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Risks of breast and ovarian cancer for women harboring pathogenic missense variants in *BRCA1* and *BRCA2* compared with those harboring protein truncating variants

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Abstract

Purpose: Germline genetic testing for *BRCA1* and *BRCA2* variants has been a part of clinical practice for >2 decades. However, no studies have compared the cancer risks associated with missense pathogenic variants (PVs) with those associated with protein truncating (PTC) variants.

Methods: We collected 582 informative pedigrees segregating 1 of 28 missense PVs in *BRCA1* and 153 pedigrees segregating 1 of 12 missense PVs in *BRCA2*. We analyzed 324 pedigrees with PTC variants in *BRCA1* and 214 pedigrees with PTC variants in *BRCA2*. Cancer risks were estimated using modified segregation analysis.

Results: Estimated breast cancer risks were markedly lower for women aged >50 years carrying *BRCA1* missense PVs than for the women carrying *BRCA1* PTC variants (hazard ratio [HR] 3.9 [2.4–6.2] for PVs vs 12.8 [5.7–28.7] for PTC variants; *P* .01), particularly for missense PVs in the BRCA1 C-terminal domain (HR 2.8 [1.4–5.6]; *P* .005). In case of *BRCA2*, for women aged >50 years, the HR was 3.9 (2.0–7.2) for those heterozygous for missense PVs compared with 7.0 (3.3–14.7) for those harboring PTC variants. *BRCA1* p.[Cys64Arg] and *BRCA2* p.[Trp2626Cys] were associated with particularly low risks of breast cancer compared with other PVs.

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Web Resources

BRCA Exchange. Accessed February 15, 2021. https://brcaexchange.org/.

HCI Priors database. Huntsman Cancer Institute. Accessed February 15, 2021. http://priors.hci.utah.edu/PRIORS/BRCA/viewer.php?gene=BRCA1.

Conflict of Interest

F.J.C. has received consulting fees from Astrazeneca. A.S. has received consulting fees from Pfizer and Astra Zeneca. T.V.O.H. has received honoraria from Pfizer. All other authors declare no conflicts of interest.

Additional Information

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Conclusion: These results have important implications for the counseling of at-risk women who harbor missense PVs in the *BRCA1/2* genes.

Keywords

BRCA1; BRCA2; Cancer risks; Missense variants

Introduction

Germline genetic testing for *BRCA1* (OMIM 113705) and *BRCA2* (OMIM 600185*)* variants has long been part of clinical practice. Initially restricted to families that met strict criteria for such testing, the advent of panel tests by a number of clinical diagnostic testing services has led to the use of these tests in a much broader group of individuals. Once a germline pathogenic variant (PV) is identified, women are counseled about their risks of breast, ovarian, and other cancers, and appropriate screening or surgical prevention strategies are discussed. In most situations, these cancer risk estimates are based on studies of large collections of families[1] or relatives of patients with breast cancer (BC) largely unselected for family history.[2] Typically, these analyses have all PVs pooled, irrespective of the variant type, under the assumption that all such variants are associated with the same risks. The vast majority of the pathogenic *BRCA* variants included in these studies were variants predicted to result in a transcript encoding a protein termination codon that is subject to nonsense-mediated decay or encoding truncated inactive protein (for simplicity, these will be hereafter referred to as protein truncating [PTC] variants). The most recent estimates for *BRCA1/2* risks associated with PVs were taken from a large prospective study of *BRCA* PV heterozygotes conducted by the IBCCS/ PROF-SC/kConFab group.[3] Cumulative risks of BC at age 70 were 66% (95% confidence interval [CI], 61–72) for *BRCA1* heterozygotes and 69% (95% CI, 55–68) for *BRCA2* heterozygotes; corresponding risks of ovarian cancer (OC) were 41% (95% CI, 33–50) and 15% (95% CI, 10–23), respectively. There is also accumulating evidence from genotype/phenotype studies that even PTC variants may not all be associated with the same risks, depending on their position within the gene.[3–6]

Analysis of missense variants poses a particular problem because most such variants are expected, a priori, to be of little clinical significance. Through the work of the Evidence-Based Network Investigating Germline Mutant Alleles (ENIGMA) Consortium[7] and others, approximately 60 missense variants have now been classified/reclassified as pathogenic by multifactorial methods[8] using lines of evidence such as cosegregation,[9] family history of index individual in clinical testing series,[10,11] and tumor histopathology[12] to classify variants of uncertain significance.[13]

