Penetrance estimates for *BRCA1*, *BRCA2* (also applied to Lynch syndrome) based on presymptomatic testing: a new unbiased method to assess risk?

Running title: Penetrance estimates from pre-symptomatic testing

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Competing Interests

Abstract (199)

Purpose:The identification of *BRCA1*, *BRCA2* or mismatch repair (MMR) pathogenic gene variants in familial breast/ovarian/colorectal cancer families facilitates predictive genetic testing of at risk relatives. However, controversy still exists regarding overall lifetime risks of cancer in individuals testing positive.

Methods:We assessed the penetrance of *BRCA1*, *BRCA2, MLH1* and *MSH2* mutations in men and women using Bayesian calculations based on ratios of positive to negative pre-symptomatic testing by 10-year age cohorts. Mutation position was also assessed for *BRCA1*/*BRCA2.*

Results:Using results from 2264 pre-symptomatic tests in First Degree Relatives (FDRs) of mutation carriers in *BRCA1* and *BRCA2* and 646 FDRs of patients with MMR mutations, we assessed overall associated cancer penetrance to age of 68 years as 73% (95% CI:61-82%) for *BRCA1*, 60% (95%CI:49-71%) for *BRCA2*, 95% (95% CI:76-99%) for *MLH1* and 61% (95% CI:49-76%) for *MSH2*. There was no evidence for significant penetrance for males in *BRCA1* or *BRCA2* families and males had equivalent penetrance to Lynch syndrome females. Mutation position and degree of family history influenced penetrance in *BRCA2* but not *BRCA1*.

*Conclusion:*We describe a new method for assessing penetrance in cancer prone syndromes. Results are in keeping with published prospective series and present modern day estimates for overall disease penetrance that bypasses retrospective series biases.

Key words: Penetrance, *BRCA1, BRCA2,* breast cancer, *MLH1, MSH2*

Introduction

Since the original identification of the *BRCA1*, *BRCA2*, *MSH2* and *MLH1* genes [1-4] between 1993-1995, much research, particularly in *BRCA1/2,* has focussed on estimations of overall lifetime cancer risk associated with mutations in these genes [5-10]. Clearly, calculated cancer risks are dependent on the method of ascertainment of the families studied. Therefore, breast/ovarian cancer risks in large familial breast/ovarian cancer kindreds with *BRCA1/BRCA2* mutations [5,9,10], were substantially greater than risks calculated from population based studies [6,7,8]. In the high-risk families that recruited to the Breast Cancer Linkage Consortium (BCLC) cohort, *BRCA1* and *BRCA2* mutations were estimated to cause a cumulative lifetime risk of breast cancer by age 70 years of 85–87% and 77–84% respectively [5,9,10]. However, estimates of breast cancer risks by age 70 years derived from previous population based studies, are much lower at 28-60% [6-8] for *BRCA1,* and 25-60% for *BRCA2*. It has been suggested that even these studies may overestimate the effect of the *BRCA1/2* mutation alone [11]. A meta-analysis of population based studies provided risks to 70 years of 57% (95% CI, 47% to 66%) for *BRCA1* and 49% (95% CI, 40% to 57%) for *BRCA2* mutation carriers[12]. These retrospective studies are subject to a number of biases [13]. By definition, family based studies require a family history of breast cancer and in the case of the BCLC, four affected relatives with breast cancer aged <60 years of age or ovarian cancer at any age. By definition therefore, each family contained an 'index' tested case and additional cases of younger onset breast cancer who would likely have been presumed carriers. Many of the analyses in these studies were based on assumed mutation status as many at risk relatives were not tested. Even after excluding index cases, most 'at risk' relatives were in previous generations. Risks of cancer in birth cohorts prior to 1937 is of little relevance to current birth cohorts as

it is clear that current risks in both the general population and *BRCA1/2* families is higher [14-16]. The most important risk to an individual, is their own personal risk rather than an averaged assessment based on the effects of *BRCA*1/*BRCA2* or *MLH1/MSH2* alone. The majority of women being tested currently still have some family history of breast cancer. In reality women with a *BRCA1* or *BRCA2* mutation may have risks in a range as large as 20-95%. Many women may be basing decision making regarding risk reducing surgery on this information and it is therefore important to try to guide them as to where they sit within that range, incorporating both family history and other risk factors [17].

Due to the inherent flaws of retrospective assessments, prospective studies are now addressing these risks. These are less biased as they represent current risks for women today and can be adjusted for degrees of family history [18,19,20]. However, these studies are also affected by shortcomings. Follow up is still short (only cloned the genes within the last 25 years) and as such, any biases in terms of failure of notification of cancers or actions that an individual may take to alter a diagnosis could affect assessments. About 30% of women undertake risk reducing mastectomy within 2 years of presymptomatic testing [17]. Two–three percent of these are identified with occult breast cancers [21] which could exaggerate risks. Additionally, if women have their first MRI scan soon after gene testing, this could result in a lead time bias by advancing the date of diagnosis [22]. Over a long follow up period of 20 years or so this would have little effect but most prospective studies still have median follow up of <7 years.

