BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk

Amanda B. Spurdle,[1,2*] Phillip J. Whiley,[1] Bryony Thompson,[1,2] Bingjian Feng,[3] Sue Healey,[1] Melissa A. Brown,[4] Christopher Pettigrew,[4] kConFab,[5] Christi J. Van Asperen,[6] Margreet G.E.M. Ausems,[7] Anna A. Kattentidt-Mouravieva,[8] Ans M.W. van den Ouweland,[8]Dutch Belgium UV Consortium,[9,10] Annika Lindblom,[11] Maritta H. Pigg,[12] Rita K. Schmutzler,[13] Christoph Engel,[14] Alfons Meindl,[15] German Consortium of Hereditary Breast and Ovarian Cancer, [9,13] Sandrine Caputo,[16] Olga M. Sinilnikova,[17,18] Rosette Lidereau,[16] French GGC Consortium,[19] Fergus J. Couch,[20] Lucia Guidugli,[20] Thomas v. O. Hansen,[21] Mads Thomassen,[22] Diana M. Eccles,[23] Kathy Tucker, [24] Javier Benitez,[25] Susan M. Domchek,[26] Amanda E. Toland,[27] Elizabeth J. van Rensburg,[28] Barbara Wappenschmidt,[13] Åke Borg,[29] Maaike P.G. Vreeswijk,[30] and David E. Goldgar,[3,31*] on behalf of the ENIGMA Consortium[9].

[1] Division of Genetics and Population Health, Queensland Institute of Medical Research, Brisbane, Queensland, 4000, Australia

[2] School of Medicine, University of Queensland, Brisbane, Queensland, 4000, Australia

[3]Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah, 84132, United States of America.

[4] School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, 4000, Australia

[5] Peter MacCallum Cancer Institute, Melbourne, Victoria, 3000, Australia

[6]Department of Clinical Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands.

[7]Department of Medical Genetics, University Medical Center Utrecht, PO Box 85090, 3508 AB Utrecht, the Netherlands

[8]Department of Clinical Genetics, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands

[9]See Appendix for full list of ENIGMA collaborators contributing to this study, operating within and outside of country consortia.

[10]Dutch Belgium UV Consortium, Co-ordinator F.B. Hogervorst, The Netherlands Cancer Institute, Amsterdam, NL

[11]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm S-17176, Sweden

[12] Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala S-75185, Sweden

[13]Department of Gynaecology and Obstetrics, Centre of Familial Breast and Ovarian Cancer and Centre for Molecular Medicine Cologne, University of Cologne, Cologne, 50931, Germany

[14]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, 04107, Germany

[15]Department of Gynaecology and Obstetrics, Technical University of Munich, Munich, 80331, Germany

[16]Sandrine Caputo, Institut Curie, Hôpital René Huguenin, Service d'Oncogénétique, 35 rue Dailly, U735 INSERM—Saint-Cloud, France

[17]Unite Mixte de Genetique Constitutionnelle des Cancers Frequents, Hospices Civils de Lyon/Centre Leon Berard, Lyon, 69002, France

[18]Laboratoire de Genetique Moleculaire, Signalisation et Cancer, Universite de Lyon, Lyon, 69361,France

[19]French GGC Consortium (Groupe Génétique et Cancer), co-ordinator Rosette Lidereau, Institut Curie, Hôpital René Huguenin, Service d'Oncogénétique, U735 INSERM—Saint-Cloud, France

[20]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, 55901, USA

[21]Center for Genomic Medicine, Rigshospitalet, Copenhagen University hospital, Copenhagen, DK-2100, Denmark

[22]Department of Clinical Genetics, Odense University Hospital, Denmark

[23]Faculty of Medicine, University of Southampton, Southampton University Hospital NHS Trust MP824, Southampton SO16 6YD, UK

[24]Hereditary Cancer Clinic, Prince of Wales Hospital, High St, Randwick, 2031, Australia

[25]Spanish National Cancer Centre, Madrid 28029, Spain

[26]Abramson Cancer Center, University of Pennsylvania, Philadelphia, 19104-3339PA, USA

[27]Division of Human Cancer Genetics, Departments of Internal Medicine and Molecular Virology, Immunology and Medical Genetics, OSU Comprehensive Cancer Center, The Ohio State University, Columbus, 43210-1273, OH, USA

[28]Department of Genetics, University of Pretoria, Hatfield 0028, South Africa

[29]Åke Borg, Department of Oncology, Lund University, Lund, S-22185, Sweden

[30]Department of Human Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands.

[31]Huntsman Cancer Institute, Salt Lake City , Utah, 84112, USA

*Correspondence:

Amanda Spurdle –; Amanda.Spurdle@qimr.edu.au (email), +617 3362 0371 (tel), +617 3362 0105 (fax), Queensland Institute of Medical Research, 300 Herston Road, Herston, Qld, 4006, Australia (postal address).

David Goldgar - david.goldgar@hsc.utah.edu (email), +1 801 585 0337 (tel), +1 801 581 6464 (fax), University of Utah School of Medicine, Salt Lake City, Utah, 84132, United States of America (postal address).

