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ORIGINAL ARTICLE

Reclassification of Hereditary Cancer Genes Variants

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OBJECTIVE

In this study, pathogenic, likely pathogenic and variant of uncertain significance/variant unknown significance (VUS) identified in the Hereditary Cancer Panel Genes between 2016 and 2017 and specified in the report are re-examined in 2022 and shown whether they have changed over time.

METHODS

Containing 26 genes in 2016-2017 variants of patients with pathogenic/likely pathogenic/VUS detected in the Hereditary Cancer Panel were analyzed again in 2022 on Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) and other databases.

RESULTS

The results of a total of 137 patients, 137 women and 2 men, were evaluated. While no pathogenic/likely pathogenic/VUS variant was detected in the results of 95 patients, at least 1 variant was detected in 42 female patients. A total of 58 variants were detected in 42 patients, and we found that 24 variants among them fell into a different class. While 12 more variants were included in the lower pathogenicity subgroup, 5 of them were higher in pathogenicity. We saw that 6 variants that were not yet identified in 2016-2017 were identified, except for 1 of them.

CONCLUSION

We have seen that the pathogenicity of the variants written in patient reports, which can cause serious changes in the patient's life, can change over time. While giving genetic counseling about these variants, it should be stated that much more comprehensive research and information should be given to the patient, this information was given to the patient under the current conditions and that there may be a possibility of change in the future.

Keywords: Hereditary breast cancer; variant of uncertain significance; variant reclassification; VUS.

INTRODUCTION

An estimated 5-10% of breast cancers are hereditary, and the genes that most frequently and with high penetrance increase the risk of hereditary breast and ovarian cancers are *BRCA1* and *BRCA2* mutations. Conventionally, New generation sequencing genetic test panels in this field have revolved around these two genes.[1]

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Multigene panel tests have become increasingly widespread as a result of developing Next Generation Sequencing Systems, and genetic tests have become cost-effective for especially hereditary cancers.[2]

The National Comprehensive Cancer Network has expanded testing criteria for high-penetrance breast and/or ovarian cancer susceptibility genes including *BRCA1, BRCA2, CDH1, PALB2, PTEN*, and *TP53*, among others that have found to be associated with hereditary risk.[3] Some are part of rare high-penetrance cancer predisposing syndromes (e.g. *BRCA1, BRCA2, TP53, CDH1, PTEN, STK11*, and *PALB2*),[4,5] while others are moderate-penetrance genes (e.g. *ATM, NBN, CHEK2*, and *BARD1*).[6] Pathogenic variants in *PALB2, ATM, CHEK2, NBN, BRIP1, RAD51C*, and *RAD51D* are associated with a ywo-fold to five-fold increase in relative risk for certain cancers. However, because of cancer syndrome heterogenity, it is often difficult for providers to determine which cancer predisposition genes to test in a patient whose family or personal history is suggestive of a hereditary cancer syndrome.[7,8]

The most effective way to identify germline mutations of different clinical significance is to analyze hereditary cancer susceptibility genes that increase the risk with the NGS method. ACMG/AMP 2015 was the first guide to variant interpretation classifying a variant as "pathogenic," "probably pathogenic," "benign," "probably benign" and "VUS."[9] Some of the databases used for variant analysis and clinical significance are:

- ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/)
- Human Gene Mutation Database (HGMD) (license required for professional version) (http://www.hgmd.org/).[10]
- Leiden Open Variation Database (LOVD) (https://www.lovd.nl/).[11]
- Simple ClinVar (http://simple-clinvar.broadinstitute.org/).[12]

VUS is a term used to describe a class of variables of uncertain clinical significance that does not provide any useful information for clinical decision making.

In recent years, the number of studies related to hereditary cancer susceptibility genes has been increasing. With updated information, reevaluation of the VUS of these genes may reveal new clinical information. This information may require changes in diagnosis and treatment protocols in hereditary cancer patients.

Indeed, some recent studies in USA, China and Korea show that there have been changes in the clinical significance of *BRCA* variants over the years.