A method of assessing penetrance that has received almost no attention to date is to assess penetrance from the results of pre-symptomatic tests in first degree relatives of a

mutation carrier who have a 50% initial *a priori* likelihood of a mutation. For

mutations without a phenotype such as cystic fibrosis heterozygotes, the likelihood of a

mutation would not alter from 50% with age unless the mutation had an effect on life

expectancy. In moderate to high penetrance conditions such as BRCA and Lynch

syndrome, the proportion of pre-symptomatic positive tests decreases with age

consistently with penetrance. It is possible to utilise this drop in proportion (using a

Bayesian calculation) to assess penetrance with age. The reverse of this has been used

in the past to assess the likelihood of an individual having a high penetrance disease

such as Huntington's disease or neurofibromatosis type 2 without symptoms at older

age without undertaking genetic testing [23]. To determine the most appropriate risks

for women attending clinical cancer genetics services we determined the cumulative

risks of breast and ovarian cancer for 1401 families with pathogenic *BRCA1*/2

mutations identified in North West and Central England covering a population of 10

million and in North West England and East Anglia (population 7 million) for 568

Lynch syndrome using results of pre-symptomatic testing.

Methods

Index cases and relatives

Breast and ovarian cancer (BRCA) and Lynch syndrome families have been tested for *BRCA1/2*, and *MLH1* and *MSH2* mutations respectively (using a whole gene analysis including a test for large deletions) since 1996 in the overlapping regions of Manchester and Birmingham in mid-north England and the geographically separate East Anglia through the East Anglian Medical Genetics Service. All cancer confirmations and genetic testing is undertaken with informed consent. The study was carried out with Local Ethical committee approval. Once a *BRCA1/2 or MSH2/MLH1* mutation is identified, further extensive attempts are made to ensure that all individuals at risk of inheriting the family mutation are represented on the pedigree. All cases of breast or abdominal cancers are confirmed by means of hospital/pathology records, from the Regional Cancer Registries (data available from 1960) or from death certification. Once a family specific pathogenic mutation is identified predictive testing is offered to all blood relatives. Where possible all those affected by breast/ovarian/colorectal or other relevant cancer are tested to establish the true extent of *BRCA1/2* or *MSH2/MLH1* involvement in the family. In many large families it is possible to establish "obligate" mutation carriers by testing for the same mutation in different branches of the family, thereby establishing that intervening relatives carry the same mutation (these were excluded from the analysis).

All *BRCA1/2* or *MLH1/MSH2* mutation carriers identified were included in this study, and their details, those of all tested relatives and first-degree untested female/male relatives were entered onto a Filemaker Pro 5 database. The initial individual in which a mutation was identified was designated the "index" case, with all other individuals

being classified as to their position in the pedigree compared to a proven mutation carrier. All men and women who have undergone pre-symptomatic testing as a first degree relative (FDR) of a proven mutation carrier were included in the present study. Individuals with breast, ovarian, prostate or pancreatic cancer were considered to be undergoing 'diagnostic' rather than pre-symptomatic testing for *BRCA1/2* and were excluded. Similarly those diagnosed with a Lynch associated malignancy including colorectal, small bowel, endometrial, ovarian, gastric, transitional cell carcinoma of the urinary tract, glioma, or prostate cancer were excluded from analysis as pre-symptomatic tests for *MLH1/MSH2*.

The proportion testing positive and negative pre-symptomatically in 10-year age cohorts: <30 years 30-39 years, 40-49 years, 50-59 years and 60+ years was recorded. Obligate carriers who were unaffected but had an affected offspring or those who were the parent of a carrier were excluded. Penetrance assessment was undertaken when the proportion testing positive dropped below 40% otherwise it was assumed that the likelihood was too close to 50% to validate an uplift in penetrance.

Penetrance estimates for related cancer incidence for each gene were derived and also by dividing each gene into the previously identified ovarian cancer cluster region (OCCR): exon 11 (nucleotides c.2281to c.4071) for *BRCA1* and exon 11 (nucleotides c.2830 to c.6401) for *BRCA2* (wide definition)[20,24]. For *BRCA1* we used the nucleotide range identified by the BCLC [20,25,26], which, although not traditionally called an OCCR it is the published region with the highest proportional risk of ovarian cancer. The Manchester scoring system was used to assess the strength of the breast/ovarian cancer history [27]. This system was devised to assess the likelihood of a

BRCA1/2 mutation and scores breast and ovarian cancers individually in the family, giving a higher score the younger the age at diagnosis [27]. Each breast and ovarian cancer in a direct lineage is scored with between one point for each gene (breast cancer aged 60+) to 6/5 points for *BRCA1/2* for a breast cancer under 30-years of age and 8/5 points for an ovarian cancer aged <60-years. A combined score of 20 reflects a 20% likelihood of identifying a *BRCA1/2* mutation.

