Guide to Genetic Testing in Breast Cancer v4.0

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Originally identified in 1994, pathogenic variants in *BRCA1* and *BRCA2* are the leading cause of hereditary breast and ovarian cancer and are responsible for 15-25% of familial cases[1 2]. Next Generation sequencing has allowed causative variants in other genes to be identified. Genes associated with an increased risk of developing breast cancer can be categorised as conferring either very high or moderate risk. However, there are many other modifying genetic factors[3] which contribute to a person's risk of developing cancer and thus an individual's family history is still an important consideration when calculating individual risk and making management decisions[4].

Definition of Risk Categories

www.nice.org.uk/conditions-and-diseases/cancer/breast-cancer

General population:	*11% lifetime chance of developing breast cancer equates to 110 women in every 1,000* *Most breast cancers develop after the age of 50.*
Moderate risk:	*17-30% lifetime chance of developing breast cancer equates to 170-300 women in every 1,000* *3 to 8% chance of breast cancer between 40 and 50 years. Equates to 30-80 women in every 1,000 developing breast cancer between the ages of 40 and 50 years.*
High risk:	*>30% lifetime chance of developing breast cancer equates to >300 women in every 1,000* ***>****8% chance of developing breast cancer between the ages of 40 and 50 equates to >80 women in every 1,000 developing breast cancer between 40 and 50years.*

Very High Risk:	*>40% lifetime chance of developing breast cancer*
	>12% chance of developing breast cancer between the ages of 40 and 50

General Principles of Breast Cancer Risk Management

All women at population risk are currently eligible for 3 yearly mammography from the age of 50 years through the National Breast Screening Programme. Women at moderate are also offered annual mammography screening from 40-49 years (or 5 years younger than the youngest age of onset in the family, although not usually below 35 years). Women at high risk are offered additional mammography screening from 35 (or 5 years younger than the youngest age of onset in the family, although not usually below 35 years). Mammography for both moderate and high risk groups is every 2 years from 35-40years. Women at high risk are offered annual mammography from 40 to 60years and then every 18months from 60-70years. Women at very high risk are offered annual MRI in addition to mammography, and starting from a younger age, due to the higher sensitivity of MRI compared to mammography[5]. Only women at high risk and above should be considered for prophylactic surgery. Chemoprevention is recommended in women at high or very high risk and can be considered in women at moderate risk.

Genes Associated with an Increased Risk of Breast Cancer

Genes in which pathogenic variants are associated with a very high risk of breast cancer development include *BRCA1*, *BRCA2* and *TP53*. *PALB2, STK11, CDH1* and *PTEN* are also now generally considered to be 'very high risk genes'.

Genes in which pathogenic variants are generally associated with a moderately increased risk include *ATM*, *CHEK2, RAD51C* and *RAD51D*. It is recognised that some families have a strong family history of cancer suggesting that the cancer risk may be higher than 'moderately increased' in some women. In all women with a variant in one of these genes, an individualised risk assessment is performed in the Genetics clinic using CanRisk (computational modelling programme) to determine eligibility to screening. Most women with a pathogenic variant in one of

these genes will be eligible for moderate risk screening but some may be eligible for high risk screening.

All pathogenic variants identified so far are dominantly inherited meaning that any offspring will have a 1 in 2 chance of inheriting the mutation. In *CHEK2* and *ATM,* only certain pathogenic variants are thought to be associated with a significantly increased risk. In addition, pathogenic variants in *CDH1* are only associated with an increased risk of lobular breast cancer, the main cancer risk in these patients being diffuse gastric cancer.

There is now reasonable data available regarding *BRCA1* and *BRCA2* mutations and associated cancer risks. However, for other genes the data are much more limited and the large confidence intervals in relation to associated lifetime risk suggests that modifying factors play a major role in cancer development in many families. This means general risk estimates can be unhelpful as a basis for discussion in the clinic. Management strategies need to be formed on a case-by-case basis after in depth discussion with the family/individual.

