Title

Frequency of mutations in 21 hereditary breast and ovarian cancer susceptibility genes among high-risk Chinese individuals

Running title

Frequency of HBOC susceptibility gene mutations

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Conflict of interest

Di Shao, Shaomin Cheng, Fengming Guo, Kunling Hu, Yuying Yuan, Zhe Wang, Xuan Meng, Xin Jin, Yun Xiong, Xianghua Chai, Hong Li, Yu Zhang, Hongyun Zhang and Mingzhi Ye are employees of BGI Genomics that produces the 21-gene panel test used in this study.

Translational relevance

Multiple high- and moderate- penetrance genes (including *BRCA1* and *BRCA2*) have been discovered as susceptibility genes for hereditary breast and ovarian cancer. Recent advances in next generation sequencing (NGS) have provided an efficient method to evaluate these susceptibility genes simultaneously. We assessed the frequency of mutations in 21 susceptibility genes in a large cohort of subjects who were referred for genetic testing. The cohort is from several comprehensive hospitals with a wide geographical distribution representing the overall situation of the Chinese population. The prevalence of mutations in this population has been rarely reported in previous studies. Our results reflect the mutation frequency in individuals defined by guidelines and have great clinical practical significance.

Abstract

Purpose: To determine the prevalence and clinical prediction factors associated with deleterious mutations among 882 high-risk Chinese individuals who underwent multigene panel testing for hereditary breast and ovarian cancer (HBOC) risk assessment.

Experimental Design: Subjects were selected from individuals referred for genetic testing using a 21-gene panel (Oseq-BRCA) between January 2015 and March 2018. The distribution and prevalence of deleterious mutations were analyzed for the full cohort as well as subtypes.

Results: Overall, 176 deleterious mutations were observed in 19.50% (n = 172) individuals. Of these, 26 mutations are not reported in public databases and literatures. In the ovarian cancer only subgroup, 115 deleterious mutations were identified in 429 patients (48.6%). Patients with ovarian cancer with mutations were enriched for a family history of breast or ovarian cancers ($p < 0.05$). In the breast cancer only subgroup, 31 deleterious mutations were identified in 261 patients. Most mutations occurred in *BRCA1* (8; 25.8%) and *BRCA2* (11; 35.5%). An additional 12 deleterious mutations (38.7%) were found in 7 other susceptibility genes. An increased frequency of mutation rate (57.9%) was observed in the subgroup of subjects with histories of both breast and ovarian cancer.

Conclusions: 19.50% of individuals carried a deleterious mutation in HBOC susceptibility genes in our cohort. Subgroup of subjects with histories of both breast and ovarian cancer had the highest prevalence of mutations. Our results highlighted the genetic heterogeneity of HBOC and the efficiency of multigene panel in performing risk assessment.

Keywords

HBOC, *BRCA1*, *BRCA2*, NGS

1 Introduction

Breast cancer is one of the most common malignant tumors in females (1). The increasing rate of morbidity in China is currently the highest in the world (2). Although ovarian cancer is less common than breast cancer, its mortality is high (2). Inherited mutations of *BRCA1* and *BRCA2* are pathogenetic in a majority of hereditary breast and ovarian cancer (HBOC) patients (3-4). In addition to *BRCA1* and *BRCA2*, the vast majority of existing studies have confirmed associations with other genes such as *ATM*, *BRIP1*, *CHEK2*, *RAD50*, *RAD51C,* and *TP53* (5-8). Moreover, it has been indicated that *TP53*, *PTEN*, *STK11,* and *CDH1* mutation carriers who may suffer from Li-Fraumeni syndrome, Cowden syndrome, Peutz–Jeghers syndrome, and hereditary diffuse gastric cancer syndrome, respectively, have high risks of breast cancer (9-13).

In consideration of associations between genes and disease, the National Comprehensive Cancer Network (NCCN) recommends genetic testing of 19 genes in breast and ovarian cancer patients (14). Timely and effective genetic testing could provide professional counseling and clinical management for patients and at-risk relatives. As *BRCA1/2* carriers display the high sensitivity to inhibitors of poly-ADP-ribose polymerase (PARP), genetic testings could also help patients in choosing therapies (15,16).

The traditional genetic testing methods, such as Sanger sequencing, polymerase chain reaction(PCR)-sequencing assay, and denaturing gradient gel electrophoresis (DGGE) mutation scanning system have been applied to detect *BRCA1/2* widely in China (8,17). However, *BRCA1/2* are high-risk tumor suppressor genes without significant mutation hotspots; as a result, some mutations would be missed by conventional approaches. Results from recent studies confirmed that NGS showed multiple advantages in cancer genetic testing in terms of time and cost effectiveness (18-20). However, there is insufficient related reports on HBOC patients of the Chinese population.

To investigate the mutation frequency among patients with a suspected HBOC risk in Chinese population, we used multi-gene testing to reveal the distribution and prevalence of deleterious germline mutations among 882 patients with a suspected HBOC risk in 21 HBOC heredity susceptive genes. Our results evaluated the benefits and limitations of multi-gene panel testing and provided insights of choosing appropriate multi-gene tests to diagnose hereditary cancer predisposition.

2 Materials and Methods

2.1 Participants

Subjects were selected from patients referred for genetic testing using a 21 gene panel Oseq-BRCA (BGI Genomics, Shenzhen, China) between January 2015 and March 2018. The patient enroll criteria of this study were based on the current National Comprehensive Cancer Network guidelines (NCCN) for

genetic risk evaluation for HBOC (14). Demographic and clinical information, including gender, personal cancer history, and family cancer history, were collected from test requisition forms (TRFs) completed by ordering clinicians at the time of testing. All patients signed informed consents approved by the Institutional Review Boards of BGI Genomics.

2.2 NGS library construction and gene capture

Genomic DNA (gDNA) was extracted from participants' peripheral blood samples using the Qiagen Blood Midi Kit (Qiagen, Hilden, Germany). DNA concentration and quality were assessed by Qubit (Life Technologies, Gaithersburg, MD, USA) and agarose gel electrophoresis. Genomic DNA (250 ng) was used for the sequencing library construction. Briefly, the gDNA was fragmented randomly by the Covaris LE220 sonicator (Woburn, MA) to generate gDNA fragments with a peak of 250 bp and then subjected to three enzymatic steps: end-repair, A-tailing, and adapter ligation. DNA libraries were purified with Agencourt Ampure XP beads (Beckman-Coulter, Indiana, USA), and PCR was performed during which a unique 8 bp barcode was added to label each sample. Five to ten PCR products were pooled equally and hybridized to a custom hereditary cancer panel (Roche NimbleGen, Madison, USA). Hybridization product was subsequently purified, amplified, and qualified. Finally, sequencing was performed with paired end and barcode on the BGISEQ-500 sequencer or Hiseq 2000 (Illumina, San Diego, CA) following the manufacturer’s protocols.

2.3 Sequencing data analysis and mutation calling

Raw fastq data generated by the sequencer was first filtered by SOAPnuke to exclude low quality reads. The clean reads were then aligned to the reference human genome (UCSC hg19) using the BWA ALN algorithm. Single nucleotide variants (SNVs) were detected by the Genome Analysis Toolkit (GATK) UnifiedGenotyper. Small insertions and deletions (InDels) were called using GATK Haplotype. Copy number variants (CNVs) were called using read-depth analysis. All above variants were further filtered by quality depth, strand bias, mapping quality, and reads position. Finally, each variant was annotated with respect to gene location and predicted function in Human Genome Variation Society (HGVS) nomenclature and was ready prepared for interpretation.