Abstract

Background: Clinical classification of rare sequence changes identified in the breast cancer susceptibility genes *BRCA1* and *BRCA2* is essential for appropriate genetic counseling of individuals carrying these variants. We previously showed that variant BRCA1 c.5096G>A p.Arg1699Gln in the BRCA1 transcriptional transactivation domain demonstrated equivocal results from a series of functional assays, and proposed that this

variant may confer low to moderate risk of cancer. ***Methods***: Measures of genetic risk (report of family history, segregation) were assessed for 68 *BRCA1* c.5096G>A p.Arg1699Gln (R1699Q) families recruited through family cancer clinics, comparing results to 34 families carrying the previously classified pathogenic *BRCA1* c.5095C>T p.Arg1699Trp (R1699W) mutation at the same residue and to 243 breast cancer families with no *BRCA1* pathogenic mutation (BRCA-X). ***Results***: Comparison of *BRCA1* carrier prediction scores of probands using the BOADICEA risk prediction tool revealed that *BRCA1* c.5096G>A p.Arg1699Gln variant carriers had family histories that were less "*BRCA1*-like" than *BRCA1* c.5095C>T p.Arg1699Trp mutation carriers ($p<0.00001$), but more "*BRCA1*-like" than BRCA-X families ($p=0.0004$). Further, modified segregation analysis of the subset of 30 families with additional genotyping showed that *BRCA1* c.5096G>A p.Arg1699Gln had reduced penetrance compared to the average truncating *BRCA1* mutation penetrance ($p=0.0002$), with estimated cumulative risks to age 70 of breast or ovarian cancer of 24%. ***Conclusion***: Our results provide substantial evidence that the *BRCA1* c.5096G>A p.Arg1699Gln (R1699Q) variant, demonstrating ambiguous functional deficiency across multiple assays, is associated with intermediate risk of breast and ovarian cancer, highlighting challenges for risk modeling and clinical management of patients of this and other potential moderate-risk variants.

INTRODUCTION

The clinical classification of rare sequence changes identified in the high risk breast cancer susceptibility genes *BRCA1* and *BRCA2* is essential for appropriate genetic counseling of individuals carrying these variants. Classification of *BRCA1* and *BRCA2*

variants was facilitated by the development of a multifactorial likelihood model[1], which provides a quantitative estimate of pathogenicity by assessing measures of genetic and other features of variant carriers relative to characteristics observed for classical high-risk mutations. Moreover, this quantitative assessment of risk has been linked to clinical management guidelines to provide a basis for standardized variant reporting, variant classification and management of families with such variants[2]. The multifactorial likelihood methodology has been applied in multiple studies[1, 3-15], with more than 200 *BRCA1* or *BRCA2* variants now classified using this approach[16]. However, the multifactorial approach is designed to distinguish high-risk mutations from variants with no or little clinical significance, and it is likely that additional methods are required to detect and validate *BRCA1* or *BRCA2* rare variants associated with more modest risks than the average penetrance reported for classical mutations in these genes i.e. 65% risk of breast cancer and 39% risk of ovarian cancer to age 70 years for *BRCA1* mutations, and 45% risk of breast cancer and 11% risk of ovarian cancer to age 70 years for *BRCA2* mutations [17].

We previously showed that the variant BRCA1 R1699Q (c.5096G>A p.Arg1699Gln) located in the BRCA1 carboxyl terminal (BRCT) regions of the transcriptional transactivation domain (TAD) demonstrated equivocal results from a series of functional assays, when compared to wildtype control and known pathogenic missense mutation BRCA1 A1708E (c.5123C>A p.Ala1708Glu) which was null in all assays[8]. In particular, this variant displayed intermediate transcriptional transactivation activity in human 293T and T47D cell lines and wild type centrosome amplification function, but

behaved as a deleterious mutation when assayed for formation of nuclear foci and trypsin sensitivity. There is also inconsistency in assay results from other functional studies, including discrepancies between yeast and mammalian transcriptional transactivation assay results in a single report[18], and categorization of R1699Q as a variant with strong functional effect due to compromised peptide binding activity and specificity, and compromised transcriptional activity in yet another study[19]. Most recently, Chang et al.[20] performed an extensive study of the R1699Q substitution using mouse embryonic stem (ES) cell-based functional assays, and demonstrated that this variant affected mouse ES cell survival and differentiation and was unable to rescue embryonic lethality of *Brca1*-null mice. However, this study *also* demonstrated that the variant did not cause significant cell cycle defects and had no effect on genomic stability, but it was suggested that abrogated repression of oncomir miR-155 was the underlying mechanism for BRCA1-mediated tumor suppression. The equivocal behavior of this variant can be explained at a protein level, as demonstrated by protein modeling predictions shown in Supplementary Figure 1. R1699 is located in the linker connecting the BRCT repeat domain, and participates in a salt bridge between the BRCT repeats[21]. The loss of salt-bridging interactions and steric strain associated with accommodating a tryptophan substitution contributes to conformational instability of the R1699W (c.5095C>T p.Arg1699Trp) pathogenic mutation and subsequently disrupts transcriptional transactivation function. In contrast, substitutions with no/little effect on structure such as R1699Q may be fully or partially active in these assays. Moreover, R1699 lies in a conserved phosphopeptide binding groove of the BRCA1 repeat and plays an important role in phosphopeptide recognition through its interaction. Specifically, our protein

modeling results directly comparing R1699Q and R1699W show that the volume of R1699W is likely to cause steric clashes with the phosphopeptide, whereas the smaller surface and volume presentation of R1699Q will not cause steric clashes but may modestly alter phosphopeptide recognition (Supplementary Figure 1). These modeling predictions explain the experimental results from biophysical assays of BACH1 binding affinity which demonstrated that R1699W leads to a significant 160-fold reduction in affinity compared to wild type, whereas the reduction is only 24-fold for R1699Q [22].