Breast Cancer panel mutation analysis

BRCA1, *BRCA2*, *TP53*, *PALB2*, *ATM*, *CHEK2*, *PTEN, RAD51C, RAD51D* and *STK11* make up the breast cancer gene panel which is accessible to all eligible families in Scotland via Clinical Genetics.

Single gene testing is no longer undertaken but it is possible to request that only a single gene in the panel is reported. *CDH1* is not on the breast cancer panel. It can be requested independently in those individuals/families with a confirmed history of invasive lobular breast cancer **and** diffuse gastric cancer. Testing is also considered in women with bilateral lobular breast cancer under 70 years or families with 2 lobular breast cancers under the age of 50 years even if there is no gastric cancer in the family. The most common route for women with a pathogenic variant in *CDH1* to be seen in a breast clinic is following predictive testing after identification in a relative with gastric cancer.

At present, all breast cancer panel requests in the East of Scotland are run and reported by the Aberdeen molecular diagnostic laboratory. Blood samples and lab request forms are sent via the local lab for DNA extraction.

The eligibility criteria are as follows:

Patient diagnosed with;

- Breast cancer, under age 40 years
- Bilateral breast cancer, average age <60 years
- Triple negative breast cancer < 60years (this criterion is currently being reviewed with the possibility of an age limit being applied)
- Breast AND ovarian cancer, any age
- Male breast cancer, any age
- Non mucinous ovarian cancer, any age
- Breast cancer with a family history meeting a Manchester Score of 15 or more (see Appendix) or CanRisk score of 10% or more.

Turnaround time for breast cancer gene panel testing is currently 8-9 weeks. However, if a result is needed to aid management decisions regarding surgery or chemotherapy, urgent testing can be requested and delivered in 2-4 weeks.

Limitations of Genetic Testing

In many families with a strong family history suggestive of an inherited cancer predisposition, a causative variant is not identified. In this situation, there is no genetic test available to relatives to clarify their risk further. In such families, risk assessment is based on family history and individuals will be offered additional screening and may wish to consider prophylactic surgery if they are considered high risk.

It is not uncommon to identify a variant on panel testing, the significance of which is unclear. These are referred to as Variants of Uncertain Significance (VUS). As more information accumulates, these variants may be able to be classified as either conferring no increased risk or as a pathogenic variant. It is not possible to determine risk by the presence or absence of a VUS.