How to treat cancer in pathogenic and benign variants in hereditary cancer genes is certain. However, VUSs are in the gray zone and clinicians remain undecided about how to treat that cancer. In this study, we investigated the change in clinical significance of VUSs over time (2022) in patients whose hereditary cancer panel was studied in 2016-2017. As a result, the approach to the uncertain situation in VUS carriers will be evaluated.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of Memorial Hospitals (Report No: 709/22-005) Patients who applied to the Memorial Hospital Genetic Diagnosis Center in 2016 and 2017 with the request of "Hereditary Cancer Panel Test" were included in the study. The criteria for requesting tests from patients are as follows; (a) patients with breast cancer under the age of 50, (b) triple negative breast cancer, (c) people with a family history of 2 or more *BRCA*-dependent cancers, (d) male breast cancers, (e) patients with bilateral breast cancer, and (f) patients diagnosed with ovarian cancer.

A total of 137 female and two male breast cancer patients were included in the study. The mean age for women was 37.8. Men's ages were 48 and 56.

All patients selected according to these criteria were informed about the test, and consent forms were signed. DNA isolation was performed with automated systems and standard protocols from peripheral blood samples collected in EDTA tubes. As part of the test, genes were sequenced with the Multiplicom *BRCA* hereditary MASTR Plus kit. Next generation sequencing was done with Illumina MiSeq platform, variant calling and bioinformatics analysis were done with genomize-Seq (http://seq.genomize.com) (Analysis Version = BWA-Freebayes-Trimmomatic primer trim (v.9), Annotation Version= Ensembl).

In the panel analyzed within the scope of hereditary breast cancer, 6 were high (*BRCA1, BRCA2, TP53, STK11, PTEN*, and *CDH1*) and 9 were moderate (*ATM, NBN, MUTYH, CHEK2, BLM, MLH1, MSH2, MSH6*, and *PMS2*), and 11 were low risk (*BARD1, BRIP1, RAD50, RAD51C, RAD51D, MRE11A, EPCAM, FAM175A, PALB2, MEN1*, and *XRCC2*), a total of 26 genes are sequenced.

Only "Pathogenic (P)," "Likely Pathogenic (LP)" and "Variant of Unknown Significance (VUS)" have been reported. During the analysis, changes other than these variants were not reported according to the current scientific information in 2016-2017.

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Table 1 Hereditary cancer panel variants of all patients included in the study