We previously proposed that the R1699Q variant has partial abrogation of BRCA1 functions and may confer low to moderate risk of cancer that would be better measured using pooled family studies[8]. In a study assessing pathogenicity of 1433 variants based on family history, co-occurrence and co-segregation data from a large dataset derived from clinical testing at Myriad Genetic Laboratories, the combined odds that *BRCA1* R1699Q was a pathogenic variant compared to neutral/no clinical significance was 2.5:1, based on a sample of 16 family histories with co-segregation data on only 3 of these[11]. By contrast, *BRCA1* R1699W at the same residue was classified as pathogenic with odds in favor of pathogenicity of 39,978:1[11]. Bioinformatic analysis shows that the arginine at position 1699 is conserved through tunicate, but the severity of the amino acid substitution is much less marked for glutamine (Grantham deviation 43) compared to tryptophan (Grantham deviation 101). Accordingly, the Align-GVGD algorithm (http://brca.iarc.fr) classifies R1699Q as a C35, while R1699W falls in the most severe C65 category for missense alterations. Based on an analysis of the same Myriad data set,

C35 variants were estimated to have a prior probability of pathogenicity of 0.66, while C65 were associated with a prior probability of 0.81[11, 23].

Mohammadi et al. assessed the likelihood of causality by co-segregation analysis of a single family, and reported a likelihood ratio of 1.4 for R1699Q[24]. In another genetic study of several *BRCA1/2* sequence variants, Gomez-Garcia et al.[25] examined the R1699Q and R1699W variants as part of a model-building exercise that incorporated family history, and estimated the probability of pathogenicity to be 0.87 for R1699Q and >0.99 for R1699W. Although this model classified both variants as pathogenic mutations, the authors noted that R1699Q did not co-segregate completely with disease in one of three of the families in which such data were available.

In summary, a number of different studies to date indicate that the R1699Q variant demonstrates inconsistent or inconclusive results at the functional and genetic level. In an extension of our previous study[8], we confirmed the intermediate transcriptional transactivation activity of BRCA1 R1699Q in the 293T cell line relative to pathogenic variant R1699W at the same residue, and then initiated large-scale genetic studies to assess if this intermediate function might translate to the lower risk of breast and ovarian cancer in families for R1699Q compared to R1699W.

METHODS

Confirmation of Transcriptional Transactivation Activity

Using methods previously described[8], we first compared transcriptional transactivation activity of BRCA1 R1699Q in the 293T cell line to that of pathogenic variant R1699W at the same residue, and also to pathogenic control A1708E, and confirmed our original findings that this variant displayed intermediate function compared to wildtype sequence and known pathogenic TAD variants (Supplementary Figure 2).

Genetic Analyses

With ethical approval from the relevant institutional review boards, we then initiated large-scale genetic studies to assess if this intermediate function might translate to the risk of breast and ovarian cancer in families. Informed consent was obtained from all participants. Through collaboration facilitated in part by the ENIGMA consortium[26], we ascertained sufficient information from multiple clinical cancer genetics centers around the world (Table 1) to compare family history and risk profiles of families in which the R1699Q variant had been identified, to families with the known pathogenic mutation R1699W at the same residue. For an additional reference group, we also collected a set of pedigrees that had been tested clinically for *BRCA1* and *BRCA2* mutations from the same centers within the same time frame as the R1699Q and R1699W families but for which no pathogenic mutation or any other unclassified variant had been found (BRCA-X). The time-frame was determined by center to ensure that a similar

criterion for testing was used. The proband in each instance was defined as the individual initially screened for *BRCA1/2* mutations.

Table 1. Number of families included in the family history and penetrance analyses.

Group	# sites*	BRCA-X	R1699Q		R1699W	
		Family History	Family History	Penetrance	Family History	Penetrance
Netherlands & Belgium	8	42	15	5	11	4
Germany	13	39	5	1	13	8
France	6	4	5	3	2	1
Spain	1	28	0	0	1	0
Denmark	2	30	10	4	0	0
Mayo Clinic, USA	1	8	7	5	0	0
Sweden	3	14	14	5	5	5
Ohio, USA	1	8	1	1	0	0
Australia	3	44	6	2	0	0
Pennsylvania, USA	1	2	1	1	0	0
United Kingdom	4	24	4	2	2	1
South Africa	1	0	1	1	0	0
TOTAL		243	68	30	34	19

** Number of individual clinic sites for each contributing country or region.*

Family history analysis: As a measure of how each family fit the characteristics of a *BRCA1* mutation-positive family, we used the BOADICEA risk prediction algorithm [27-30] to calculate the probability that the proband from each family was a carrier of a *BRCA1* mutation based on the pedigree structure and the phenotypes of individuals in the pedigree. BOADICEA uses a full pedigree likelihood approach and incorporates ages at diagnosis of breast and ovarian cancer, presence of pancreatic and prostate cancer, the age at last follow-up for unaffected individuals, and the year of birth to account for cohort effects in penetrance. The model estimates the simultaneous effects of the high risk genes *BRCA1* and *BRCA2* using the age-specific penetrance estimates derived from 22 population-based studies [17], while allowing for unknown genetic effects that explain

the residual familial clustering of breast cancer. The residual familial clustering is explained by a polygenic component with variance that decreases linearly with age.

