Gene-panel sequencing for predicting breast cancer risk: a note of caution

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Introduction

Recent advances in sequencing technology have made multigene testing, or "panel testing", for genetic variants that may be associated with breast cancer risk a practical option. In addition, in June 2013, the Supreme Court of the Unites States[1] invalidated specific claims made by Myriad, with respect to the patenting of the genomic DNA sequence of *BRCA1* and *BRCA2*. Other companies immediately began to offer breast cancer gene panel testing that included *BRCA1* and *BRCA2*.

The subsequent flourishing of gene panel testing services (e.g. Table 1 and Table S1) has generated much interest both within the clinical genetics community and in the popular press[2]. These panels cover, in total, more than 100 genes - for 21 of which breast cancer is specifically mentioned as an indication. However, just because the technology is available does not imply that such tests are appropriate or desirable.

According to the ACCE framework, genetic tests should be evaluated on the basis of four criteria: 1) Analytic validity, 2) Clinical validity, 3) Clinical utility, and 4) Ethical, legal and social issues[3]. Analytic validity refers to how accurately a test detects the presence or absence of a mutation. Here, however, we focus here on the key question of clinical validity: are the variants being tested for associated with disease risk, and are these risks well estimated? The validity of the risk estimates is a key determinant of the clinical utility of panel testing, which in turn should inform decisions regarding adoption into clinical practice. We do not consider in detail who should be tested, the level of risk associated with any given variant that might be considered clinically useful or how that risk might be managed. However, broadly comparable guidelines for the management of women with a family history of breast cancer exist in several countries (Table 2). These guidelines are based on the stratification of women according to levels of risk, and provide guidance on the identification of women who should be offered screening by mammography or magnetic resonance imaging (MRI), risk reducing medication and risk reducing surgery (for example, prophylactic mastectomy and/or

oophorectomy reduce mortality in women carrying *BRCA1* or *BRCA2* mutations). These recommendations could be modified to reflect the identification of risk variants through the use of gene-panel testing. Whatever the management recommendations, the guidelines should be underpinned by reliable cancer risk estimates.

Before developing management guidelines, the appropriateness of the tests themselves needs to be considered. Analytical validity for laboratory-developed diagnostic tests falls under the remit of the Clinical Laboratory Improvement Amendments (1988) (CLIA), but neither clinical validity nor clinical utility form part of the assessment process. Therefore, whereas new drugs without clinical utility will not be approved by the US Food and Drug Administration, gene panel tests can be adopted without any review of data regarding their clinical utility[4,5].

Key issues and general principles

Several key questions have to be addressed in order to establish clinical validity: (i) Are variants in the gene associated with breast cancer risk? (ii) Which variants, or classes of variants, are risk-associated? (iii) What is the magnitude of those risks? (iv) How precisely have those risks been estimated?

We will concentrate on those genes in which rare variants have been proposed to confer a "moderate" or "high" risk of breast cancer. For the purpose of this review we consider moderate risk to imply an average increased risk of 2-4 fold compared to population incidence and high-risk to imply an increased risk of more than 4-fold[6]. We leave aside the separate question of risk prediction using profiling based on genotyping of common polymorphisms (SNPs) [Box 1]. We will restrict attention to risk prediction in women unaffected with the disease, although somewhat analogous issues apply to testing in affected women. We concentrate on the question of breast cancer risk, but similar considerations apply to other cancers. Indeed, some of the genes considered here also

predispose to ovarian, pancreatic and other cancers and some of the available panels also include genes putatively involved in a wider range of cancers (Table 3 and Table S2) We also leave aside the use of panel testing for diagnosis or in the management of women affected with cancer, for example in the selection of patients for clinical trials.

Types of genetic variant - Most panel testing involves the sequencing of the coding sequence and splice-junctions of the genes of interest, often combined with alternative methods for detecting large genomic rearrangements[33]. Most variants identified will be single base substitutions, small insertions or small deletions (indels). We will follow the normal practice of grouping all nonsense substitutions, frameshift indels and variants affecting splicing as "protein-truncating" variants. For the large majority of genes, most of the evidence on breast cancer risk relates to protein-truncating variants assumed to result in loss of function.

Statistical significance and burden tests - Stringent levels of statistical significance, which have become well-established for genome-wide association studies of common variants, are equally important here. Although it would be ideal to have specific evidence for every variant detected, most variants suspected of being associated with high disease risks are rare and the sample sizes to establish allele-specific risk association are not feasible. Consequently, some form of burden testing is frequently used, in which association between a specific class of variants and disease is evaluated. A potential problem with this method is that it will not be known if a specific variant identified is disease associated or not. It is often assumed that all protein-truncating variants are equally pathogenic; however, not all these variants will necessarily confer the same risks. For missense variants, the situation is even more problematic (see below).

Strength of statistical evidence for association -The issue of appropriate significance levels has been extensively discussed for genome-wide association studies but has been less thoroughly reviewed in

the case of targeted sequencing. An "exome-wide" significance level of $P<2.5x10^{-6}$ is often used for whole exome studies (based on a Bonferroni correction for ~20,000 genes). Since most breast cancer susceptibility genes are involved in DNA repair, a class involving less than 500 genes, more liberal significance levels, of the order of $P<10^{-4}$, might be appropriate for genes in this pathway. Bayesian arguments lead to similar thresholds (see Online Methods). While the above significance thresholds may be appropriate for a single burden test, more stringent significance levels would be required for individual variants.

A related question is the precision in the risk estimate. It is clearly undesirable for a counselee to be given a risk estimate that then changes substantially with further data. For the purpose of this review, we consider that a given risk is likely to be above (or below) a given threshold if the 90% confidence limit on the risk estimate exceeds (or is less than) the threshold.

Definition of Risk- We have chosen to present estimates here primarily in terms of the average relative risks. In doing this, we recognize that for counselling purposes absolute risk estimates (over the next few years, or lifetime) are more useful. However, almost all studies estimate relative rather than absolute risk, and moreover absolute risks are more strongly influenced by other factors (see below). For a rare variant conferring a relative risk of 2 or 4, this would correspond to absolute risks of breast cancer of ~18% and 32% by age 80, respectively, based on recent United Kingdom incidence rates[32], in the absence of other causes of death. These risks correspond, approximately, to the definitions of moderate and high risk familiar to the clinical genetics community (see e.g.[34]).
It follows from this that the identification of a variant conferring a relative risk >4 can, in the absence of any other data, place a woman in the high risk category. In contrast, a variant conferring a relative risk of 2-4 will only place a woman in the high-risk category if her risk is also increased by other factors.

Note that, for some genes (notably *BRCA1*[7], *CHEK2*[28] and *ATM*[24]) there is evidence that the rate ratio declines with age. The published overall relative risk estimates can then give a misleading estimate

for the lifetime risk. Ideally, age-specific estimates are required, but the data at older ages are often very limited.

Study Design – appropriate study design is critical both for the identification of disease-associated alleles and in the derivation of reliable risk estimates. Several study designs are available (see Table 4). Risk estimation using case-control studies in the context of rare variants can be problematic; family-based methods provide an alternative, but these methods also have pitfalls. Furthermore, many studies are based on a few variants that are restricted to specific populations; while it is generally assumed that the risk estimates for different truncating variants observed in other populations are similar, this is usually impossible to test.