2.4 Data interpretation

Interpretation was focused on variants in the selected 21 HBOC susceptibility genes (Table 1). These 21 genes were selected through NCCN guidelines and published research articles, and they include core genes in the FA pathway and homologous recombination genes (14). Variants were classified into the following 5 categories according to the American College of Medical Genetics (ACMG) recommendations: class 1, benign; class 2, likely benign; class 3, variant of uncertain significance (VUS); class 4, likely pathogenic（LP）; and

class 5, pathogenic (P) (21). Population allele frequencies were collected from NCBI dbSNP, HapMap, 1000 human genome dataset, and an internal database of 100 Chinese healthy adults. Individuals with likely pathogenic or pathogenic variants were defined as having deleterious variants. Every deleterious variant was validated by qPCR, Sanger sequencing, or time of flight mass spectrometry.

3 Results

3.1 Participant Characteristics

A total of 1,175 individuals were referred to our clinical test center for Oseq-BRCA multigene testing. After applying exclusion criteria of *BRCA1/2* TESTING in NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian, 882 participants were included in our study. Demographics for these 882 subjects are shown in Table 2. Age at diagnosis ranged from 13 to 80, with an average age of 47 years old. The majority of the subjects were women, 261 subjects with a personal history of breast cancer, 429 subjects had a personal history of ovarian cancer, and 19 had personal histories of both breast and ovarian cancer. Only 173 subjects were unaffected by cancer. Regarding family history information, 108 (12.2%) had at least one first- or second-degree relative with breast cancer only, and 96 (10.9%) had a relative with ovarian cancer only.

3.2 Deleterious mutations identified in this cohort

Exons and splice sites of 21 HBOC susceptibility genes were examined for mutations by Oseq-BRCA in all 882 recruited participates. Overall, 176 deleterious (LP/P) mutations were observed in 19.50% (n = 172) individuals (Table 3). Of all these mutations, 89 (50.6%) were found in *BRCA1*, 49 (27.8%) in *BRCA2*, and 38 (21.6%) mutations in 14 other susceptibility genes (Figure 1A, Figure 2). In addition, 2 individuals with ovarian cancer carried mutations in both *BRCA1* and another gene (*TP53* or *MRE11A*). Additionally, 2 individuals with breast cancer had mutations in both *CHEK2* and another gene (*BRCA2* or *TP53*). Deleterious mutations were identified in all individual genes, except *ATM*, *PTEN*, *CDH1*, *BARD*1 and *PMS2*.

In the breast cancer only subgroup (n = 261), 31 deleterious mutations were identified in 261 patients (Table 3). Most mutations occurred in *BRCA1* (66; 57.4%) and in *BRCA2* (33; 28.7%). An additional 16 mutations (13.9%) were found in 9 other susceptibility genes (Figure 1B). Deleterious *BRCA1* mutations consisted of 8 truncating (2 deletion, 2 frameshift, 2 nonsense, and 2 splice) mutations. Deleterious *BRCA2* mutations (2.7%) were 11 truncating mutations (9 frameshift, 2 nonsense). Among the other HR pathway genes, mutations were most commonly found in *CHEK2* (n = 5; 1.92%) and *BRIP1* (n = 2; 0.77%). In addition, mutations were also observed in *RAD51C, PALB2,* and *MRE11A* in 1 individual per gene. Only one Lynch syndrome gene mutation was identified in *PMS1* in the breast cancer subgroup. Among the other highly penetrant

genes, mutations were found in *TP53* (n = 1; 0.38%) while no mutations were identified in *STK11*, *PTEN,* and *CDH1*.

In the ovarian cancer only subgroup (n = 429), overall, 115 deleterious mutations were identified in 26.3% (n = 113) individuals (Table 3). Of these, 66 (57%) occurred in *BRCA1*, 33 (29%) in *BRCA2*, and 16 (14%) in 9 of 19 other susceptibility genes (Figure 1C). Deleterious *BRCA1* mutations consisted of 61 truncating (5 deletion, 35 frameshift, 16 nonsense and 5 splice) mutations and 5 known deleterious missense mutations. The 33 deleterious *BRCA2* mutations consisted of 30 truncating mutations (1 deletion,19 frameshift, 7 nonsense, and 3 splice mutations) and 3 known deleterious missense mutations. Among the HR pathway genes, mutations were found most frequently in *BRIP1* (n = 5; 1.17%), and *RAD51C* (n = 2; 0.47%). Mutations were also identified in *CHEK2*, *MRE11A* and *RAD50* in 1 individual per gene. As for genes associated with Lynch syndromes genes (*MLH1, MSH2, MSH6, PMS1, PMS2*), deleterious mutations were identified in *MSH2* (n = 3) and *MSH6* (n = 1), accounting for 3.5% (n = 4) of all mutations in the ovarian cancer subgroup. Among the other highly penetrant genes, mutations were found in *TP53* (n = 1; 0.23%) and *STK11* (n = 1; 0.23%).

In the subgroup of subjects with disease histories of both breast and ovarian cancer (n = 19) (Table 3), a higher frequency of mutation rate was observed. Eleven (57.9%) subjects in this subgroup had a mutation, including 10 with a mutation in *BRCA1*, and 1 with a *MUTYH* mutation (Figure 1D).

Further, 173 subjects were unaffected by cancer. They were recruited due to family cancer history. In this subgroup, only 19 mutations were identified in 21 cancer susceptibility genes with a prevalence of 11.0% (Table 3), in which 10 had mutation in *BRCA1/2* genes, 2 in *BRIP1*, 2 in *MLH1*, 1 in *CHEK2*, 1 in *MRE11A*, 1 in *NBN*, 1 in *RAD51C,* and 1 in *MUTYH*. No mutations were found in *PALB2*, *RAD50, STK11*, *TP53*, *MSH2*, *MSH6,* and *PMS1* genes.

3.3 Recurrent mutations, founder mutations, and novel mutations

In our cohort, recurrent mutations (n ≥ 3) were found in *BRCA1* p.Ile1824AspfsX3, *CHEK2* p.His371Tyr, *BRCA1* p.Glu1257GlyfsX9, and *BRCA2* p.Ser2670Leu (Table S1). And *BRCA1* p.Ile1824AspfsX3 was also one of Chinese founder mutations. The other Chinese founder mutations included *BRCA1* p.Cys328*, *BRCA2* p.Thr3033Asnfs*11, and *BRCA2* p.Gln1037Ter. No Ashkenazi Jewish or European founder mutations were observed. We confirmed 26 novel mutations that are not reported in public databases (ClinVar, UMD, LOVD, BIC) and literature. Of these, 7 in *BRCA1* (p.Val14Glyfs*3, p.Asn298LysfsX2, p.Asn599Ilefs*13, p.Phe901Leufs*99, p.Glu1288Glnfs*18, p.Arg1753Ter, p.Glu1849Ter), 9 in BRCA2 (p.Ser942Glnfs*18, p.Asn1066Lysfs*1, p.Asn1287LysfsX6, p.Lys1765Glnfs*13, p.Asp1868Valfs*5, p.Thr2125Asnfs*4, p.Pro2827Leufs*36, p.Ser3080CysfsX30, p.Asn3124Glnfs*26) (Figure 2), 3 in BRIP1 (p.Ser206Ter, p.Ser230Ter,

p.Lys998AsnfsX5), 3 in RAD51C (p.Ser231Ter, p.Gln62Ter, p.Val41Glyfs*18), and 1 in CHEK2 (Leu303_E8splice), MSH2 (p.Asn412Metfs*22), NBN (p.Asn639Argfs*6), PMS1 (p.Tyr90*), respectively.

