

Modifiers of breast and ovarian cancer risks for *BRCA1* and *BRCA2* mutation carriers

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Abstract

Pathogenic mutations in *BRCA1* and *BRCA2* are associated with high risks of breast and ovarian cancer. However, penetrance estimates for mutation carriers have been found to vary substantially between studies, and the observed differences in risk are consistent with the hypothesis that genetic and environmental factors modify cancer risks for women with these mutations. Direct evidence that this is the case has emerged in the past decade, through large-scale international collaborative efforts. Here, we describe the methodological challenges in the identification and characterisation of these risk-modifying factors, review the latest evidence on genetic and lifestyle/hormonal risk factors that modify breast and ovarian cancer risks for women with *BRCA1* and *BRCA2* mutations and outline the implications of these findings for cancer risk prediction. We also review the unresolved issues in this area of research and identify strategies of clinical implementation so that women with *BRCA1* and *BRCA2* mutations are no longer counselled on the basis of 'average' risk estimates.

Key Words

- ▶ *BRCA1*
- ▶ *BRCA2*
- ▶ breast cancer
- ▶ ovarian cancer
- ▶ modifier
- ▶ common genetic variants
- ▶ lifestyle/hormonal risk factors
- ▶ genotype–phenotype
- ▶ risk prediction

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Introduction

Pathogenic mutations in *BRCA1* and *BRCA2* are associated with high risks of breast and ovarian cancer. Based on conservative estimates (Antoniou *et al.* 2008a), approximately 1 in 240 individuals in the population carry one of these mutations. *BRCA1* and *BRCA2* mutations explain 5–10% of breast cancers diagnosed in women before age 40 years (Peto *et al.* 1999, Anglian Breast Cancer Study Group 2000) and ~11–14% of high-grade serous ovarian cancers (Alsop *et al.* 2012, Song *et al.* 2014). They account for 17–20% of the familial risk of breast cancer (Peto *et al.* 1999, Anglian Breast Cancer Study Group 2000, Mavaddat *et al.* 2010) and

~24% of the familial risk of ovarian cancer (Jervis *et al.* 2014). Genetic testing for *BRCA1* and *BRCA2* mutations is widely available and has become an integral part of genetic counselling and oncologic and gynaecologic practice (Karlan *et al.* 2007). Test results are often used to inform recommendations about the most appropriate treatment or clinical management options for women. To provide optimal advice to women found to carry mutations in these genes, particularly given that prevention options can have significant side effects, precise estimates of associated age-specific cancer risks are required.

Estimates of the cumulative risk of cancer (to age 70 years) for *BRCA1* and *BRCA2* mutation carriers have been found to vary substantially between studies. Retrospective studies have reported estimates for breast cancer that range from 40 to 87% for *BRCA1* mutation carriers and from 27 to 84% for *BRCA2* mutation carriers (Easton *et al.* 1995, Ford *et al.* 1998, Hopper *et al.* 1999, Antoniou *et al.* 2003, 2005b, 2008a,b, Chen *et al.* 2006, Begg *et al.* 2008, Milne *et al.* 2008, Brohet *et al.* 2014, Gabai-Kapara *et al.* 2014). The corresponding ovarian cancer risk estimates vary from 16 to 68% for *BRCA1* mutation carriers and from 11 to 27% for *BRCA2* mutation carriers. Although the observed variability in risk estimates could be partly due to different sampling schemes across studies, the risk estimates within studies have been found to vary by the age of diagnosis and type of cancer in close relatives (Antoniou *et al.* 2003, Begg *et al.* 2008) and to be higher for women born in more recent decades (Antoniou *et al.* 2003, Simchoni *et al.* 2006, Brohet *et al.* 2014). These observations suggest that genetic factors, including genotype–phenotype correlations (i.e. mutation-specific risks), and environmental or reproductive/lifestyle factors, modify cancer risks for mutation carriers.

Retrospective, family-based studies have inherent limitations including ascertainment biases and biases due to inaccuracies in the reporting of family history. Many of these are overcome by prospective studies, but those published to date have been based on relatively small sample sizes (with fewer than 65 incident cancers); as a result, estimates remain relatively imprecise (Mavaddat *et al.* 2013, Senst *et al.* 2013, Evans *et al.* 2014). These prospective studies were enriched for families that met high- or moderate-risk screening criteria presenting to genetic clinics, and the risk estimates were generally higher than those from retrospective population-based studies. This observation is also consistent with the existence of genetic modifiers that aggregate in multiple-case families.

In the past decade, through large international collaborative efforts and advances in genotyping technologies, direct evidence has emerged that there are genetic and lifestyle/hormonal factors that modify breast and ovarian cancer risks for *BRCA1* and *BRCA2* mutation carriers. These findings suggest that it is no longer appropriate to counsel *BRCA1* and *BRCA2* mutation carriers on the basis of ‘average’ risk estimates. Moreover, the development of cost-effective sequencing technologies and gene panel testing are likely to enable more widespread *BRCA1* and *BRCA2* mutation screening, not necessarily restricted to those with a strong family history of breast or ovarian cancer, as exemplified by

the 100,000 genomes project in the United Kingdom (<http://www.genomicsengland.co.uk>). Thus, it is critical that we improve our ability to estimate cancer risks for carriers in all contexts. We should aim to be able to provide comprehensive counselling based on estimates that consider: the gene mutated and the position and functional effect of the mutation, as well as family history of cancer and all genetic and lifestyle/hormonal factors that modify risk for mutation carriers.

Here, we review the latest evidence on genetic and lifestyle/hormonal risk factors that modify breast and ovarian cancer risks for women with *BRCA1* and *BRCA2* mutations and outline their implications for cancer risk prediction. However, first we summarise the methodological challenges in the identification and characterisation of such modifiers of risk.