To date, all predicted *BRCA1* and *BRCA2* missense substitution variants that have been classified on the basis of genetic data as PVs (excluding those that act by disruption of normal splicing) reside in 1 of 3 domains of these proteins: the RING domain of BRCA1 (nucleotides 4–294), the BRCA1-C-Terminal (BRCT) repeats in *BRCA1* (nucleotides 4987–5577), and the DNA-binding domain (DBD) of BRCA2 (nucleotides 7669–9558). However, it is not clear whether pathogenic missense variants in these important domains are associated with the same levels of risk of BCs and OCs as the PTC/null variants

that have been the subject of most studies designed to estimate these risks. Indeed, the *BRCA1* variant c.5096G>A, p.[Arg1699Gln] was shown to be associated with lower risks of BC (approximately 20% by age 70)[14,15] and had reduced function in a transcriptional activation assay in 293T cell line (78% of wild-type activity compared with 45% of the p.[Arg1699Trp] variant activity included in this study).[14] Similarly, functional and case-control analyses have identified *BRCA2* p.[Tyr3035Ser] variant as a hypomorphic allele associated with only a 2.5-fold increased risk of BC (95% CI, 1.05–6.05).[16]

To comprehensively examine the risks associated with established (eg, those classified as such in ClinVar,[17] BRCA Challenge,[18] ENIGMA[7]) missense PVs in *BRCA1* and *BRCA2*, a large series of pedigrees segregating missense PVs were collected through the ENIGMA and Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA)[19] consortia and compared with a set of pedigrees segregating PTC variants using detailed statistical and genetic analyses. In addition, the histopathological profiles of breast tumors of patients carrying *BRCA1* PVs and PTC variants were compared.

Here we show that pathogenic missense variants, especially in *BRCA1*, are associated with a reduced risk of BC compared with PTC variants, particularly for older women.

Material and Methods

Through the ENIGMA[7] Consortium we put out a broad call for pedigree and tumor pathology information from families with a *BRCA1* or *BRCA2* missense variant that, at the time of initiation of the study, had evidence in favor of pathogenicity; this initial list comprised 58 such variants. We also obtained qualifying families from the CIMBA[8] Consortium in which complete pedigree information was available. Criteria for inclusion of families for this study were as follows:

1. index individual (proband) with a pathogenic or likely pathogenic missense variant in *BRCA1* or *BRCA2*, as determined by ENIGMA through multifactorial analysis[13] or ClinVar[17] (at least 2 submitters with a review status of at least 1 * denoting it to be pathogenic/likely pathogenic with none calling it benign or likely benign) and
2. information on segregation analysis, ie, at least 1 nonproband individual tested for the variant.

As a comparison group, we also received a set of 538 pedigrees segregating PTC variants from the large German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC). These pedigrees were matched by specific clinical center and date of ascertainment/testing to the GC-HBOC missense variant pedigrees.

Variant selection

Pedigrees in which the index individual had 1 of 58 selected missense variants were contributed to the study. Of these, 18 variants were excluded from the analysis because they were determined at the time of analysis to not meet the specified criteria either because they were found to be acting primarily through disruption of normal splicing

and/or other evidence that cast doubt on their classification as pathogenic missense variant. The remaining 40 variants consisted of 11 variants in the BRCA1 RING domain, 17 in the BRCA1 BRCT domain, and 12 in the BRCA2 DBD. Supplemental Table 1 provides detailed genetic and functional information regarding the 40 included variants. One *BRCA1* missense variant (p.[Cys64Gly]) and 2 *BRCA2* missense variants (p.[Glu2663Val] and p.[Asp2723Gly]) show experimental and bioinformatics evidence of pathogenicity through both protein effects and potential splicing effects.[20,21] Because these variants showed strong deleterious effects in functional assays[22–24] and only partial splicing defects,[25,26] they were included in our analyses.

Family data

A total of 1146 missense PV pedigrees and 543 PTC variant pedigrees from 45 collaborators representing 17 countries from around the world were contributed for this study. The requested information included pedigree data, age at testing, age at death, current age, age at diagnosis of BC, and age at diagnosis of OC; any available information on breast tumor grade, estrogen receptor (ER), progesterone receptor, and HER2 status; and prophylactic surgeries. All pedigrees segregating PTC variants (326 *BRCA1* and 217 *BRCA2*) and 527 of the missense variant pedigrees (including 279 c.181T>G, p.[Cys61Gly]) were contributed by the GC-HBOC network. In addition, we also had relatively large contributions of 2 founder variants: (*BRCA1*:c.190T>C, p.[Cys64Arg])[27] with 51 informative families submitted from Italy and (*BRCA1*:c.5212G>A, p.[Gly1738Arg])[28] 21 informative families submitted from Greece. Of the total 1689 contributed families, 416 were excluded from all analyses because they proved to be not informative (308 families) for the planned analyses, did not have 1 of the 40 final selected variants (93 families), or had unclear pedigree structures or multiple variants (15 families).