The estimated probabilities of the proband carrying a pathogenic *BRCA1* mutation based on the BOADICEA prediction model, B_i, were then transformed in order to better fit a Gaussian distribution using a logit transformation $b_i=\text{logit}(B_i)=\ln(B_i/(1-B_i))$, so that standard statistical methods could be used. For each center that contributed R1699Q/W families, we calculated the mean and standard deviation of the probabilities calculated for the BRCA-X families from this center. This distribution was used to create z-scores as $Z_{ij}=(b_{ij} - Xj)/S_j$, where b_{ij} is the logit of the BOADICEA predicted probability of a *BRCA1* mutation in the ith BRCA-X family in the jth Center, X_j and S_j are the sample mean and standard deviation of the logit-transformed B_i from the jth center. For families with the sequence variants of interest, R1699Q and R1699W, these Z_{ij} thus represent the position of family histories of probands carrying an R1699Q or R1699W variant within the distribution of families tested negative for *BRCA1/2* mutations in the same centers and time frame. Letting Z_{Qi} be the standardized logit score of the *i*th R1699Q family and and Z_{Wi} represent the corresponding score for the *i*th R1699W family. Assuming further that the Zqi and Zwi are Normally distributed with means μ_Q and μ_W and variances σ^2_Q and σ^2_W respectively, these scores can then used to test the following two hypotheses:

(1) The family histories of R1699Q probands are more *BRCA1*-like than those of matched BRCA-X. That is, we test the null hypothesis $\mu_Q=0$ versus the alternative $\mu_Q>0$ with a one-sample t-test. Rejection of the null hypothesis indicates that the

R1699Q families have proband/family histories more compatible with a pathogenic *BRCA1* mutation than the center matched BRCA-X families.

(2) The family histories of R1699Q are less "*BRCA1*-like" than those of R1699W mutations. This is tested by a two sample t-test of the null hypothesis $\mu_Q=\mu_W$ against the one-sided alternative $\mu_Q<\mu_W$.

If both these null hypotheses are rejected, this indicates that R1699Q variants are in some sense intermediate in terms of their *BRCA1* family history profile compared to BRCA-X and *BRCA1* R1699W families.

Segregation Analyses: Risk was analyzed more directly through analysis of co-segregation of the R1699Q/W genotypes in the relatives of probands presenting with R1699Q/W variants [31]. Analyses included 30 R1699Q informative families with 111 total tested individuals and 19 R1699W families with 80 tested individuals. Risks were estimated by examining the likelihood of the genotypes of the family members (both women affected with breast or ovarian cancer, and healthy women) as a function of *BRCA1* penetrance, conditional on the proband's genotype and all pedigree phenotypes. The conditioning is needed to account for the fact that families were ascertained on the basis of the cancer phenotypes in the entire family, and the fact that the proband carried the variant. In this situation, most information about penetrance derives from the distribution of variant genotypes among unaffected women. Because there was insufficient additional genotyping in these families to reliably estimate age-specific risk ratios for each age group, we examined the risk associated with the R1699Q/W variants relative to those associated with the "average pathogenic *BRCA1* mutation" as found in

much larger studies of predominantly truncating mutations[17]. In these analyses the age-specific hazard ratio (by decade) was assumed to be a constant multiple of the estimate of Antoniou et al.[17], with cumulative penetrances re-estimated at each trial value of the multiplier. This allowed for a similar pattern of age-specific effects as in *BRCA1*, but only required estimation of a single parameter. We also repeated the analyses allowing for separate penetrance multipliers for breast cancer and ovarian cancer to allow for the possibility that the functional effects of R1699Q or R1699W might be more relevant to cancer risk for one but not both of these cancers. We varied the multiplier of the assumed standard penetrance of *BRCA1* from 0.05 to 2, in increments of 0.01 in order to find the value that maximized the likelihood of the observed data (and to obtain confidence intervals). If under a particular model, a given value of the penetrance implied risks of cancer in carriers lower in a given age group than in non-carriers, these were constrained to be the same as the non-carrier rates.

The analysis of penetrance was done using the LINKAGE package of programs [32] to calculate pedigree likelihoods and the other statistical analyses were performed using STATA v11.0 (StatCorp, College Station TX).

RESULTS

Table 2 shows the results of the analyses comparing family history scores of probands from R1699Q families, R1699W families, and families with no *BRCA1* pathogenic mutation. Of note is the ordered progression of the BOADICEA raw scores showing

clear differences between all three groups of families, and the tests of significance between groups. The Z-scores for R1699Q (adjusted for the mean and standard deviation of the BRCA-X families from the same centers) were significantly greater than 0 (p=0.0004), indicating that carriers of the R1699Q variant have more "*BRCA1*-like" family histories than families that test negative for both genes, and that they have some of the characteristics of family history (e.g, ovarian cancer) of *BRCA1*. However, they are also clearly less "*BRCA1*-like" than family histories of probands carrying the previously classified pathogenic R1699W mutation (p<0.00001).