Challenges in the identification and characterisation of risk modifiers for mutation carriers

In contrast to epidemiological and genetic association studies in the general population, identifying and characterising genetic and lifestyle cancer risk-modifying factors for *BRCA1* and *BRCA2* mutation carriers pose a number of methodological and analytical challenges. The optimal study design for studying factors that modify cancer risks is a prospective cohort in which unaffected mutation carriers are followed up over time to observe prospectively who goes on to develop cancer. This study design overcomes issues of ascertainment, recall and testing bias (Antoniou *et al.* 2005a, Whittemore 2007, Heemskerk-Gerritsen *et al.* 2015b), but many years of follow-up are required for a sufficient number of incident cancer cases to occur to obtain adequately precise risk estimates. Furthermore, a large fraction of mutation carriers opt for risk-reducing surgery and hence are removed from the ‘at-risk’ cohort. To date, findings from only a limited number of prospective studies have been reported, and these were based on small sample sizes (Mavaddat *et al.* 2013, Senst *et al.* 2013, Evans *et al.* 2014). An alternative approach would be to screen for *BRCA1* and *BRCA2* mutations in large-scale population-based case-control studies. However, *BRCA1* and *BRCA2* mutations are rare in the population, and large sample sizes of affected and unaffected individuals are required. Until recently, such designs have been prohibitively expensive, with the exception of studies of founder mutations in the Ashkenazi, Icelandic and Polish populations. However, with advances in sequencing technologies,

population-based case-control studies are likely to become feasible in the context of large international consortia such as the Breast/Ovarian Cancer Association Consortia (BCAC: <http://bcac.ccge.medschl.cam.ac.uk>; OCAC: <http://apps.ccge.medschl.cam.ac.uk/consortia/ocac/>).

Given these challenges, most published studies to date have identified affected and unaffected *BRCA1* and *BRCA2* mutation carriers through ongoing genetic testing programmes. However, genetic testing is targeted at women with a strong family history of cancer and young affected women are more likely than unaffected women to get tested first. Therefore, the sampling of mutation carriers is not random with respect to their disease status and standard methods of analysis can yield biased relative risk estimates (Antoniou *et al.* 2005a, Barnes *et al.* 2012). A number of different analytical approaches have been proposed to adjust for the sampling frame (Antoniou *et al.* 2005a, Whittemore 2007, Barnes *et al.* 2012), and these have been applied in several, but not all, large-scale association analyses.

Retrospective epidemiological risk factor studies may be subject to selection bias, information bias or confounding. In retrospective studies of mutation carriers, selection bias may particularly be problematic for the assessment of reproductive history; the decision a woman makes about being tested may be influenced by both whether or not she has children and whether she has been diagnosed with breast or ovarian cancer (Antoniou *et al.* 2009a). As most of the studies performed to date on lifestyle/hormonal risk modifiers for *BRCA1* and *BRCA2* mutation carriers have been retrospective and based on relatively small sample sizes, these limitations apply to practically all published findings. In general, studies of genetic modifiers of cancer risk for mutation carriers are less likely to be susceptible to such biases. However, in studies in which family history or the disease phenotypes play a role in the sample selection, some biases may arise in the relative risk estimates if these factors are not correctly accounted for in the analysis.

Genetic modifiers

Until relatively recently, the approach taken to investigate common polymorphisms that modify breast and ovarian cancer risk for mutation carriers was to conduct hypothesis-based association studies focused on genes considered biologically likely to be involved in disease aetiology. These studies assessed putative functional variants in genes in candidate pathways such as DNA repair, steroid hormone metabolism and

environmental carcinogen detoxification. The advantage of this approach is that the biological interpretation of positive findings is relatively straightforward. However, analogous to research into common breast and ovarian cancer susceptibility variants in the general population, these candidate gene studies generally gave contradictory results across multiple small-scale studies and have not been convincingly replicated in more adequately powered studies (Breast Cancer Association Consortium 2006, Chenevix-Trench *et al.* 2007). Another limitation of this candidate gene approach is that it was based on what would now be considered a narrow consideration of what might be a functional variant, focused primarily on the coding sequence of genes.

The Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) was established to evaluate reliably the common genetic modifiers of breast and ovarian cancer risk for mutation carriers. With advances in technology and concurrent dramatic reduction in the cost of large-scale genotyping, candidate gene approaches to study the genetic epidemiology of complex disease have largely been replaced by hypothesis-free genome-wide association studies (GWAS). Genotypes at many millions of common variants across the genome can be genotyped or imputed with high accuracy using large-scale genotyping arrays, using reference panels from the 1000 Genomes Project (Auton *et al.* 2015). This approach has been applied with great success in cancer epidemiology in the general population, with GWAS having identified more than 100 common susceptibility variants for breast cancer (Cox *et al.* 2007, Easton *et al.* 2007, Hunter *et al.* 2007, Stacey *et al.* 2007, 2008, Ahmed *et al.* 2009, Thomas *et al.* 2009, Antoniou *et al.* 2010b, Turnbull *et al.* 2010, Cai *et al.* 2011, Fletcher *et al.* 2011, Haiman *et al.* 2011, Ghousaini *et al.* 2012, Hein *et al.* 2012, Long *et al.* 2012, Siddiq *et al.* 2012, Bojesen *et al.* 2013, French *et al.* 2013, Garcia-Closas *et al.* 2013, Gaudet *et al.* 2013, Meyer *et al.* 2013, Michailidou *et al.* 2013, 2015, Cai *et al.* 2014, Milne *et al.* 2014a, Orr *et al.* 2015, Couch *et al.* 2016, Dunning *et al.* 2016, Lawrenson *et al.* 2016, Zheng *et al.* 2009) and 22 for ovarian cancer (Song *et al.* 2009, Bolton *et al.* 2010, Goode *et al.* 2010, Bojesen *et al.* 2013, Permuth-Wey *et al.* 2013, Pharoah *et al.* 2013, Shen *et al.* 2013, Kuchenbaecker *et al.* 2015).

