Analysis of BRCA1/2 variants of unknown significance in patients with breast cancer from a prospective KOHBRA study

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Abstract

Background Genetic testing for BRCA1 and BRCA2 genes is crucial for diagnosing hereditary breast and ovarian cancer syndromes (HBOC). Testing for such genes has been on the rise due to the development of multigene panel tests. However, results classified as variants of uncertain significance (VUS) present challenges to clinicians trying to interpret their functions and choose appropriate management plans.

Methods We reviewed a total of 676 breast cancer patients who had VUS on BRCA mutation tests between November 2007 and April 2013 in the KOHBRA study. These results were compared to the ClinVar database. We calculated the incidence and odds ratios for these variants using the Korean Reference Genome Database (KRGDB).

Results A total of 58 and 91 distinct VUS in BRCA1 and 2 were identified in the KOHBRA study (comprising 258 and 453 patients, respectively). A total of 27 variants in the KOHBRA study were not registered in the SNP database. Of the BRCA1 VUS, 20 variants were reclassified as benign or likely benign, 4 variants were reclassified as pathogenic or likely pathogenic, and 8 variants remained as VUS according to the ClinVar database. Of the BRCA2 VUS variants, 25 variants were reclassified as benign or likely benign, two variants were reclassified as pathogenic or likely pathogenic, and 33 variants remained as VUS according to the Clinvar database. There were 12 variants with conflicting interpretations of pathogenicity for BRCA1 and 18 variants for BRCA2. Among them, p.Leu1780Pro showed a particularly high odds ratio.

Conclusions Six pathogenic variants and one conflicting variant identified using ClinVar could be reclassified as pathogenic variants in this study. Using updated ClinVar and calculating odds ratios can be helpful when reclassifying VUS in BRCA1/2.

Background

Hereditary breast and ovarian cancer syndrome (HBOC) is related to germline mutations in BRCA1 and BRCA2 [1]. Demands for genetic testing to identify pathogenic mutations in BRCA1 and BRCA2 have steadily increased since the identification of these genes [2]. A BRCA genetic test shows 4 possible results: no mutation detected, pathogenic mutation, benign mutation, or variant of uncertain significance (VUS). A VUS is an alteration in the gene sequence that has an unknown effect on the function of the gene product.

Identification of a pathogenic BRCA mutation in a patient diagnosed with breast cancer affects treatment and prognosis, but also enables prevention of other cancers [3]. Guidelines for the management of pathogenic variants in BRCA1 and BRCA2 genes recommend consideration of risk-reducing medications or surgeries [4, 5]. Therefore, identifying the function of the mutation is important for patients and their families. Furthermore, the cost of genetic testing has rapidly decreased due to recent technological progress and the use of large-scale next-generation sequencing (NGS) requiring shorter processing times [2].

A major problem with genetic testing for BRCA is the interpretation and management of VUSs. This leaves patients with uncertainty due to the lack of interpretation in the clinical context, as well as the lack of specific guidelines regarding genetic counseling or prophylactic management in mutation carriers and their relatives [6].

Overall, a VUS rate of 7–15% in women who received BRCA testing has been reported [7]. The frequency of VUS reports varies worldwide depending on testing prevalence and population ancestry [7, 8]. Researchers reported that

the frequency of VUSs was 21% in African-Americans, 5–6% in individuals of European ancestry in the USA, and 15% in European laboratories [9, 10]. Myriad Genetic Inc. (Salt Lake City, UT, USA) reported that they decreased the proportion of VUS to 2.1% using their accumulated data [11]. However, these databases are not public or accessible.

In this study we aimed to explore the prevalence of VUS and reclassify these variants using the ClinVar database and the Korean Reference Genome Database (KRGDB) in the Korean population.

Methods

Subjects

The study population was obtained from the Korean Hereditary Breast Cancer (KOHBRA) study [12]. The KOHBRA study is a multicenter prospective cohort study to identify the prevalence and cause of hereditary breast cancer in the Korean population. Through the study, 3,015 subjects were recruited between May 2007 and December 2013 from 36 institutions [13]. The eligibility criteria were as follows: (1) breast cancer patients with a family history of breast or ovarian cancer; (2) breast cancer patients without a family history of breast or ovarian cancer (non-familial) who were 40 or younger at diagnosis, and were diagnosed with bilateral breast cancer or another primary malignancy; (3) male breast cancer patients; and (4) family members of *BRCA 1/2* mutation carriers. After excluding several subjects, a total of 2,953 subjects (1,228 familial breast cancer patients, 1,175 non-familial breast cancer patients, and 550 family members of affected carriers) were evaluated. We identified 676 breast cancer patients with VUSs on BRCA mutation tests.