3.4 Variants of uncertain significance (VUS)

The total uncertain genetic effects rate in our cohort was 38.55% (n = 340), and 406 (339 missense, 58 splice, 4 inframe, 2 frameshift, 2 duplication, 1 deletion) VUS mutations were detected in 882 individuals. *ATM* accounted for up to 53, followed by 29 VUS in *BRCA1* and 42 VUS in *BRCA2* (Figure 3). All the genes detected with mutation got a certain amount of data in VUS. In the mutation classification, except for *BRCA1* and *BRCA2*, other genes have insufficient interpretation of pathogenicity and benign polymorphism, resulting in a high proportion of VUS. Of these mutations, VUS were found most frequently in *MRE11A* p.Met157Val (n = 8), *BRIP1* p.Gln944Glu (n = 8), and *ATM* p.His42Arg (n = 8). In addition, *PMS1* p.Arg919Cys and *MSH6* p.Pro1082Ser were occurred in 6 individuals, respectively.

3.5 Mutation frequency in subgroup with different family histories and ages at diagnosis

The deleterious mutation rate for each subgroup according to age at diagnosis is detailed in Table 4. In the breast cancer only subgroup, the average age of diagnosis among mutation positive probands was 39 years old compared to 40 years old for mutation negative probands ($p = 0.66$). In the ovarian cancer only cohort, the average age of ovarian cancer diagnosis was 53 among positive probands and 54 among mutation negative probands ($p = 0.90$). In the breast and ovarian cancer cohorts, age of diagnosis was slightly older among mutation negative individuals compared to those positive for a mutation, however, the difference was not significant ($p = 0.41$) (Table 4). We also evaluated whether patient subjects with deleterious mutations in the 21 susceptibility genes were associated with a greater family history of breast and/or ovarian cancers than nonmutated patient subjects (Table 5). Patient subjects of breast cancer with mutations were not significantly associated with a family history for either breast or ovarian cancer. However, patient subjects of ovarian with mutations were enriched for a family history of breast or ovarian cancers ($p < 0.05$) (Table 5).

4 Discussion

4.1 The contribution of this study

Using a HBOC multi-gene panel, we revealed the distribution and prevalence of deleterious germline mutations among 882 subjects who were high-risk individuals and referred for Oseq-BRCA testing. This test utilizes liquid solution hybridization-based target enrichment and next generation sequencing to identify all types of variants in 21 HBOC genes. Our results support the views that the panel testing could increase the diagnostic detection rate of deleterious germline mutation compared with testing for *BRCA1/2* mutations alone. In our

cohort, 172 (19.50%) subjects had a deleterious mutation, and 21.6% of deleterious mutations were in genes other than *BRCA1* and *BRCA2*. Previous studies have reported on the frequency of deleterious mutations with NGS based multi-gene testing. Our study is distinguished from other studies in the following ways. First, our large-size cohort is from several comprehensive hospitals with a wide geographical distribution representing the overall situation of the Chinese population. The prevalence of mutations in this population was rarely reported in previous studies. In addition, our cohort was selected according to the NCCN guidelines, including breast cancer patients, ovarian cancer patients, and high-risk volunteers. Our results reflect the mutation frequency in individuals defined by the guidelines and have great clinical practical significance.

4.2 Comparison of mutation frequency with previous studies

In the breast cancer only subgroup, the prevalence of *BRCA1* and *BRCA2* deleterious mutations were 3.07% and 4.21%, respectively. In a previous Chinese population-based study, Jie Sun et al. (28) reported that *BRCA1/2* deleterious mutations frequencies were 4.24% and 6.60% in early-onset breast cancer and familial breast cancer cohort, which is similar to our subgroup and those observed in other studies in China (29-30). However, the prevalence of *BRCA1/2* deleterious variants in breast cancer in other countries ranges from 9.3% to 18% (31-33). Among African Americans women, Churpek et al. (33) reported that the prevalence in deleterious mutations in *BRCA1 and BRCA2* genes was 10% and 8%, respectively. Differences in the definition of early-onset or familial breast cancer and genetic testing methods for hereditary breast cancer between studies may influence results. Previous studies demonstrated that 4%-5% patients carried deleterious mutations beyond *BRCA1*/*2*, which was consistent with our finding that 4.21% subgroup carried deleterious variants in neither *BRCA1*nor *BRCA2*. The third commonly mutated gene was *CHEK2* in our study, which encodes a checkpoint kinase 2 interacting with cell cycle regulators and DNA repair proteins. And the deleterious mutation of CHEK2 would increase the risk of breast cancer (14). Five patients carried *CHEK2* deleterious mutations, 4 in p.His371Tyr and 1 in c.908+2T>A. Although the recurrent mutation p.His371Tyr in *CHEK2* marked as variant uncertain significance in ClinVar database, we interpreted as likely pathogenic variants. This mutation results in the change of a Histidine to a Tyrosine at position 371 of the CHEK2-encoded protein. Baloch A H et al. (40) found that the mutation occurred in a region of protein kinase activity that plays an important role in DNA damage repair. The mutation is a suspected disease-causing mutation with 1 strong pathogenicity (PV3: functional studies supportive of a damaging effect) (6) and 1 moderate pathogenicity (PM2 low frequency in 1000 Genomes Project). Noteworthy, our study found only 1 breast cancer patient carried *PALB2* but none of the patients carried ATM, comparing *ATM* and *PALB2* also commonly identified in other studies.

The frequency of *BRCA1/2* mutations was 23.07% in the ovarian cancer only subgroup, 15.38% for *BRCA1* and 7.69% for *BRCA2*. Overall, *BRCA1/2* accounted for 86% in hereditary ovarian cancer, and the *BRCA1* mutation rate was more pronounced than the *BRCA2* mutation rate in ovarian cancer patients, similar to both Ang Li et al. (22) (2018, n = 1331, *BRCA1* for 17.1% and *BRCA2* for 5.3%) and Norquist et al. (23) (2016, n = 1915, *BRCA1* for 9.5% and *BRCA2* for 5.1%). Interestingly and noteworthy, ovarian cancer carried *BRCA1/2* mutations in China was slightly higher than expected. As patients with tumors of ovarian origin in other country that found *BRCA1/2* deleterious mutations rates from 13% to 15% (23-25). Beyond B*RCA1/2*, 0.9% of the subgroup carried *BRIP1* (BRCA1-interacting protein C-terminal helicase 1), which is similar to other studies ranging from 0.8% to 1.5% (23,26). BRIP1, a member of the BRCA-Fanconi anemia DNA repair pathway, is one of ovarian cancer moderate-risk genes that mutations in the gene increased lifetime ovarian cancer risk of 10%-15% (27). Reviewing 5 patients with *BRIP1* deleterious mutations, 100% subjects had a family history of cancer (ovarian cancer, breast cancer, pancreatic cancer, colon cancer, gallbladder cancer) compared to 48.68% of all subjects had a family history of a cancer-carried deleterious mutation. This data suggests that *BRIP1* mutation may be the pathogenic cause in ovarian cancer patients with a family history of cancer. In reviewing the mutations in mismatch repair genes (MMR; *MLH1*, *MSH2*, *MSH6*, *PMS2*), mainly causing Lynch syndrome, were of low frequency in our subgroup (n = 4; 0.93%). However, in our cohort, MMR mutations only occurred on the *MSH2* and *MSH6* genes, and no mutations in *MLH1* were found, which is different from the spectrum of hereditary colorectal cancer. This phenomenon also occurred in the study of Norquist et al. (23) (7 of 8 MMR mutations occurred in *PMS2* or *MSH6*). In addition, although there is few *CHEK2*, *MRE11A*, *TP53,* and *RAD50* reported with increased risk of ovarian cancer in the NCCN genetic testing criteria, but we found one patient, respectively, in our study that carried the above mutations. Although the risk values of these genes are unknown, we cannot completely rule out these genes when doing genetic testings in ovarian cancer.