In this context, within CIMBA, four approaches have been applied to identify loci associated with breast and ovarian cancer for mutation carriers: (i) GWAS for breast and ovarian cancer specifically performed in samples of *BRCA1* and *BRCA2* mutation carriers (Antoniou *et al.* 2010b, Couch *et al.* 2013, Gaudet *et al.* 2013); (ii) association

studies to assess common breast and ovarian cancer susceptibility alleles identified in the general population as potential modifiers of risk for mutation carriers (Antoniou *et al.* 2008b, 2009b, 2010a, 2011, 2012, Ramus *et al.* 2010, Couch *et al.* 2012, Ramus *et al.* 2012, Couch *et al.* 2013, Kuchenbaecker *et al.* 2014); (iii) meta-analyses of GWAS performed in *BRCA1* and *BRCA2* mutation carriers with GWAS of related phenotypes in the general population (for example, combining studies of breast cancer risk for *BRCA1* mutation carriers with those of oestrogen receptor-negative breast cancer in general population or studies of ovarian cancer risk for *BRCA1* and *BRCA2* mutation carriers with GWAS of serous ovarian cancer in the general population) (Kuchenbaecker *et al.* 2015, Couch *et al.* 2016); and iv) fine-scale mapping of risk-modifying loci identified through GWAS approaches to fully characterise the associations with all genetic variants at these loci (Bojesen *et al.* 2013, Dunning *et al.* 2016, Lawrenson *et al.* 2016).

The third of these approaches stems from results from the first two, suggesting that many of the loci found to modify cancer risks for mutation carriers coincide with those found in GWAS of the general population (predominantly non-carriers) (Garcia-Closas *et al.* 2013, Michailidou *et al.* 2013). More specifically, susceptibility loci for overall and oestrogen receptor (ER)-positive breast cancer for women in the general population tend to be associated with overall breast cancer risk for *BRCA2* mutation carriers (who mostly (70–80%) develop ER-positive disease (Mavaddat *et al.* 2012)), whereas those for ER-negative breast cancer tend to be associated with overall risk for *BRCA1* mutation carriers (who mostly (70–80%) develop ER-negative disease (Mavaddat *et al.* 2012)) (Milne & Antoniou 2011, Kuchenbaecker *et al.* 2014). A systematic evaluation of the associations of 74 known breast cancer susceptibility alleles found that their associations with ER-positive breast cancer for *BRCA1* and *BRCA2* mutation carriers were more consistent with the associations of these SNPs with ER-positive breast cancer in the general population. Furthermore, the associations of these SNPs with ER-negative breast cancer for *BRCA1* and *BRCA2* mutation carriers were more consistent with the associations of the SNPs with ER-negative breast cancer in the general population (Kuchenbaecker *et al.* 2014). Similarly, common variants associated with the risk of serous ovarian cancer in the general population are associated with overall ovarian cancer risk for carriers of mutations in both genes (approximately two-thirds of whom develop serous disease) (Mavaddat *et al.* 2012). These observations have two important implications. The first underpins approach (ii) listed above, and is

that common genetic modifiers of breast and ovarian cancer risk for *BRCA1* and *BRCA2* mutation carriers can be identified through GWAS of overall disease or disease subtypes in the general population, which have far greater statistical power due to much greater sample sizes. Therefore, common genetic variants identified from population-based GWAS have a high prior probability of association with risk for mutation carriers and more liberal significance thresholds can be used to assess the associations of such variants in mutation carriers. The second implication informs approach (iii) above, and is that an optimal strategy for the identification of novel genetic modifiers is meta-analysis of GWAS of these related phenotypes (Kuchenbaecker *et al.* 2015, Couch *et al.* 2016). Nevertheless, there appear to be some loci that modify breast or ovarian cancer risk specifically for *BRCA1* and *BRCA2* mutation carriers, showing no evidence of association with risk in the general population (Couch *et al.* 2013, Gaudet *et al.* 2013). As summarised in Table 1, to date, a total of 26 and 16 single nucleotide polymorphisms (SNPs) associated with breast cancer risk for *BRCA1* and *BRCA2* mutation carriers, respectively, have been identified. The corresponding numbers for ovarian cancer risk are 11 and 13. The associated effect sizes are small, with estimated relative risks per copy of the minor allele in the range 1.05–1.26 for breast cancer and 1.03–1.48 for ovarian cancer. These genetic modifiers are estimated to account for a relatively small proportion (<10%) of the modifying genetic variance for *BRCA1* and *BRCA2* mutation carriers (based on estimates of the modifying variance from segregation analyses), and it is predicted that residual family history remains an important risk-modifying factor (Couch *et al.* 2013, 2016, Gaudet *et al.* 2013, Kuchenbaecker *et al.* 2015), as recently demonstrated in the general population (Mavaddat *et al.* 2015). However, the joint effects of SNPs and family history have not been estimated for *BRCA1* and *BRCA2* mutation carriers. These are required before these genetic susceptibility findings can be implemented in the genetic counselling process.

One of the limitations of the hypothesis-free approach underlying GWAS is that they identify the associations with genetic markers, with no a priori knowledge of what functional variants might explain these associations or the genes or genetic pathways through which they might act. Thus, establishing the biological mechanisms underpinning GWAS associations has proven to be particularly challenging. Fine-mapping studies, in which a much denser selection of SNPs in susceptibility loci is genotyped and analysed using multivariable (conditional)

Table 1 Approaches adopted to identify independent genetic modifiers of breast and ovarian cancer risk for *BRCA1* and *BRCA2* mutation carriers.