Penetrance assessment of unaffected FDR relatives undertaking predictive genetic testing

The penetrance assessment assumes that there is a 50% likelihood of inheriting the family mutation for siblings and offspring (FDRs) of a proven mutation carrier. If it is assumed for 100 FDRs that half develop an associated cancer by a certain age then of 50 assumed carriers, 25 will have developed cancer. The ratio of mutation positive to negative will be 25:50 or 1:2 with a 33% chance of testing positive if unaffected at that age. For 20% penetrance, the ratio of positive to negative is 0.8:1 while for 33% penetrance the ratio is 0.67:1. For 67% penetrance means that 25% women are positive or a 1:3 ratio.

Penetrance estimates are not provided for *MSH6* and *PMS2* as the numbers were too small.

Results

A total of 2264 unaffected first degree relatives from 1401 index families have undertaken pre-symptomatic testing for *BRCA1* and *BRCA2* gene mutations between 1996-2017. In *BRCA1* families, 364/839 (43.4%) women and 161/307 (52.4%) men tested positive, with 383/817 (46.9%) of women and 159/301 (52.8%) of men testing positive in *BRCA2* families (Table 1). The range (median) of birth years for those testing positive was 1914-1998 (1969).

Whilst the proportion of women testing positive for *BRCA1* dropped below 40% aged 40-49 years (p=0.026), this did not occur in *BRCA2* until aged 50-59 (p=0.12) and was not significantly below 50% until aged 60+ (p=0.01). Assessment of penetrance in women based on breast or ovarian cancer diagnosis is presented in table 2. The median age for those testing age 60+ was 68 for both genes equating to cancer penetrance of 70% for *BRCA1* and 56% for *BRCA2* by age 68 years.

Amongst men (unaffected by breast, pancreas or prostate cancer) the ratio testing positive never dropped significantly below 50% with the older *BRCA2* tests actually having a ratio above 50%. Nevertheless we are yet to see a *BRCA2* related prospective cancer in the 46 testing positive aged 60+ years.

Proportions using Manchester score (Manchester cases only)

For females, the proportion testing positive for *BRCA1* with Manchester score below 20 and 20+ decreased after age 50 years with non-significantly higher proportion testing negative for families with score <20. As for *BRCA1*, the proportion testing positive for *BRCA2* with Manchester score of 20+ also decreased after age 50 years which was not seen for those from families with scores of <20. Proportional differences for BRCA2 were significantly different at age 60+ (p=0.01)(table 3). Penetrance estimates in *BRCA2* families with a Manchester score of 20+ were equivalent to *BRCA1* with a penetrance estimate of 71% by age 68.

10

Prospective cancers

In total, 892 women have had a positive pre-symptomatic test, including 747 FDRs of a proven carrier included in the analysis above. Sixty women (6.6%) have been diagnosed with breast cancer since pre-symptomatic testing (*BRCA1*=31; *BRCA2*=29). Nineteen (32%) were diagnosed within 12 months of predictive testing (*BRCA1*=8; *BRCA2*=11) and nine within 6-months. None were symptomatic at time of diagnosis and six were picked up with a prevalence MRI scan with breast cancers of 10mm in size or less. Twenty women had undergone bilateral risk reducing mastectomy (BRRM) 0.7-16.2 (median 3.6) years prior to pre-symptomatic testing. A further 251 underwent BRRM after testing but 28 as a result of a prospective breast cancer diagnosis. Five of the remaining 223 (2.2%) had an occult breast cancer at surgery. The rate of breast cancer in the first year post test was 2.8% compared to 1.5% and 1.8% in years two and three post testing when censored at last follow up, mastectomy or breast cancer diagnosis. Seven prospective (*BRCA1*=4; *BRCA2*=3) ovarian cancers have occurred with two identified as occult at risk reducing surgery within one year of genetic testing. Sixty two women had undergone risk reducing oophorectomy (0.2-42.7; median 5.76 years) before testing. Seven *BRCA1* and 7 *BRCA2* carriers testing positive including an 86 year old *BRCA1* carrier (surgery 42.7 years before) had undergone oophorectomy >5 years before testing. Information on those testing negative was not complete with only 24 recorded as having surgery before testing and only 3 >5 years before in the >60 year group. After testing a further 254 have already undertaken risk reducing oophorectomy 143 within 12-months.

OCCR region

Ratios for those tested after 50 years when rates of positives have dropped for both genes showed some evidence for an OCCR effect on penetrance. For *BRCA1* 14/62 (22.6%) tested positive in the OCCR with a median age of 58.18 years and 42/126 (33.3%) of those from families outside the region at a median age of 57.95 (p=0.17). For *BRCA2* 37/86 (43%) tested positive in the OCCR at a median age of 60.13 whereas this fell to 39/126 (30.9%) at a median age of 57.5 (p=0.06).

Proportion of FDR testing negative

No adjustment was made for those FDRs that had previously developed breast cancer and tested negative. Overall 33/292 (11.4%) of FDRs (index excluded) tested negative for the family *BRCA1* mutation and 41/342 (12%) for *BRCA2*. The rates for ovarian cancer were 3/151 (2%) and 2/65 (3%) respectively.