Gene Specific Information & Guidelines

BRCA1 and *BRCA2*

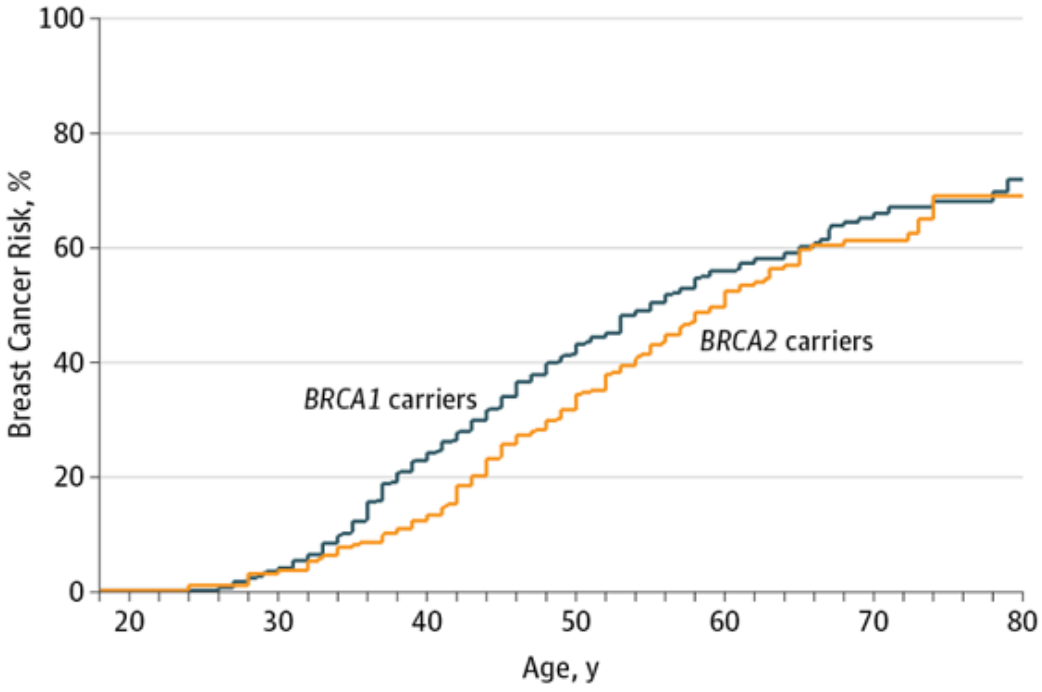
Most inherited cases of breast cancer are associated with a pathogenic variant in two genes: *BRCA1* (BReast CAncer gene One) and *BRCA2* (BReast CAncer gene Two) and together they account for approximately 20% of familial breast cancer[1]. Most pathogenic variants are truncating and are scattered throughout the gene. There are specific founder variants that occur in certain populations such as Ashkenazi Jewish and Icelandic populations[6]. BRCA1 and BRCA2 are tumour suppressor

proteins with a fundamental role in the cellular response to DNA damage through activation of specific repair processes (homologous recombination). The majority of variants are truncating variants predicted to result in a reduction in BRCA1 and BRCA2 protein and thus impeding the cells ability to repair DNA[7].

The risk of developing breast cancer in women with a pathogenic *BRCA1* and *BRCA2* variant significantly increases from the age of 30years (Figure 1)[8]. From this age, women have a 2-3% annual risk of breast cancer. Those who have had a young onset primary cancer (<50years) and a strong family history of breast cancer, have a 50% lifetime risk of developing a second primary breast cancer within 15years[9]. Cumulative risk of developing contralateral breast cancer by age 70years is approximately 80% for *BRCA1* and 60% for *BRCA2* carriers[10]. Higher incidences of *BRCA1/2* pathogenic variants are found in women with triple-negative breast cancer[11].

Women with a pathogenic variant in BRCA1 or BRCA2 are eligible for very high risk screening from the age of 30years. However some women may be eligible for screening from 25years if they have an >8% 10 year risk before the age of 30 (not yet formally adopted in NHS Lothian).

Figure 1: Cumulative risk of breast cancer among *BRCA1* and *BRCA2* mutation carriers (Kuchenbaecker *et al*, 2017 JAMA).



Recently the *BRCA1* c.5096G>A (p.Arg1699Gln) variant has been identified as conferring a lower risk of breast cancer than expected for *BRCA1* variants with a cumulative risk of 20% (moderately increased risk)[12]. A consensus statement from the UK Cancer Genetics Group advises that screening should be based on an individualised risk assessment (CanRisk) recognising that some women with a strong family history may be eligible for high risk screening, chemoprevention and/or risk reducing surgery. Noncarriers from BRCA1 R1699Q families may still be eligible for screening based on their family history.

TP53

TP53 is a critical tumour suppressor protein often referred to as 'the guardian of the genome' due to its fundamental role in ensuring genome stability[13]. Inheriting a*TP53* pathogenic variant causes Li-Fraumeni syndrome, a disorder that is associated with the development of soft tissue cancers at a young age[14]. People with this rare syndrome have a high lifetime risk of a diverse spectrum of malignancies, including breast, adrenocortical, and brain tumours as well as sarcomas (cancer of the bones or connective tissue). The cancer risk in women with a *TP53* mutation is up to nearly 100%. In men, it is up to ~70%. This gender difference is mostly due to the high breast cancer risk in women. *TP53* is particularly associated with early onset breast cancer and all women who develop breast cancer below the age of 30years should be offered *TP53* testing[15].