Approach	P value threshold	Breast cancer		Ovarian cancer	
		BRCA1	BRCA2	BRCA1	BRCA2
GWAS of mutation carriers	* $P < 5 \times 10^{-8}$	2 SNPs ^{a,b} MAF (0.26–0.47) HR (1.14–1.26)	1 SNP ^c MAF (0.35) HR (1.18)	2 SNPs ^b MAF (0.20–0.48) HR (1.20–1.26)	None
Candidates from other breast cancer GWAS	# $P < 0.05$	16 SNPs ^{d,e,f,g,h,i} MAF (0.08–0.47) HR (1.05–1.21)	15 SNPs ^{d,e,f,g,h,i} MAF (0.08–0.49) HR (1.06–1.24)	4 SNPs ^{j,k,l} MAF (0.08–0.13) HR (1.11–1.25)	7 SNPs ^{b,j,k,l} MAF (0.08–0.30) HR (1.16–1.48)
Meta-analysis	† $P < 5 \times 10^{-8}$	2 SNPs ^m MAF (0.24–0.34) HR (1.08–1.09)	Not done	5 SNPs ⁿ MAF (0.15–0.31) HR (1.08–1.15)	6 SNPs ⁿ MAF (0.15–0.31) HR (1.03–1.35)
Fine-mapping	* $P < 5 \times 10^{-4}$	6 SNPs ^{o,p,q} MAF (0.07–0.50) HR (1.07–1.12)	Not done	Not done	Not done

BRCA1, *BRCA1* mutation carriers; BRCA2, *BRCA2* mutation carriers; SNP, single nucleotide polymorphism; MAF, range of minor allele frequencies; HR, range of hazard ratio estimates.

*Classical genome-wide statistical significance; #Nominal statistical significance, given the high prior probability of association based on evidence (at $P < 5 \times 10^{-8}$) from other GWAS; †Genome-wide statistical significance in meta-analysis and HR estimate consistent in direction with findings for non-carriers; ‡Statistical significance assessed in mutation carriers after adjustment for the top hit(s).

^aAntoniou *et al.* (2010b); ^bCouch *et al.* (2013); ^cGaudet *et al.* (2013); ^dAntoniou *et al.* (2012); ^eKuchenbaecker *et al.* (2014); ^fAntoniou *et al.* (2011); ^gAntoniou *et al.* (2010a); ^hAntoniou *et al.* (2009b); ⁱAntoniou *et al.* (2008b); ^jRamus *et al.* (2012); ^kCouch *et al.* (2012); ^lRamus *et al.* (2010); ^mCouch *et al.* (2016); ⁿKuchenbaecker *et al.* (2015); ^oDunning *et al.* (2016); ^pLawrenson *et al.* (2016); ^qBojesen *et al.* (2013).

models, can highlight potentially causal variants, although extensive laboratory work is often required to demonstrate their function. These experiments are not feasible for all loci, and those that are, are often laborious and sometimes equivocal in identifying a single causal variant or even a single target gene (Dunning *et al.* 2016, Lawrenson *et al.* 2016). These fine-mapping studies often identify additional independent associations.

Genotype-phenotype correlations

Soon after the discovery of *BRCA1* and *BRCA2*, observations in families carrying mutations provided evidence that breast and ovarian cancer risks for *BRCA1* and *BRCA2* mutations may vary depending on the location of the mutation in each gene (Gayther *et al.* 1995, 1997). Using a larger dataset, the Breast Cancer Linkage Consortium (BCLC) (Thompson *et al.* 2002) reported that the ratio of ovarian to breast cancers associated with mutations in a central region of *BRCA1* was significantly higher than that for mutations outside this region. This was attributed to a lower risk of breast cancer associated with mutations in this region, compared with mutations outside the region, and to a lower ovarian cancer risk for mutations in the 3' end up to nucleotide 4191 region, compared to mutations in the rest of the gene. A study of probands with ovarian cancer (Risch *et al.* 2001) reported that the risk of breast cancer for *BRCA1* mutation carriers increases with mutation

position, from 5' to 3'. Similarly, mutations in a central region of exon 11 (the "Ovarian Cancer Cluster Region" – OCCR) of *BRCA2* were found by the BCLC to be associated with a higher ratio of ovarian to breast cancer. Mutations in the OCCR were found to be associated with both a lower risk of breast cancer and a higher risk of ovarian cancer relative to *BRCA2* mutations outside this region (Thompson *et al.* 2001). Risch and coworkers (Risch *et al.* 2001) found that only mutations outside the OCCR of *BRCA2* were associated with increased breast cancer risk. Subsequent penetrance studies for *BRCA1* and *BRCA2* mutation carriers found supporting, but non-significant, evidence for the risk patterns observed in the BCLC analyses (Antoniou *et al.* 2003, Brohet *et al.* 2014), although one did not observe differences in risk (Milne *et al.* 2008).

Using the largest dataset analysed to date, CIMBA found results that were consistent with those of the BCLC for both *BRCA1* and *BRCA2* mutation carriers (Rebbeck *et al.* 2015). This study also identified multiple breast cancer cluster regions (BCCRs) in *BRCA1* and *BRCA2* and two further OCCRs in *BRCA2*. The analysis also showed that mutations conferring nonsense-mediated decay are associated with different breast and ovarian cancer risks. Although clear differences in risks were demonstrated by mutation location and function, it was not possible to estimate absolute risks of developing breast or ovarian cancer by mutation characteristics. To obtain valid absolute

cancer risk estimates, it is necessary to perform studies which explicitly adjust for the ascertainment of mutation carriers on the basis of family history and which take into account the competing risks of breast and ovarian cancer and the population prevalence of specific mutations.