After the exclusions described earlier, the data used for the analyses in this study included 582 informative pedigrees with pathogenic missense variants in *BRCA1*, 153 informative pedigrees with pathogenic missense variants in *BRCA2*, 324 informative pedigrees with PTC variants in *BRCA1*, and 214 informative pedigrees with PTC variants in *BRCA2*. Summary of the characteristics of the set of families included in the study for each of the included variants is shown in Table 1.

Imputation of missing ages and censoring

To avoid any biases owing to differential information provided by submitting center (and hence *BRCA* variant), we imputed missing ages at last follow up/death and missing years of birth using the program PedPro[29] that uses the ages/ years of birth of close relatives to assign an age or year of birth to individuals missing such information. Those with imputed ages ≥90 years were assumed to be aged 65 years because these were likely in older generations without reliable data, and those imputed to be 80–89 were censored at age 80 years. To be conservative, we assumed that 324 cases with missing ages at diagnosis of BC or OC were diagnosed at the age at their last follow up/age at death (including imputed ages). Women with BC and OC were considered to have both cancers if BC occurred first, whereas women with BC after OC were considered to have only OC and were censored at the age of diagnosis of OC. Age at bilateral prophylactic mastectomy (BPM) was available in the

GC-HBOC data set, in several smaller centers, and in the 36 families contributed from the CIMBA consortium but were not available in the remainder of the data sets. Women without BC or OC were censored at their age at last observation or, if deceased, at the age at death. When such information was provided, women known to have had a prophylactic mastectomy were censored at the age at surgery. Because only a very small subset of the contributed families provided data on risk reducing salpingo-oophorectomy, we chose not to censor women at this age; this may lead to an underestimation of OC risk, but this effect is expected to be the same for the PTC variant pedigrees as well as the missense PV set, particularly for the p.[Cys61Gly] variant, which has been known to be pathogenic for >20 years.

Estimation of risk

BC and OC risks were estimated by maximum likelihood analysis using modified segregation analysis with the MENDEL package of programs[30] as implemented for BC, OC, and other cancers in the recent analyses of families with PVs in *PALB2.*[31] In the present analyses, we estimated the risks for BC and OC jointly, censoring each affected individual at their age at the first occurrence of BC or OC. For each data set, we estimated the hazard ratio (HR) associated with development of BC in the following 2 age groups: <50 and ≥50 years. Because of the more limited data for OC in these data sets, we assumed a constant HR across ages. Thus, in each analysis 3 parameters were estimated simultaneously. For both BC and OC analyses, the baseline population incidence rates specified in the model were assumed to be those for the United Kingdom. To account for temporal trends we utilized rates for 8 10-year birth cohorts (Cancer Incidence in Five Continents Reports).[32] To adjust for the ascertainment process of the families, the likelihood of observing the pedigree phenotypes and variant genotypes was calculated conditional on the pedigree phenotypes and the genotype of the index individual; all information for risk estimation thus comes from the distribution of genotypes (ie, genotype status) among the phenotypes of the family members of the proband. From the resulting parameter estimates of BC and OC relative risks, age-specific cumulative risk estimates were calculated from the cumulative incidence $\Lambda(t)$: $F(t)\ 1 - \exp(-\Lambda(t))$, assuming the United Kingdom rates for the cohort between 1970 and 1979; the corresponding CIs were calculated using a parametric bootstrap. Heterogeneity of BC or OC risk between variants in the same domain was tested by a likelihood ratio test of model likelihoods. Comparisons of parameters estimate from the missense variants (or groups of variants) pedigrees with those from PTC variant pedigrees were done by constructing a Z-test on the basis of the parameter estimates and their respective SEs.

Results

BRCA1 and *BRCA2* risk estimates

Table 2 shows the results of the modified segregation analyses in which we estimated the risks of BC and OC relative to United Kingdom population incidence rates. Based on the estimated HRs, the biggest difference between pathogenic missense variants and PTC variants was in the risk of BC for women aged >50 years. For example, for women aged ≥50 years, the HR for *BRCA1* RING domain variants was roughly half the corresponding