Mastectomy is recommended rather than breast conserving surgery in women with breast cancer and a pathogenic variant in *TP53* so that radiotherapy can be avoided as well as reducing the risk of a second primary breast cancer[14 15].

PALB2

PALB2 (partner and localizer of BRCA2) encodes a protein that binds/colocalises with BRCA2 in response to DNA damage facilitating repair[7]. Research published in 2014 found that a *PALB2* mutation increases breast cancer risk 5 to 9 times higher than average, almost as high as a *BRCA1* or *BRCA2* mutation[16]. Women with a *PALB2* mutation have a 33% to 58% lifetime risk of developing breast cancer. For women with a *PALB2* pathogenic variant, the lifetime risk of breast cancer is 33% for those with no family history compared with 58% for those with a strong family history. Therefore, risk reducing surgery is often only considered in those individuals with a strong family history.

PTEN

PTEN helps regulate cell growth by blocking PI3K signalling inhibiting cell survival, growth and proliferation[17]. Mutations in *PTEN* cause multiple hamartoma tumour syndrome also known as Cowden syndrome[18]. Affected individuals have a higher incidence of both benign and malignant breast lumps, as well as lesions in the digestive tract, thyroid, uterus, and ovaries. The lifetime breast cancer risk for women with Cowden syndrome was previously thought to be 25-50% prior to gene identification. However, a recent study estimates lifetime risk to be as high as 85% with highest risk between 40 and 60years[19].

At least 20% of all individuals who meet the diagnostic criteria of Cowden syndrome do not have a mutation in *PTEN* indicating marked genetic heterogeneity[18].

STK11

The STK11 serine/threonine kinase is part of the mTOR signalling pathway that serves as a central regulator of cell metabolism, growth, proliferation and survival[20]. Pathogenic variants in *STK11* cause Peutz-Jeghers syndrome, in which people develop hamartomatous polyps, mostly in the small intestine but also in the stomach and colon, along with characteristic mucocutaneous pigmentation[21]. The highest risks are associated with gastrointestinal cancers, but there is also a significant risk of developing breast, uterine and ovarian tumours[22].

ATM

ATM initiates the DNA damage repair in response to DNA double strand breaks[7]. Inheriting two pathogenic variants in this gene causes the disease Ataxia-Telangiectasia, a rare disease characterised by cerebellar degeneration, telangiectasia, immunodeficiency and cancer predisposition[23].

1-2% of the adult population carry a heterozygous pathogenic variant (present in one copy of the gene) in *ATM*. Women carriers have a 20-30% lifetime risk of breast cancer and are therefore considered at moderate risk[24]. The increase in risk may be more significant at a younger age. In the UK only truncating variants in *ATM* are reported as part of the breast cancer gene panel due to concerns with interpretation of missense variants in moderate risk genes[25]. However, women heterozygous for the missense variant, c.7271T>G, have a considerably higher breast cancer risk and thus should be managed as very high

risk[26 27]. Individuals homozygous (the variant is present in both gene copies) for a pathogenic *ATM* variant should be managed as very high risk.

Those affected with Ataxia telangiectasia syndrome exhibit sensitivity to ionizing radiation and thus should be avoided where possible[23]. The risk of radiation toxicity in *ATM* carriers (heterozygous individuals) has been less clear[24]. However, a systematic review of the literature concluded that radiation for diagnostic purposes and radiation therapy at conventional doses is not contraindicated and therefore ATM status should not influence decision making with regards to breast cancer management[28].