Results of classification of Lynch patients by *MLH1* and *MSH2* gene and age cohort:

In total 646 FDRs in *MLH1* (n=299; 154 female) and *MSH2* (n=347; 202 female) from a total of 568 MMR families have undergone pre-symptomatic testing in Manchester and Cambridge. The range of birth years of those testing positive was 1922-1996 (median 1972). There was evidence to support higher penetrance for *MLH1* as this was statistically significant for tests age 60 years and over and for all tests after age 40 years (table 4). Results for females and males separately are shown supplementary table 1. These showed similar penetrance levels of 76% in females and 67% in males by age 68 years. There were insufficient numbers to break these down by gene. There were also insufficient tests in *MSH6* (n=83) or *PMS2* (n=42) families to obtain useful results although testing ratios for *MSH6* were 18:9 postive:negative and for *PMS2* 14:10 aged >50years indicating no substantial penetrance.

Discussion

The present report demonstrates that it is possible to derive penetrance estimates for cancer genes on the basis of results of pre-symptomatic tests in FDRs stratified by age. We have shown that this confirms high penetrance consistent with estimates from recent prospective series [18-20] for *BRCA1* and *BRCA2* and for *MLH1* [28]. The results were not as convincing for *MSH2* with the ratio only dropping well below 50% in the 60+ year group. The results would be considerably strengthened by substantially increasing the number of test results in those over 50 years of age.

We confirmed evidence for greater overall penetrance for those with *BRCA2* mutations outside the OCCR but of borderline significance[20]. A large collaborative effort using prospective risks from a number of countries, estimated risks of breast cancer to 80 years of age of 72% (95%CI:65-79%) for *BRCA1* and 69% (95%CI:61-77%) for *BRCA2* carriers. *BRCA2* mutations outside the OCCR region were associated with a significantly greater breast cancer risk compared to those in the broad OCCR definition: HR=1.93 (95% CI:1.36-2.74)[20]. *BRCA1* mutations located outside the region bounded by positions c.2282 to c.4071 were associated with a significantly higher breast cancer risk compared to mutations within the region (HR=1.46 (95%CI:1.11-1.93), p=0.007[20]. This is at odds with our finding of a lower rate of positive predictive tests in *BRCA1* carriers within the region. This was, however, not significant and based on small numbers. Whilst there were large numbers in the collaborative study (5046 for both genes at baseline), the median follow up was only 5 years. We have demonstrated a substantial elevation in cancers in the first year post genetic test compared to the following two years. This is likely to be due to a lead time effect of baseline MRI along with (to a lesser extent) the identification of occult cancers at BRRM. It is not clear if such effects were excluded in the collaborative study [20]. Furthermore the end of the follow up period was classified by a variety of means including questionnaires with variable intervals, or cancer registry checks and some women were lost to follow up. It is likely that there is a margin of error with this short follow up time. Nonetheless, we also demonstrated an effect of family history on risks in particular in *BRCA2*. The collaborative study found a 1.91 (95%CI:1.08-3.37) fold risk of breast cancer for *BRCA2* carriers with a significant family history (two affected FDRs) (cumulative risks to age 70: 65% (95%CI: 56-74%) vs 39% (95%CI: 25-56%) for those without such history. We were unable to confirm the 1.99 fold relative risk for

BRCA1. Interestingly we found no evidence for substantial penetrance in males for either *BRCA1* or *BRCA2.* Although substantial increased risks of breast cancer, prostate cancer and pancreatic cancer are reported for *BRCA2* [29,30] these are less convincing for *BRCA1* [29,31].

The results for Lynch syndrome are in keeping with the higher penetrance of *MLH1* for colorectal cancer in both men and women [28,32]. The higher risks for extra-intestinal cancers for *MSH2* probably does not equate to similar combined penetrance aged <70 years [28,33]. Overall penetrance appears similar for men and women.

The results of the present study should not be taken as a replacement for prospective studies but, where a new gene is identified with a reasonably high frequency, less biased estimates for penetrance could be derived in a few years if substantial pre-symptomatic testing takes place and precedes prospective analysis. Whilst using pre-symptomatic test ratios in FDRs can only assess combined risks of relevant gene associated cancers rather than individual cancers, this is less problematic if there is an association with a single cancer type only. For example a gene such as *PALB2* (with mainly a breast cancer risk although there is a small pancreatic cancer risk) could produce unbiased penetrance estimates from pre-symptomatic testing in FDRs before prospective studies have matured sufficiently. The method would particularly suit a specific cancer predisposition that is rare in the general population.