Risk over-estimation - The related problems of publication bias, where negative studies are not published, and winner's curse, whereby an initial study identifying an association tends to overestimate the risk, should be noted[36]. Furthermore, many gene discovery studies oversample for early onset cases or cases with a family history. This approach improves power but leads to seriously biased risk estimates, unless the ascertainment is allowed for in the analysis. Moreover, risk estimates based on data from highly selected families may not reflect the true "average" risk for all carriers of pathogenic variants, because such biased sampling results in a selection of individuals that are non-random with respect to other modifiers of risk.

Evidence for specific genes

In this section, we consider the evidence for association for specific genes for which there is some reported evidence for a breast cancer association. We concentrate first on functionally damaging variants. A full summary of the genes we have considered is given in Table 3 (for those genes with established evidence of association), Table S2 (for other genes) and Table S3 (See Online Methods for the methods used to derive summary risk estimates).

BRCA1 and *BRCA2* - The clinical validity and utility of testing for variants in *BRCA1* and *BRCA2* are well-established. There is overwhelming evidence that most protein-truncating variants in these genes are associated with a high risk of breast and other cancers[7,9,10]. Even among protein-truncating variants, however, variant-specific differences in risk have been observed[37]. Furthermore, a polymorphic nonsense variant at the carboxyl terminus of *BRCA2*, p.Lys3326Ter has been reported to be associated with relative risk for breast cancer of 1.4 (90% CI 1.2 - 1.7) [38] - substantially lower than the risks conferred by more proximal truncating variants.

TP53, *CDH1, PTEN*, *STK11* and *NF1* - These genes cause pleiotropic tumor syndromes, of which breast cancer is only one feature. Germline mutations in *TP53* (both protein truncating and missense) are responsible for Li-Fraumeni syndrome, in which carriers are predisposed to childhood sarcomas, brain tumours, adrenocortical carcinoma and other rare malignancies, in addition to breast cancer[39]. While the association with breast cancer is uncontroversial, reliable risk estimates are lacking; most studies are based on pedigrees with Li-Fraumeni features and hence subject to ascertainment bias. However, a study based on *TP53* mutation carriers identified through childhood sarcoma probands has also demonstrated a high breast cancer risk[12]. Similar ascertainment biases apply to mutations in *PTEN*, which are associated with Cowden syndrome, in which breast cancer is a characteristic of the clinical phenotype [13,40], and in *STK11*, associated with Peutz-Jeghers syndrome and an increased breast cancer risk[16]. Protein-truncating variants in *CDH1*, known to be associated with diffuse type gastric cancer, are also thought to be associated with an increased risk of breast cancer (specifically of lobular subtype) with a reported relative risk of 6.6 (90% CI 2.2 – 19.9, *P*=0.004)[15]. Recent cohort studies have demonstrated an elevated breast cancer risk in women with Neurofibromatosis Type I (OR 2.6, 90% CI 2.1-3.2).

Other DNA double strand break repair genes - There is strong evidence that protein-truncating variants in four other genes involved in DNA repair confer increased risks of breast cancer; these are the best established "moderate risk" breast cancer genes to date. The risks appear to be highest for *PALB2*: the largest family-based study estimated a risk of approximately 6 fold[19], although two case-control studies based on the Finnish founder variant c.1592delT estimated somewhat lower risks[20,22]. Based on a meta-analysis of these estimates, the combined relative risk is 5.3 (90%CI 3.0-9.4). Thus, while *PALB2* mutations may fall into the high-risk (>4-fold) category, the confidence limits are too wide to be certain. Most of the data for *CHEK2* relate to the c.1100delC variant that is relatively frequent in Northern European populations[27]. Two large-combined analyses of case-control studies have been published, which give a combined estimated relative risk of 3.0 (90% CI, 2.6 to 3.5)[28,29] . Truncating variants in *ATM* have been evaluated both in case-control studies (though of selected cases) [23] and through cohort studies in relatives of ataxia-telangiectasia patients[24-26]. A meta-analyses of the three largest cohort studies gives an estimated relative risk of 2.8 (90% 2.2-3.7, P = 4.7×10^{-11}), similar to that for *CHEK2* truncating variants.

For *NBN*, a protein-truncating variant, c.657del5, is sufficiently common in some eastern European populations to evaluate it using a case-control design. A meta-analysis of ten studies found strong evidence of association for this variant with breast cancer risk (pooled 2.7, 90 % CI 1.9-3.7, P = 5×10^{-7})[31]. More limited evidence is available for two other DNA repair genes, *MRE11A* and *RAD50*, which encode proteins forming an evolutionarily conserved complex with NBN[41-46].

Mutations in three other DNA repair genes*, RAD51C*, *RAD51D* and *BRIP1*, have shown clear evidence for association with ovarian cancer[47-51]. However, in each case the evidence for association with breast cancer is limited. Recent exome and targeted sequencing studies have suggested breast cancer associations for deleterious variants in *FANCC*[52], *FANCM*[53] and *XRCC2*[54]. In none of these instances, however, does the evidence reach the level of evidence threshold we propose.

Other genes - Current cancer gene panels contain many other genes, mostly included by virtue of their relevance to rare Mendelian cancer syndromes. Variants in some of these may also be associated with breast cancer. Mutations in DNA mismatch repair genes (*MLH1*, *MSH2, MSH6*, *PMS2*) may be associated with breast cancer, but a recent review by Win et al [55] concluded that the evidence was equivocal. *MUTYH* variants that predispose to polyposis colorectal cancer have also been suggested to predispose to breast cancer, but a recent case-control study found no association[56]. A recent study suggested that carriers of *MEN1* mutations may be at increased risk of breast cancer[57]. Finally, a recent case-control study has shown an association between rare variants in *PPM1D* and breast cancer[58]. However, this association does not reach the proposed significance threshold, and moreover the sequence variants are observed as mosaics in lymphocytes and are not inherited. To our knowledge, there is currently no clear evidence for association with breast cancer for any other gene.

Missense variants

With the exception of *TP53*, the situation with regard to missense variants in the above genes is much more problematic. It is well established that missense variants in specific domains of *BRCA1* and *BRCA2* confer high risks of breast and ovarian cancer, but that the great majority do not[59,60]. For these genes, algorithms based on conservation, pedigree data and tumor pathology can be used to predict the pathogenicity of some variants[59 61,62]. Similar considerations may apply to *ATM* and *CHEK2*: missense variants falling in key functional domains and at positions that show a high degree of species conservation are more likely to be associated with increased risk[63]. However, even for *BRCA1* and *BRCA2*, the breast cancer risk associated with the large majority of missense variants remains unknown; such variants are referred to as variants of unknown significance (VUS). Moreover, clearly pathogenic missense variants need not be associated with the same risk as truncating variants. For example, the *CHEK2* missense variant, p.Ile157Thr confers lower risks of

breast cancer than the *CHEK2* c.1100delC truncating variant[30], while *ATM* p.Val2424Gly appears to be associated with a higher risk of breast cancer than truncating variants (8.0, 90% CI, 2.8 to 22.5; P = 0.0005)[64]. A more systematic approach to this problem would involve defining risks based on variant classes defined by *in-silico* prediction algorithms. However, while the existing data provide good evidence that missense variants falling at highly conserved positions in several genes confer disease risk, and that such variants may make an important contribution the heritability of breast cancer[65], there is currently no agreed system for classifying variants such as to define risk estimates that could be used clinically.