These results were reclassified using the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/) based on refSNP (RS) numbers. The odds ratio (OR) for each variant was calculated using Korean population data from the KRGDB, which was established by conducting whole genome sequencing of 1,722 Koreans [14]. Variants that are not registered in the Single Nucleotide Polymorphism (SNP) database are shown in Table 1. In this study, variations without RS numbers were also included in the denominator when checking frequency.

BRCA 1/2 mutation analysis

BRCA 1/2 genetic testing was performed using genomic DNA from the peripheral blood by fluorescence-based confirmation sensitive gel electrophoresis, denaturing high performance liquid chromatography, or direct sequencing, based on the procedure of the DNA testing laboratories linked to each institution. All *BRCA 1/2* variants were categorized as pathogenic, variants of unknown significance (VUSs), or polymorphic (benign).

Mutation nomenclature

All sequence variations are described according to HUGO-approved systematic nomenclature (http://www.hgvs.org/mutnomen/) using GenBank reference sequences (NM_007294.2 for *BRCA 1* and NM_000059.3 for *BRCA 2*). The breast cancer information core nomenclature is also described for convenience.

This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University. (IRB# 4-2017-0255)

Results

Among 2,403 breast cancer patients, more than a quarter, 676 (28.13%) patients, had mutations that were classified as VUS in the KOHBRA study. Simultaneous mutations of BRCA1 and BRCA2 were observed in 55 (55/676, 8.14%) patients. Of the 676 subjects, 278 patients had VUS in BRCA1 and 453 patients had VUS in BRCA2 (Fig. 1). RS numbers were reviewed for 262 and 440 subjects with BRCA1 and BRCA2 mutations, respectively.

Table 1 shows the reclassification results for VUS according to the ClinVar database. We classified the results into four groups: benign/likely benign, VUS, conflicting in interpretations of pathogenicity, and pathogenic/likely pathogenic. “Benign/likely benign” was the most common reclassification in both BRCA1 and BRCA2 VUS. Table 2 shows the rank of VUS genes based on the number of patients.

Of the 278 patients with BRCA1 VUS, 58 types of VUS were identified and 44 types had RS numbers. 20 of these, found in 193 patients, were classified as “benign/likely benign;” the least common VUS was classified as “pathogenic/likely pathogenic” and comprised 4 types in 6 patients (Table 1).

Of the 453 patients with BRCA2 VUS, 91 types of VUS were identified and 78 types had RS numbers. The most common type of VUS was “benign/likely benign”, with 25 types in 328 patients, and “pathogenic/likely pathogenic” was the least common and included 2 types of VUS in 3 patients.

In this study, six gene mutations previously classified as VUS were reclassified as “likely pathogenic” based on ClinVar review. The variants were BRCA1 c.5089T > C (p.Cys1697Arg), BRCA1 c.5509T > C (p. Trp1837Arg), BRCA1 c.5516T > C (p.Leu1839Ser), BRCA1 c.81-9C > G, BRCA2 c.8023A > G (p.Ile2675Val), and BRCA2 c.9004G > A (p.Glu3002Lys).

We calculated ORs for each variant using the KRGDB as shown in Fig. 2. The OR of BRCA1 c.5339T > C (p.Leu1780Pro) was significantly high, although BRCA1 c.5339T > C had conflicting pathogenicity interpretations based on ClinVar.

Discussion

We re-evaluated genetic results in patients with VUS in BRCA1 and BRCA2 using the ClinVar database. Because genetic characteristics can vary by ethnicity [7, 9, 10]. this study aimed to identify the prevalence of VUS in the Korean population and to re-classify the results of initial genetic testing. The KOHBRA study is a multicenter prospective cohort study that aims to identify the prevalence and cause of hereditary breast cancer in the Korean population [12]. Although the initial genetic testing results showed 676 patients with VUS (278 patients for BRCA1 and 453 patients for BRCA2) among 2,403 breast cancer patients (28.13%, 676/2,403), re-evaluation showed a decreased frequency of VUS (8.03%, 193/2,403). About a third of the variants that were originally classified as VUS in the KOHBRA study were reclassified as benign/likely benign or pathogenic/likely pathogenic, accounting for two-thirds of all VUS patients (71.45%, 483/676). This result suggests that a re-classification approach using the ClinVar database can reduce the frequency of VUS in the Korean population.