The subgroup of subjects with breast cancer complicated with ovarian cancer identified *BRCA1* and *MUTYH* deleterious mutations in our population. Ava Kwong et al. (34) (2018, n = 20) reported Chinese patients with breast cancer complicated with ovarian cancer carried 40% and 20% mutations in *BRCA1* and *BRCA2*, respectively. In Shulman, LP et al. (35), *BRCA1* and *BRCA2* mutation frequencies were 38.71% and 22.58%, and there were three other subjects carried *BRIP1*, *CHEK2*, and *MRE11A* mutations, respectively. Although the incidence of personal histories of both breast and ovarian is relatively low in domestic and international research, it is suggested that the frequency of *BRCA1* mutations in this type of concurrent population is higher than that of carrying *BRCA2* but more evidence is needed to support this point.

4.3 Variants of uncertain significance

The rate of uncertain genetic effects in a similar multi-gene panel study (27 genes) was 32.7% (38). Gardner, S. A et al. (38) considered panels with fewer genes that include *BRCA1/2*, and they tended to have a higher positive rate and a lower VUS rate presumably due to a narrower range of indications guiding test uptake. However, VUS rate in our cohort was 38.55%, which is slightly higher than the results of foreign studies. It is possible that the incidence of breast cancer and ovarian cancer in the Chinese population is lower than that in foreign countries, and the variants are relatively sporadic.

ATM has the most frequent VUS detected due to the long transcript length. When we exclude the length of CDS to make comparisons, it shows that *RAD51C* has the greatest number of VUS in per 1000 base, up to 14.15 (Table S2). According to the NCCN guidelines, *RAD51C* specifically increases the risk of ovarian cancer. In our ovarian cancer patients, only one mutation of *RAD51C* was detected, which is relatively low compared with foreign populations (36). This may due to the lack of reports on *RAD51C* in Chinese population, and some variants in our result need to be adjusted to the level of the disease-causing gene.

We amended *BRCA1 (*c.5357T>C, c.5504G>A, c.5089T>C, c.101C>A) from uncertain significance to likely pathogenic through a combination of ClinVar reports, examinees' phenotypes, and references (37), However, the evidence for these variants is not currently sufficient, and it would be better to do more functional tests. In addition, we amended *BRCA1*(c.5347A>C, c.266T>C, c.154C>T) to be likely benign variants.

4.4 Clinical impact of deleterious mutations detection

The identification of a deleterious variants in a cancer susceptibility gene allows identification of eligible patients for surveillance screening, and it may provide targeted therapy and prevention strategies for both patients and family members. Clinical interventions and recommendations of *BRCA1* and *BRCA2* mutation carriers have been well established and widely used in clinical practice. Most genes in our panels (*CDH1*, *MSH2*, *MLH1*, *MSH6*, *PMS2*, *PTEN*, *STK11,* and *TP53*) had corresponding current management suggestions in the NCCN guidelines. However, management guidelines for other moderate penetrance genes (*BARD1*, *RAD50*, *ATM*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD51C*) are not available, mutation in these genes were found in 2.60% subjects. When encountering these mutations, it is a big challenge for clinicians. It is necessary to combine the family history and personal history to make a medical decision. Therefore, guidelines recommend that multi-gene testing is ideal in the context of professional genetic expertise for pre- and post-test counseling.

4.5 Conclusion

Here, we reported the successful utility of multiple gene testing for identification of HBOC relevant risk gene mutations in a large number of individuals referred for genetic testing. This is the first clinical investigation of the mutation spectrum with a multiple gene panel among high-risk Chinese individuals with a suspected HBOC risk. Results of this study indicated that multi-gene panel testing can identify more individuals with relevant cancer risk gene mutations than *BRCA1/2* genetic testing alone. Although current NCCN guidelines recommend the management of patients with mutations in the majority of risk genes, the clinicians should be prepared to deal with the VUS and mutations in moderate penetrance genes. Our findings provide insights for the clinician to consider multi-gene tests to diagnose cancer predisposition in clinical practice.

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Tables

Table 1 List of the 21 tested genes

Risk Category	Genes	Gene names
BRCA1/2	2	*BRCA1*, *BRCA2*
BRCA Pathway/Moderate Risk	9	*ATM*, *BARD1*, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51C*
High Penetrant	4	*CDH1*, *PTEN*, *STK11*, *TP53*
Lynch Syndrome	5	*MLH1*, *MSH2*, *MSH6*, *PMS1*, *PMS2*
Other Moderate Risk gene	1	*MUTYH*

Table 2 Demography and clinical characteristics

	Total (n = 882)	BC (n = 261)	OC (n = 429)	BC & OC (n = 19)	FHx (n = 173)
Sex					
Male	29 (3.3%)	-	-	-	29 (16.8%)
Female	853 (96.7%)	261 (100%)	429 (100%)	19 (100%)	144 (83.2%)
Age at testing (yr)					
[-,35]	170	78	22	0	70
(35,50)	369	165	130	5	69
[50, +]	340	17	276	14	33
NA	3	1	1	0	1
Mean(±SD)	47.0(±12)	39.8(±7)	53.5(±10)	57.1(±10)	40.4(±12)
Median	47	40	54	57	38.5
Range	13-80	20-62	24-79	39-80	13-78
Family history					
BC	108	30	31	0	47
OC	96	3	25	2	66
BC & OC	21	2	3	0	16

Abbreviations:

BC, Breast cancer; OC, Ovarian cancer; BC & OC, Breast and ovarian cancer; FHx, subjects recruited based on family cancer history; SD, standard deviation; NA, unknown