Risk modifiers and absolute risks

For genetic counselling purposes, relative risks need to be converted into absolute risks. For an "average" mutation carrier, this can be done straightforwardly by applying the estimated relative risk to population incidence rates. This is illustrated in Figure 1 for *PALB2* and *CHEK2* mutation carriers.

However, these absolute risks depend critically on how the risk associated with a given variant combine with other genetic and lifestyle risk factors, including family history. There is strong evidence that the absolute risk of breast cancer in *BRCA1*, *BRCA2*, *PALB2* and *CHEK2* carriers is higher in women with a strong family history of breast cancer[7,19,27,66]. It has also been shown that the absolute risk of breast cancer in *BRCA1* and *BRCA2* carriers depends on a profile of risk SNPs[67]. A broader question is how the risks associated with genetic variants should be combined with other lifestyle risk factors. Evidence for common SNPs indicates that these combine multiplicatively with other risk factors[68-70], and this would be a plausible assumption for the rare moderate/high-risk variants. However, there is currently limited, and conflicting, evidence on this question for *BRCA1* and *BRCA2*[71], and no evidence for the other genes. In addition, absolute risks need to be adjusted for competing mortality, which may be significant for genes associated with other cancers.

Almost all the available data relate to women of European ancestry. At present it is unclear whether the available relative risk estimates can be safely extrapolated to women from other ancestries, or to populations with different background incidences.

Concluding Remarks

We have discussed some of the difficulties of assigning risks to rare variants and summarized those genes for which the evidence of association with breast cancer is sufficiently robust to be incorporated into personalised risk prediction. Variants predicted to truncate *BRCA1* and *BRCA2* (together with a subset of missense variants) confer a high risk of breast cancer; *PALB2* and perhaps *PTEN* may also fall in this category, but the evidence is insufficient to place them confidently in this (rather than the moderate-risk) category. For *TP53*, both missense and protein-truncating variants are associated with substantially increased risks for breast cancer. The relative risks fall into the moderate risk (2-4 fold) category for *CHEK2*, *ATM* and *NF1*. There is clear evidence for association for *STK11*, *CDH1* and *NBN*, but the risk estimates are too imprecise for categorization. Estimates for *PTEN*, *STK11* and *CDH1* derive entirely from clinic-based families and may be seriously overestimated. We found insufficient evidence to establish any other genes as breast cancer predisposition genes and would caution against their use in breast cancer risk prediction. As the costs of sequencing fall, it is inevitable that gene panel testing, and indeed whole-exome and whole-genome sequencing, will become widespread. Therefore, there is an urgent need for much larger, well designed population- and family-based studies in diverse populations that can provide reliable risk estimates for counselling. The systematic collection of data from ongoing panel testing, linked to the epidemiological and clinical data, may also make an important contribution. Further breast cancer susceptibility genes (and perhaps rarer variants in non-coding sequences) will probably be identified and could be added to genetic testing panels. Panel testing can make a useful contribution to predicting a woman's risk of breast cancer, but end-users need to be aware of their limitations.

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Box 1: SNPs vs. rare variants

Approximately 100 independent common variants (SNPs) associated with breast cancer risk have been identified, through large-scale genotyping studies. These typically have minor allele frequencies >1%, and all confer relative risks of less than 1.5 fold; almost all occur in non-coding sequences. Some commercial panels also include a subset of these SNPs. Thus at present there is a reasonably clear distinction between the low-risk susceptibility SNPs and moderate/high risk variants identified through sequencing (though some sequence variants in "high" or "moderate" risk genes confer risks that place them in the "low-risk" category – examples include *BRCA2* p.Lys3326Ter and *CHEK2 p.*Ile157Thr (see main text). Note that we refer to variants conferring risks of less than 2-fold as "low-risk", a term in widespread use, but this is not a particularly helpful term for counselling purposes since carriers of such variants are still at elevated risk.

Table 1. Examples of multi-gene cancer testing panels

Company	Test	Website	Genes included[1]
Ambry Genetics	Breastnext	http://www.ambrygen.com/tests/breastnext	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, TP53*
Breast Health UK	BreastGene	https://www.breasthealthuk.com/screening-services/genetic-testing/breastgene	*ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, PALB2, PTEN, STK11, TP53*
Centogene	Breast Ovarian Cancer Panel	https://www.centogene.com/	*ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MSH6, NBN, PALB2, PTEN, RAD51, RAD51C, STK11, TP53*
Emory genetics	High risk breast cancer panel	http://genetics.emory.edu/	*BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53*
Fulgent Clinical Diagnostics	Breast-ovarian cancer NGS panel	http://fulgent-therapeutics.com/testing/tests-offered/breast-ovarian-cancer-ngs-panel/	*APC, ATM, ATR, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNB1, EPCAM, FANCC, HOXB13, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PALLD, PMS2, PTEN, RAD50, RAD51, RAD51C, RAD51D, SMAD4, STK11, TP53, VHL, XRCC2, XRCC3*
Gene Dx	OncogeneDx	http://www.genedx.com/test-catalog/available-tests/breastovarian-cancer-panel/	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53, XRCC2*
Illumina	TruSight Cancer	http://www.illumina.com/clinical/translational_genomics/panels/kits.html/	94 genes plus 287 risk SNPs
Invitae	High-risk hereditary breast cancers	https://www.invitae.com	*BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53*
Myriad Genetics[1]	myRisk	https://www.myriad.com/products/myrisk-hereditary-cancer-panel/	*ATM*, *BARD1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, NBN, *PALB2*, *PTEN*, *RAD51C*, STK11, *TP53*
CD Genomics		http://www.cd-genomics.com/Genetic-Testing-for-the-Cancer-Susceptibility.html	Not specified
University of Washington[1]	BROCA – Cancer Risk Panel	http://web.labmed.washington.edu/tests/genetics/BROCA	*AKT1, ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, GEN1, MRE11A, MUTYH, NBN, PALB2, PIK3CA, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2*

[1]Only those genes where breast cancer risk is given as an indication are listed here – for a full list see Table S1. In several cases the panels include additional genes, and several companies also offer larger panels. Thus, even if the primary purpose of the test is breast cancer risk prediction, results will often be available (and need to be interpreted) for a larger set of genes than listed here.

Table 2: Guidelines regarding management of *BRCA1* and *BRCA2* mutation carriers[1]

	NCCN[2] US	NICE[3] UK	GC-HBOC/AGO[4] Germany	EviQ[5] Australia	IKNL/KiMS[6] Netherlands
Age range (years) and frequency for mammography	25-75, "or individualized based on earliest age of onset in the family".	40-69, annually; 70+, biennially Consider 30-39, annually.	40 -50 every 1-2 years if density ACRIII/IV[7] (If density ACRIII/IV[7], then biannual US[8]).	BRCA1: 30-50 years, annually (+/- US) BRCA2: 30-50 years, annually (+/- US). >50 year annual MMG +/- US[8] + CBE[9] If family member diagnosed under 35 years recommendation may be individualised if family member diagnosed at age under 35 years.	Annually; because of the elevated risk of radiation-induced tumours in young women, a starting age of 30 is advised for this group.
Age range and frequency for breast magnetic resonance imaging	25-75, "or individualized based on earliest age of onset in the family".	30-49, annually, unless "dense breast pattern", in which case 50-69.	25-69, if density > ACRI[7]; annually.	BRCA1: 30-50 years, annually (+/- US). BRCA2: 30-50 years, annually (+/- US). >50 year annual MMG +/- US[8] + CBE[9]. Recommendation may be individualised if family member diagnosed at age under 35 years.	Annually, starting age 25.
Recommended age for considering preventive mastectomy	Not stated, "degree of protection and risks" should be discussed	Not stated; but discussions of the potential benefits of surgery should take into account the woman's current age	Not stated, degree of protection and risks should be discussed".	$\leq$ 40 years	From age 25; residual breast cancer risk considered <5% and discussed as such with counselee.