About two thirds of the VUS in the KOHBRA study were reclassified as benign or likely benign (193/278 (69.42%) of BRCA1 patients, 328/453 (72.41%) of BRCA 2 patients, and 471/676 (69.67%) of all patients). A third of the mutation types classified as VUS in the KOHBRA study were downgraded to “benign or likely benign” (20/58 mutations in BRCA1 and 25/91 in BRCA2, 30.2%). These results were consistent with previous studies [1, 15, 16]. So et al. reported that 30/75 (40%) VUS patients were reclassified to “benign or likely benign” [17]. In our study, six patients with BRCA1 VUS (4 mutations) and three patients with BRCA2 VUS (2 mutations) were reclassified as “pathogenic or likely pathogenic”.

Pathogenic/ likely pathogenic mutations

In this study, six mutations in nine patients were reclassified from VUS to “pathogenic or likely pathogenic”. BRCA1 c.5089T>C (p.Cys1697Arg), BRCA1 c.5509T>C (p. Trp1837Arg), BRCA1 c.5516T>C (p.Leu1839Ser), and BRCA1 c.81-9C>G were interpreted as likely pathogenic in BRCA1. BRCA2 c.8023A>G (p.Ile2675Val) and BRCA2 c.9004G>A (p.Glu3002Lys) were interpreted as pathogenic/ likely pathogenic in BRCA2 based on the ClinVar database.

Because all six mutations have sufficient evidence in functional studies (PS3) [18-20], have not been reported in the genomic database from the general population (PM2), are classified as pathogenic in ClinVar (PP5), and are deleterious mutations (PP3) in an *in silico* study [21], they should be reclassified as likely pathogenic or pathogenic mutations. For patients with these mutations, additional genetic counseling and proper management such as familial genetic testing, risk-reducing medications, or risk-reducing surgery are needed for prevention of cancer.

Conflicting interpretations of pathogenicity

Calculating ORs

Interestingly, we identified several cases with “conflicting interpretations of pathogenicity” and reviewed the current status of reported evidence (Table 3). Calculating ORs can help to reclassify VUS in BRCA1/2. The KRGDB is built for precision medicine research and is a large-scale, single-race database collected from 1,722 Koreans. We calculated ORs using the KRGDB (Figure 2). Several mutations (7 BRCA1 and 9 BRCA2 mutations, orange colored dots) were evaluated. Most of them showed no significance. However, BRCA1 c.5339T>C (p.Leu1780Pro) showed possible pathogenicity, while BRCA1 c.154C>T (p.Leu52Phe) and BRCA2 c.964A>C (p.Lys322Gln) were potentially benign. BRCA 1 c.5014_5016delCAC, BRCA 1 c.5332G>A, BRCA2 c.182T>C, BRCA 2 c.1909+22delT, BRCA 2 c.8486A>G, and BRCA2 c.8954-5A>G have no reports in the general population; they should remain classified as VUS because there are no reports on their deleterious function.

Functional study

The interpretation of VUS is still complex. Functional studies for reclassifying VUS could be a promising approach. Traditionally, VUS interpretation depended on inductive conclusions based on information from individual patients [22]. However, many potential variants in BRCA have a low frequency and the phenotype is incompletely penetrant.

Findlay et al. reported the application of saturation genome editing (SGE) to measure the functional outcomes of all possible single nucleotide variants (SNVs) in key areas of BRCA1 [18]. Functional effects were almost concordant with established assessments of pathogenicity. Function scores using SGE could help interpret the significance of VUS by providing functional classification and assessment of ambiguous or newly-discovered variants.