Lifestyle/hormonal risk factors

As explained previously, environmental/lifestyle factors may be important in explaining some of the variation in breast and ovarian cancer risk for *BRCA1* and *BRCA2* mutation carriers. The vast majority of data used to assess the associations has been retrospective and from selected individuals from multiple-case breast and ovarian cancer families. Moreover, different studies have used different methods to estimate the relative risks associated with potential risk factors, each with their strengths and weaknesses in terms of statistical power and adjustment for possible biases. Although prospective studies of mutation carriers unaffected at recruitment are optimal to overcome information, selection or ascertainment biases, they are faced with substantial challenges, including small sample sizes, limited prospective follow-up period (accrual of incident cases), gaps in the information available on risk factors between follow-ups, and loss to follow-up. The latter is further influenced by women undergoing prophylactic interventions (bilateral oophorectomy and/or bilateral mastectomy). Until findings from large-scale prospective studies become available, we must assess the evidence from retrospective studies on its merits, to be able to inform mutation carriers, clinicians and genetic counsellors about individual cancer risk for mutation carriers and what can be done to reduce it.

Unfortunately, very few consistent findings have been reported across over 40 publications based on analyses of retrospective case-control data from mutation carriers (reviewed in (Friebel *et al.* 2014)). Most consistent across studies have been findings for tamoxifen use and reduced breast cancer risk for both *BRCA1* and *BRCA2* mutation carriers, with reported relative risk (RR) estimates ranging between 0.33 and 0.63 (Narod *et al.* 2000, Gronwald *et al.* 2006b, Phillips *et al.* 2013), and oral contraceptive use and protection from ovarian cancer risk for *BRCA1* mutation carriers (RR estimates: 0.40–0.56) (Narod *et al.* 2001, Gronwald *et al.* 2006a, McLaughlin *et al.* 2007, Antoniou *et al.* 2009a). However, there was potential overlap in some of the data (Narod *et al.* 2000, 2001, Gronwald *et al.* 2006a,b, McLaughlin *et al.* 2007) and one study investigated only the association between tamoxifen and risk of contralateral breast cancer in affected women

(Phillips *et al.* 2013). Both these findings are consistent with the associations of these risk factors with cancer risks in the general population. The findings that exposure to chest X-rays at young ages is associated with an increased risk of breast cancer for mutation carriers were somewhat consistent (Andrieu *et al.* 2006, Lecarpentier *et al.* 2011, Pipje *et al.* 2012), although there was overlap between these studies and a subsequent smaller study found no evidence of association (John *et al.* 2013).

The reported findings for *BRCA1* mutation carriers in relation to reproductive history and breast cancer risk have also been relatively consistent across studies. In line with the established associations for the general population, breast feeding for at least 1 year has been found to be protective (RR: 0.50–0.68) (Jernstrom *et al.* 2004, Andrieu *et al.* 2006, Gronwald *et al.* 2006a, Kotsopoulos *et al.* 2012a,b), as has later age at menarche (Chang-Claude *et al.* 2007, Kotsopoulos *et al.* 2005, 2012a), (RR: 0.91 per year; Kotsopoulos *et al.* 2012a). Later age at first full-term pregnancy has also consistently been reported to be associated with reduced risk of breast cancer for *BRCA1* mutation carriers (Andrieu *et al.* 2006, Antoniou *et al.* 2006, Milne *et al.* 2010b, Lecarpentier *et al.* 2012) (RR: 0.65 for age ≥ 30 years vs < 30 years; Friebel *et al.* 2014), which is in contrast to what is known about the association with risk for overall breast cancer in the general population. It is not clear why this would be the case, although there is evidence that the association in the general population differs by disease subtype and the protective effect of early childbirth is not observed for triple-negative breast cancer (Yang *et al.* 2011, Barnard *et al.* 2015), which comprises approximately two-thirds of all tumours diagnosed in *BRCA1* mutation carriers (Mavaddat *et al.* 2012). Findings for these factors and breast cancer risk for *BRCA2* mutation carriers have been null or inconclusive (Friebel *et al.* 2014). Observations from studies of parity and breast cancer risk have also been largely consistent for *BRCA1* and *BRCA2* mutation carriers, and the general population (Kelsey *et al.* 1993), with more full-term pregnancies associated with protection from breast cancer (Andrieu *et al.* 2006, Antoniou *et al.* 2006, Milne *et al.* 2010b, Lecarpentier *et al.* 2012) (RR: 0.83 per pregnancy, Friebel *et al.* 2014), although contradictory findings have also been published (Jernstrom *et al.* 1999, Cullinane *et al.* 2005, Kotsopoulos *et al.* 2012a).

Findings for *BRCA1* mutation carriers in relation to oral contraceptive use and smoking have been inconsistent (Brunet *et al.* 1998, Narod *et al.* 2002, Ghadirian *et al.* 2004, Colilla *et al.* 2006, Haile *et al.* 2006, Brohet *et al.* 2007, Breast Cancer Family Registry 2008, Ginsburg *et al.* 2009, Bernholtz *et al.* 2011, Lecarpentier *et al.* 2011), even

within reports from the same research groups (Brunet *et al.* 1998, Narod *et al.* 2002, Ghadirian *et al.* 2004, Gronwald *et al.* 2006a, Ginsburg *et al.* 2009). For *BRCA2* mutation carriers, published evidence is more consistent with oral contraceptive use being associated with an increased risk of breast cancer (Haile *et al.* 2006, Brohet *et al.* 2007, Bernholtz *et al.* 2011). There is insufficient published evidence on associations with breast cancer risk for other factors such as hormone therapy and with ovarian cancer risk in *BRCA2* mutation carriers for lifestyle/hormonal factors in general, in the latter case, predominantly due to small sample sizes. There is similarly little or no evidence on the effects on cancer risk for mutation carriers associated with obesity, physical activity, alcohol consumption and diet, so that, as for smoking, general population recommendations for healthy living should apply.