	NCCN[2] US	NICE[3] UK	GC-HBOC/AGO[4] Germany	EviQ[5] Australia	IKNL/KiMS[6] Netherlands
Recommended age for considering preventive oophorectomy	"Ideally between ages 35-40".	Not stated.	Salpingo-oophorectomy: recommended age around 40 for *BRCA1* mutation carrier, around 45 for *BRCA2* mutation carrier.	"Preferably at age ≤ 40 years".	From age 35 for BRCA1 carriers, 40 for BRCA2 carriers.
Use of the oral contraceptive pill	No clear directive.	No clear directives – conflicting data "should be discussed".	No clear directive.	Combination oral contraceptive pill is not contra-indicted.	No clear directives; a non-systemic form of anticonception could be discussed.
Use of chemoprevention	No clear directive.	Offer Tamoxifen to women at high breast cancer risk, but BRCA1/2 status not discussed	Not stated.	Individualised consideration with professional recommended.	Not stated.
Use of hormonal therapy (estrogen/progesterone)	No clear directive.	Not discussed.	Excluded for *BRCA1/2* mutation carrier without RRSO[10].	If RRSO[10] prior to menopause HRT should be considered until time of natural menopause.	Not stated.
Consideration of screening for other organs potentially at risk	Prostate cancer screening recommended for *BRCA2* mutation carriers from age 40 (consider for *BRCA1* mutation carriers).	Not discussed as document focused on familial breast cancer only.	Prostate cancer screening recommended for *BRCA2* mutation carrier from age 45-50.	Male carriers: consider annual PSA[11] = digital rectal exam from early 40s.	Not stated.

[1]For a summary of available guidelines, see http://www.cancer.gov/cancertopics/pdq/genetics/breast-and-ovarian/HealthProfessional/page4#_2665_toc

[2]NCCN – National Comprehensive Cancer Network (USA); NCCN.org - NCCN Guidelines Version 2.2014 Updates Genetic/Familial High-Risk Assessment: Breast and Ovarian, original document – Hereditary Breast and/or Ovarian Cancer syndrome (HBOC-1).

[3]NICE – National Institute for Health Care and Excellence (UK); guidance.nice.org.uk/cg164 - Familial breast cancer
Classification and care of people at risk of familial breast cancer and management of breast cancer and related risks in people with a family history of breast cancer, issued: June 2013.

[4]GC-HOBC – see http://www.konsortium-familiaerer-brustkrebs.de/

[5]EviQ Cancer Treatments Online, see https://www.eviq.org.au ("Risk management for an unaffected female BRCA1 mutation carrier", "Risk management for an unaffected female BRCA2 mutation carrier" and "Risk management for an unaffected male BRCA1 or BRCA2 mutation carrier").

[6]http://richtlijnendatabase.nl/en/richtlijn/breast_cancer/screening.html

[7]American College of Radiologists categorisation of breast density.

[8]Ultrasound.

[9]Breast self-examination.

[9]Risk reducing salpingo-oophorectomy

[10]Prostate-specific antigen.

Table 3. Genes with an established association between protein-truncating variants and breast cancer risk.

Gene	Magnitude of relative risk associated with truncating variants		Risk associated missense variants[3]	Estimated relative risks (90% CI)[1]	*P*-value	Absolute risk by age 80[4]	Comments	Other associated cancers	References
	>2 fold risk[2]	>4 fold risk[2]							
BRCA1	Yes	Yes	Yes	11.4		75%	Estimates based on the BOADICEA model for woman born in 1960.	Ovary	7-10
BRCA2	Yes	Yes	Yes	11.7		76%	Estimates based on the BOADICEA model for woman born in 1960. p.Lys3326Ter in the carboxyl terminus is associated with a lower increase in risk	Ovary, prostate, pancreas	7-10
TP53[5]	Yes	Yes	Yes	105 (62-165)			Most published risk estimates subject to ascertainment bias	Childhood sarcoma, adrenocortical carcinoma, brain tumours	11,12
PTEN	Unknown	Unknown	Yes	[6]			Published risk estimates subject to ascertainment bias	Thyroid, endometrial	13,14
CDH1	Likely	Unknown	Unknown	6.6 (2.2-19.9)	0.004	53%	Lobular breast cancer specific	Diffuse gastric	15
STK11	Unknown	Unknown	Unknown	[7]			Published risk estimates subject to ascertainment bias	Colon, pancreas, ovarian sex cord-stromal tumors	16
NF1	Likely	Unlikely	Unknown	2.6 (2.1-3.2)	$2.3x10^{-13}$	26%	Based on cohort studies of patients with Neurofibromatosis Type I[8].	Malignant peripheral nerve sheath, brain and central nervous system tumors	17,18
PALB2	Likely	Unknown	Unknown	5.3 (3.0-9.4)	$4x10^{-10}$	45%		Pancreas	19-22
ATM	Likely	Unlikely	Yes	2.8 (2.2-3.7)	$5 x 10^{-11}$	27%	p.Val2424Gly is associated with higher risk		23-26
CHEK2	Likely	Unlikely	Yes	3.0 (2.6 to 3.5)	$8x10^{-37}$	29%	Most data are limited to c.1100delC		27-30

Gene	Magnitude of relative risk associated with truncating variants		Risk associated missense variants[3]	Estimated relative risks (90% CI)[1]	*P*-value	Absolute risk by age 80[4]	Comments	Other associated cancers	References
	>2 fold risk[2]	>4 fold risk[2]							
NBN	Likely	Unlikely	Unknown	2.7 (1.9-3.7)	5×10^{-7}	23%	p.Ile157Thr associated with ~1.3-fold risk Almost all data pertain to c.657del5 in Slavic populations		[31]

[1] Typical relative risk, where reliable estimates could be identified. Estimates were obtained from the BOADICEA risk model for *BRCA1* and *BRCA2*, a single study for *TP53* and *CDH1*, and a meta-analysis of multiple studies for the other genes (see Online Methods and Table S3 for further details). Note that there is evidence that the relative risk declines with age for *BRCA1*[7], *CHEK2*[28] and *ATM*[24], and weaker evidence for *PALB2*[19]. These "average" relative risks may therefore underestimate the relative risk at younger ages and overestimate the relative risk at older ages. The estimates relate for protein truncating variants, except as noted (see Online Methods).

[2] Where a quantitative analysis has been possible, "likely" and "unlikely" are taken here to imply that the lower 90% confidence limit on the relative risk estimate exceeds the threshold, or the upper 90% confidence limit is lower than the threshold, respectively.

[3]Indicates whether any missense substitutions have been definitively established as breast cancer associated (typically, a small fraction of all missense variants).

[4] Absolute risks in the absence of other causes of death. Adjusted estimates allowing for competing mortality will be lower, especially where the risk of other cancers is high (e.g. *BRCA1* and *BRCA2*). Unless otherwise indicated, these have been estimated by applying the estimated relative risks to breast cancer incidence rates for England (CI5 volume X, 2003-2007, http://ci5.iarc.fr)[32].