The four BRCA1 mutations (c.5089T>C (p.Cys1697Arg), c.5509T>C (p. Trp1837Arg), c.5516T>C (p.Leu1839Ser), and c.81-9C>G) identified as “likely pathogenic” using the ClinVar database were identified as non-functional in Findlay's study [18]. The c.5339T> C (p.Leu1780Pro) variant identified as “conflicting interpretations of pathogenicity” in the ClinVar database was also identified as “non-functional” in the functional study results, which suggests that this variant is pathogenic [18]. On the other hand, other variants with conflicting interpretations of pathogenicity in the ClinVar database (including c.154C>T (p.Leu52Phe), c.5068A>C (p.Lys1690Gln), and c.5332G>A (p.Asp1778Asn)) were categorized as functional or intermediate [18]. The other BRCA1 variants with conflicting interpretations of pathogenicity in the ClinVar database could not be evaluated according to the Findlay’s study because it analyzed only RING and BRCT domains as targets [18].

Patients whose results indicate "conflicting interpretations of pathogenicity," should be managed with a multi-disciplinary team approach.

L1780P interpretation

With regard to c.5339T>C (p.Leu1780Pro), four reports interpret this as “pathogenic or likely pathogenic” and one report stated that it was of “uncertain significance”.

When ORs were calculated using the KRGDB for all KOHBRA data, the OR of BRCA1 c.5339T>C (p.Leu1780Pro) was found to be significantly elevated (Fig. 2). This variant was also identified as non-functional in Findlay’s study. In addition, several studies have suggested that this mutation is pathogenic based on other evidence including strong family history of breast and ovarian cancer, absence in general population data, impaired function demonstrated by *in silico* studies, and triple negativity in clinicopathologic features [16, 17, 23]. It is advisable to make management decisions based on ‘likely pathogenic’ variants. Previous studies have used a similar approach to reclassify some variants [24, 25].

Limitation

One limitation of the study is that we reviewed VUSs by assigning them to a database based on a mostly Caucasian population. Researchers contributing to the SNP database or ClinVar tend to be concentrated in Western countries, leading to a lack of registration of major variants in the Asian population or a lack of interpretation of variants such as L1780P. Nevertheless, it is meaningful that we confirmed VUS status in Koreans using a prospective study and laid the groundwork for broadening our understanding of VUS and conducting further research. Another limitation of the study is that there is missing information in the KOHBRA data that is necessary to reclassify VUS (e.g. 27 variants not submitted to the SNP database). However, lacking data comprised only 4.29% of the cohort and is unlikely to weaken the power of the current study.

Conclusions

Taken together, most of the mutations that were classified as VUS in the KOHBRA study were reclassified as benign. Six patients with BRCA1 VUS (4 mutations) and three patients with BRCA2 VUS (2 mutations) were reclassified as “pathogenic or likely pathogenic”. When ORs were calculated using the KRGDB for all KOHBRA data, the OR of BRCA1 c.5339T > C (p.Leu1780Pro) was significantly high, although ClinVar considered BRCA1 c.5339T > C to have conflicting interpretations of pathogenicity. These seven mutations could be reclassified as likely pathogenic or pathogenic mutations according to the American College of Medical Genetics (ACMG) guidelines. Mutations classified as benign in ClinVar have a high normal frequency, so it is desirable to judge them as benign.

However, still some VUSs have been reported as having conflicting interpretations of pathogenicity rather than being assessed as benign or pathogenic. Their character is expected to be more clearly distinguished when more information is gathered. When VUSs are reclassified as pathogenic/likely pathogenic, appropriate management including risk-reducing medication and surgery should be discussed with patients and their families. In addition to collecting individual data, functional studies using genetic techniques such as SGE will also contribute to the functional classification and assessment of VUS.

Abbreviations

KOHBRA: Korean Hereditary Breast Cancer; HBOC: hereditary breast and ovarian cancer syndromes; VUS: variants of uncertain significance; KRGDB: Korean Reference Genome Database; NGS: next-generation sequencing; SNP: Single Nucleotide Polymorphism; RS: refSNP; OR: odds ratio; SGE: saturation genome editing; SNVs: single nucleotide variants

Declarations

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Authors' contributions

JHK, SP, HSP and JSP designed and supervised the study. S-WK, JWL, MHL, SKP, W-CN, DHC, WSH and SHJ generated the data. SP and S-TL analyzed the data. JHK wrote the manuscript with contributions from SP, HSP, JSP and S-TL. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset supporting the conclusions of this article is available upon reasonable request. Requests should be made to Dr. HS Park (imgenius@yuhs.ac).