No	Gene variant	2016	2022	Up down new	Variant type	Sift-polyphen-mutaster
1	*BRCA2*:c.8456A>T	VUS	VUS		Missense	Harmful
2	*BRCA1*:c.66dupA	Pathogenic	Pathogenic		Insertion, Frameshift	none
	BRCA1:c.4434G>T	VUS	VUS		Missense	benign
3	*BRCA1*:c.547+14delG	VUS	VUS		Intronic	none
4	*BRCA1*:c.4697C>G	LP	VUS	D	Missense	Harmful
5	*TP53*:c.524G>A	Pathogenic	Pathogenic		Missense	Harmful
6	*BRCA1*:c.4766G>A	VUS	VUS		Missense	none
7	*BRCA2*:c.*135G>A	None	None		none	none
8	*BRCA2*:c.6935A>t	VUS	Benign	D	Missense splice region	Harmful
9	*BRCA1*:c.4801A>C	Pathogenic	VUS	D	none	Harmful
	NBN:c.415A>G	LP	LB	D	Missense	Harmful
10	*BRCA2*:c.8T>G	LP	LP		Missense	none
	MSH2:c.55T>C	VUS	VUS		Missense	Harmful
	MSH2:c.50T>G	VUS	LP	U	Missense	
	STK11:c.551T>C	None	VUS	N	Missense	none
	STK11:c.590T>G	None	VUS	N	Missense	none
11	*BRCA2*:c.6821G>T	VUS	VUS		Missense	Harmful
	MSH2:c.260C>G	VUS	VUS		Missense	none
12	*BRCA2*:c.67+82C>G	LP	VUS	D	Intronic	none
13	*BRCA1*:c.20G>A	VUS	VUS		Missense	none
	ATM:c.3576G>A	Pathogenic	Pathogenic		Splice Region	none
14	*BRCA2*:c.8452G>A	VUS	VUS		Missense	none
15	*BRCA1*:c.–86C>T	VUS	VUS		5'UTR	none
16	*BRCA1*:c.3708T>G	VUS	Benign	D	Missense	none
	BRCA1:c.850C>T	Pathogenic	Pathogenic		Stop Gained	none
	BRCA2:c.8749C>T	VUS	LP	U	Missense	none
17	*BRCA1*:c.3541G>A	VUS	LB	D	Missense	Harmful
18	*BRCA2*:c.1181A>C	VUS	Benign	D	5'UTR	none
19	*MLH1*:c.1321G>A	VUS	Benign	D	Missense	none
20	*MLH1*:c.108T>G	VUS	VUS		Missense	none
21	*MLH1*:c.1039–1G>A	Pathogenic	Pathogenic		Splice Acceptor	none
22	*MSH2*:c.2005+43 2005+44delTT	None	VUS	N	Deletion	none
	NBN:c.880A>T	VUS	VUS		Missense	none
23	*MLH1*:c.108T>G	VUS	VUS		Missense	none
	TP53:c.1079G>C	VUS	VUS		Missense	none
24	*MEN1*:c.655-4delT	None	VUS	N	Spice region, deletion	none
25	*BLM*:c.934T>G	VUS	VUS		Missense	none
	MSH2:c.50T>G	VUS	LP	U	Missense	none
26	*MSH2*:c.55T>C	LP	VUS	D	Missense	Harmful
27	*MUTYH*:c1187G>A	Pathogenic	VUS	D	Stop Gained	Harmful
28	*BRCA2*:c.*135G>A	VUS	VUS		none	none
29	*MSH6*:c.2503C>G	VUS	VUS		none	none
30	*BRCA2*:c.10095C>T	None	Benign	N	Missense	none
31	*MLH1*:c.36C>A	None	LP	N	Missense	none
32	*BRCA2*:c.67+82C>G	VUS	VUS		Intronic	none
	BRCA2:c.1415delA	VUS	VUS		Frameshift	none
33	*BRCA1*:c.-86C>T	VUS	VUS		5'utr	none
34	*ATM*:c.3257G>A	VUS	VUS		Missense	Harmful
	MSH6:c.435T>G	VUS	VUS		Missense	Harmful
	RAD51:c.263+2T>G	LP	VUS	D	Splice Donor	none

Table 1 Cont.

No	Gene variant	2016	2022	Up down new	Variant type	Sift-polyphen-mutaster
35	*STK11*:c.1211C>T	VUS	VUS		Missense	none
36	*RAD50*:c.1421–1439del	VUS	VUS		Frameshift	none
37	*PALB2*:c.1043A>G	VUS	LP	U	Missense	Harmful
38	*TP53*:c.1010G>C	LP	VUS	D	none	none
39	*BRCA2*:c.9682delA	Pathogenic	VUS	D	Frameshift	Harmful
40	*PALB2*:c.1196C>T	VUS	LP	U	Missense	none
41	*MSH2*:c.382C>G	VUS	VUS		Missense	Harmful
42	*BRCA2*:c.6131G>C	VUS	VUS		Missense	Benign

VUS: Variant unknown significance; LP: Likely pathogenic; LB: Likely benign; D: Downregulated (Pathogenic→Likely Pathogenic→VUS→Likely Benign→Benign); U: Upregulated (Benign→Likely Benign→VUS→Likely Pathogenic→Pathogenic); N: New variant (There is no knowledge in the databases)

In January 2022, the variants of these patients were re-controlled from Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/), ACMG, and other databases, (https://search.ngscloud.com/) and the changings were recorded. The changing of variants was evaluated in the approximately 5-year period between the two dates.

RESULTS

At least 1 pathogenic, LP or VUS variant of the genes found in the panel was detected in 42/137 (30.65%) women and 0/2 men. No variant (Patogenic, LP or VUS) was found in 95 people (69.35%). The variants of all genes included in the Hereditary Cancer panel are shown in Table 1-without gene discrimination.