CHEK2

CHEK2 is a tumour suppressor gene encoding a cell cycle checkpoint kinase implicated in cell cycle arrest in response to DNA damage, DNA repair and apoptosis[29]. Currently only heterozygous truncating variants in *CHEK2* are considered to confer a moderate increase in breast cancer risk with the risk being highest in those with a significant family history[30]. Individuals homozygous for *CHEK2* pathogenic variants also have a *BRCA* equivalent risk for breast cancer.

RAD51C & RAD51D

RAD51C and *RAD51D* encode proteins required for efficient repair of DNA damage through homologous recombination. Pathogenic variants in these genes are associated with a significantly increased risk of ovarian cancer (lifetime risk 6-21%) and affected women are offered prophylactic surgery following completion of their families (age also guided by family history). It has been recently established that pathogenic variants in these genes are also associated with a moderately increased risk of breast cancer (Lifetime risk is estimated at 15-29%) especially triple negative or ER-negative breast cancer[31]. However, as with pathogenic variants in *ATM* and *CHEK2,* some women may be eligible for high risk screening following an individualised risk assessment if they have a strong family history of breast cancer.

CDH1

The *CDH1* gene makes a protein called E cadherin which resides in the membrane of epithelial cells. A mutated *CDH1* gene increases the risk of gastric linitus plastica at an early age causing Hereditary Diffuse Gastric Cancer syndrome[32]. The lifetime risk is up to 80%. Women with

a *CDH1* mutation also have a 23% to 68% lifetime risk of invasive lobular breast cancer[33]. Only individuals with a personal or family history suggestive of a *CDH1* mutation will be offered testing. MRI is recommended for screening in *CDH1* carriers due to the low sensitivity of mammography in detecting lobular breast cancer[33].

Gene	***Lifetime Risk***	***Features***	***Other associated cancers***	***Screening offered***
BRCA1	65-79%	Peak incidence in 40-49yr age group	Ovarian	30-49yrs: Annual MRI & Mammograms 50-69yrs: Annual mammograms (& annual MRI to 59 yrs if dense breast pattern) >70yrs: NBSP
BRCA2	61-77% (8% lifetime risk for men)		Ovarian, pancreatic, prostate	
PALB2	44-63%	Wide variability between families	Pancreatic, ovarian	30-49yrs: Annual MRI & Mammograms 50-69yrs: Annual mammograms (& annual MRI to 59 yrs if dense breast pattern) >70yrs: NBSP
TP53 *Li Fraumeni Syndrome*	85% by 60years	Young age of onset	Wide ranging include osteosarcoma, soft tissue sarcomas, brain tumours, phyllodes, Wilms and adrenocortical tumours	Annual Breast MRI from 20-69 years Annual whole body MRI under consideration but not currently available >70yrs: NBSP
STK11 *Peutz-Jeghers*	27-68%	Younger age of	Gastrointestinal, pancreatic,	30-49yrs: annual MRI &

Syndrome		onset, ductal & lobular cancer	gynecological and lung	mammograms 50-69yrs: Annual mammograms (& annual MRI to 59 yrs if dense breast pattern) >70yrs: NBSP Plus OGD & Colonoscopy 2-3yrly age dependent
PTEN *Cowden syndrome*	25-85%	High incidence of fibrocystic breast disease	Epithelial (non-medullary) thyroid, endometrial, renal cell and colorectal	30-49yrs: annual MRI & mammograms 50-69yrs: Annual mammograms (& annual MRI to 59 yrs if dense breast pattern) >70yrs: NBSP Plus annual thyroid from 16yrs, annual renal USS from 40yrs, Colonoscopy at 35 & 55yrs
ATM Het c.7271C>G variant or homozygotes	>30%			As for *BRCA1/2* carriers
CHEK homozygotes	>30%			As for *BRCA1/2* carriers
Het ATM, CHEK2, RAD51C or RAD51D (high risk*)	>30%		Ovarian cancer (RAD51C & RAD51D only)	40**-60yrs: Annual Mammograms 50yrs onward: NBSP