The outline above highlights that, despite an accumulating body of research, we know relatively little about how lifestyle factors modify breast and ovarian cancer risk for a woman with a *BRCA1* or *BRCA2* mutation. This places limitations on both the degree to which we can individualise risk prediction for mutation carriers and the advice that can be given to mutation carriers about how they can modify their behaviour to reduce their risk. With the exception of age at first full-term pregnancy for *BRCA1* mutation carriers, there is little evidence that the risk factors for mutation carriers differ from those in the general population. As in the general population, the benefits of oral contraceptive use in terms of reducing risk of ovarian cancer must be weighed against the potential harms in terms of breast cancer risk.

Another risk-reducing intervention that has been assessed in several studies is risk-reducing salpingo-oophorectomy (RRSO), which has benefits in terms of ovarian cancer prevention and has been reported to be associated with a reduction in breast cancer risk of approximately 50% for *BRCA1* and *BRCA2* mutation carriers (Rebbeck *et al.* 1999, 2002, Eisen *et al.* 2005, Kramer *et al.* 2005, Domchek *et al.* 2006, Kauff *et al.* 2008, Domchek *et al.* 2010). However, a recent publication has highlighted that the observed protection may be overestimated due to several biases inherent in the design and analytical approaches of these observational studies (Heemskerk-Gerritsen *et al.* 2015b). Although still the subject of debate (Chai *et al.* 2015, Heemskerk-Gerritsen *et al.* 2015a), two analyses have now shown that after accounting analytically for these biases, in particular the exclusion of prevalent breast cancer cases from the analysis and the allocation of immortal person-time to the non-RRSO comparison group, no protective effect of

RRSO for breast cancer is observed (Heemskerk-Gerritsen *et al.* 2015b). It is important that this issue is resolved so that *BRCA1* and *BRCA2* mutation carriers contemplating RRSO are aware of both the impact of doing so on their risk of breast cancer and what other measures they might take to reduce that risk. On the other hand, a recent comprehensive review of the literature on the role of bilateral risk-reducing mastectomy (BRRM) has found consistent evidence in both retrospective and prospective studies that BRRM is associated with a >90% reduction in breast cancer risk for women with *BRCA1* or *BRCA2* mutations (Hartmann & Lindor 2016).

Mammographic density

Mammographic density is one of the strongest risk factors for breast cancer in the general population. A meta-analysis of published studies estimated that the risk for women with mammographic density $\geq 75\%$ is 4.64 times greater than that for women with $< 5\%$ mammographic density (McCormack & dos Santos Silva 2006). Data from population-based studies also suggest that mammographic density is a risk factor for both ER-positive and ER-negative breast cancer (Bertrand *et al.* 2013), although some studies found associations only with ER-positive disease. Only three studies have investigated the association between mammographic density and breast cancer risk for *BRCA1* and *BRCA2* mutation carriers (Mitchell *et al.* 2006, Passaperuma *et al.* 2010, Ramon *et al.* 2015). The two largest studies, both retrospective (Mitchell *et al.* 2006, Ramon *et al.* 2015) with sample sizes of 206 and 691 mutation carriers (including 96 and 248 affected women, respectively) found that mammographic density is an independent risk factor for breast cancer in both *BRCA1* and *BRCA2* mutation carriers with similar magnitudes of association to those observed in the general population (RR: 2.30 for density $\geq 50\%$ vs $< 50\%$). However, a nested case-control study of mutation carriers that included a much smaller number ($N=46$) of cases, all incident (Passaperuma *et al.* 2010), found no evidence of association. Although the balance of evidence suggests that mammographic density is likely to also be a breast cancer risk factor for *BRCA1* and *BRCA2* mutation carriers, additional and larger studies are required to fully characterise the associations.

Implications for risk prediction

The genetic and lifestyle/hormonal modifiers of breast or ovarian cancer risk for *BRCA1* and *BRCA2* mutation

carriers described previously, with modest associated relative risks, are likely to be of limited utility individually in terms of cancer risk prediction. However, the relative risks associated with several common genetic variants and/or lifestyle/hormonal factors in combination are much larger. Further, because women with *BRCA1* and *BRCA2* mutations are already at high risk of developing breast or ovarian cancer, the combined effects of SNPs and lifestyle/hormonal risk factors translate into large differences in the absolute risks of developing the diseases (Antoniou *et al.* 2008b, 2010a).

Analyses of data from the Breast Cancer Association Consortium have demonstrated that in the general population, the common breast cancer genetic susceptibility variants combine multiplicatively on the risk of developing breast cancer (Mavaddat *et al.* 2015); no evidence of interactions between SNPs has been observed (Milne *et al.* 2014b). In mutation carriers, a systematic assessment of the pairwise interactions between all SNPs that are known to modify cancer risks is currently ongoing. However, analyses based on smaller subsets of SNPs suggest no evidence of departure from the multiplicative model for the joint effects of SNPs (Antoniou *et al.* 2008b, 2010a), as observed in the general population. Given the observed differences between *BRCA1* and *BRCA2* mutation carriers in the association patterns of the common genetic variants with breast cancer risk and their consistency with associations with ER-negative and ER-positive disease, respectively, the most likely underlying model of genetic susceptibility to breast cancer is one where the associated effects of common susceptibility variants and of *BRCA1* and *BRCA2* mutations on breast cancer risk would be multiplicative, after taking into account tumour ER status (Kuchenbaecker *et al.* 2014).

This multiplicative model can be applied to identify groups of mutation carriers who are at substantially different levels of risk. For example, on the basis of 10 variants associated with breast cancer risk for *BRCA1* mutation carriers, the lifetime risk of developing breast cancer for the 5% of *BRCA1* carriers at lowest risk is predicted to be 28–50%, compared to 81–100% for the 5% at highest risk (Couch *et al.* 2013). Similarly, based on the distribution of seven common genetic variants found to modify ovarian cancer risk for mutation carriers, the 5% of *BRCA1* mutation carriers at lowest risk will have a lifetime risk of developing ovarian cancer less than 30%, whereas the 5% at highest risk will have a lifetime risk greater than 60% (Couch *et al.* 2013). These differences in cancer risks may have practical implications for the clinical management of

mutation carriers, for example in deciding the timing of preventative interventions.