[5]Pathogenic mutations for *TP53* are mostly missense.

[6]Relative risks for breast cancer in *PTEN* mutation carriers of 39.1 (90% CI 26.7 to 54.9) and 25.4 (90% CI, 20.6-30.8) have been reported[13,14]. However, estimates were based on selected families with Cowden or related syndromes, which would result in an overestimate of risk.

[7]The cumulative breast cancer risk for women with Peutz-Jeghers syndrome has been reported to be 45 per cent by age 70 (90% CI 29 – 64 percent)[16] . This estimate, however, did not allow for ascertainment, which would result in an overestimate of risk even in high risk families. Furthermore the data included Peutz-Jeghers syndrome patients in whom no *STK11* variant had been identified.

[8]Risk estimates are based on follow-up of patients with Neurofibromatosis Type I, which is caused by both truncating and missense mutations in *NF1* (though the majority are protein truncating). There are no published risk estimates for *NF1* mutations subdivided by mutation type.

Table 4. Study designs for estimating risks associated with rare variants

Method	Description	Advantages	Disadvantages	Example
Population-based case-control	Screen for variants in unselected cases of disease and population-matched controls	Provides direct estimates of the relative risk (odds ratio), not biased by other familial factors.	Need to be very large as variants are typically rare. Biases arise if controls are not appropriately population-matched (large differences in allele frequency among populations). To provide valid tests and estimates, cases and controls need to be assayed in the same way, typically screening the full coding sequencing in all cases and controls. Large biases can arise if only variants identified in the cases are tested in the controls.	*CHEK2*[28]
Family-based case-control	Case-control studies in which cases are enriched for family history	Improvement in power due to higher frequency of variants in familial cases.	Risk estimates biased. Correction of the bias depends on additional assumptions about modifying effects of other familial factors	*CHEK2*[27]
Kin-cohort	Data on cancer occurrence in relatives of carriers in population-based series used to estimate maximum likelihood[35]	Provides estimates without the need to screen controls. Genotype data in relatives can also be incorporated but not required.	Limited by the accuracy of the family history. Risks overestimated if familial factors not accounted for.	*BRCA1/2*, *PALB2*[7,19]
Segregation in families		Can be applied in families oversampled for a strong family history. Controls not required.	Requires samples on multiple individuals from the same family. Power typically very limited.	*CHEK2*[27]
Prospective cohort		Provides direct estimates of absolute risk	Long-term investment required. Prohibitively large except for high-risk variants. Risk estimates altered by management (e.g. prophylactic	*BRCA1/2*[10]

	surgery). Risk estimates affected by other familial factors.

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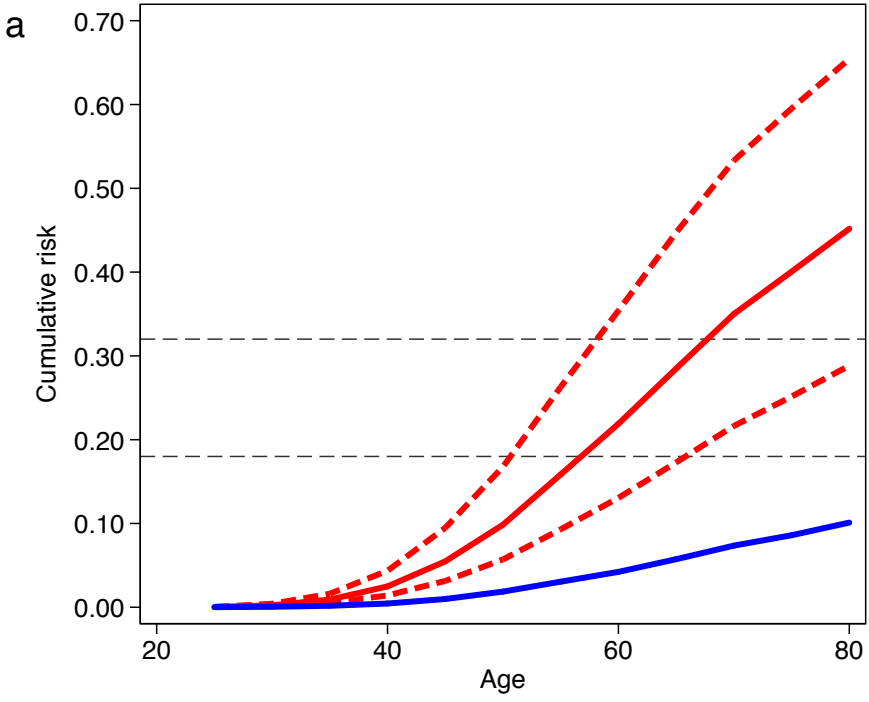
Figure 1. Predicted cumulative risk of breast cancer for carrier of a deleterious mutaton in *PALB2* mutation (panel a) or a deleterious mutation in *CHEK2* mutation (panel b). Solid red lines are the summary estimates, red dotted lines are the upper and lower 90% confidence limits. The absolute risks were estimated by applying the relative risk estimates to the breast cancer incidence rates for England and Wales 2003-07 (CI5 volume X)[32]. The solid blue lines are the cumulative risks based on these population incidence rates (i.e. corresponding to a relative risk of 1). Estimates ignore competing mortality (i.e. are the cumulative risks in the absence of death from another cause). The horizontal dotted lines represent lifetime risks that are twofold and fourfold greater than the population average. Thus an average *CHEK2* mutation carrier is likely to fall into the "moderate risk" category. The best estimate for *PALB2* place carriers in the "high-risk" category, but the confidence interval is such that it may fall in the "moderate-risk" category. These estimates are average cumulative risks (for a women not selected for other factors) and will be modified by other risk factors, including family history (see text).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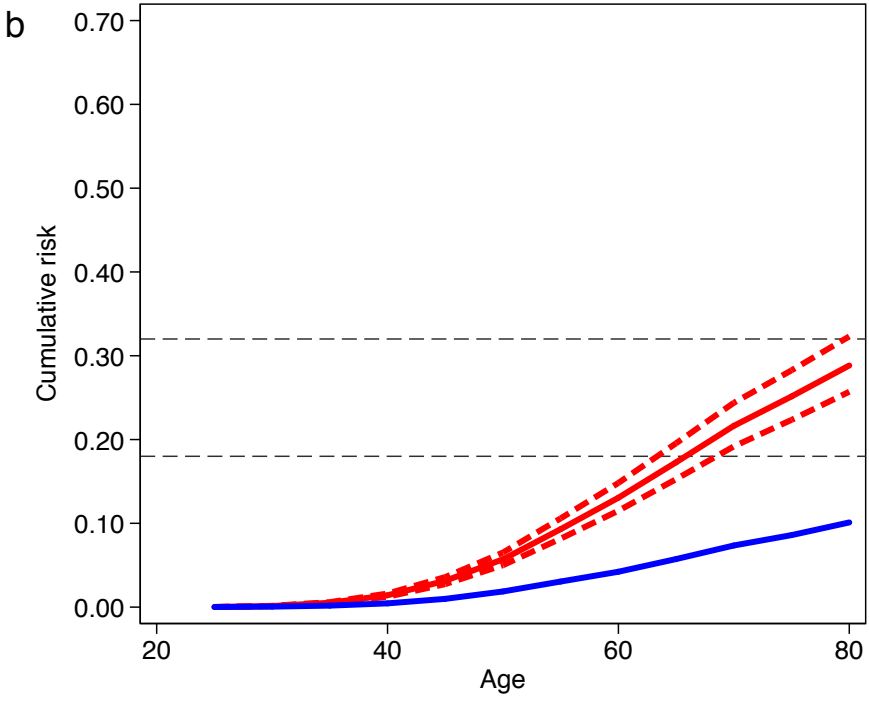
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Supplementary Appendix