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University. (IRB# 4-2017-0255)

Consent for publication

Not applicable

Competing interests

none

References

1. Park KS, Cho EY, Nam SJ, Ki CS, Kim JW. Comparative analysis of BRCA1 and BRCA2 variants of uncertain significance in patients with breast cancer: a multifactorial probability-based model versus ACMG standards and guidelines for interpreting sequence variants. Genetics in medicine: official journal of the American College of Medical Genetics. 2016;18(12):1250–7.
2. Grindedal EM, Heramb C, Karsrud I, Ariansen SL, Maehle L, Undlien DE, Norum J, Schlichting E. Current guidelines for BRCA testing of breast cancer patients are insufficient to detect all mutation carriers. BMC Cancer. 2017;17(1):438.
3. Kotsopoulos J. **BRCA Mutations and Breast Cancer Prevention**. *Cancers* 2018, 10(12).
4. Practice **Bulletin No 182**: **Hereditary Breast and Ovarian Cancer Syndrome**. *Obstetrics and gynecology* 2017, **130**(3):e110-e126.
5. Krontiras H, Farmer M, Whatley J. Breast Cancer Genetics and Indications for Prophylactic Mastectomy. Surg Clin North Am. 2018;98(4):677–85.
6. Richter S, Haroun I, Graham TC, Eisen A, Kiss A, Warner E. Variants of unknown significance in BRCA testing: impact on risk perception, worry, prevention and counseling. Annals of oncology: official journal of the European Society for Medical Oncology. 2013;24(Suppl 8):viii69–74.
7. Calo V, Bruno L, La Paglia L, Perez M, Margarese N, Di Gaudio F, Russo A. The Clinical Significance of Unknown Sequence Variants in BRCA Genes. Cancers. 2010;2(3):1644–60.
8. Kurian AW. BRCA1 and BRCA2 mutations across race and ethnicity: distribution and clinical implications. Curr Opin Obst Gynecol. 2010;22(1):72–8.
9. Lindor NM, Goldgar DE, Tavtigian SV, Plon SE, Couch FJ. BRCA1/2 sequence variants of uncertain significance: a primer for providers to assist in discussions and in medical management. Oncologist.

2013;18(5):518–24.

10. Ready K, Gutierrez-Barrera AM, Amos C, Meric-Bernstam F, Lu K, Hortobagyi G, Arun B. Cancer risk management decisions of women with BRCA1 or BRCA2 variants of uncertain significance. The breast journal. 2011;17(2):210–2.
11. Eggington JM, Bowles KR, Moyes K, Manley S, Esterling L, Sizemore S, Rosenthal E, Theisen A, Saam J, Arnell C, et al. A comprehensive laboratory-based program for classification of variants of uncertain significance in hereditary cancer genes. Clin Genet. 2014;86(3):229–37.
12. Han SA, Park SK, Ahn SH, Lee MH, Noh DY, Kim LS, Noh WC, Jung Y, Kim KS, Kim SW. The Korean Hereditary Breast Cancer (KOHBRA) study: protocols and interim report. Clin Oncol (R Coll Radiol (G B)). 2011;23(7):434–41.
13. Kang E, Seong MW, Park SK, Lee JW, Lee J, Kim LS, Lee JE, Kim SY, Jeong J, Han SA, et al. The prevalence and spectrum of BRCA1 and BRCA2 mutations in Korean population: recent update of the Korean Hereditary Breast Cancer (KOHBRA) study. Breast cancer research treatment. 2015;151(1):157–68.
14. Jung KS, Hong KW, Jo HY, Choi J, Ban HJ, Cho SB, Chung M: **KRGDB**: **the large-scale variant database of 1722 Koreans based on whole genome sequencing**. *Database: the journal of biological databases and curation* 2020, **2020**.
15. Macklin S, Durand N, Atwal P, Hines S. Observed frequency and challenges of variant reclassification in a hereditary cancer clinic. Genetics in medicine: official journal of the American College of Medical Genetics. 2018;20(3):346–50.
16. Park JS, Nam EJ, Park HS, Han JW, Lee JY, Kim J, Kim TI, Lee ST. Identification of a Novel BRCA1 Pathogenic Mutation in Korean Patients Following Reclassification of BRCA1 and BRCA2 Variants According to the ACMG Standards and Guidelines Using Relevant Ethnic Controls. Cancer research treatment: official journal of Korean Cancer Association. 2017;49(4):1012–21.
17. So MK, Jeong TD, Lim W, Moon BI, Paik NS, Kim SC, Huh J. Reinterpretation of BRCA1 and BRCA2 variants of uncertain significance in patients with hereditary breast/ovarian cancer using the ACMG/AMP 2015 guidelines. Breast cancer (Tokyo Japan). 2019;26(4):510–9.
18. Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, Janizek JD, Huang X, Starita LM, Shendure J. Accurate classification of BRCA1 variants with saturation genome editing. Nature. 2018;562(7726):217–22.
19. Biswas K, Das R, Eggington JM, Qiao H, North SL, Stauffer S, Burkett SS, Martin BK, Southon E, Sizemore SC, et al. Functional evaluation of BRCA2 variants mapping to the PALB2-binding and C-terminal DNA-binding domains using a mouse ES cell-based assay. Human molecular genetics. 2012;21(18):3993–4006.
20. Bonnet C, Krieger S, Vezain M, Rousselin A, Tournier I, Martins A, Berthet P, Chevrier A, Dugast C, Layet V, et al. Screening BRCA1 and BRCA2 unclassified variants for splicing mutations using reverse transcription PCR on patient RNA and an ex vivo assay based on a splicing reporter minigene. J Med Genet. 2008;45(7):438–46.
21. Lee JS, Oh S, Park SK, Lee MH, Lee JW, Kim SW, Son BH, Noh DY, Lee JE, Park HL, et al. Reclassification of BRCA1 and BRCA2 variants of uncertain significance: a multifactorial analysis of multicentre prospective cohort. J Med Genet. 2018;55(12):794–802.
22. Borg A, Haile RW, Malone KE, Capanu M, Diep A, Torngren 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. 2010;31(3):E1200–40.