There are some potential weaknesses of the current study. It has been assumed that the ratio of positive to negative mutation results is 50% at birth. There is currently no evidence to oppose this assumption. We have also not taken into account the protective

14

effects of risk reducing surgery. The number of patients undergoing risk reducing mastectomy prior to testing were small and are unlikely to have significantly altered the results; however seven *BRCA1* and 7 *BRCA2* mutation carriers aged >60 years at testing had undergone oophorectomy greater than 5 years prior to their predictive test. Therefore the contribution of ovarian cancer to penetrance could have been underestimated. Unfortunately we do not have complete data on those individuals testing negative for their familial *BRCA1/2* mutation to adjust for these cases. It has been assumed there is no testing bias in asymptomatic FDRs. It is possible that some apparently 'asymptomatic' individuals attending for pre-symptomatic testing harbour concerns about symptoms that could represent cancer. However, the cancers presenting in the first year post testing were all occult detected on surveillance. If there were such an effect this would also influence early results for prospective studies. We have also not taken into account cancer penetrance in those testing negative for the family mutation. The risk of breast cancer in the general population to age 68 years is about 7-8%, but <1% will have developed ovarian cancer. We have demonstrated both here and previously that rates may be higher in FDRs in highly ascertained families [34]. If anything, adjustment for this should have increased penetrance at age 68. For Lynch syndrome the combined effect of colorectal, endometrial ovarian and other associated cancers will have been lower. Table 5 shows adjustment for a 9% population risk of BRCA related cancers in women and 4% risk in both sexes of Lynch syndrome cancers in those testing negative. Overall the confidence intervals are still large in the oldest category where the final penetrance figures would be obtained, but this could be addressed with large-scale collaborative studies.

Conclusion

A new approach to assess penetrance using ratios of positive to negative pre-symptomatic test results in FDRs is presented. This demonstrates high predicted penetrance of 70% for *BRCA1*, 57% for *BRCA2* and 95% for *MLH1* by age 68 years although confidence intervals are large. More robust estimates could be obtained by accessing larger number of tests in individuals >70 years of age.

Author Contributions
Conception **Evans DG,**
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Data analysis **Evans DG, Harkness E**
Manuscript writing **All**
Approval of final version **All**

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References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994; 266(5182):66-71.
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995; 378(6559):789-792.
3. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell. 1993;75(5):1027-38
4. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature. 1994;368(6468):258-61.
5. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998; 62(3):676-689.
6. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997; 336(20):1401-1408.
7. Warner E, Foulkes W, Goodwin P, Meschino W, Blondal J, Paterson C, Ozcelik H, Goss P, Allingham-Hawkins D, Hamel N, Di Prospero L, Contiga V, Serruya C, Klein M, Moslehi R, Honeyford J, Liede A, Glendon G, Brunet JS, Narod S. Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. J Natl Cancer Inst 1999; 91(14):1241-1247.
8. Hopper JL, Southey MC, Dite GS, Jolley DJ, Giles GG, McCredie MR, Easton DF, Venter DJ. Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in BRCA1 and BRCA2. Australian Breast Cancer Family Study. Cancer Epidemiol Biomarkers Prev 1999; 8(9):741-747.
9. Cancer risks in BRCA2 mutation carriers. The Breast Cancer Linkage Consortium. J Natl Cancer Inst 1999; 91(15):1310-1316.
10. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994; 343(8899):692-695.
11. Begg CB. On the use of familial aggregation in population-based case probands for calculating penetrance. J Natl Cancer Inst 2002; 94(16):1221-1226
12. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol. 2007 Apr 10;25(11):1329-33.
13. Vos JR, Hsu L, Brohet RM, Mourits MJ, de Vries J, Malone KE, Oosterwijk JC, de Bock GH. Bias Correction Methods Explain Much of the Variation

Seen in Breast Cancer Risks of BRCA1/2 Mutation Carriers. J Clin Oncol. 2015;33(23):2553-62.

14. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643-6.

15. Tryggvadottir L, Sigvaldason H, Olafsdottir GH, Jonasson JG, Jonsson T, Tulinius H, Eyfjord JE. Population-based study of changing breast cancer risk in Icelandic BRCA2 mutation carriers, 1920-2000. J Natl Cancer Inst. 2006; 98(2):116-22.

16. Evans DG, Shenton A, Woodward E, Lalloo F, A Howell, Maher ER. Penetrance estimates for BRCA1 and BRCA2 based on genetic testing in a Clinical Cancer Genetics service setting. BMC Cancer 2008;8(1):155

17. Evans DG, Lalloo F, Ashcroft L, Shenton A, Clancy T, Baildam AD, Brain A, Hopwood P, Howell A. Uptake of risk reducing surgery in unaffected women at high risk of breast and ovarian cancer is risk, age and time dependent. Cancer Epid Biomarkers Prev 2009;18(8):2318-24

18. Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, Evans DG, Izatt L, Eeles RA, Adlard J, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Tischkowitz M, Douglas F, Hodgson S, Walker L, Porteous ME, Morrison PJ, Side LE, Kennedy MJ, Houghton C, Donaldson A, Rogers MT, Dorkins H, Miedzybrodzka Z, Gregory H, Eason J, Barwell J, McCann E, Murray A, Antoniou AC, Easton DF. Cancer Risks for BRCA1 and BRCA2 Mutation Carriers: Results From Prospective Analysis of EMBRACE. J Natl Cancer Inst. 2013;105(11):812-22

19. Evans DG, Harkness E, Lalloo F, Howell A. Long-term prospective clinical follow-up after BRCA1/2 presymptomatic testing: BRCA2 risks higher than in adjusted retrospective studies. J Med Genet. 2014;51(9):573-80.