HR estimated for the *BRCA1* PTC variants. Of note, the estimated HR for c.181T>G, p.[Cys61Gly] in women aged ≥50 for the subset of 217 informative pedigrees with this variant ascertained from the GC-HBOC clinical network was significantly different from the corresponding HR for pedigrees with *BRCA1* PTC variant ascertained from the same GC-HBOC clinical centers ($P = .049$). The 3 groups of *BRCA1* RING domain variants, as shown in Table 2, did not reveal statistically significant heterogeneity in the estimated HRs ($\chi^2 = 10.0$, 6 degrees of freedom; $P = .12$) by likelihood ratio test). For women aged ≥50 years, the HR for *BRCA1* BRCT domain missense variants was significantly reduced compared with the HR for PTC variant heterozygotes (2.0 vs 12.8; $P = .005$). There was no evidence of significant heterogeneity between variants within the BRCT domain, and there was no significant difference between the overall estimated models for the RING and BRCT domains ($\chi^2 = 5.48$; 3 degrees of freedom; $P = .14$).

No significant difference was observed in the HRs for the combined set *BRCA2* missense PVs in the DBD compared with the estimated cancer risks for PTC variants. However, there was some evidence of heterogeneity associated with the *BRCA2* variants in the DBD with HRs for women aged >50 years varying from 1.7 (95% CI, 0.4–6.9) for c.7878G>C, p.[Trp2626Cys] to 5.9 (95% CI, 2.2–16.3) for the well-established missense PV p.[Asp2723His] and 7.0 (95% CI, 3.3–14.7) for the *BRCA2* PTC variants.

When we examined the risks in women aged >60 years (data not shown), we observed an even larger difference in risks for BC than for PTC variants, although reduction in sample size increased the SEs, resulting in wider CIs. For example, for the RING and BRCT domain PV heterozygotes, the HRs were 2.8 (95% CI, 1.1–7.3) and 2.0 (0.6–6.6), respectively, for these women versus 16.5 (95% CI, 4.3–63.2) for the *BRCA1* PTC variants in our study. For women aged >60 years, the HR for *BRCA2* pathogenic DBD missense variants was 2.0 (95% CI, 0.6–6.3) compared with 5.3 (95% CI, 1.6–17.9) for the *BRCA2* PTC variants.

In general, the estimated HRs for OCs were quite similar in the various pathogenic missense variant analyses, although risks were somewhat reduced for the *BRCA1* BRCT variants, but this was not statistically significant. Estimated cumulative risks of BC at age 50 years and 70 years and of OC at age 70 years for the primary sets of variants are shown in Table 3. Globally, risks at age 50 years did not vary substantially between missense PVs and their PTC variant counterparts. However, the estimated risks at age 70 years were more variable owing to the HRs for ages >50 years being, in general, lower for the missense variants.

Estrogen receptor status of breast tumors with BRCA1 variants

ER status was available for 361 breast tumors from women harboring a *BRCA1* pathogenic missense variant. These included 250 tumors from missense variants in the RING domain (220 of which were from p.[Cys61Gly] heterozygotes) and 111 tumors in the BRCT domain compared with 210 tumors from *BRCA1* PTC variants heterozygotes (Table 4). There was no significant difference in the frequency of ER negative tumors among women with BCs associated with *BRCA1* missense PV compared with the tumors among women heterozygous for a PTC variant. Individually, only p.[Ala1708Glu] variant had a nominally significantly lower frequency of ER negative tumors (52% vs 72%; $P = .027$) than PTC

variants, although this was not significant when corrected for the number of comparisons made.

BC risk associated with specific variants

We found that the BC (but not OC) risks associated with 2 specific missense PVs were lower than that for other *BRCA1* missense PVs. The HR associated with the missense PVs, p.[Cys64Arg] for women aged <50 years was 3.7 (95% CI, 1.4–9.5) and that for women aged ≥50 years was 4.1 (95% CI, 0.9–18.3). In particular, the estimated HR for younger women with this missense PV was substantially lower than that for both other RING domain PVs and BRCT domain PVs. We tested this variant against the estimated risk for the p.[Cys61Gly] variant assuming a constant HR and found it to be significantly different (Z-test; *P* .015). These lower estimated risks were consistent with the observation of 10 BC cases in these families who tested negative for the variant (mean age at diagnosis was 48.6 years) compared with 14 nonproband cases who tested positive (mean age at diagnosis was 43.9 years). Moreover, among 13 women unaffected with BC or OC aged >60 years who were tested for the variant, almost half (6/13) were found to be positive, also indicating a lower penetrance. Thus, the evidence suggests that this *BRCA1* missense PV has substantially reduced BC risk (but not necessarily OC) compared with other PVs but still higher than the population incidence rates. The risks associated with the *BRCA2* p.[Trp2626Cys] missense variant for BC and OC were significantly reduced compared with both *BRCA2* PTC variants and other *BRCA2* missense PVs analyzed in this study (Table 2). Among the 31 female relatives who tested positive for the p.[Trp2626Cys] variant in the 34 pedigrees, there were only 3 cases of BC and 1 case of OC. Moreover, among the 22 unaffected women aged >60 years who were tested for the variant, 13 were found to be positive for the p.[Trp.2626Cys] variant, also indicating that this variant has reduced penetrance.