Table 2. Analysis of family history scores.

Group	**Number of Families** [a]	**Mean BOADICEA Score** [b]	**Z-Scores**[c]	
			Mean	**Standard Error**
BRCA-X	243	0.085	0.0	0.06
R1699Q	67	0.12	0.48	0.14
R1699W	34	0.36	1.81	0.20
H0: Q=0	*t= 3.51, 66 df, p=0.0004*			
H0: Q=W	*t= -5.5, 99 df, p=1.3 x 10^{-7}*			

[a] Analysis includes 67 families in which the proband carried a R1699Q variant and for which there was at least 1 matching BRCA-X family, and the 34 matching families in which the proband had a R1699W variant. One R1699Q family was excluded because there was no BRCA-X family available for comparison. For another R1699Q family with only a single matched BRCA-X family, the standard deviation could not be determined so the average standard deviation across all centers was used for analysis. Families were matched by country, as shown in Table 1.

[b] All BOADICEA scores represent "pre-test" calculations based on family history information only. Scores ranges were as follows:- Q: 0.0006 to 0.88; W: 0.0026 to 0.97; X: 0.0002 to 0.92.

[c] See text for definition of Z-scores

Although the above analyses indicate that families carrying R1699Q are different in terms of their personal and family history from both BRCA-X families and families carrying the R1699W variant, these analyses do not directly address the question of cancer risks conferred by these mutations. They also do not provide a level of evidence that the variant is pathogenic as in the typical assessment of co-segregation within the framework of the multifactorial model [1, 33]. Segregation analyses were thus undertaken. For R1699W, the maximum likelihood estimate of the relative proportion of the standard *BRCA1* penetrance was 0.24 (95% CI=0.06-1.10), which was not significantly different from 1.0 (Likelihood Ratio (LR) X_1^2=3.44; p=0.06). The odds in favor of pathogenicity at this value of the penetrance multiplier were 314,100:1. When we allowed the possibility that there were different multipliers for breast and ovarian cancer, the estimates were 0.11 for breast cancer and 2.35 for ovarian cancer, with corresponding odds of 2,420,000:1 in favor of pathogenicity. The LR test provided some evidence for difference from a single value (X_1^2 = 4.08; p=0.043) and for a difference from standard penetrance (X_2^2 =7.53; p=0.023).

For R1699Q, the maximum likelihood estimate of the penetrance multiplier parameter was 0.20 (95% CI=0.09-0.45), significantly reduced compared to the standard model (X_1^2 =14.2; p=0.0002). The odds in favor of pathogenicity were 6226:1 for R1699Q at this value of the multiplier, whereas they were only 5:1 under the standard model. In contrast to R1699W, allowing separate multipliers for breast and ovarian cancer did not result in a big difference in likelihood, with estimated parameters of 0.18 for breast cancer and 0.30

for ovarian cancer (odds of 6787:1), which was not significantly different from a single value of 0.20 ($p=0.7$) for breast and ovarian cancer.

Clearly there is a reduced penetrance for this variant, compared to the standard penetrance of *BRCA1* as estimated by Antoniou et al.[17]. To represent these estimated parameters in terms of absolute risks which are perhaps more clinically relevant, we can translate the penetrance multipliers into age-specific relative risks of breast and ovarian cancer and use these to obtain cumulative risks of breast and/or ovarian cancer by age, based on the age-specific relative risks in Antoniou et al.[17]. Figure 1 shows the predicted cumulative risks of developing either breast or ovarian cancer based on the maximum likelihood parameter estimates of the breast and ovarian relative risk multiplier parameters for R1699Q and R1699W compared to the standard model and population rates. Similar figures for breast cancer risk and ovarian cancer risk individually are provided in the supplementary Figure 3. If our model is correct, the risk of breast or ovarian cancer to age 70 is 24% (95% CI 10%-40%) for carriers of *BRCA1* R1699Q, and 58% (95% CI 7%-72%) for carriers of *BRCA1* R1699W assuming the best fitting model of separate risk multipliers for breast and ovarian. This compares to 4.6% for women in the general population, and 68% for the carriers of an average pathogenic mutation. The risks for R1699Q are higher than that conferred by family history alone, but still lower than that conferred by *BRCA2* and *PALB2* mutations.

DISCUSSION

Although we have presented results of analyses examining risk, our goal was not to estimate penetrance *per se*, but rather to compare these two specific variants to the penetrance of the "average" *BRCA1* mutation (the vast majority of which are truncating), both in terms of family histories of probands carrying these variants and in terms of co-segregation of the variants within families. Here we provide, for the first time, significant evidence that a *BRCA1* variant can be associated with reduced risks of breast cancer compared to the "average" pathogenic mutation. It is of particular relevance and consequence for future studies, since the variant R1699Q was selected for study due to its behavior in a variety of functional assays. Depending on the assay, this missense variant has demonstrated either wildtype function, abrogated function akin to known pathogenic mutations, or functional activity intermediate between that observed for wild type BRCA1 and known truncating pathogenic and missense pathogenic mutations.