These predictions were based on a limited number of SNPs. Several more have since been shown to be associated with breast and ovarian cancer risk for mutation carriers. In addition, a much larger number of common genetic variants are now known to be associated with breast and ovarian cancer risk in the general population. Given the effect sizes associated with individual SNPs, the power to detect associations with each of these SNPs in mutation carriers is limited by the currently available sample sizes. However, greater statistical power can be achieved by investigating their combined effects, modelled as a polygenic risk score (PRS) based on associations observed in the general population (Mavaddat *et al.* 2015). These studies are currently underway within CIMBA. PRSs based on large numbers of SNPs are expected to result in even larger differences in the absolute cancer risks estimated for mutation carriers at the extremes of the combined SNP distributions, compared with the limited SNP profiles investigated so far.

Furthermore, integrating the effects of the common genetic variants with other lifestyle/hormonal risk factors, family history and mammographic density, may enable the identification of groups of *BRCA1* and *BRCA2* mutation carriers with sufficiently different cancer risks to enable more effective stratified clinical management, as recently demonstrated for the general population (Garcia-Closas *et al.* 2014). As described previously, the associations for several of the risk factors still remain to be clarified. However, as an illustration of the potential for cancer risk stratification for *BRCA1* and *BRCA2* mutation carriers, we provide projected breast cancer risks for *BRCA2* mutation carriers assuming that all common genetic factors (including risk-modifying SNPs and the OCCR effect), lifestyle/hormonal factors and mammographic density act multiplicatively on the risk of developing breast cancer. We also assumed that these risk factors have similar distributions in mutation carriers to those observed for overall breast cancer risk in the general population, as described in Garcia-Closas and coworkers (2014). This seems a reasonable assumption based on the observed association patterns for *BRCA2* mutation carriers described previously. Given the differences in the association patterns of the genetic (and other) risk factors between *BRCA1* and *BRCA2* mutation carriers, and the fact that the associations for *BRCA1* mutation carriers are more similar to the associations for ER-negative breast cancer in the general population, constructing an equivalent figure for *BRCA1* mutation carriers would require data on the

joint risk factor distributions with respect to ER-negative breast cancer risk which are not currently available. Figure 1 shows the predicted 5-year breast cancer risks for *BRCA2* mutation carriers at the bottom percentiles of the combined risk factor distribution. These demonstrate large differences in the absolute risk of developing breast cancer, which may have implications for decisions about cancer prevention. For example, no established risk thresholds exist for recommending anti-oestrogens for primary breast cancer prevention in *BRCA1* and *BRCA2* mutation carriers. In the United States, the approved chemoprevention threshold for the general population is a 5-year breast cancer risk of 1.7%. On the basis of the average breast cancer risk for *BRCA2* mutation carriers, this threshold would be reached at age 28 years. However, the assumptions for the combined effects of risk factors indicate that 5% of *BRCA2* carriers at the lowest risk would not reach that threshold until age 40 years (Fig. 1). Similarly, no accepted risk thresholds for risk-reducing mastectomy exist, but the benefit from, and acceptability of, this aggressive procedure would be limited for women in the lower risk categories. These findings demonstrate that by integrating the effects of genetic, lifestyle/hormonal and other risk factors we can identify mutation carriers at substantially different levels of risk, which will be informative in the genetic counselling process to allow female mutation carriers to make more informed choices about the type and timing of cancer control. However, these projected risks remain theoretical

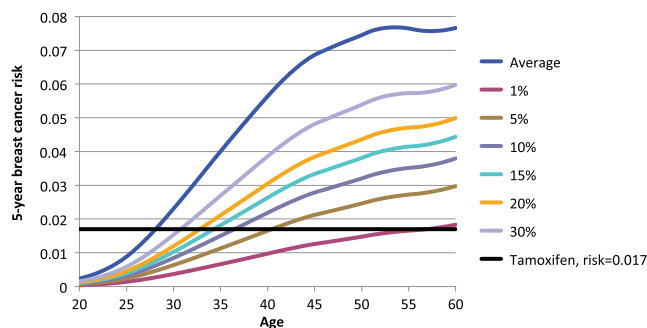


Figure 1
Predicted 5-year breast cancer risks for *BRCA2* mutation carriers in the lower percentiles of the combined risk factor distribution. We assumed that all common genetic variants (including the OCCR effect), lifestyle/hormonal factors and mammographic density interact multiplicatively on the risk of developing breast cancer and that they have similar distributions to those observed in the general population, as described in Garcia-Closas *et al.* (2014). Absolute risks were calculated using the methods described in Antoniou *et al.* (2010a). The horizontal black line shows the absolute 5-year breast cancer risk at 1.7%, which is the threshold applied in the United States for recommending anti-oestrogens for primary breast cancer prevention in the general population.

at this point and the joint effects of all genetic, lifestyle/hormonal and other risk factors will need to be evaluated in empirical studies. Further, the risk estimates will need to be validated in prospective cohorts of mutation carriers.

The clinical management of healthy women with *BRCA1* and *BRCA2* mutations often involves a combination of screening, prophylactic surgery and other risk-reduction strategies (Clark & Domchek 2011). Prevention options include risk-reducing salpingo-oophorectomy (RRSO), risk-reducing mastectomy (RRM) and chemoprevention. However, these are invasive, have side effects and are associated with adverse psychosocial effects (recently reviewed in (Hartmann & Lindor 2016)). Most women opt for RRSO, which results in premature menopause and is associated with adverse effects such as increased risks of cardiovascular disease and osteoporosis, as well as cognitive impairment and mortality from neurological diseases (Parker *et al.* 2009, Rivera *et al.* 2009a,b). Moreover, young mutation carriers make decisions about RRSO/RRM at a time in their lives that often coincides with family planning. The findings on genetic modifiers and calculations integrating their associated effects with other risk factors indicate that there is the potential to identify low- and moderate-risk groups of *BRCA1* and *BRCA2* mutation carriers, who will be appropriate for studies of less-intensive interventions such as salpingectomy (Falconer *et al.* 2015) and who may choose to avoid or delay preventive surgery.