A total of 58 different gene variants were found in 42 patients. The number of genes with clinical significance changes was 24/58 (41.37%) between the mentioned years (Table 1).

Of the Variants (5) detected as Benign in 2022, 4 were previously identified as VUS and one was Undefined in 2016. In 2022, 2 Likely Benign Variant were detected. *BRCA1*:c.3541G>A was previously VUS but was defined as Likely Benign in 2022. *NBN*:c.415A>G was previously likely pathogenic interestingly it is defined as Likely Benign in 2022.

When variant changes were evaluated without gene discrimination, 13 of the 24 changes were downgrade, 5 were upgrade, and 6 were new variants that were not yet defined in the variant databases in 2016 (Table 1).

Table 2 shows the variant's clinical significance changes between 2016 and 2022. The remarkable thing in this table was that all but 1 of the previously undefined variants were identified. (4 VUS, 1 LP, 1 Benign). This shows the speed with which the clinical significance of novel variants is interpreted.

Table 2 Comparison of the classification of pathogenicity of 58 variants we detected in 42 patients in 2016-2017 compared to 2022

Variant type	2016-2017	2022
VUS	36	38
Likely pathogenic	7	7
Pathogenic	8	5
None	7	1
Benign		5
Likely benign		2
Total	58	58

VUS: Variant unknown significance

DISCUSSION

Genetic testing for hereditary breast cancer risk may be important in surgical decision making and the use of new drugs (such as platinums) for newly diagnosed cancer patients. *BRCA* mutation carriers have been shown to have a higher rate of developing contralateral breast cancer and those choosing bilateral mastectomy are less likely to die from breast cancer than women with unilateral mastectomy.[13] Other genes have been shown to have a lower, but still contralateral, risk of breast cancer. Therefore, it can be said that the pathogenicity of the variants in the hereditary cancer genes determines the treatment method. In a meta-analysis by Li et al.[14] in which they evaluated 109 studies between 1999*2019, they evaluated changes in the *BRCA* 1-2 genes. About 8.3% found that 112/1,351 variants changed into different categories on reclassification.

About two-thirds of VUSs in the KOHBRA study by Kim et al.[15] were reclassified as benign or likely benign [193/278] *BRCA1* patients (69.42%), *BRCA2* patients 328/453 (72.41%), and all patients 471/676

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(69.67%). One-third of the mutation types classified as VUS in the KOHBRA study were downgraded as benign or likely benign (20/58 mutations in *BRCA1* and 25/91 mutations in *BRCA2*).

Mighton et al.[16] identified 1209 *BRCA1* and *BRCA2* variants between 2012 and 2017. During this period, 12.4% (150/1209) of variants were reclassified. The majority of reclassified variants were downgraded (74.7%). Of the reclassified variants, 63.3% (95/150) were reclassified to benign, 20.7% to likely benign, 10.0% to variant of uncertain significance, 2.0% to likely pathogenic, and 4.0% to pathogenic. Discordant ClinVar submissions were found for 40.4% (488/1209) of variants. In our study, the 14/24 (33.6%) variant was changed to downgrade and the 5/24 (20.83%) variant to upgrade, as in other studies. In addition, 6 novel variants that were not previously available in databases were identified. Macklin et al.[17] also reported that over the years, most of the VUSs (29/40) were converted to benign or LB variants, just like in our study.

As seen in all reclassification studies, including ours, most VUSs transform into B or LB variants over time. Therefore, treating VUSs as pathogenic variants may increase the rate of unnecessary bilateral mastectomy. Re-analysis of this variant at regular intervals in patients with VUS will be important in terms of monitoring and guiding the treatment.

CONCLUSION

A limitation of this study is that not all variants belong to the same gene and different genes are included in the classification.

This study represents relatively few variants and consists of data from one laboratory whose policies may differ from other laboratories, limiting the generalizability of these results. Despite these limitations, this study provides inspiration about the speed and importance of ClinVar variants reassortment in genes that increase breast cancer risk. This should be taken into account when arranging the treatment of patients, and overly aggressive attitudes in prophylaxis and treatments should be avoided. Future research should consider more broadly the impact of variant reclassifications on patients and the healthcare system.

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