likely benign

BP benign supporting evidence, *BS* benign strong evidence, *PM* pathogenic moderate evidence, *PP* pathogenic supporting evidence, *PS* vpathogenic strong evidence, *VUS* variant of uncertain significance

[a]GRCh37 reference (hg19) was used for the chromosome locus

[b]Initial report at author's lab

[c]Reinterpretation according to ACMG/AMP 2015 guidelines

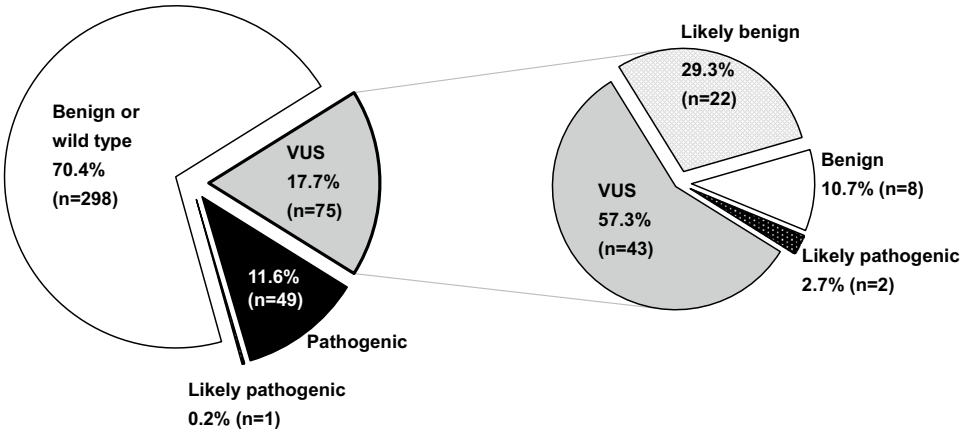

Fig. 2 Reclassification of patients with previously reported variants of uncertain significance (VUS) based on ACMG/AMP 2015 guidelines according to reinterpreted *BRCA1/2* genetic testing

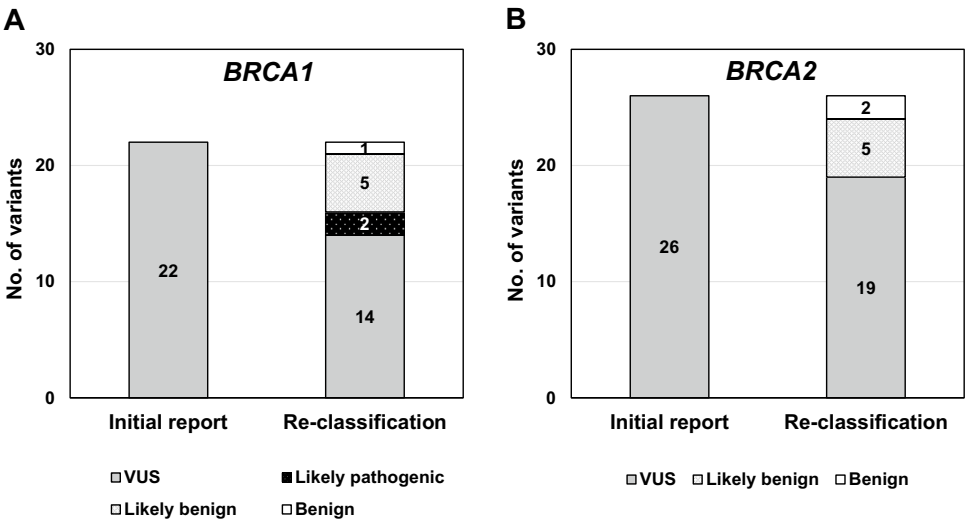

Fig. 3 Reclassification of previously reported variant of uncertain significance (VUS) based on ACMG/AMP 2015 guidelines, stratified by sequence variants. **a** Among 22 *BRCA1* variants previously reported as VUS, eight (8/22, 36.3%) were reclassified into specific categories: two variants were reclassified as "likely pathogenic" and six variants were reclassified as either "benign" or "likely benign". **b** Among 26 VUS in *BRCA2*, seven (7/26, 26.9%) were reclassified as either "benign" or "likely benign"

Discussion

Here we re-analyzed the genetic testing results in patients with VUS in *BRCA1/2* according to the ACMG/AMP 2015 guidelines with updated information to assess the possibility of reclassifying VUS under a specific category. Among the 75 patients carrying VUS, 32 patients (32/75, 42.7%) had variants that were reclassified after the reinterpretation, of whom 30 (30/32, 93.8%) were downgraded to "likely benign" or "benign" variants and two (2/32, 6.3%) were upgraded to "likely pathogenic". Our results were consistent with several previous studies [11, 26–28]. In a previous study, 44.9% of women with a previously identified VUS were reclassified to another category after reinterpretation [11]. Approximately 90% of these women carried variants that were downgraded to polymorphisms or benign variants, while roughly 10% carried variants that were upgraded to deleterious or suspected deleterious variants [11]. Other studies have similarly shown that a large number of reclassified variants were downgraded, while some were upgraded [26–29].