Table3 Frequency of mutations by personal cancer history

	No. of Individuals (%)				
Gene	Total (n =882)	BC (n =261)	OC (n =429)	BC & OC (n =19)	FHx (n =173)
BRCA1/2					
BRCA1	89 (10.09)[cd]	8 (3.07)	66 (15.38)[cd]	10 (52.63)	5 (2.89)
BRCA2	49 (5.56)[a]	11 (4.21)[a]	33 (7.69)	-	5 (2.89)
BRCA Pathway/Moderate Risk					
BRIP1	9 (1.02)	2 (0.77)	5 (1.17)	-	2 (1.16)
CHEK2	7 (0.79)[ab]	5 (1.92)[ab]	1 (0.23)	-	1 (0.58)
MRE11A	3 (0.34)[c]	1 (0.38)	1 (0.23)[c]	-	1 (0.58)
NBN	1 (0.11)	-	-	-	1 (0.58)
PALB2	1 (0.11)	1 (0.38)	-	-	-
RAD50	1 (0.11)	-	1 (0.23)	-	-
RAD51C	4 (0.45)	1 (0.38)	2 (0.47)	-	1 (0.58)
High Penetrant					
STK11	1 (0.11)	-	1 (0.23)	-	-
TP53	2 (0.23)[bd]	1 (0.38)[b]	1 (0.23)[d]	-	-
Lynch Syndrome					
MLH1	2 (0.23)	-	-	-	2 (1.16)
MSH2	3 (0.34)	-	3 (0.70)	-	-
MSH6	1 (0.11)	-	1 (0.23)	-	-
PMS1	1 (0.11)	1 (0.38)	-	-	-
Other Moderate Risk gene					
MUTYH	2 (0.23)	-	-	1 (5.26)	1 (0.58)
TOTAL	176(19.95)	31(11.88)	115(26.81)	11(57.89)	19(10.98)

[a]one subject had both *BRCA2* and *CHEK2* mutation

[b]one subject had both *CHEK2* and *TP53* mutation

[c]one subject had both *BRCA1* and *MRE11A* mutation

[d]one subject had both *BRCA1* and *TP53* mutation

Table 4 Correlation between deleterious mutations and age in subgroup

	BC		OC		BC & OC		FHx	
Age (yr)	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
mean	39.7	39.8	53.6	53.4	54.8	60.3	41.1	40.4
median	39	40	53	54	52	59	35	39
range	26-56	20-62	27-76	24-79	44-68	39-80	27-71	13-78
p-value	0.6643		0.9033		0.4083		0.9027	

Pos = positive, carried deleterious mutation

Neg = negative, no deleterious mutation

Table 5 Correlation between deleterious mutations and HBOC family history in subgroup

	BC		OC	
	Pos	Neg	Pos	Neg
with HBOC family history	6	29	37	22
without HBOC family history	23	203	76	294
p-value	0.246		<0.05	

Figure legends

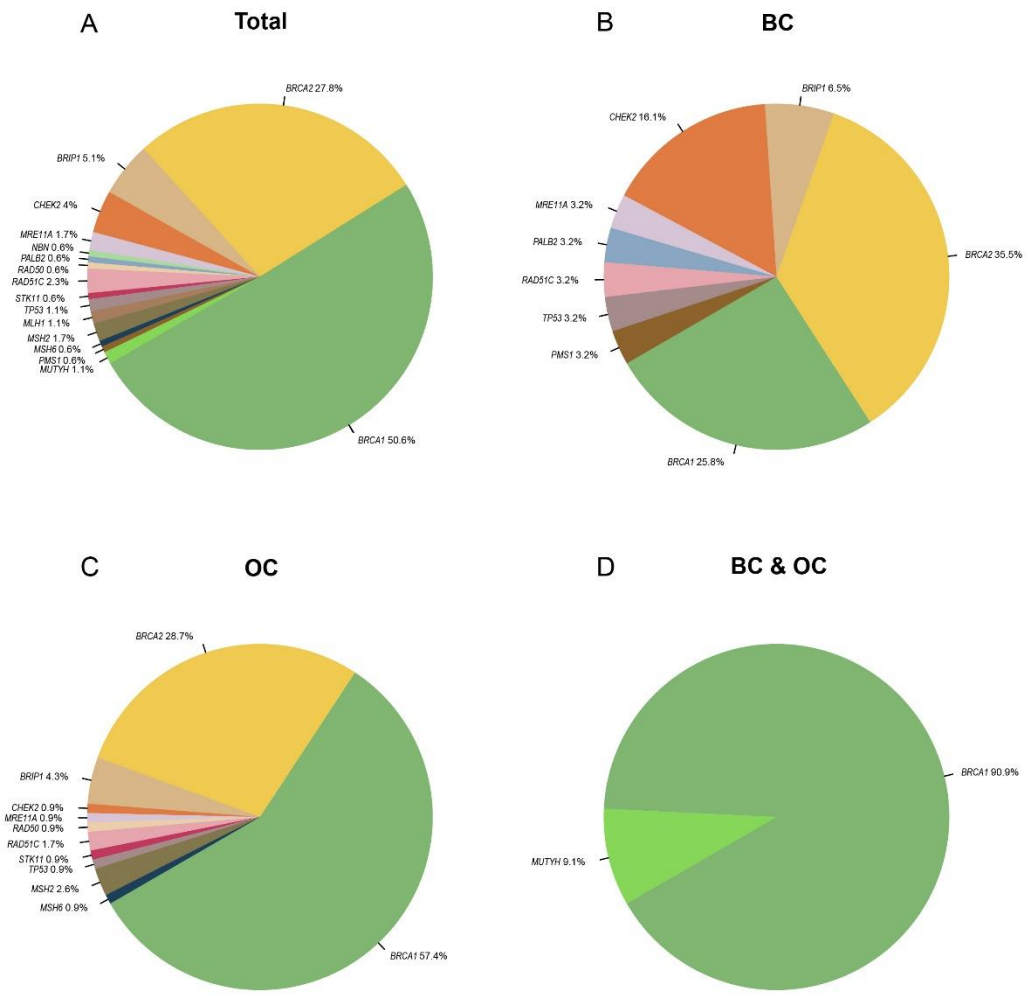


Figure 1 Deleterious mutation distribution in 21 HBOC susceptibility genes.

(A) Overall 176 deleterious mutations were distributed in 21 genes among 172 patients of 882 individuals. Note that due to rounding, the sum is over 100%. (B) In 261 patients with breast cancer only, distribution of 31 deleterious mutations in 21 genes was identified. (C) In 429 patients with ovarian cancer only, distribution of 115 deleterious mutations in 21 genes was identified. (D) Distribution of 11 deleterious mutations distribution in 21 genes was identified in 19 patients with both breast and ovarian cancer.