Interestingly there was also evidence that the R1699W variant was associated with significantly lower breast cancer risk and a markedly increased risk of ovarian cancer. We recognize that the estimation of breast and ovarian parameters separately is somewhat difficult given the necessity of conditioning the data on all pedigree phenotypes, but the results nevertheless raise the question that differences in risk of breast versus ovarian cancer may be a characteristic of some missense mutations in the BRCT repeat domains. In this regard, we note that in the 34 R1699W families there were an average of 2.24 breast cancers and 1.48 ovarian cancers, while in the 68 R1699Q families there were 2.35

breast cancers and 0.85 ovarian cancers per family, consistent with the higher estimated risk of ovarian cancer in these families. Further study of a large number of such variants will be necessary to address such an intriguing possibility that would have clear clinical implications.

Using the standard multifactorial model, the posterior probability for R1699Q is calculated to be 0.79 from the available data, namely: prior probability of pathogenicity of 0.66 based on the A-GVGD class C35 [23]; segregation odds of 5:1 in favor of pathogenicity from *this* study of 30 families; likelihood ratios from Easton et al.[11] of 8:1 against pathogenicity for family history, and 3:1 in favor of pathogenicity for co-occurrence data. That is, using the model developed based on the characteristics of *BRCA1* pathogenic mutations of "average" penetrance, R1699Q would be classified as IARC Class 3 "uncertain".

Our conclusive finding that *BRCA1* c.5096G>A R1699Q can be shown to both have intermediate functional deficiency in several assays, and is associated with breast and ovarian cancer risk at significantly lower levels than truncating *BRCA1* mutations, has a number of consequences. Our findings suggest that results from a battery of functional assays may highlight other variants with intermediate or equivocal results for investigation as potential moderate risk variants. Indeed, the variant BRCA1 A1708V showed abrogated centrosome amplification but normal nuclear foci formation and trypsin sensitivity equivocal results from a series of functional assays in our original report[8], and is a candidate for further investigation as a potential moderate risk variant.

If this observation of intermediate function translating to intermediate risk is a general finding, it is likely that there will be a subset of variants that are difficult to classify using the standard multifactorial likelihood approaches that are based on comparing data for a particular variant under the hypothesis that it is a fully penetrant pathogenic *BRCA1* mutation against the hypothesis that it is neutral or of no clinical significance with respect to risk. As shown for the R1699Q variant with more families available for analysis than will likely be achieved for most other rare variants, the standard co-segregation analysis yielded odds of only 5:1 in favor of the variant being pathogenic compared to the >6000:1 odds when a lower penetrance was allowed. Further, and more importantly, we must now face the question of how these women should be counseled in terms of cancer risk and the management of that risk. We do not propose that counseling be any different for R1699W, although results from the two parameter analyses suggest that particular attention should perhaps be paid to ovarian cancer for this known pathogenic variant. We emphasize however that the confidence intervals are wide, particularly for cancer site-specific risks and future studies are necessary to confirm the markedly increased ovarian cancer risk observed in our dataset. While there is certainly significant evidence that R1699Q carriers are at increased risk over population rates, this risk is markedly lower than that observed for the average *BRCA1* mutation. The findings presented here are likely to provide impetus for research studies considering approaches to clinical management of patients with cancer risks intermediate to those conferred by *BRCA1/2* mutations and those from family history alone. In the case of R1699Q, counseling could be similar to that for other moderate-penetrance genes such as *PALB2*, *CHEK2* and

RAD51C, although that may change if ovarian cancer screening improves given the increased rate of ovarian cancer over the general population. In all these cases, the incorporation of the now 30+ common breast cancer susceptibility alleles into comprehensive risk prediction models will be of great value in allowing women and their providers to make informed management decisions. In addition, it would be interesting to specifically explore if *BRCA1* haplotypes altering promoter activity [34, 35] or potentially altering 3'UTR microRNA binding [36] influence the level of function of R1699Q *in vivo*, and explain in part the variable presentation of families.

In summary, we provide evidence that a *BRCA1* variant demonstrating equivocal functional deficiency across multiple assays is associated with intermediate risk of breast and ovarian cancer, highlighting challenges for risk modeling and clinical management of patients of this and other potential moderate-risk variants.

Contributor Statement

All authors made a significant contribution to data collection, data analysis, writing, and critical assessment of this study. Specifically: DEG and ABS were responsible for study concept and design. DEG performed the statistical analyses. DEG, ABS and MPGV wrote the manuscript. PJW, SH, and BT were responsible for data collection and management.CJVA, AAKM, MGEMA, RKS,CE,AM, SC,OS, RL, FJC, LG, TVOH, MT, DME, KT,JB, SMD,AET ,EJVT, BW, AB, MPGV provided the family data analyzed in this manuscript; MAB and CP performed functional analyses, and SC performed structural protein modeling.All authors have approved the final draft submitted.