Sensitivity analyses for imputed age

Reanalysis of the data assuming all individuals with missing year of birth were born between 1960 and 1969 (thus eliminating cohort differences in incidence rates for these individuals) resulted in only a minimal effect on the estimates because the vast majority of the missing ages were in individuals who were not tested or closely related to a tested individual and thus did not contribute heavily to the analysis. Individuals with missing current age/age at death were excluded in these analyses as well. Results of these analyses are shown in Supplemental Table 2. For example, for the *BRCA1* PTC set of pedigrees, the HR for BC for women aged <50 years, HR for BC for women aged ≥50 years, and HRs for OC were 16.4, 12.1, and 26.3, respectively, without the missing age imputation and 17.0, 12.8, and 27.1, respectively (Table 2), using imputed age data.

Sensitivity analyses of population incidence rates

For the analyses we considered age- and cohort-specific cancer incidence rates for the United Kingdom population. To see how our results and conclusions could be affected by possible differences in rates, we reanalyzed larger subsets of pedigrees considering rates that were 20% higher and 20% lower than these (across all ages and birth cohorts). Given that the vast majority of the pedigrees submitted came from Western European clinical centers, this should account for the incidence rate differences among the various populations

included here. The parameter estimates for the *BRCA1* BRCT domain variants, RING domain variants, and PTC variants from the GC-HBOC resource were on average 5% higher when incidence rates were assumed to be 20% lower and estimates were 4% lower when rates were set to be 20% higher. The largest differences were seen for the ln(HR) estimates for OC, which were 11% higher for the set of *BRCA1* RING domain variants when incidence rates were assumed to be 20% lower. On the basis of these analyses, differences in rates from our assumed incidence rates in the United Kingdom population are an unlikely explanation for the findings.

Discussion

Here we report a large international collaborative study examining the risks of BC and OC for women harboring pathogenic missense variants in the *BRCA1* and *BRCA2* genes. We benefited from a large national study of hereditary BC and OC in Germany (GC-HBOC), which contributed approximately half of the missense PV families and a series of families that segregated PTC variants in *BRCA1* and *BRCA2* to form comparison groups for the missense variants in each gene. The results shown in Tables 2 and 3 clearly indicate that missense PVs in both functionally important domains (RING and BRCT) in *BRCA1* are associated with lower risks of BC than PTC variants. Although data are limited, it appears that OC risks are comparable (though on average perhaps slightly lower) for *BRCA1* with those found in published risk estimates for PTC variants[1–3] and also comparable with those estimated from the PTC variant pedigrees from GC-HBOC. Although associated with lower BC risks, missense PVs in *BRCA1* had similar hormone receptor status profiles as their PTC counterpart; importantly, the families studied here were largely tested before hormone receptor triple negative status was a stand-alone criterion for *BRCA* testing, suggesting a lack of ascertainment bias based on pathology. Most *BRCA2* pathogenic missense variants appeared to be associated with lower risks than the *BRCA2* PTC variants in families in the age group >50 years (Table 2); however, these differences were not statistically significant and were of smaller magnitude than the parallel comparisons for *BRCA1* variants.

Clinical considerations

Our results provide evidence of the convergence of moderate-to high-risk classes that no longer seem to be distinct but rather describe a risk continuum. We have previously shown that the hypomorphic *BRCA1* variant p[Arg1699Gln] is associated with reduced levels of BC risk compared with the average *BRCA1* truncating variant.[15,16] This raises the question of what clinical measures to offer for different levels of risk. In women aged 70 years, the estimated cumulative risks of 70% for *BRCA1* PTC variants compared with 39% for the combined group of missense PVs in the BRCT repeat domains (primarily p.[Ala1708Glu] and p.[Arg1699Trp]) are significantly different and may very well affect a woman’s choice of prevention/screening options, in particular, BPM. Furthermore, these risks will depend on family history, their polygenic risk score,[33] and lifestyle factors.[34] We believe that these new risk estimates for *BRCA1* missense variants should be incorporated into comprehensive risk prediction tools such as BOADICEA.[35] Although on average slightly lower, the OC risks associated with both *BRCA1* and *BRCA2* missense PVs did not seem to differ markedly from that for the PTC variants (with the possible exception of PVs in the BRCT domain

