Role of genetic testing in familial breast cancer – outside of *BRCA1* and *BRCA2*

Introduction

- Most commonly diagnosed cancer in South African women and the second most commonly diagnosed cancer in Black women (after cervical cancer).
- The current reported lifetime risk : 1 in 11 (White), 1 in 15 (Asian), 1 in 19 (mixed ancestry), and 1 in 53 (Black)
- The overall lifetime risk is 1 in 27
- Ovarian : 1/387; White : 1/134; Black : 1/777; Coloured : 1/457; Asian : 1/248
- Different incidence rates - may well be representative of the historical socio-economic structure in SA.

(South African National Cancer Registry (2017). Cancer Statistics for 2012).

BC is a significant clinical and public health issue in SA

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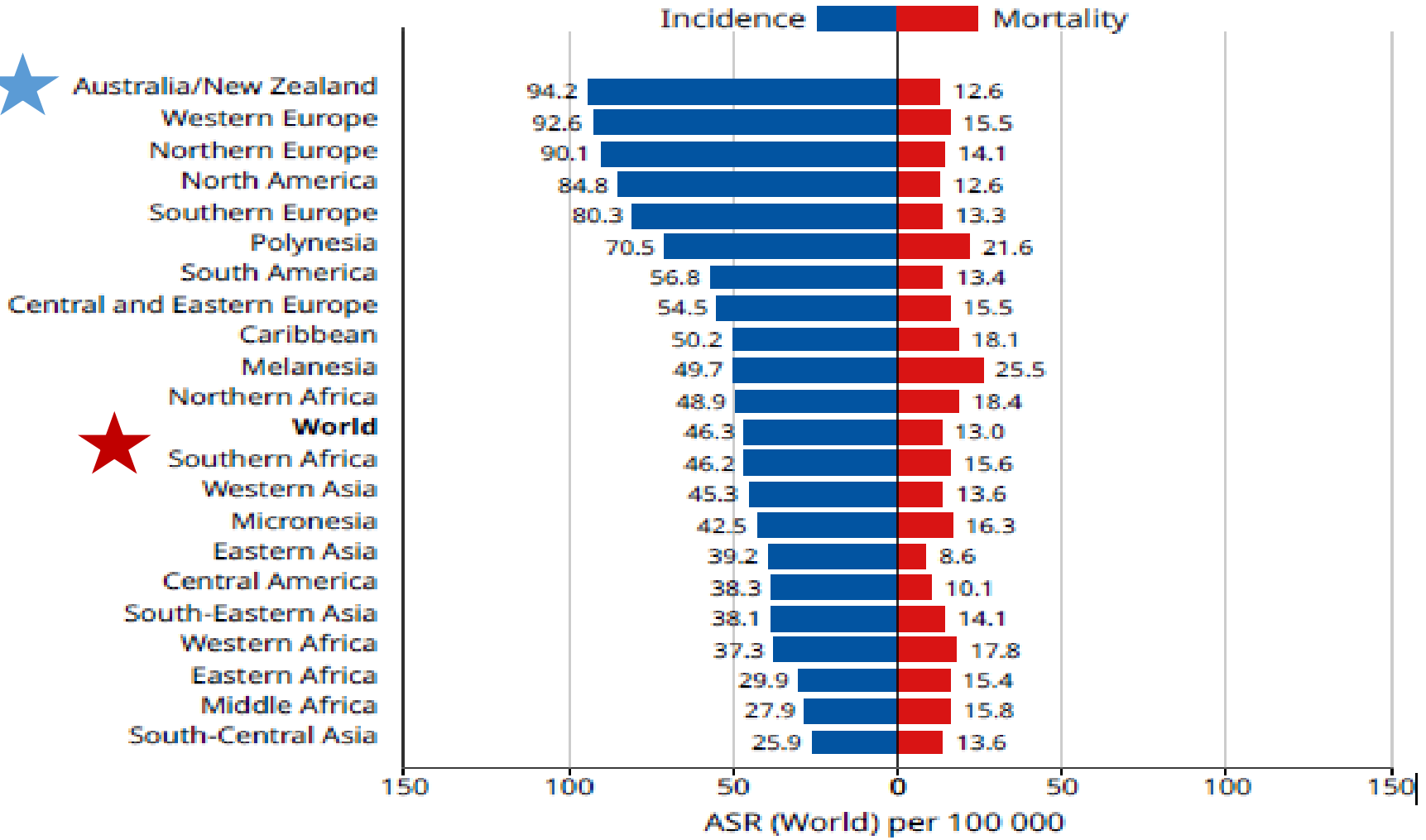
World Health Organization

Age standardized (World) incidence and mortality rates, breast



Notably:

- The SA national cancer registry is pathology based, and not population based, and it relies on voluntary reporting from pathology laboratories on invasive cancers; therefore, figures are underestimated, providing the lowest estimate of breast cancer in South Africa.

- No national mammography screening programme

- gene screening programs for familial breast cancer exclude the majority of South African women; tests are based on founder mutations that are predominant in small groups in the South African population, who are of European ancestry.