Although reinterpretation of most of the cases resulted in variant downgrades (i.e., from VUS to benign or likely benign) could have little clinical impact, only a small number of upgraded variants (i.e., from VUS to likely pathogenic) could provide important information in clinical decision-making. For examples, the clinician could choice additional treatment options such as PARPi or RRSO. Furthermore, downgraded variants can help reduce the anxiety of the patient and their family, and it would not be necessary to take a family genetic test [12, 13]. Thus, these findings emphasize the clinical significance for reclassification of VUS in *BRCA1/2*.

Over time, the prevalence of VUS decreases due to accumulations of the data helpful to accurate interpretation and classification of the variants [9]. In the present study, we divided the data into two groups before and after July 2016, when the ACMG / AMP 2015 guidelines were applied. Based on the time point, the pre- and post-prevalence of

VUS using the time point were 22.8% (58/254) and 10.1% (17/169), respectively. In the early period, there was a lack of relevant data aided to interpret the clinical significance of sequence variants, so more variants would have been classified as VUS. After then the VUS tended to decrease over time with evolving over the 5–7 year period of this study. In total study period, reclassified VUS gave a revised prevalence of 10.2% (43/423) changed from 17.7% (75/423). These results can be explained by the evidence that the prevalence of VUS decreased as *BRCA1/2* gene-related data accumulated. Therefore, it is necessary to periodically reinterpret the VUS results.

In this study, two cases were reclassified from VUS to likely pathogenic category. First case carried a missense mutation in *BRCA1* (c.5339T>C, p.Leu1780Pro). The evidences of the first case based on the ACMG/AMP 2015 guidelines were as follows: PS3 (presence of functional studies supportive of a damaging effect) [20, 21], PS4 (that the odds ratio of an epidemiological study was significantly higher than that of the population control) [26], PM2 (variant was absent or present at extremely low frequency in controls), and PP3 (multiple lines of computational evidence support a deleterious effect). The supporting data was also found in other studies [30, 31]. The patient was consequently enrolled in a clinical trial for olaparib target therapy and underwent RRSO.

Second case was a variant leading to an in-frame deletion of codon 1673 in *BRCA1* (c.5017_5019delCAC, p.His1673del). The evidences according to the ACMG/AMP 2015 guideline were as follows: PM2, PM4 (protein length changes as a result of in-frame deletions/insertions), and lastly, PS4 (prevalence of the variant in affected individuals is significantly higher compared with that in controls) could be applied based on literature [32]. The patient showed no evidence of recurrence after breast conserving surgery and concurrent radiation therapy and chemotherapy. Further action has not yet been taken since the reinterpretation of the VUS results.

The ACMG first published guidelines for the classification of sequence variants in 2000 [33]. This guideline was revised in 2007 and 2015 as the analysis and interpretation of genetic testing became more complex [7, 14]. The most recently published ACMG/AMP 2015 guidelines recommend classifying variants into five categories [7]. The guidelines recommend that variants are classified by a certified expert using various types of variant evidence, including population, computational, functional, and segregation data. This evidence-based interpretation is still open to subjective judgment [34]. In a previous study, the BP1 criterion, which is a "missense variant in a gene for which primarily truncating variants are known to cause disease" and serves as supporting evidence of benign impact, was applied to all missense variants when evaluating *BRCA1/2* variants [28]. As the BP1 criterion was applied to all missense variants, a large number of VUS may be downgraded to "benign" or "likely benign". However, although mutations in *BRCA1/2* that result in premature termination codons commonly cause disease, several single nucleotide variations that cause amino acid changes have also been reported to cause disease, e.g., c.181T>G (p.Cys61Gly) [35] in *BRCA1* and c.8167G>C (p.Asp2723His) [29] in *BRCA2*. Thus, we concluded that it would be inappropriate to apply the BP1 criterion to all missense variants. For this reason, the BP1 evidence was not applied in our analysis. As such, the subjective opinion of the individual who interpret the genetic testing results may influence how each type of evidence is applied.

There are several limitations in this study. First, this was a retrospective study by reviewing medical records in which variant interpretations were performed using only *BRCA1/2* genetic test results of the patients, without sufficient family history or segregation analysis. Thus, the pathogenicity evidence criteria, PS2, PM3, PP1, and PP4, as well as a benign evidence criterion, BS4, were limited. These evidence criteria may be applied when a sufficient family history of the patient is available. There are, however, some instances where laboratories have no choice, but to report results without being able to determine the patient's family history. For more informative interpretation according to the ACMG/AMP 2015 guidelines, the family history of patients should be determined and used as evidence. Second, we thought that it could be helpful to periodically reanalyze genetic variants that were initially found to lack established clinical significance, such as VUS. However, this study does not provide information on how frequently reinterpretation should be performed. Although the ACMG/AMP 2015 guidelines indicate the need for an ongoing effort for reclassifications of VUS, a detailed agreement and guideline on when and how often reinterpretation must be performed have not been developed [7].

In conclusion, there is always a possibility for *BRCA1/2* VUS to ultimately be reclassified under another category when it is reanalyzed using data that have been expanded and updated. Genetic testing is important in medical decision-making, and thus a continued effort is required to clarify ambiguous results with unknown clinical significance, such as those for VUS.

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Compliance with ethical standards

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