of *BRCA1*). For cancer-free women aged >50 years, many of the *BRCA1* pathogenic missense variants are associated with relative BC risks closer to those estimated for PVs in genes such as *ATM*, *CHEK2*, and other moderate risk genes, indicating that surveillance might be an optimal approach. However, given the high OC risks of 36% by age 70 for RING domain missense PVs, we recommend that women heterozygous for these variants should be counseled the same with respect to OC as women with *BRCA1* PTC variants in terms of recommendations for risk reducing salpingo-oophorectomy. Clinically, these data strengthen the importance of communicating not only the cumulative lifetime risks but also risks within a manageable timespan, eg, 10-year risks. For the *BRCA1* p.[Cys64Arg] variants we propose counseling heterozygotes similarly to those proposed for the *BRCA1* p.[Arg1699Gln] hypomorphic variant.[15] The p.[Cys64Arg] variant has been classified as pathogenic by the majority of clinical laboratories, and therefore, it will be important to collect more genetic data to clarify the status of this variant and its associated cancer risks.

We also particularly noted the low BC risk estimates for *BRCA2* p.[Trp2626Cys] variant. The functional effects of this variant have been examined, and although it was classified as functionally deleterious, it was near the boundary between deleterious and intermediate function.[36] This variant is denoted in ClinVar as pathogenic by 9 clinical laboratories and as likely pathogenic by 3 others, with a single lab reporting it in ClinVar as a variant of uncertain significance. This variant has also been evaluated in a number of other recent studies. On the basis of the family histories of 12 individuals heterozygous for p.[Trp2626Cys] in a large clinical testing set, we estimated odds of 16:1 against pathogenicity,[11] and in the large OncoArray[37] BC case-control series, this variant was identified in 16 of 75,350 cases and 5 of 52,793 controls (odds ratio = 2.2; 95% CI, 0.8–7.8; P =.1), which is consistent with the estimate reported here (K. Michailidou, personal communication). Taken together, the available evidence indicates that caution should be exercised in counseling individuals harboring this variant as a pathogenic *BRCA2* variant until further genetic and functional studies can be performed.

Limitations and caveats

The primary limitation inherent in our study is that families were submitted from a wide variety of countries and clinical centers that could vary widely with respect to ascertainment criteria, cascade testing, and prospective follow up. Although in our analysis, we adjusted for the phenotypes of all pedigree members and relied only on the genotype status of nonproband family members, it is possible that different practices in different centers could affect the risk estimates. However, the consistency of our results together with the direct comparison of p.[Cys61Gly] with PTC variant pedigrees from the GC-HBOC resource make it highly unlikely that systematic biases could explain our findings.

A second limitation of this study is the lack of complete data on prophylactic surgery and potentially differing rates of uptake across countries. To ensure that the observed differences in risk were not due to the censoring of women at BPM in some of the data sets and not others, we repeated analyses ignoring the BPM information. In particular, we were concerned about the effect in the *BRCA1* and *BRCA2* reference sets because they were used for comparison with the missense PVs. The mean difference between age at BPM and

current age/age at death was only 2.9 years (95% CI, 1.9–3.9), indicating that differential information with regard to BPM would not alter our conclusions.

Conclusion

Our analyses of approximately 1250 informative pedigrees have shown that *BRCA1* missense PVs are associated with smaller increased risks of BC in women aged >50 years when compared with variants predicted to result in complete loss of function. The risk reduction was less pronounced for *BRCA2* in such women. We have also shown that specific variants are associated with particularly lower risks than other *BRCA* PVs: *BRCA1* p.[Cys64Arg] and *BRCA2* p.[Trp2626Cys] for BC and possibly *BRCA1* p.[Ala1708-Glu] for OC. Interestingly, the histopathology of BCs from patients carrying pathogenic missense variants in *BRCA1* showed similar rates of ER negative/triple negative status as that from patients carrying PTC variants. Future studies should focus on the functional basis for the reduction in relative risks for older women who harbor a pathogenic missense variant in *BRCA1* and, to a lesser extent, *BRCA2*.

Data Availability

Requests for the data files used in the analysis should be made to D.E.G., although European General Data Protection Regulation (GDPR) regulations may not permit pedigree data to be transferred outside the European Union.

Supplementary Material

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Ethics Declaration

This study was covered under Amendment to Project P1051 (PI: A.BS.) approved by the Human Research Ethics Committee at QIMR Berghofer, Brisbane, Australia, on October 7, 2020. All patients gave consent to have their data used for research purposes, and all studies received local Ethics Committee approvals. Data from all centers were de-identified before analysis at the coordinating center.