20. Kuchenbaecker KB, Hopper JL, Barnes DR, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, Jervis S, van Leeuwen FE, Milne RL, Andrieu N, Goldgar DE, Terry MB, Rookus MA, Easton DF, Antoniou AC; and the BRCA1 and BRCA2 Cohort Consortium, McGuffog L, Evans DG, Barrowdale D, Frost D, Adlard J, Ong KR, Izatt L, Tischkowitz M, Eeles R, Davidson R, Hodgson S, Ellis S, Nogues C, Lasset C, Stoppa-Lyonnet D, Fricker JP, Faivre L, Berthet P, Hooning MJ, van der Kolk LE, Kets CM, Adank MA, John EM, Chung WK, Andrulis IL, Southey M, Daly MB, Buys SS, Osorio A, Engel C, Kast K, Schmutzler RK, Caldes T, Jakubowska A, Simard J, Friedlander ML, McLachlan SA, Machackova E, Foretova L, Tan YY, Singer CF, Olah E, Gerdes AM, Arver B, Olsson H.. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017 Jun 20;317(23):2402-2416.

21. Evans DGR, Baildam AD, Anderson E Brain A, Shenton A, Vasen HF, Eccles D, Lucassen A, Pichert G, Hamed H, Moller P, Maehle L, Morrison PJ, Stoppat-Lyonnet D, Gregory H, Smyth E, Niederacher D, Nestle-Krämling C, Campbell J, Hopwood P, Lalloo F, Howell A. Risk reducing mastectomy: outcomes in 10 European Centres. J Med Genet 2009; 46(4):254-8

22. Evans DGR, Lennard F, Pointon LJ, Ramus SJ, Gayther SA, Sodha N, Kwan-Lim GE, Leach MO, Warren R, Thompson D, Easton DF, Eeles R; UK Study of MRI Screening for Breast Cancer in Women at High Risk (MARIBS). Eligibility for MRI screening in the UK: Effect of strict selection criteria and anonymous DNA testing on breast cancer incidence in the MARIBS study. Cancer Epid Biomarkers Prev 2009;18(7):2123-31

23. Evans DGR, Newton V, Neary W, Baser ME, Wallace A, Macleod R, Jenkins JP, Gillespie J, Ramsden RT. Use of MRI and audiological tests in pre-symptomatic diagnosis of type 2 neurofibromatosis. J Med Genet 2000; 37:944-7.
24. Lubinski J, Phelan CM, Ghadirian P, Lynch HT, Garber J, Weber B, Tung N, Horsman D, Isaacs C, Monteiro AN, Sun P, Narod SA. Cancer variation associated with the position of the mutation in the BRCA2 gene. Fam Cancer. 2004;3(1):1-10
25. Thompson D, Easton D; Breast Cancer Linkage Consortium. Variation in BRCA1 cancer risks by mutation position. Cancer Epidemiol Biomarkers Prev. 2002;11(4):329-36.
26. Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, Mazoyer S, Chenevix-Trench G, Easton DF, Antoniou AC, Nathanson KL; CIMBA Consortium, Laitman Y, Kushnir A, Paluch-Shimon S, Berger R, Zidan J, Friedman E, Ehrencrona H, Stenmark-Askmalm M, Einbeigi Z, Loman N, Harbst K, Rantala J, Melin B, Huo D, Olopade OI, Seldon J, Ganz PA, Nussbaum RL, Chan SB, Odunsi K, Gayther SA, Domchek SM, Arun BK, Lu KH, Mitchell G, Karlan BY, Walsh C, Lester J, Godwin AK, Pathak H, Ross E, Daly MB, Whittemore AS, John EM, Miron A, Terry MB, Chung WK, Goldgar DE, Buys SS, Janavicius R, Tihomirova L, Tung N, Dorfling CM, van Rensburg EJ, Steele L, Neuhausen SL, Ding YC, Ejlertsen B, Gerdes AM, Hansen Tv, Ramón y Cajal T, Osorio A, Benitez J, Godino J, Tejada MI, Duran M, Weitzel JN, Bobolis KA, Sand SR, Fontaine A, Savarese A, Pasini B, Peissel B, Bonanni B, Zaffaroni D, Vignolo-Lutati F, Scuvera G, Giannini G, Bernard L, Genuardi M, Radice P, Dolcetti R, Manoukian S, Pensotti V, Gismondi V, Yannoukakos D, Fostira F, Garber J, Torres D, Rashid MU, Hamann U, Peock S, Frost D, Platte R, Evans DG, Eeles R, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Hodgson S, Morrison PJ, Walker L, Porteous ME, Kennedy MJ, Izatt L, Adlard J, Donaldson A, Ellis S, Sharma P, Schmutzler RK, Wappenschmidt B, Becker A, Rhiem K, Hahnen E, Engel C, Meindl A, Engert S, Ditsch N, Arnold N, Plendl HJ, Mundhenke C, Niederacher D, Fleisch M, Sutter C, Bartram CR, Dikow N, Wang-Gohrke S, Gadzicki D, Steinemann D, Kast K, Beer M, Varon-Mateeva R, Gehrig A, Weber BH, Stoppa-Lyonnet D, Sinilnikova OM, Mazoyer S, Houdayer C, Belotti M, Gauthier-Villars M, Damiola F, Boutry-Kryza N, Lasset C, Sobol H, Peyrat JP, Muller D, Fricker JP, Collonge-Rame MA, Mortemousque I, Nogues C, Rouleau E, Isaacs C, De Paepe A, Poppe B, Claes K, De Leeneer K, Piedmonte M, Rodriguez G, Wakely K, Boggess J, Blank SV, Basil J, Azodi M, Phillips KA, Caldes T, de la Hoya M, Romero A, Nevanlinna H, Aittomäki K, van der Hout AH, Hogervorst FB, Verhoef S, Collée JM, Seynaeve C, Oosterwijk JC, Gille JJ, Wijnen JT, Gómez Garcia EB, Kets CM, Ausems MG, Aalfs CM, Devilee P, Mensenkamp AR, Kwong A, Olah E, Papp J, Diez O, Lazaro C, Darder E, Blanco I, Salinas M, Jakubowska A, Lubinski J, Gronwald J, Jaworska-Bieniek K, Durda K, Sukiennicki G, Huzarski T, Byrski T, Cybulski C, Toloczko-Grabarek A, Złowocka-Perłowska E, Menkiszak J, Arason A, Barkardottir RB, Simard J, Laframboise R, Montagna M, Agata S, Alducci E, Peixoto A, Teixeira MR, Spurdle AB, Lee MH, Park SK, Kim SW, Friebel TM, Couch FJ, Lindor NM, Pankratz VS, Guidugli L, Wang X, Tischkowitz M, Foretova L, Vijai J, Offit K, Robson M, Rau-Murthy R, Kauff N, Fink-Retter A, Singer CF, Rappaport C,