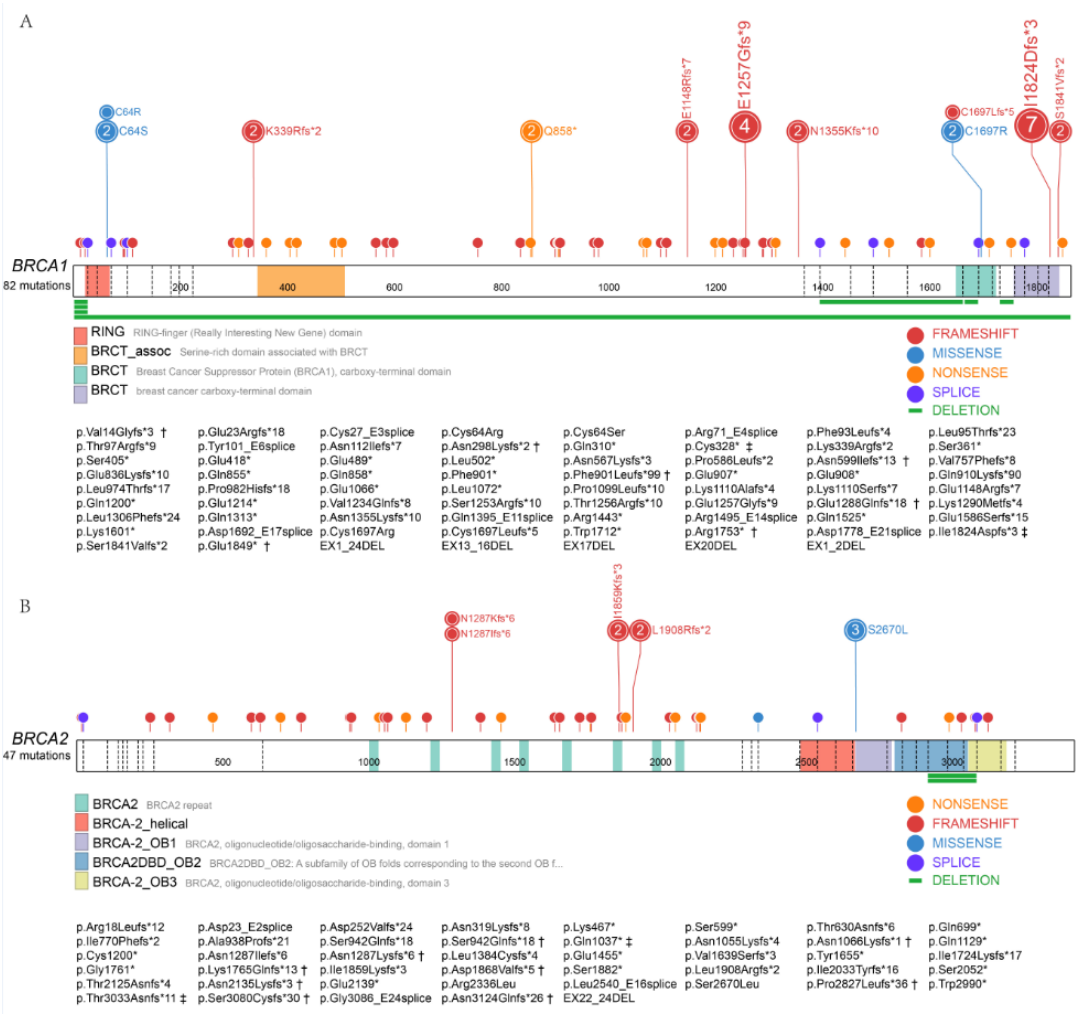


Figure 2 Spectrum of deleterious mutations detected in 882 individuals.

(A) The spectrum of deleterious mutations in *BRCA1* gene. (B) The spectrum of deleterious mutations in *BRCA2* gene. Sticks represent mutation positions. The number represents the number of samples with the mutation (the unmarked represents 1). The green bars represent the deletion locations, and each segment represents a sample. The figure was made using ProteinPaint. (39) † novel mutation; ‡ Chinese founder mutation

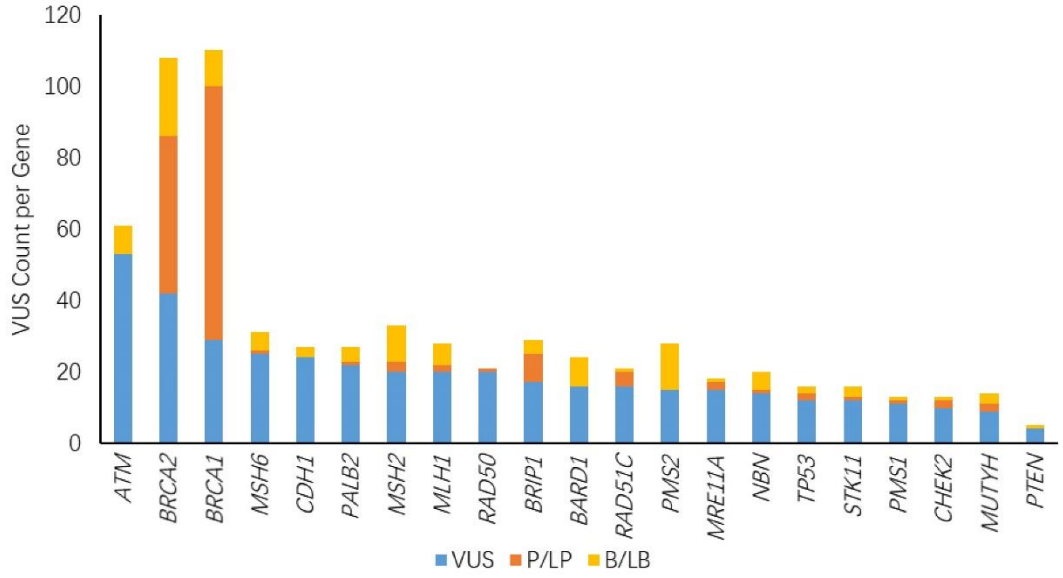


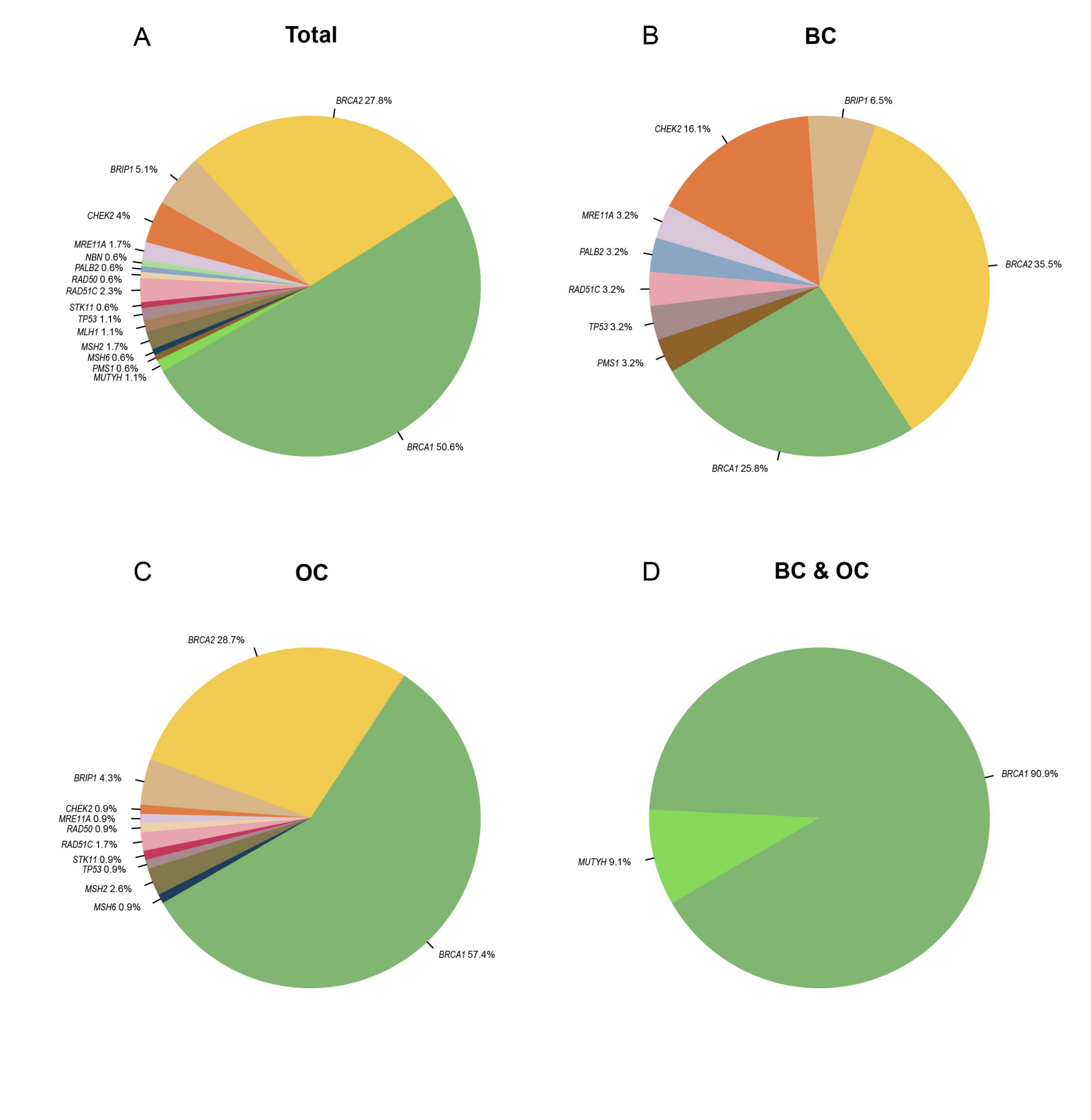
Figure 3 Overall proportion of VUS in 21 cancer susceptibility genes. VUS: variant of uncertain significance; P/LP: pathogenic or likely pathogenic; B/LB: benign or likely benign