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Table 1

Description of variants and families included in the risk estimation analyses

Variant/Group	Informative Families	Breast Cancer			Ovarian Cancer		
		Heterozygous	Wild Type	Unknown	Heterozygous	Wild Type	Unknown
BRCA1							
GC-HBOC *BRCA1* PTC	324	85	7	495	22	2	125
RING domain							
c.181T>G, p.[Cys61Gly]	316	114	14	490	20	0	87
c.190T>C, p.[Cys64Arg]	55	14	10	116	4	0	33
Other RING domain[a]	34	13	3	55	7	0	23
All RING domain	405	141	27	662	31	0	143
GC-HBOC c.181T>G, p.[Cys61Gly]	277	81	9	422	9	0	73
BRCT domain							
c.5095C>T, p.[Arg1699Trp]	43	11	2	45	13	0	36
c.5123C>A, p.[Ala1708Glu]	56	17	0	84	3	1	19
c.5212G>A, p.[Gly1738Arg]	36	25	2	39	12	0	9
Other BRCT domain[b]	42	21	6	59	10	0	14
All BRCT domain	177	74	10	227	38	1	78
All *BRCA1* nissense	582	215	37	889	69	1	221
BRCA2							
GC-HBOC *BRCA2* PTC	214	48	9	376	5	0	38
DBD							
c.7878G>C, p.[Trp2626Cys]	34	3	0	44	0	1	9
c.8167G>C, p.[Asp2723His]	33	18	3	66	2	0	12
c.9154C>T, p.[Arg3052Trp]	20	9	2	39	0	0	8
Other B2 DBD[c]	66	19	6	121	6	1	18
All DBD Missense	153	49	11	270	8	2	47

Index individuals are excluded from tabulations.

B2, *BRCA2*; *DBD*, DNA binding domain; *GC-HBOC*, German Consortium for Hereditary Breast and Ovarian Cancer; *PTC*, protein truncating.

[a] c.53T>C, p.[Met18Thr]; c.65T>C, p.[Leu22Ser]; c.110C>A, p.[Thr37Lys]; c.115T>C, p.[Cys39Arg]; c.116G>A, p.[Cys39Tyr]; c.130T>A, p.[Cys44Ser]; c.131G>T, p.[Cys44Phe]; c.190T>G, p.[Cys64Gly]; c.191G>A, p.[Cys64Tyr].

[b] c.5053A>G, p.[Thr1685Ala]; c.5054C>T, p.[Thr1685Ile]; c.5089T>C, p.[Cys1697Arg]; c.5117G>A, p.[Gly1706Glu); c.5143A>C, p.[Ser1715Arg]; c.5213G>A, p.[Gly1738Glu]; c.5216A>T, p.[Asp1739Val]; c.5291T>C, p.[Leu1764Pro]; c.5297T>G, p.[Ile1766Ser]; c.5309G>T, p.[Gly1770Val]; c.5324T>A, p.[Met1775Lys]; c.5324T>G, (p.Met1775Arg]; c.5363G>T, p.[Gly1788Val]; c.5513T>A, p.[Val1838Glu].

[c] c.7879A>T, p.[Ile2627Phe]; c.7940T>C, p.[Leu2647Pro]; c.7958T>C, p.[Leu2653Pro]; c.7988A>T p.[Glu2663Val]; c.8165C>G, p.[Thr2722Arg]; c.8168G>A, p.[Asp2723Gly]; c.8243G>A, p.[Gly2748Asp]; c.9004G>A, p.[Glu3002Lys]; c.9227G>A, p.[Gly3076Glu].