Unresolved issues in the identification and characterisation of risk-modifying factors

Several important challenges remain in the identification of breast and ovarian risk-modifying factors for *BRCA1* and *BRCA2* mutation carriers. The first is sample size. Even for the study of common genetic modifiers, for which retrospective studies have proven to be adequate, combining data from >35,000 mutation carriers through the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) (Chenevix-Trench *et al.* 2007), has proven to be insufficient to detect associations with individual genetic variants that have small effects on risk. Instead, studies of carriers now focus primarily on replicating associations observed in the general population and evaluating their combined effects (as polygenic risk scores) in risk prediction for these high-risk women. Much more of a challenge is the assessment of lifestyle/hormonal modifiers, for which study design is a far more important issue and less data have been collected. Although it is clear that prospective studies are required, sample size is

an even greater limitation for these, and even studies with extensive follow-up periods have substantial censoring due to women undergoing prophylactic interventions. Multi-consortium collaborations, such as that formed by the International BRCA1/2 Carrier Cohort Study (<http://www.ibccs.nl/>), the kConFab Clinical Follow-Up Study (Phillips *et al.* 2005) and the Breast Cancer Family Registries (http://epi.grants.cancer.gov/CFR/about_breast.html) will be essential to overcome these challenges.

Sample size impacts to an even greater extent on the estimation of combined effects of genetic and lifestyle factors on cancer risk for mutation carriers, even with the use of combined data through consortia. The general approach to work around this problem has been to assume that these factors act independently in modifying risk, unless evidence to the contrary is observed. Although statistical power to detect deviations from log-additive combined effects is limited, studies in the general population have consistently found very little evidence of these (Milne *et al.* 2010a,b, Travis *et al.* 2010, Campa *et al.* 2011, Nickels *et al.* 2013, Rudolph *et al.* 2015). Prospective studies will be essential to assess the validity of this assumption and of the risk prediction models more generally. Sample size similarly limits the capacity to identify interactions between *BRCA1* and *BRCA2* and other variants, particularly rare variants.

Future work will require several other issues to be addressed, including appropriate analytical consideration of the fact that *BRCA1* and *BRCA2* increase the risk of cancers other than breast and ovarian; risk prediction models are based primarily on data for mutation carriers that present at family cancer clinics and may be less relevant to those identified through primary care or population screening; the development of risk prediction tools that are easy to use and to understand; and cancer risk modifiers and risk prediction for male mutation carriers. Although multiple common genetic modifiers of breast or ovarian cancer risks for *BRCA1* and *BRCA2* mutation carriers have been identified, relatively few of these have been subjected to fine-scale mapping studies to fully characterise the associations with all the genetic variants at those regions and to identify plausible candidate causal variants. It will be important to carry out these studies because they will inform downstream functional studies to better understand the biological basis of cancer risk modification. They may also lead to the discovery of novel therapeutic targets for mutation carriers.

The vast majority of the studies on risk-modifying factors for *BRCA1* and *BRCA2* mutation carriers have focused on women of European ancestry from Western

populations. The findings therefore may not be applicable to populations from other countries with different breast and ovarian cancer incidence patterns and different risk factor distributions or to mutation carriers from ethnic minorities in Western countries. There are a number of ongoing efforts at the moment to pool data from other populations (e.g. in Asian countries (Kwong *et al.* 2016, Nakamura *et al.* 2016)), but further large-scale studies are required in such populations to comprehensively assess the effects of genetic risk-modifying factors for women with *BRCA1* and *BRCA2* mutations.

Strategies for implementation in clinical practice

Given the large differences in absolute risks by the combined distribution of genetic, lifestyle/hormonal and risk factors for mutation carriers, women with *BRCA1* and *BRCA2* mutations could be one of the first groups to benefit from clinical applications of findings from GWAS and epidemiological studies. However, parallel to the analytical and methodological work required to develop cancer risk prediction models and risk assessment tools specifically for *BRCA1* and *BRCA2* mutation carriers, clinical implementation studies will also be required before personalised risk prediction can be provided to mutation carriers on routine basis. It will be necessary to perform risk communication and acceptability studies to assess the attitudes of women with *BRCA1* and *BRCA2* mutations to risk stratification and to breast and ovarian cancer risk-stratified management. It will also be essential to assess the attitudes of health care professionals to providing personalised cancer risk estimates. The provision of more personalised risk predictions on the basis of polygenic risk scores or comprehensive risk prediction models will require feasibility studies to be performed to assess the uptake of personalised cancer risk prediction and the uptake of the different risk management options (screening/risk-reducing surgery/chemoprevention), as well as the psychosocial impact and cost-effectiveness of these. It will also be necessary to educate health care providers in the provision of personalised cancer risk predictions for mutation carriers on the basis of factors modifying risks.

We have come a long way in understanding and more precisely estimating the average breast and ovarian cancer risks for *BRCA1* and *BRCA2* mutation carriers. Large-scale consortia have led international collaboration, harnessing advances in genotyping technologies, to identify many common genetic factors that modify these risks, and

polygenic risk scores based on these show great promise in affording more personalised genetic counselling and cancer prevention based on stratified risk prediction. Further work is required to establish the relative risks for mutation carriers associated with lifestyle/hormonal and other risk factors and to incorporate these into risk prediction models to achieve even greater precision. Multidisciplinary collaboration is required to overcome the various challenges inherent to this important and potentially transformative work.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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