Supplementary Data

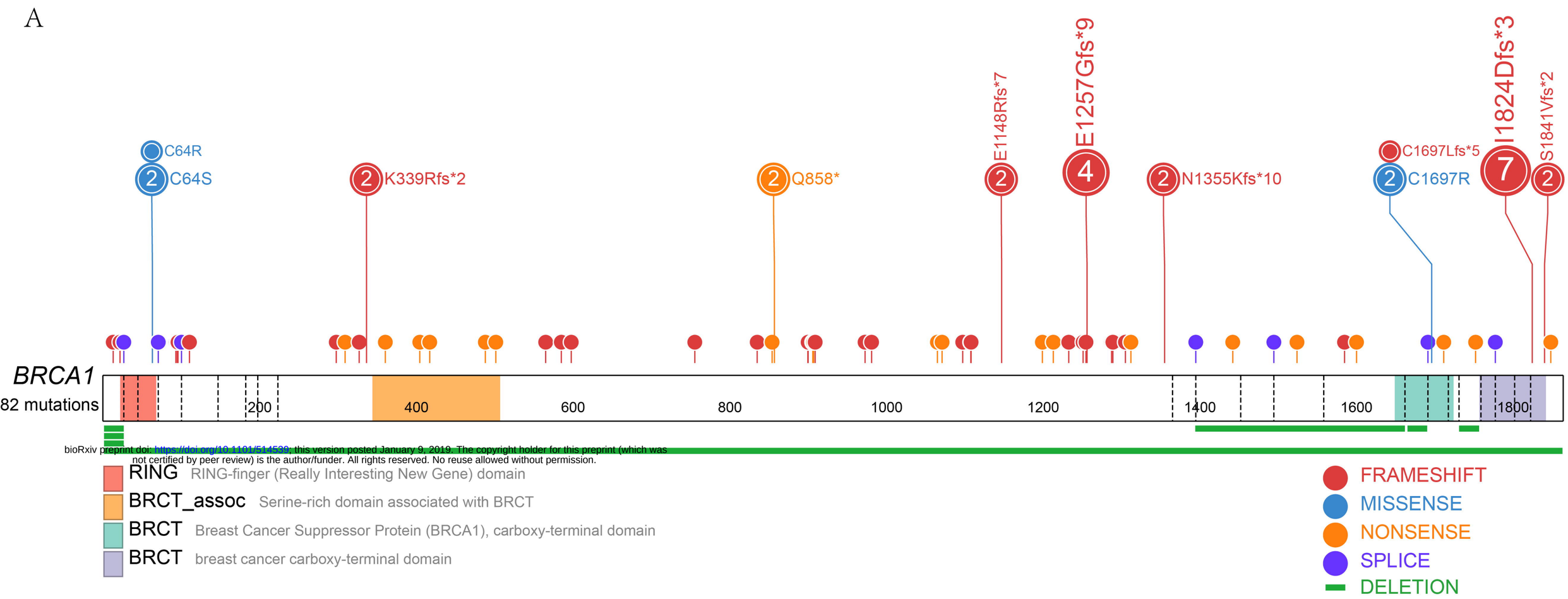
Supplement Table S1 List of deleterious mutations

Supplement Table S2 Number of VUS mutations per kb in 21 genes

Supplement Figure S1 Distribution of *BRCA1* and *BRCA2* deleterious mutations

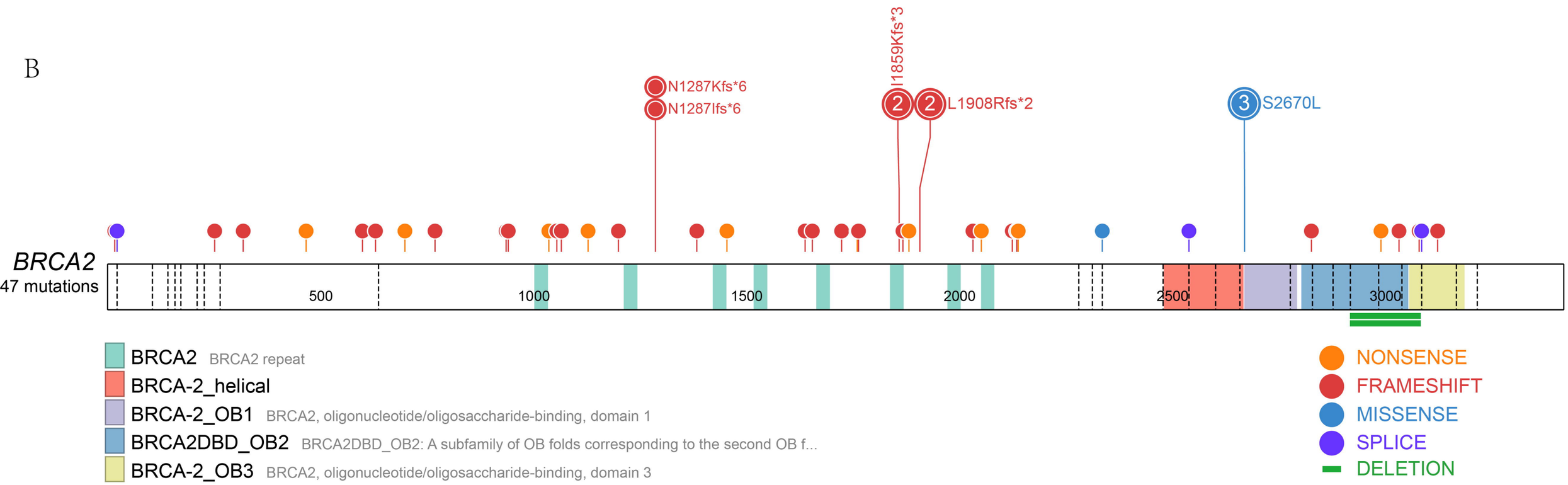

A
Total
BRCA2 27.8%
BRIP1 5.1%
CHEK2 4%
MRE11A 1.7%
NBN 0.6%
PALB2 0.6%
RAD50 0.6%
RAD51C 2.3%
STK11 0.6%
TP53 1.1%
MLH1 1.1%
MSH2 1.7%
MSH6 0.6%
PMS1 0.6%
MUTYH 1.1%
BRCA1 50.6%
B
BC
BRIP1 6.5%
CHEK2 16.1%
MRE11A 3.2%
PALB2 3.2%
RAD51C 3.2%
TP53 3.2%
PMS1 3.2%
BRCA2 35.5%
BRCA1 25.8%
C
OC
BRCA2 28.7%
BRIP1 4.3%
CHEK2 0.9%
MRE11A 0.9%
RAD50 0.9%
RAD51C 1.7%
STK11 0.9%
TP53 0.9%
MSH2 2.6%
MSH6 0.9%
BRCA1 57.4%
D
BC & OC
BRCA1 90.9%
MUTYH 9.1%