Gschwantler-Kaulich D, Pfeiler G, Tea MK, Berger A, Greene MH, Mai PL, Imyanitov EN, Toland AE, Senter L, Bojesen A, Pedersen IS, Skytte AB, Sunde L, Thomassen M, Moeller ST, Kruse TA, Jensen UB, Caligo MA, Aretini P, Teo SH, Selkirk CG, Hulick PJ, Andrulis I. Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. JAMA. 2015;313(13):1347-61

27. Evans DGR, Eccles DM, Rahman N, Young K, Bulman M, Amir E, Shenton A, Howell A, Lalloo F. A new scoring system for the chances of identifying a BRCA1/2 mutation, outperforms existing models including BRCAPRO. J Med Genet 2004;41(6):474-480.

28. Møller P, Seppälä TT, Bernstein I, Holinski-Feder E, Sala P, Gareth Evans D, Lindblom A, Macrae F, Blanco I, Sijmons RH, Jeffries J, Vasen HFA, Burn J, Nakken S, Hovig E, Rødland EA, Tharmaratnam K, de Vos Tot Nederveen Cappel WH, Hill J, Wijnen JT, Jenkins MA, Green K, Lalloo F, Sunde L, Mints M, Bertario L, Pineda M, Navarro M, Morak M, Renkonen-Sinisalo L, Valentin MD, Frayling IM, Plazzer JP, Pylvanainen K, Genuardi M, Mecklin JP, Moeslein G, Sampson JR, Capella G; Mallorca Group. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. Gut. 2017 Jul 28. doi: 10.1136/gutjnl-2017-314057

29. Moran A, O'Hara C, Khan S, Shack L, Woodward E, Maher ER, Lalloo F, Evans DG. Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. Fam Cancer. 2012;11(2):235-42.

30. van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, Hoogerbrugge N, Verhoef S, Vasen HF, Ausems MG, Menko FH, Gomez Garcia EB, Klijn JG, Hogervorst FB, van Houwelingen JC, van't Veer LJ, Rookus MA, van Leeuwen FE; Netherlands Collaborative Group on Hereditary Breast Cancer (HEBON). Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. J Med Genet. 2005;42(9):711-9.

31. Streff H, Profato J, Ye Y, Nebgen D, Peterson SK, Singletary C, Arun BK, Litton JK. Cancer Incidence in First- and Second-Degree Relatives of BRCA1 and BRCA2 Mutation Carriers. Oncologist. 2016; 21(7):869-74.

32. Barrow E, Aldhuaj W, Robinson L, Shenton A, Clancy T, Lalloo F, Hill J, Evans DG. Colorectal cancer in HNPCC: Cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. Clin Genet 2008; 74(3):233-42

33. Barrow E, Robinson L, Aldhuaj W, Shenton A, Clancy T, Lalloo F, Hill J, Evans DG. Extracolonic cancers in HNPCC: Cumulative lifetime incidence and tumour distribution. A report of 121 families. Clin Genet 2009;75(2):141-9.