Table 2

HR estimates by variant/group of variants

Variant/Variant Group	Breast Cancer Dx <50 HR (95% CI)	Breast Cancer Dx ≥50HR (95% CI)	Ovarian Cancer HR (95% CI)
BRCA1			
GC-HBOC *BRCA1* PTC	17.0 (9.4–30.5)	12.8 (5.7–28.7)	27.1 (8.6–85.2)
RING domain			
p.[Cys61Gly]	14.6 (8.8–24.3)	7.6 (3.6–16.4)	41.2 (19.0–89.3)
p.[Cys64Arg]	3.7 (1.4–9.5)[a]	4.1 (0.9–18.3)	99.9 (37.8–264)
Other RING domain	12.0 (3.2–37.4)	2.9 (0.5–17.0)	23.9 (4.6–119)
All RING Domain	11.5 (7.2–18.3)	5.8 (3.0–11.3)	41.0 (20.9–80.4)
GC-HBOC p.[Cys61Gly]	15.3 (8.3–28.2)	3.7 (1.5–9.4)[a]	27.1 (7.2–102.8)
BRCT domain			
p.[Arg1699Trp]	10.4 (2.7–39.4)	2.0 (0.2–21.8)	31.5 (5.1–195)
p.[Ala1708Glu]	12.1 (4.3–34.0)	4.9 (1.3–18.3)	5.2 (0.8–33.8)
p.[Gly1738Arg]	22.8 (9.2–56.9)	3.6 (1.4–8.9)	14.7 (3.8–57.8)
Other BRCT domain	15.0 (6.0–37.5)	2.1 (0.7–6.2)[b]	18.8 (6.3–56.2)
All BRCT domain	14.8 (8.7–25.1)	2.8 (1.4–5.6)[b]	15.2 (7.6–30.4)
All *BRCA1* missense	13.1 (9.2–18.9)	3.9 (2.4–6.2)[b]	21.7 (12.4–38.0)
BRCA2			
GC-HBOC *BRCA2* PTC	10.4 (5.9–19.8)	7.0 (3.3–14.7)	13.1 (3.7–45.6)
DBD			
p.[Trp2626Cys]	5.0 (0.3–92.0)	1.7 (0.4–6.9)	2.1 (0.1–32.8)
p.[Asp2723His]	8.5 (2.5–28.7)	5.2 (1.5–18.6)	15.0 (2.1–109)
p.[Arg3052Trp]	8.1 (0.9–73.3)	3.5 (0.4–30.0)	4.1 (0.3–115)
Other DBD	9.5 (3.8–23.7)	5.3 (2.1–13.2)	5.6 (1.4–21.8)
All DBD	8.3 (2.2–30.8)	3.9 (2.0–7.2)	5.5 (2.0–14.8)

BRCT, BRCA1-C-Terminal; *CI*, confidence interval; *DBD*, DNA binding domain; *Dx*, Age at diagnosis of breast cancer; *GC-HBOC*, German Consortium for Hereditary Breast and Ovarian Cancer; *HR*, hazard ratio; *PTC*, protein truncating.

[a] $P < .05$ for test of parameter vs corresponding parameter for loss of function variants.

[b] $P < .01$ for test of parameter vs corresponding parameter for loss of function variants.

Table 3

Theoretical cumulative risks for selected groups of variants based on HR estimates and United Kingdom incidence rates (1960–1969)

	Breast Cancer		Ovarian Cancer
Variant Group	**Risk at Age 50 (95% CI)**	**Risk at Age 70 (95% CI)**	**Risk at Age 70 (95% CI)**
GC-HBOC *BRCA1* PTC	0.33 (0.20–0.51)	0.70 (0.51–0.89)	0.28 (0.09–0.61)
All RING Domain	0.21 (0.14–0.31)	0.46 (0.33–0.61)	0.36 (0.20–0.58)
All BRCT Domain	0.30 (0.18–0.44)	0.39 (0.27–0.54)	0.16 (0.08–0.28)
All *BRCA1* missense	0.24 (0.17–0.32)	0.40 (0.32–0.49)	0.21 (0.12–0.33)
GC-HBOC *BRCA2* PTC	0.22 (0.12–0.37)	0.51 (0.34–0.69)	0.15 (0.04–0.39)
All DBD	0.18 (0.09–0.31)	0.36 (0.24–0.51)	0.065 (0.02–0.15)

BRCT, BRCA1-C-Terminal; *CI*, confidence interval; *DBD*, DNA binding domain; *GC-HBOC*, German Consortium for Hereditary Breast and Ovarian Cancer; *HR*, hazard ratio; *PTC*, protein truncating.

Table 4

ER status in tumors of *BRCA1* pathogenic variant heterozygotes

Variant/Group	No. of Tumors	No. of ER Positive (%)	No. of ER Negative (%)
GC-HBOC BRCA1 PTC	210	59 (28)	151 (72)
BRCA1 missense PVs			
p.[Cys61Gly]	216	55 (25)	161 (75)
p.[Cys64Arg]	21	4(19)	17 (81)
Other RING Domain	11	2 (18)	9 (82)
p.[Arg1699Trp]	26	8(31)	18 (69)
p.[Ala1708Glu]	29	14 (48)	15 (52)
p.[Gly1738Arg]	33	9 (27)	24 (73)
Other BRCT Domain	23	6 (26)	17 (74)
All *BRCA1* missense	359	98 (27)	261 (73)

BRCT, BRCA1-C-Terminal; *ER*, estrogen receptor; *GC-HBOC*, German Consortium for Hereditary Breast and Ovarian Cancer; *PTC*, protein-truncating.