23. Yoon KA, Park B, Lee BI, Yang MJ, Kong SY, Lee ES. Clinically Significant Unclassified Variants in BRCA1 and BRCA2 genes among Korean Breast Cancer Patients. Cancer research treatment: official journal of Korean Cancer Association. 2017;49(3):627–34.
24. Minucci A, Lalle M, De Leo R, Mazzuccato G, Scambia G, Urbani A, Fagotti A, Concolino P, Capoluongo E: **Additional molecular and clinical evidence open the way to definitive IARC classification of the BRCA1 c.5332G > A (p.Asp1778Asn) variant**. *Clinical biochemistry* 2019, **63**:54–58.
25. Zuntini R, Cortesi L, Calistri D, Pippucci T, Martelli PL, Casadio R, Capizzi E, Santini D, Miccoli S, Medici V, et al. BRCA1 p.His1673del is a pathogenic mutation associated with a predominant ovarian cancer phenotype. Oncotarget. 2017;8(14):22640–8.

Tables

Table 1. Reclassification of patients diagnosed with VUS based on ClinVar data

BRCA1	58 mutations (n= 278)
benign/ likely benign	20 mutations (n= 193)
VUS	8 mutations (n= 8)
conflicting in interpretations of pathogenicity	12 mutations (n= 55)
pathogenic/ likely pathogenic	4 mutations (n= 6)
variants not registered in SNP database	14 mutations (n=16)

BRCA2	91 mutations (n= 453)
benign/ likely benign	25 mutations (n= 328)
VUS	33 mutations (n= 58)
conflicting in interpretations of pathogenicity	18 mutations (n= 51)
pathogenic/ likely pathogenic	2 mutations (n= 3)
variants not registered in SNP database	13 mutations (n=13)

Table 2. Top 10 high-frequency mutations based on the number of patients

BRCA1	RS_number	ClinVar	No. of patients	OR	CI
c.4883T>C	rs4986854	Benign	57	0.8649	0.5865-1.2754
c.4484+14A>G	rs80358022	Benign	41	0.5823	0.3845-0.8818
c.2566T>C	rs80356892	Benign	40	0.3659	0.2491-0.5374
c.3113A>G	rs16941	Benign	17	0.0079	0.0049-0.0128
c.154C>T	rs80357084	Conflicting interpretations of pathogenicity	15	0.4646	0.2421-0.8914
c.5339T>C	rs80357474	Conflicting interpretations of pathogenicity	12	8.5181	1.1192-64.8277
c.4729T>C	rs80356909	Conflicting interpretations of pathogenicity	9	1.2874	0.4314-3.8417
c.3448C>T	rs80357272	Benign	8	0.6349	0.2448-1.6464
c.3548A>G	rs16942	Benign	8	0.0046	0.0025-0.0086
c.547+14delG	rs273902771	Conflicting interpretations of pathogenicity	6		