34. Smith A, Moran A, Boyd MC, Bulman M, Shenton A, Smith L, Iddenden I, Woodward E, Lalloo F, Rahman N, Maher ER, Evans DGR. The trouble with phenocopies: are those testing negative for a family BRCA1/2 mutation really at population risk? J Med Genet 2007;44(1):10-15;

Table 1: Number of presymptomatic unaffected FDR females and males undertaking predictive genetic testing for *BRCA1* and *BRCA2* by five age cohort

Predictive test result by age	*BRCA1* positive	*BRCA1* negative	% positive *BRCA1*	*BRCA2* Positive	*BRCA2* Negative	% positive *BRCA2*
FEMALES						
18-30 yrs	96	94	50.53%	73	76	48.99%
30-39 yrs	134	122	52.34%	134	112	54.47%
40-49 yrs	80	126	38.83%	101	111	47.64%
50-59 yrs	35	70	33.33%	48	73	39.67%
60+	19	63	23.17%	27	62	30.34%
total	364	475	364/839 43.4%	383	434	383/817 46.88%
MALES						
18-30 yrs	19	9	67.86%	15	13	53.57%
30-39 yrs	30	27	52.63%	27	25	51.92%
40-49 yrs	27	34	44.26%	37	36	50.68%
50-59 yrs	53	41	56.38%	34	40	45.95%
60+	32	35	47.76%	46	28	62.16%
Total	161	146	161/307 52.44%	159	142	159/301 52.82%

Table 2: Penetrance of *BRCA1* and *BRCA2* genes assessed among presymptomatic unaffected FDR females undertaking predictive genetic testing by gene and age cohort.

Predictive test result by age	*BRCA1* positive	*BRCA1* negative	Positive to negative ratio	Penetrance	*BRCA2* Positive	*BRCA2* Negative	Positive to negative ratio	penetrance	P value
18-30 yrs	96	94	1.02	0	73	76	0.96	0	-
30-39 yrs	134	122	1.10	0	134	112	1.20	0	-
40-49 yrs	80	126	0.63	0.37	101	111	0.91	0.09*	0.07
50-59 yrs	35	70	0.5	0.5	48	73	0.66	0.34	0.33
60+	19	63	0.30	0.70	27	62	0.43	0.57	0.30
Total	364	475			383	434			

*Penetrance estimate is provided but as the proportion testing positive was >40% it is unlikely that this differs substantially from 0% (see methods)

Table 3: proportion of predictive tests positive in unaffected FDR females of five age cohort by family Manchester score 20+ and <20 for *BRCA1* and *BRCA2* genes

BRCA1	Positive <20	Negative <20	Proportion positive <20 %	Positive 20+	Negative20+	Proportion positive 20+ %	P value
18-30 yrs	25	18	58%	63	67	48%	Ns
30-39 yrs	38	18	68%	77	97	44%	Ns
40-49 yrs	14	16	47%	59	103	36%	Ns
50-59 yrs	4	18	18%	27	45	38%	Ns
60+	4	13	24%	10	41	20%	Ns
BRCA2	Positive <20	Negative <20	Proportion +ve <20 %	Positive 20+	Negative 20+	Proportion +ve 20+ %	P value
18-30 yrs	23	19	55%	46	54	46%	Ns
30-39 yrs	39	31	56%	87	75	54%	Ns
40-49 yrs	27	15	64%	72	86	46%	Ns
50-59 yrs	18	19	49%	26	50	34%	0.15
60+	12	13	48%	10	35	22%	0.05

Table 4: Number of pre-symptomatic unaffected FDR females and males undertaking predictive genetic testing for *MLH1* and *MSH2* genes by age cohort

Predictive test result by age	Number positive *MLH1*	Number negative *MLH1*	Proportion positive *MLH1* %	Ratio	Penetrance estimate
18-30 yrs	36	43	45.56%	0.84	
30-39 yrs	42	41	50.60%	1.02	
40-49 yrs	24	52	32.89%	0.46	0.54
50-59 yrs	14	26	37.5%	0.54	0.46
60+	1	19	5%	0.052	0.95 P=0.034*
Total	117	181	117/298		
	Number positive *MSH2*	Number negative *MSH2*	Proportion positive *MSH2* %	Ratio	
18-30 yrs	46	41	52.87%	1.12	
30-39 yrs	44	47	48.35%	0.94	
40-49 yrs	28	39	41.79%	0.72	
50-59 yrs	22	19	53.66%	1.16	
60+	17	44	27.87%	0.39	0.61
Total	157	190	157/190		

For positive pre-symptomatic tests >40 years of age *MLH1* significantly less likely to be positive p=0.05

Table 5: Adjusted penetrance estimates to 68 years of age for *BRCA1, BRCA2, MLH1* and *MSH2* genes taking into account cancer incidence in negative testing group

Predictive test result by age	Number positive *MLH1*	Number negative *MLH1*	Ratio	Penetrance estimate	95% CI
BRCA1	19	69	0.27	0.73	0.61 – 0.82
BRCA2	27	68	0.40	0.60	0.49 – 0.71
BRCA2 MSS 20+	10	38	0.26	0.74	0.58 – 0.85
MLH1	1	20	0.05	0.95	0.76 - 0.99
MSH2	17	46	0.39	0.61	0.49 – 0.76

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