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Appendix A. ENIGMA collaborators (excluding those named in the author list)

Dutch Belgium UV Consortium:	
Frans B. Hogervorst	The Netherlands Cancer Institute, Amsterdam, NL (Coordinating center)
Rogier A. Oldenburg	Erasmus Medical Center, Rotterdam, NL
Juul T. Wijnen, Peter Devilee	Leiden University Medical Center, Leiden, NL
Rob B. van der Luijt	University Medical Center Utrecht, Utrecht, NL
Johan J.P. Gille, Muriel A. Adank	VU University Medical Center, Amsterdam, NL
Encarna B. Gomez-Garcia, Marinus J. Blok	University Hospital Maastricht, Maastricht, NL
Jan C. Oosterwijk, A.H. van der Hout	University Medical Center Groningen University, NL
Genevieve Michils, Eric Legius	University of Leuven, Leuven, Belgium
Erik Teugels, Jacques de Grève	Familial Cancer Clinic, UZ Brussel, Brussels, Belgium
German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC):	
Norbert Arnold	Department of Gynaecology and Obstetrics, University Hospital of Schleswig-Holstein, Christian-Albrechts Campus Kiel, Kiel, Germany
Helmut Deisler	Department of Obstetrics and Gynaecology, University of Ulm, Ulm, Germany
Dorothea Gadzicki	Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany
Andrea Gehrig	Centre of Familial Breast and Ovarian Cancer, Department of Medical Genetics, Institute of Human Genetics, University Würzburg, Germany
Wolfram Heinritz	Institute of Human Genetics, University of Leipzig, Leipzig, Germany
Karin Kast	Department of Gynecology and Obstetrics, University Hospital, Technical University of Dresden, Dresden, Germany
Dieter Niederacher	Department of Gynaecology and Obstetrics, University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Germany
Sabine Preisler-Adams	Institute of Human Genetics, University of Münster, Vesaliusweg, Germany
Christian Sutter	Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany
Raymonda Varon-Mateeva	Institute of Human Genetics, Campus Virchov Klinikum, Charite Berlin, Germany

Bernhard H. Weber	Institute of Human Genetics, University of Regensburg, Regensburg, Germany
French BRCA GGC Consortium:	
Nicolas Sévenet, Françoise Bonnet, Michel Longy:	Institut Bergonié - Bordeaux
Agnès Hardouin, Dominique Vaur, Sophie Krieger	Centre François Baclesse - Caen
Nancy Uhrhammer, Yves-Jean Bignon	Centre Jean Perrin - Clermont-Ferrand
Jean-Philippe Peyrat, Françoise Revillion, Joëlle Fournier	Centre Oscar Lambret - Lille
Sylvie Mazoyer	Centre de Recherche en Cancérologie de Lyon – Lyon
Mélanie Léone	Hospices Civils de Lyon and Centre Léon Bérard – Lyon
Hagay Sobol, Tetsuro Noguchi, Violaine Bourdon, Audrey Remenieras	Institut Paoli-Calmettes - Marseille
Jean-Marc Rey	Laboratoire de Biologie Cellulaire et Hormonale (CHU Arnaud de Villeneuve) - Montpellier
Myriam Bronner, Joanna Sokolowska-Gillois, Philippe Jonveaux	CHU de Nancy-Brabois - Vandœuvre-lès-Nancy
Capucine Delnatte	CHU - Institut de Biologie - Hôtel Dieu - Nantes
Florence Coulet	Groupe hospitalier Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Université Pierre et Marie Curie, Laboratoire d'Oncogénétique et Angiogénétique moléculaire - Paris
Laurent Castera, Virginie Caux-Moncoutier, Claude Houdayer, Dominique Stoppa-Lyonnet	Institut Curie - Paris
Chantal Delvincourt, Marie-Claude Gorisse	CHU et Institut Jean Godinot - Reims
Ivan Bièche, Cédrick Lefol, Etienne Rouleau	Institut Curie - Hôpital René Huguenin, Service d'Oncogénétique - Saint Cloud
Joseph Abecassis, Danièle Muller	Centre Paul Strauss - Strasbourg
Christine Toulas	Institut Claudius Régaud - Toulouse
Marine Guillaud-Bataille, Brigitte Bressac-de Paillerets	Institut Gustave Roussy - Villejuif
ENIGMA collaborators operating outside of a country consortium:	
Richard Barber	West Midlands Regional Genetics Laboratory,

	Birmingham Women's NHS, Foundation Trust, Edgbaston, Birmingham, UK
Tina Bedenham	Oxford Molecular Genetic Laboratory, Oxford University Hospital NHS Trust, The Churchill Hospital, Oxford, UK
Lucy Burgess	West Midlands Regional Clinical Genetics Service, Birmingham, UK
Joanna Campbell	DNA laboratory, Guy's Hospital, London, UK
Jackie Cook	Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Western Bank, Sheffield, UK
Andrew Devereau	NGRL Manchester, Genetic Medicine, St Mary's Hospital, Manchester, UK
Bent Ejlertsen	Department of Oncology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
Mike Fields	Familial Cancer Service, Royal North Shore Hospital, Sydney, Australia
Anne-Marie Gerdes	Department of Clinical Genetics, Rigshospital and Copenhagen University, Denmark
Elizabeth Johnston	West Midlands Regional Genetics Laboratory, Birmingham Women's NHS, Foundation Trust, Edgbaston, Birmingham, UK
Torben A. Kruse	Department of Clinical Genetics, Odense University Hospital, Denmark
Anita Luharia	West Midlands Regional Genetics Laboratory, Birmingham Women's NHS, Foundation Trust, Edgbaston, Birmingham, UK
Carole Mckeown	West Midlands Regional Clinical Genetics Service, Birmingham, UK
Kate Nathanson	Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA
Finn C. Nielsen	Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark
Leigha Senter	Clinical Cancer Genetics Program, Division of Human Genetics, Department of Internal Medicine, OSU Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA
Barbara Stayner	Oxford Regional Genetics Service, Oxford , UK
Kevin Sweet	Clinical Cancer Genetics Program, Division of Human Genetics, Department of Internal Medicine, OSU Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA
Simon Thomas	Wessex Regional Genetics Laboratory, UK
Yvonne Wallis	West Midlands Regional Genetics Laboratory, Birmingham Women's NHS, Foundation Trust, Edgbaston, Birmingham, UK