Gene	***Lifetime Risk***	***Features***	***Other associated cancers***	***Screening offered***
Het *ATM*, CHEK2, RAD51C, RAD51D (moderate risk*)	17-30%		Ovarian cancer (RAD51C & RAD51D only)	40**-49yrs: Annual mammograms 50yrs onwards: NBSP
BRCA1 R1699Q	20%			40**-49yrs: Annual mammograms 50yrs onwards: NBSP
CDH1	39-52%	Lobular breast cancer only	Diffuse gastric cancer, colon cancer	30-69yrs: annual MRI and mammography >70yrs: NBSP Annual gastroscopy if not had gastrectomy, colonoscopy if positive family history

Table 1. Cancer risks, features and corresponding screening guidelines associated with breast cancer predisposition genes. * NBSP: National Breast Screening Programme. *as evaluated by CanRisk risk assessment. **screening may start at 5 years before first diagnosis in the family but not before 35yrs.

Additional Information Given to Affected Families

Testing Relatives

If a pathogenic variant is identified in an individual, then relatives also at risk of inheriting the variant can be offered testing regardless of whether they have had cancer or not. This is termed 'cascade screening' and is undertaken by the Clinical Genetics team.

Pre-implantation Genetic Diagnosis (PGD)

Some high-risk pathogenic variant carriers (both female and male) might consider the option of Preimplantation Genetic Diagnosis (PGD) to avoid passing the variant onto their children. PGD is a process that involves '*in vitro* fertilisation' (IVF) to create embryos from the couple in the laboratory, which are then tested at an early stage for the familial variant. Access to PGD is through the Clinical Genetics Service.

Insurance and genetic test results

In 2018 the Association of British Insurers agreed to an open-ended moratorium which means unaffected carriers of a pathogenic variant will not have to disclose the results of their predictive genetic test if taking out life or critical illness insurance after genetic testing. The Moratorium will be reviewed by the Department of Health and the ABI every 3 years.

Those who are having genetic testing as part of their diagnostic process (e.g. a breast cancer patient) would be having a diagnostic genetic test. Insurers can use this to inform subsequent applications for life or critical illness insurance. However, the key element of insurance evaluation is likely to be the cancer diagnosis.

Appendix: Manchester Scoring System

A score of 15 or more equates to a 10% probability of identifying a pathogenic variant in *BRCA1* or *BRCA2*.

Family history Age at Diagnosis	BRCA 1 & 2 score
Female breast cancer < 30 years	11
Female breast cancer 30 – 39 years	8
Female breast cancer 40 – 49 years	6
Female breast cancer 50 – 59 years	4
Female breast cancer >60 years	2
Grade 3 Triple negative tumor	4
Male breast cancer < 60 years	13
Male breast cancer > 59 years	10
Ovarian cancer < 60 years	13
Ovarian cancer > 60 years	10
Pancreatic cancer	1
Prostate cancer < 60 years	2
Prostate cancer > 59 years	1
BRCA Pathology Index case	
LCIS (no invasive cancer)	-4
HER 2+ve	-4
If above completed no further adjustment needed	
ER -ve	1
ER +ve	-1
Grade 3 Invasive ductal cancer	2
Grade 2 Invasive ductal cancer	0
Grade 1 Invasive ductal cancer	-2
DCIS (no invasive cancer)	-1
Lobular	-2

Ovarian Exclude mucinous, borderline and germ call tumours except granulosa cell (adapted from Evans *et al,* 2011)

Examples:

This family is not eligible:

- Maternal grandmother diagnosed aged 62 years breast cancer score = 2
- Mother diagnosed aged 52 years breast cancer score = 4
- Sister diagnosed aged 54 years breast cancer score = 4

Total score = 10

This family is eligible:

- Maternal grandmother diagnosed aged 60 years breast cancer score = 2
- Mother diagnosed aged diagnosed 59 years ovarian cancer score= 13

Total score = 15

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