BRCA2	RS_number	ClinVar	No. of patients	OR	CI
c.10234A>G	rs1801426	Benign	67	0.6443	0.4627-0.8972
c.8187G>T	rs80359065	Benign	57	0.7962	0.5445-1.1643
c.9649-19G>A	rs11571830	Benign	40	0.5462	0.3609-0.8266
c.5785A>G	rs79538375	Benign	33	0.8118	0.4922-1.339
c.2971A>G	rs1799944	Benign	29	0.0432	0.0299-0.0623
c.2350A>G	rs11571653	Benign	28	0.4624	0.2868-0.7456
c.1744A>C	rs80358457	Benign	24	0.7135	0.4046-1.2581
c.7052C>G	rs80358932	Benign/Likely benign	12	0.6115	0.2826-1.3231
c.6325G>A	rs79456940	Conflicting interpretations of pathogenicity	10	0.6488	0.2754-1.5285
c.623T>G	rs80358865	Uncertain significance	9	1.2867	0.4313-3.8389

Table 3. Conflicting interpretations of pathogenicity

BRCA1	RS_number	(likely) pathogenic	uncertain significance	(likely) benign	No. of patients	OR	CI
c.1357G>C, p.Glu453Gln	rs768054411		3	1	1		
c.154C>T p.Leu52Phe	rs80357084		5	3	15	0.4646	0.2421-0.8914
c.2481A>C p.Glu827Asp	rs397508970		2	1	1	0.7082	0.0446-11.2359
c.2726A>T p.Asn909Ile	rs80357127		8	1	2		
c.4729T>C p.Ser1577Pro	rs80356909		1	7	9	1.2874	0.4314-3.8417
c.5014_5016delCAC p.His1673del	rs80358343	3	4		1		
c.5068A>C p.Lys1690Gln	rs397507239		8	1	1	0.2358	0.0246-2.2601
c.5332G>A p.Asp1778Asn	rs80357112	2	3		1		
c.5339T>C p.Leu1780Pro	rs80357474	4	1		12	8.5181	1.1192-64.8277
c.547+14delG c.547+14delG	rs273902771		2	4	6		
c.671-8A>G c.671-8A>G	rs80358144		1	4	5	0.8852	0.2385-3.2849
c.811G>A p.Val271Met	rs80357244		1	11	2	0.3537	0.065-1.9254

BRCA2	RS_number	(likely) pathogenic	uncertain significance	(likely) benign	No. of patients	OR	CI
c.10150C>T p.Arg3384Ter	rs397507568		1	5	2	0.4717	0.0791-2.8122
c.1568A>G p.His523Arg	rs80358443		2	9	2	0.2855	0.0554-1.4714
c.1817C>T p.Pro606Leu	rs80358469		5	1	1	0.7145	0.0448-11.4046
c.182T>C p.Leu61Pro	rs1555280374		2	1	1		
c.1909+22delT	rs276174816		1	7	1		
c.317-10A>G	rs81002824		1	1	1		
c.3256A>G p.Ile1086Val	rs80358571		5	2	3		
c.4599A>C p.Lys1533Asn	rs80358694		3	5	1		
c.5554G>A p.Val1852Ile	rs80358777		4	2	1	0.7078	0.0446-11.225
c.5969A>C p.Asp1990Ala	rs148618542		6	1	7	1.2391	0.3642-4.216
c.6101G>A p.Arg2034His	rs80358849		2	1	1		
c.6131G>T p.Gly2044Val	rs56191579		1	8	4	0.4078	0.1194-1.3933
c.6325G>A p.Val2109Ile	rs79456940		2	10	10	0.6488	0.2754-1.5285
c.7522G>A p.Gly2508Ser	rs80358978		7	6	9	0.5353	0.2254-1.2713
c.8092G>A p.Ala2698Thr	rs80359052		6	4	1		
c.8486A>G p.Gln2829Arg	rs80359100	2	1		1		
c.8954-5A>G	rs886040949	5	1		1		