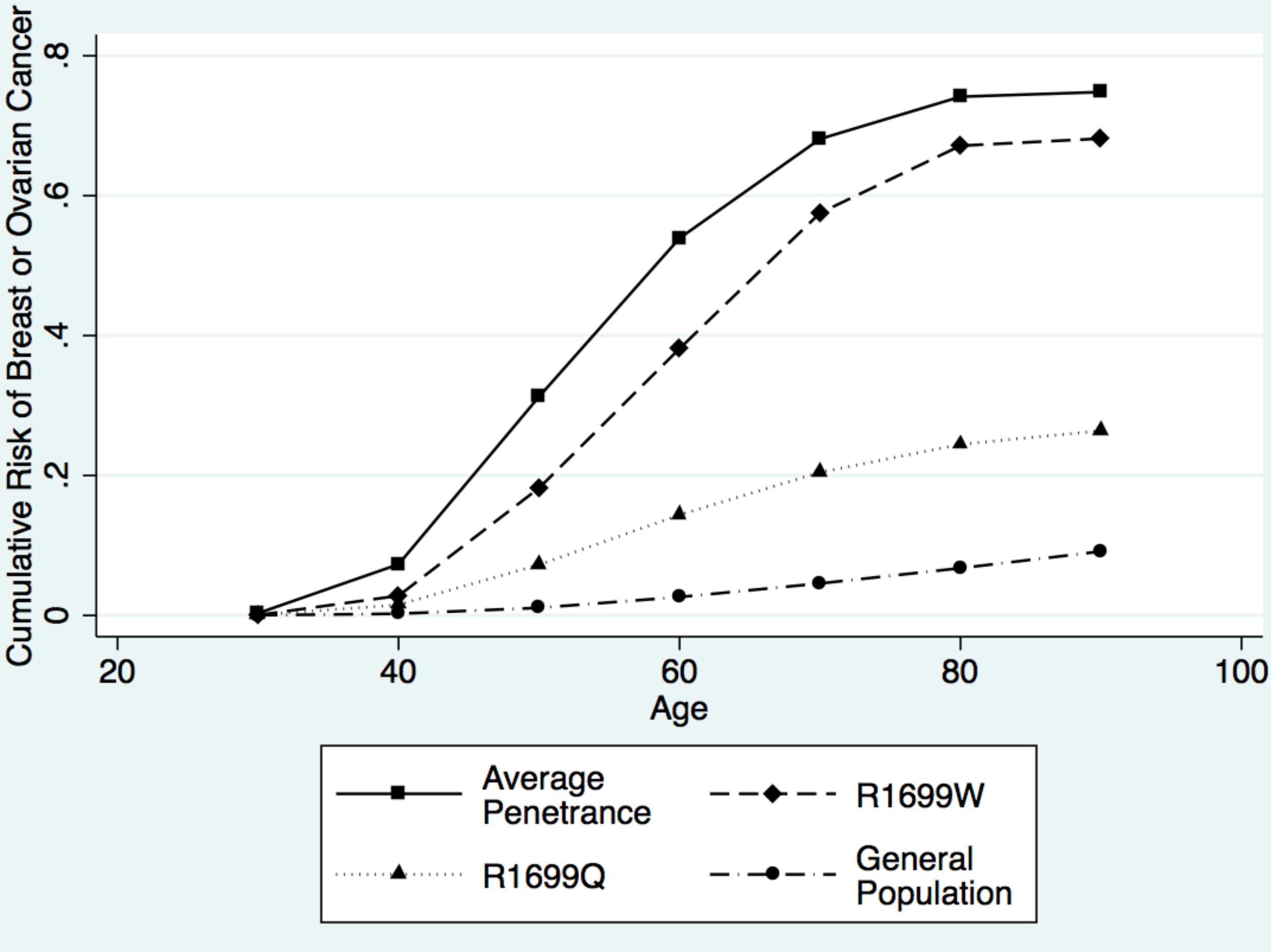
Sally Watts	Clinical Genetics Department, Guy's Hospital, London, UK

Competing Interests:

The authors declare there are no conflicts of interest.

Figure Legends

Figure 1: Cumulative risk of breast or ovarian cancer by age, assuming the best fitting models of penetrance for R1699Q (dotted line) and R1699W (dashed line). The corresponding curves for the standard penetrance (solid line) and for the general population (dash-dotted line) are also shown.


Cumulative Risk of Breast or Ovarian Cancer
0
.2
.4
.6
.8
20
40
60
80
100
Age
Average Penetrance
R1699W
R1699Q
General Population