Gene-panel sequencing for predicting breast cancer risk: a note of caution

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Online Methods

We attempted to evaluate the evidence for breast cancer association for the genes on the 10 panels in table 1, with the exceptions that (a) for the Myriad and University of Washington panels, we considered only those genes that where breast cancer risk was listed as one of the indications (b) we did not consider all the genes on the Illumina panel, which is a general cancer predisposition panel. We also considered 5 additional genes for which predisposition to breast cancer has been suggested: *FANCM*, *PPM1D, MEN1*, *NF1* and *PPM1D* (*FANCM*, *PPM1D* and *MEN1* are on the Myriad and/or University of Washington panels but breast cancer is not listed as an indication). The 48 genes evaluated are listed in Tables 1 and 2, and the full list of genes on the panels is given in Supplementary Table 1. We then attempted to identify all studies that had attempted to estimate the relative risk of breast cancer associated with protein truncating variants, through case-control, cohort or segregation analysis (see Supplementary Table 3; 21 genes).

For definiteness, we use *relative risk* here in the sense of the rate ratio (or hazard ratio) for carriers of the variant relative to non-carriers. For case-control studies, odds ratio estimates were used. (Note that the term "average" relative risk used here refers to the relative risk that is obtained after averaging over all other factors (genetic or lifestyle) that may also affect the disease risk – that is, the usual relative risk that would be estimated in a population based case-control or cohort study, without consideration of other risk factors. Note that this can differ substantially from the *conditional* relative risk (that is, the relative risk to an individual, given a particular set of risk factors, see e.g.[1]). Where more than one estimate was available (12 genes), these estimates were then combined in a fixed effects meta-analysis to derive an overall effect size and test of association. We have presented uncertainty in the relative risk estimates in terms of 90% confidence intervals (CIs). 90% CIs have been provided rather than the more usual 95% CIs since, in practice, once an increased risk has been established, counselling will be based on a point estimate. The CIs provide an

indication as to whether to risk estimates are likely to change markedly with additional data (such the estimate would fall in a different category), but 90% CIs should be sufficiently stringent for this purpose. In Table 3 we use the terms “likely” and “unlikely” to indicate that the lower 90% confidence limit on the relative risk estimate exceeds the indicated threshold (2 or 4), or that the upper 90% confidence limit is lower than the threshold, respectively. To provide consistent reporting, published 95%CIs have also been converted to 90% CIs for presentation (by first computing the standard error of the log(relative risk)). Where risk estimates and confidence limits were not published in case-control studies, we derived odds ratio estimates from the observed genotype frequencies. We excluded missense variants, unless the published results were only available for truncating variants combined with rare missense variants at evolutionary conserved positions. A key exception is *TP53*, for which the majority of deleterious mutations are missense: for this gene, an estimate was obtained from a segregation analysis in families ascertained through sarcoma patients carrying presumed deleterious variants. For some genes, estimates were based on cohorts of cases, or relatives of cases, ascertained through a specific phenotype. In these cases the estimates refer to variants that predispose to the characteristic phenotype: these include *NF1, ATM* (based on relatives of patients with Ataxia-Telangiectasia) and *MEN1*. In each case the majority of pathogenic variants (but not all) are protein truncating. Where possible, we excluded case-control studies based on selected familial cases, unless the familial aggregation had been specifically adjusted for in the analysis; however, the results from these studies were included in the meta-analysis for the test of association. For high- risk genes, we excluded retrospective cohort studies in high-risk families, unless it was clear that the phenotypes on which the cohort was not ascertained did not include breast cancer. We did not attempt to re-derive estimates for *BRCA1* and *BRCA2* since these have been thoroughly investigated in multiple studies and two meta-analyses. The estimates in Table 4 were derived from the BOADICEA model, based on the average relative risks up to age 80 for a women born in 1960[2].The evidence for breast cancer risk in carriers of mutations in the mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) was thoroughly reviewed recently in Win et

al, and we did not therefore attempt to derive estimates for these genes[3]. For *NBN* we utilised the meta-analysis of Zhang et al[4] since this included all available studies. Uncertainty in the relative risk estimates has been presented in terms of 90% confidence intervals. Specific considerations for other individual genes are listed in Supplementary Table 3. For the genes for which the evidence of association was strong (Table 4), we estimated the cumulative absolute risk of breast cancer in carriers up age 80 years, in the absence of other causes of death, based on the estimate relative risk and the breast cancer incidence rates for England 2003-2007[5].

In general we used a significance level of $P<10^{-4}$ for susceptibility genes involved in DNA repair, and an "exome-wide" significance level of $P<2.5\times10^{-6}$ (based on a Bonferroni correction for ~20,000 genes) for other genes. An alternative approach is to use a Bayesian argument based on determining the posterior probability that an association is true given the prior probability that a given class of genes is associated[6,7]. Assuming for example, that 10 truly associated genes are typically detectable studies of the sample sizes that are currently available (which seems reasonable since the genes with readily detectable effects (*CHEK2*, *PALB2*, *ATM*) each explain 1-2% of the familial risk of breast cancer), the prior probability of a true association would be 1 in 2,000. To obtain a conditional error probability of approximately 10%, the required significance level would be around $P<10^{-6}$ for an unselected gene, or $P<10^{-4}$ for a DNA repair gene, i.e. similar thresholds to those given by the Bonferroni argument[7,8]

Table S1. List of all genes on breast cancer testing panels.