A

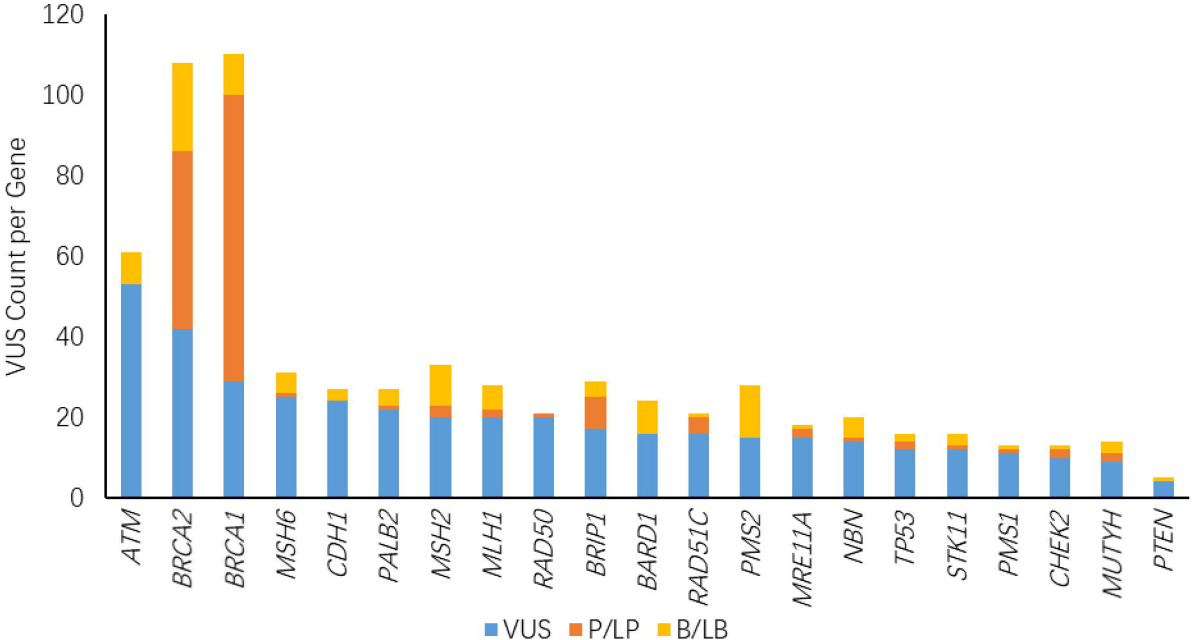


p.Val14Glyfs*3 †	p.Glu23Argfs*18	p.Cys27_E3splice	p.Cys64Arg	p.Cys64Ser	p.Arg71_E4splice	p.Phe93Leufs*4	p.Leu95Thrfs*23
p.Thr97Argfs*9	p.Tyr101_E6splice	p.Asn112Ilefs*7	p.Asn298Lysfs*2 †	p.Gln310*	p.Cys328* ‡	p.Lys339Argfs*2	p.Ser361*
p.Ser405*	p.Glu418*	p.Glu489*	p.Leu502*	p.Asn567Lysfs*3	p.Pro586Leufs*2	p.Asn599Ilefs*13 †	p.Val757Phefs*8
p.Glu836Lysfs*10	p.Gln855*	p.Gln858*	p.Phe901*	p.Phe901Leufs*99 †	p.Glu907*	p.Glu908*	p.Gln910Lysfs*90
p.Leu974Thrfs*17	p.Pro982Hisfs*18	p.Glu1066*	p.Leu1072*	p.Pro1099Leufs*10	p.Lys1110Alafs*4	p.Lys1110Serfs*7	p.Glu1148Argfs*7
p.Gln1200*	p.Glu1214*	p.Val1234Glnfs*8	p.Ser1253Argfs*10	p.Thr1256Argfs*10	p.Glu1257Glyfs*9	p.Glu1288Glnfs*18 †	p.Lys1290Metfs*4
p.Leu1306Phefs*24	p.Gln1313*	p.Asn1355Lysfs*10	p.Gln1395_E11splice	p.Arg1443*	p.Arg1495_E14splice	p.Gln1525*	p.Glu1586Serfs*15
p.Lys1601*	p.Asp1692_E17splice	p.Cys1697Arg	p.Cys1697Leufs*5	p.Trp1712*	p.Arg1753* †	p.Asp1778_E21splice	p.Ile1824Aspfs*3 ‡
p.Ser1841Valfs*2	p.Glu1849* †	EX1_24DEL	EX13_16DEL	EX17DEL	EX20DEL	EX1_2DEL	

B



p.Arg18Leufs*12	p.Asp23_E2splice	p.Asp252Valfs*24	p.Asn319Lysfs*8	p.Lys467*	p.Ser599*	p.Thr630Asnfs*6	p.Gln699*
p.Ile770Phefs*2	p.Ala938Profs*21	p.Ser942Glnfs*18	p.Ser942Glnfs*18 †	p.Gln1037* ‡	p.Asn1055Lysfs*4	p.Asn1066Lysfs*1 †	p.Gln1129*
p.Cys1200*	p.Asn1287Ilefs*6	p.Asn1287Lysfs*6 †	p.Leu1384Cysfs*4	p.Glu1455*	p.Val1639Serfs*3	p.Tyr1655*	p.Ile1724Lysfs*17
p.Gly1761*	p.Lys1765Glnfs*13 †	p.Ile1859Lysfs*3	p.Asp1868Valfs*5 †	p.Ser1882*	p.Leu1908Argfs*2	p.Ile2033Tyrfs*16	p.Ser2052*
p.Thr2125Asnfs*4	p.Asn2135Lysfs*3 †	p.Glu2139*	p.Arg2336Leu	p.Leu2540_E16splice	p.Ser2670Leu	p.Pro2827Leufs*36 †	p.Trp2990*
p.Thr3033Asnfs*11 ‡	p.Ser3080Cysfs*30 †	p.Gly3086_E24splice	p.Asn3124Glnfs*26 †	EX22_24DEL			


VUS Count per Gene
120
100
80
60
40
20
0
ATM
BRCA2
BRCA1
MSH6
CDH1
PALB2
MSH2
MLH1
RAD50
BRIP1
BARD1
RAD51C
PMS2
MRE11A
NBN
TP53
STK11
PMS1
CHEK2
MUTYH
PTEN
VUS
P/LP
B/LB