c.964A>C p.Lys322Gln	rs11571640	3	9	4	0.2375	0.0766-0.7367

Figures

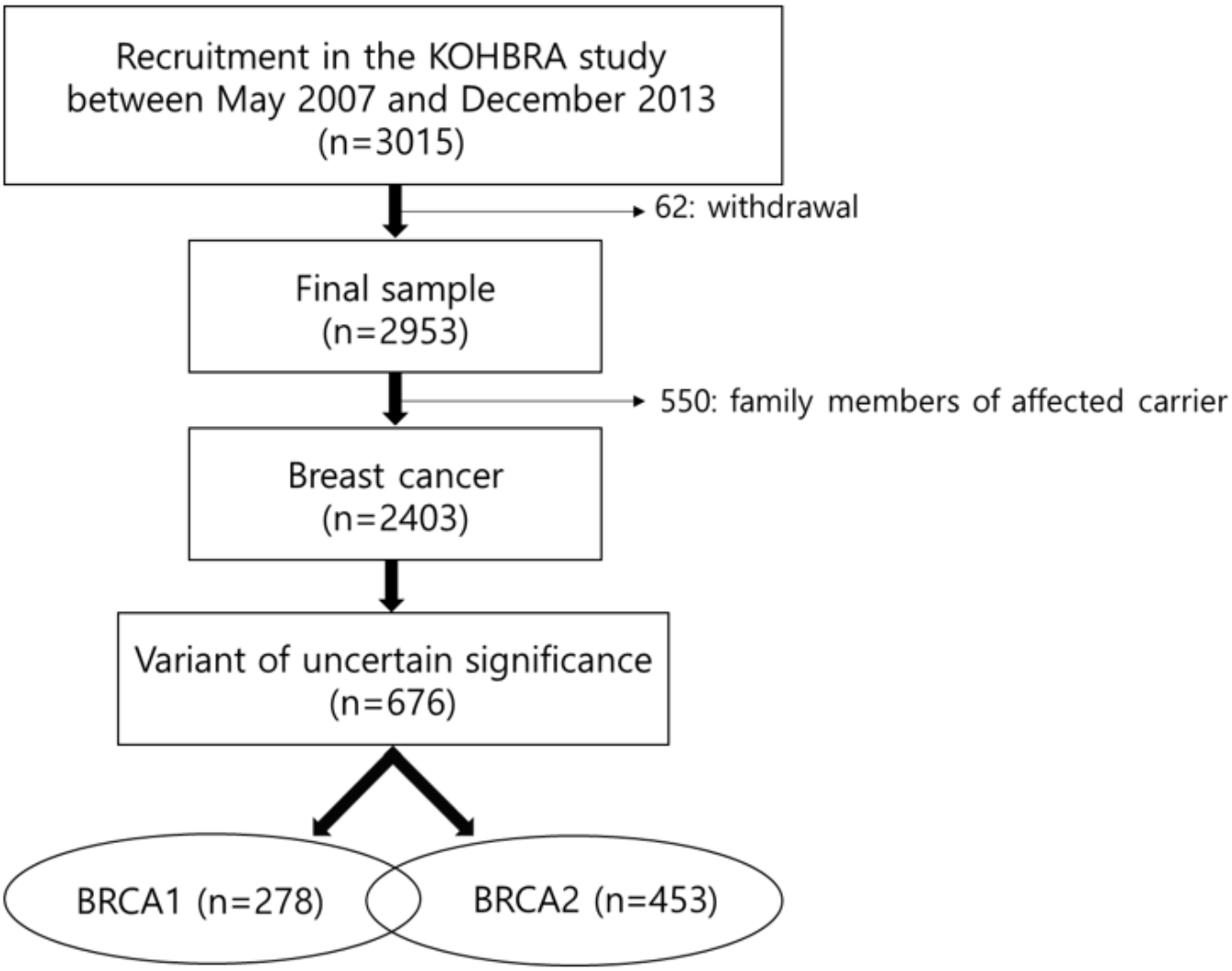

Figure 1

Schematic diagram of patient selection: BRCA1 and 2 (n=55)

Figure 2

Odd ratios (ORs) using Korean population data from KRGDB.