Supplier	Trucating Breast Cancer Associated	Truncating RR>2	Truncating RR>4	Missense Risk Associated	Ambry-Genetics BreastNext	Breast Health UK	Centogene	Emory-genetics	Fulgent Clinical Diagnostics	Gene Dx	Illumina TruSeq	Invitae	Myriad	Myriad - breast cancer related	University of Washington	BROCA - breast cancer related	Breast cancer any panel	Table	Analysed	Estimate_possible	P_value	Studies
Panel name					Breastnext	BreastGene	Breast-Ovarian Cancer-Panel	Breast Ovarian Cancer Panel	Breast-ovarian cancer NGS panel	OncogeneDx	TruSight Cancer	High-risk hereditary breast cancers	myRisk		BROCA Cancer Risk Panel							
AIP											yes											
ALK											yes											
AKT1															yes	yes	yes	2	yes	No		
APC									yes		yes		yes		yes		yes	2	yes	No		
ATM	YES	LIKELY	UNLIKELY	YES	yes	yes	yes		yes	yes	yes		yes	yes	yes	yes	yes	1	yes	Yes		3
ATR									yes						yes		yes	2	yes	No		
AXIN1									yes								yes	2	yes	No		
BAP1									yes		yes				yes		yes	2	yes	No		
BARD1					yes		yes		yes	yes			yes	yes	yes	yes	yes	2	yes	No		
BLM									yes		yes						yes	2	yes	Yes		Multiple
BMPR1A									yes		yes		yes		yes		yes	2	yes	No		
BRCA1	YES	DEFINITE	DEFINITE	YES	yes	yes		yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes	Yes		Many
BRCA2	YES	DEFINITE	DEFINITE	YES	yes	yes		yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes	Yes		Many
BRIP1					yes	yes	yes		yes	yes	yes		yes	yes	yes	yes	yes	2	yes	Yes		1
BUB1B											yes											
CDC73											yes											
CDH1	YES	LIKELY	UNKNOWN	UNKNOWN	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes	Yes		1
CDK4									yes		yes		yes		yes		yes	2	yes	No		
CDKN1C											yes											
CDKN2A									yes		yes		yes		yes		yes	2	yes	No		
CEBPA											yes											
CEP57											yes											
CHEK1															yes							
CHEK2	YES	LIKELY	UNLIKELY	YES	yes	yes	yes		yes	yes	yes		yes	yes	yes	yes	yes	1	yes	Yes		Multiple
CTNNA1															yes							
CTNNB1									yes								yes	2	yes	No		
CYLD											yes											
DDB2											yes											
DICER1											yes											
DIS3L2											yes											
EGFR											yes											
EPCAM									yes	yes	yes		yes		yes		yes	2	yes	No		
ERCC2											yes											
ERCC3											yes											
ERCC4											yes											
ERCC5											yes											
EXT1											yes											
EXT2											yes											
EZH2											yes											
FAM175A											yes				yes	yes	yes	2	yes	No		
FANCA											yes											
FANCB											yes											
FANCC									yes	yes	yes						yes	2	yes	No		
FANCD2											yes											
FANCE											yes											
FANCF											yes											
FANCG											yes											
FANCI											yes											
FANCL											yes											
FANCM											yes							2	yes	Yes		1
FH											yes											
FLCN											yes											
GALNT12															yes							
GATA2											yes											
GPC3											yes											
GEN1															yes	yes	yes	2	yes	Yes		1
GREM1															yes							
HNF1A											yes											
HOXB13									yes						yes		yes	2	yes	Yes		3
HRAS											yes											
KIT											yes											
MAX											yes											
MEN1											yes				yes			2	yes	Yes		2
MET											yes											
MLH1									yes	yes	yes		yes		yes		yes	2	yes	No		
MRE11A					yes		yes		yes						yes	yes	yes	2	yes	No		
MSH2									yes	yes	yes		yes		yes		yes	2	yes	No		
MSH6							yes		yes	yes	yes		yes		yes		yes	2	yes	No		

MUTYH					yes				yes		yes		yes		yes		yes	2	yes	Yes		1
NBN	YES	LIKELY	UNLIKELY	UNKNOWN	yes		yes		yes	yes	yes		yes	yes	yes	yes	yes	1	yes	Yes		Multiple
NF1	YES	LIKELY	UNLIKELY	UNKNOWN	yes						yes							2	yes	Yes		2
NF2											yes											
NSD1											yes											
PALB2	YES	LIKELY	UNKNOWN	UNKNOWN	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes	Yes		3
PALLD									yes								yes	2	yes	No		
PHOX2B											yes											
PIK3CA															yes	yes	yes	2	yes	No		
PMS1											yes											
PMS2									yes	yes			yes		yes		yes	2	yes	No		
POLD1															yes							
POLE															yes							
PPM1D																		2	yes	Yes		1
PRF1											yes											
PRKAR1A											yes											
PRSS1															yes							
PTCH1											yes											
PTEN	YES	UNKNOWN	UNKNOWN	YES	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes			
RAD50					yes		yes		yes								yes	2	yes	Yes		3
RAD51							yes		yes								yes	2	yes	No		
RAD51B															yes							
RAD51C					yes		yes		yes	yes	yes		yes	yes	yes	yes	yes	2	yes	Yes		1
RAD51D					yes				yes	yes	yes		yes		yes	yes	yes	2	yes	Yes		2
RB1											yes											
RECQL4											yes											
RET											yes				yes							
RHBDF2											yes											
RINT1																		2	yes	Yes		1
RUNX1											yes											
SBDS											yes											
SDHAF2											yes											
SDHB											yes				yes							
SDHC											yes				yes							
SDHD											yes				yes							
SLX4											yes				yes							
SMAD4									yes		yes		yes		yes		yes	2	yes	No		
SMARCB1											yes											
STK11	YES	UNKNOWN	UNKNOWN	UNKNOWN		yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes			
SUFU											yes											
TMEM127											yes											
TP53	YES	DEFINITE	DEFINITE	YES	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	1	yes	Yes		1
TP53BP1																						
TSC1											yes											
TSC2											yes											
VHL									yes		yes				yes		yes	2	yes	No		
XRCC2									yes	yes					yes	yes	yes	2	yes	No		
XRCC3									yes								yes	2	yes	No		
WRN											yes											
WT1											yes											
XPA											yes											
XPC											yes											

Table S2. Other genes for which protein-truncating variants have been suggested to be associated with breast cancer, or are present on breast cancer testing panels, but for which the association has not been established.

Gene	Comments	Estimated RR (90%CI)	*P*-value	Other associated cancers[2]	References
AKT1	Germline *AKT1* mutations predispose to rare form of Cowden like syndrome. Breast cancer risk unknown.	-			[9]
APC	No published evaluation of risk.	-		Colorectal	
ATR	No published evaluation of risk.	-			
AXIN1	No published evaluation of risk.	-		Colorectal	
BAP1	Case reports of breast cancers in families segregating germline *BAP1* mutations – no systematic study	-		Uveal and cutaneous melanoma	[10]
BARD1	Deleterious mutations found ~9/1824 triple negative cases. No published evaluation of risk.	-			[11]
BLM	Evidence relates to p.Gln548Ter in Slavic populations and c.2207_2212delATCTGAinsTAGATTC in Ashkenazim. Evidence of increased breast cancer risk in homozygotes.	2.4 (1.6-3.6),	0.0002	Colorectal	[12,13]
BMPR1A	Germline mutations predispose to Juvenile Polyposis Syndrome. No published evaluation of breast cancer risk.	-		Colorectal	
BRIP1	Single case-control study of familial cases Most data for R798X	2.0 (1.3-3.0)	0.012	Ovary	[14]
CDK4	Case reports in families – no published evaluation of risk	-		Melanoma	[15]
CDKN2A	Case reports in families – no published evaluation of risk	-		Melanoma, pancreas	
CTNNB1	No published evidence	-			
EPCAM	No evidence on truncating mutations. Suggestive evidence for association for missense variant p.Thr115Met	-		Colorectal	[16]
FAM175A	No evidence of truncating mutations in high-risk families. No published evaluation of risk.	-			[17]
FANCC	Evidence from one exome sequencing study plus replication (4/1395 cases vs. 0/2210 controls)	-	0.02		[18]
FANCM	Evidence from one exome sequencing study plus targeted genotyping of nonsense variant (p.Gln1701Ter)	1.9 (1.3-2.6)	0.002		[19]
GEN1	Most data relate to polymorphic truncating mutation c.2515_2519delAAGTT , ~4% frequency	1.1 (0.81-1.5)	0.63		[20,21]
HOXB13	Analyses relate to p.Gly84Glu prostate cancer susceptibility variant	1.6 (0.98-2.8)	0.11	Prostate	[22-24]
MEN1	Suggestive evidence from cohort *MEN1* carriers	2.0 (1.5-2.6)	$2x10^{-5}$	Pituitary, parathyroid and pancreatic neuroendocrine tumors	[25]
MLH1	Evidence from cohort analyses in lynch-syndrome families inconclusive. 3.95 (1.59- 8.13), *P*=.001 for mismatch repair gene mutations combined, in one prospective study	-		Colorectal, endometrial, ovary	[3]
MRE11A	Two mutations in 8 multiple case breast cancer families with tumors that showed loss of all	-	-		[26,27]

Gene	Comments	Estimated RR (90%CI)	*P*-value	Other associated cancers[2]	References
	three MRN proteins. Combined analysis of truncating and rare missense variants affecting key functional domains in *MRE11A*, *NBN* and *RAD50*: OR 2.88 (1.22-6.78) P=.02.				
MSH2	see *MLH1*	-		Colorectal, endometrial, ovary	[3]
MSH6	See *MLH1*	-		Colorectal, endometrial, ovary	[3]
MUTYH	Suggestive evidence for increased breast cancer risk in MAP patients homozygote for MUTYH mutations. One case-control study found no evidence of increased risk.	1.3 (0.86-2.1)	0.26	Gastro-intestinal	[28-30]
PALLD	No published evaluation of risk	-			
PIK3CA	Germline *PIK3CA* mutations predispose to rare form of Cowden-like syndrome. Breast cancer risk unknown.	-			[9]
PMS2	See *MLH1*	-		Colorectal, endometrial, ovary	[3]
PPM1D	Association in one case-control study. Genotypes mosaic lymphocytes, not inherited	15.3 (3.3-350)	0.0002	Ovary	[31]
RAD50	Analyses based on four case-control studies, three of Finnish founder variant c.697delT	2.20 (0.98-4.7)	0.11		[27,32-35]
RAD51	No evidence of association. No truncating variants found in large case-control study.	-			
RAD51C	Initial evidence for association through breast-ovarian cancer families, but little evidence for breast cancer risk after adjustment for ovarian cancer risk in family-based analysis or population-based case-control data.	0.91 (0.50-1.7)	0.79	Ovary	[36-38]
RAD51D	Evidence for association in breast-ovarian families but no evidence of breast cancer association after adjustment for ovarian cancer risk	1.3 (0.68-2.5)	0.49	Ovary	[39,40]
RINT1	Suggestive evidence from exome sequencing and targeted replication.	3.2 (1.5-7.0)	0.013		[41]
SMAD4	Germline mutations predispose to Juvenile Polyposis Syndrome. No published evaluation of breast cancer risk.	-			
VHL	No published evaluation of breast cancer risk	-			
XRCC2	Suggestive evidence exome sequencing followed by replication case-control study (truncating + rare likely deleterious missense).)	-	0.02		[42]
XRCC3	No published evaluation of breast cancer risk.	-	-		[42]

Table S3. Summary of analyses used to derive risk estimates by gene.

Gene[1]	Number of studies	Designs	Notes	References
ATM	3	Kin-cohort	Meta-analysis of estimates from three cohort studies of cancer incidence in relatives of Ataxia-Telangiectasia patients[43-45]. The case-control of Renwick et al[14] was not included as it was based on familial cases, but the point estimate, adjusting for family history, was similar (2.4, 90%CI 1.6-3.5, P = 0.0003).	[43-45]
BLM	6	Case-control	The meta-analysis combined the results of the meta-analysis of six studies reported by Prokofyeva et al[12] with the additional study of Anisimenko et al[13]. All studies pertain to the p.Gln548X variant in Slavic populations and c.2207_2212delATCTGAinsTAGATTC in Ashkenasim.	[12,13]
BRCA1		Segregation analysis	Estimates derived from BOADICEA model, based on risks to age 80 years for a woman born in 1960 (http://*ccge.medschl.cam.ac.uk/boadicea/*) [2] . Combined analyses of kin-cohort studies[46,47] and prospective studies[48] give similar estimates.	
BRCA2		Segregation analysis	See *BRCA1*	
BRIP1	1	Family-based case-control	One case-control study including familial cases, with adjustment for ascertainment. No population-based estimates yet available.	[14]
CDH1	1	Segregation	Segregation analysis in families ascertained on the basis of diffuse gastric cancer in which a *CHD1* mutation was identified. The analyses corrected for ascertainment, but since the ascertainment process was uncertain this estimate is potentially subject to bias. No population-based estimates available.	[49]
CHEK2	27	Case-control	Estimated odds ratios for the c.1100delC variant have been reported in combined analyses of case-control studies by the CHEK2 Breast Cancer Case-Control Consortium[50] and by Weischer et al[51]. In the combined analysis, five studies included in both analyses were excluded from the former meta-analysis. An estimate based on familial cases[52 53] was not included.	[50,51]
FANCM	1	Case-control	One estimate from a case-control study of a Finnish founder variant, p.Gln1701Ter, following an initial exome-sequencing study	[19]
GEN1	2	Case-control	Combined analysis of two case-control studies. The study of Kuligina et al[21] may be enriched for bilateral cases.	[20,21]

HOXB13	3	Case-control	Analyses relate the missense variant p.Gly84Glu, which is known to be associated with prostate cancer risk. Meta-analysis of three case-control studies. No analyses of truncating variants have been published.	[22-24]
MEN1	2	Kin-cohort	Combined analysis of two retrospective cohort studies of carriers in families *MEN1*.	[25]
MUTYH	1	Case-control	Estimate from one case-control study. Other family-based analyses not included as these are subject to potential ascertainment bias.	[30]
NBN	10	Case-control	Estimate from recent meta-analysis of ten case-control studies of the c.657del5 population in Slavic populations.	[4]
NF1	2	Prospective cohort, retrospective cohort	Combined analysis of two cohort studies (one prospective, one retrospective) of NF1 patients. The studies of Walker *et al.*[54] and Sharif *et al.*[55] were excluded as they may overlap significantly with the larger UK study of Seminog and Goldacre[56]	[56,57]
PALB2	3	Kin-cohort + segregation analysis, 2 case-control	Combined analysis of a family-based analysis[1] and two case-control studies (both of the Finnish founder mutation c.1592delT). The former analysis was based on analysis of population-based series of breast cancer patients, or selected families, in which truncating *PALB2* mutations were identified. The analysis corrected for ascertainment, but the estimate may not be comparable to the case-control estimates if there are *PALB2* specific modifiers. The reported absolute risk, and 95%CI, by age 70 were converted to a relative risk estimate.	
PPM1D	1	Case-control	Single estimate from case-control study of Ruark et al[31]. Estimate relates to carrying a mosaic variant. Study may be enriched for cases with a family history.	[31]
RAD50	3	Case-control	Combined analysis of three case-control studies, two of which relate to the Finnish founder variant c.687delT.	[27,32,33]
RAD51C	1	Family-based segregation analysis	Single estimate, based on family-based study adjusted for ovarian cancer risk.	[37]
RAD51D	2	Case-control, Family-based segregation analysis	Combined analysis of one case-control study[40] and one family-based study, adjusted for ovarian cancer risk[39].	[39,40]
RINT1	1	Case-control	Analysis of one case-control replication study, following initial exome sequencing study.	[41]
TP53	1	Segregation analysis	Estimate from one segregation analysis, based on families ascertained through sarcoma probands. Other studies of Li-Fraumeni families subject to ascertainment bias.	[58]

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