- Approximately 80% of women in SA do not belong to these groups.

- fewer than 25% of South Africans have access to private health care.

- lack of data affects the public health strategies which aim to reduce the burden of disease in the country – both biological and socio-economic factors must be addressed

(Moodley *et al.*, 2016) (Singh et al., 2014)..

Risks

- The aetiology of breast cancer is widely researched and as yet not fully understood, again reflecting the complexity of the disease.

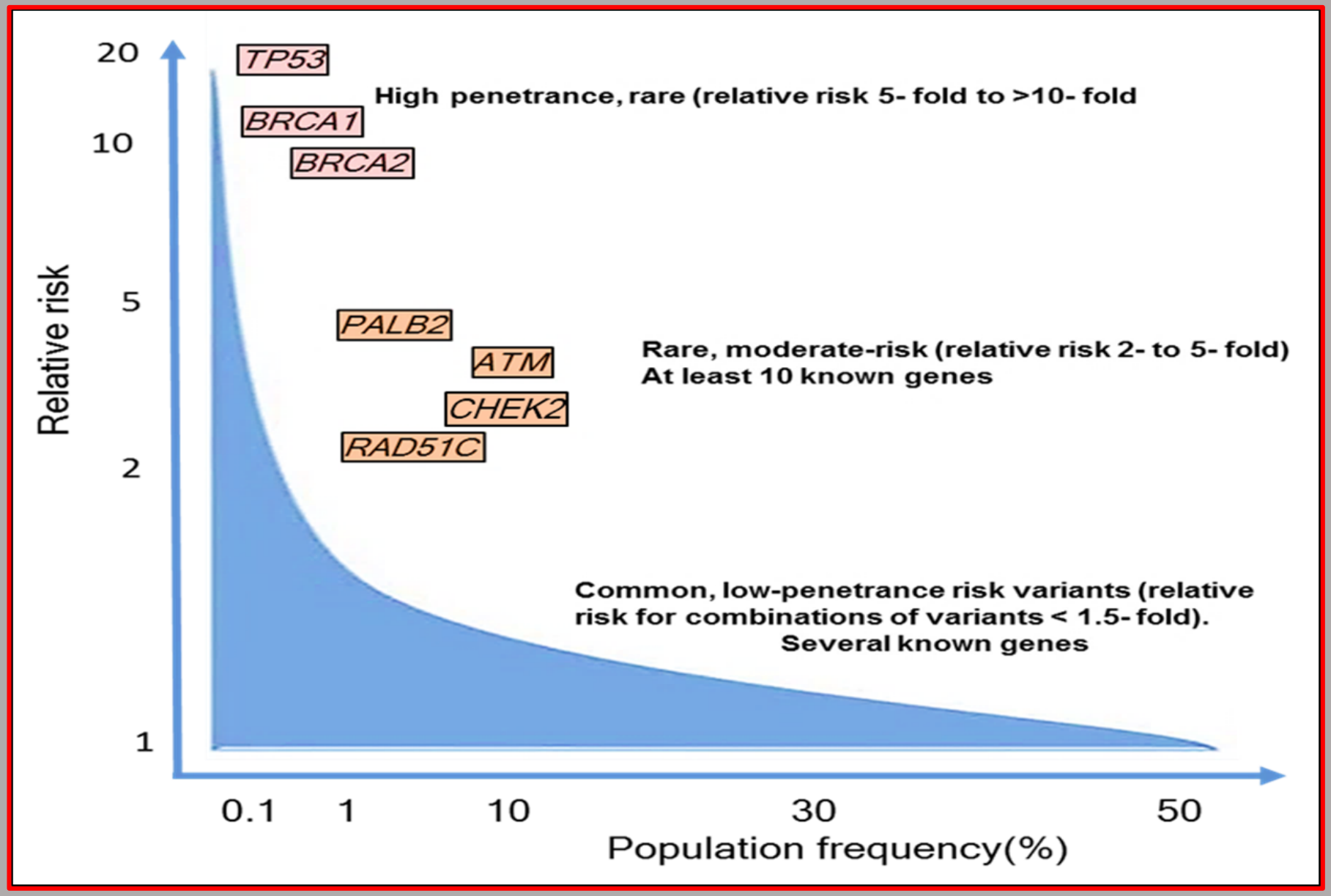
- exposure to reproductive hormones (endogenous and exogenous)
- lifestyle
- environmental risk factors.

- not yet proven that a change in behaviour significantly changes the incidence of the disease, or death as a result of breast cancer (Narod *et al.*, 2015).

Family history

Penetrance

- Three groups: high- , moderate- and low- penetrance
- inverse relationship between allele frequency in the population and the conferred risk



(Stratton and Rahman, 2008, Mavaddat *et al.*, 2010

- Currently heterozygous germline mutations in at least 20 known genes confer a high to moderate risk for developing breast cancer

- some cause distinct cancer syndromes that include breast cancer, and breast cancer risk is established only (or predominantly) in the context of these syndromes.

- founder mutations places them in the moderate to high risk group.

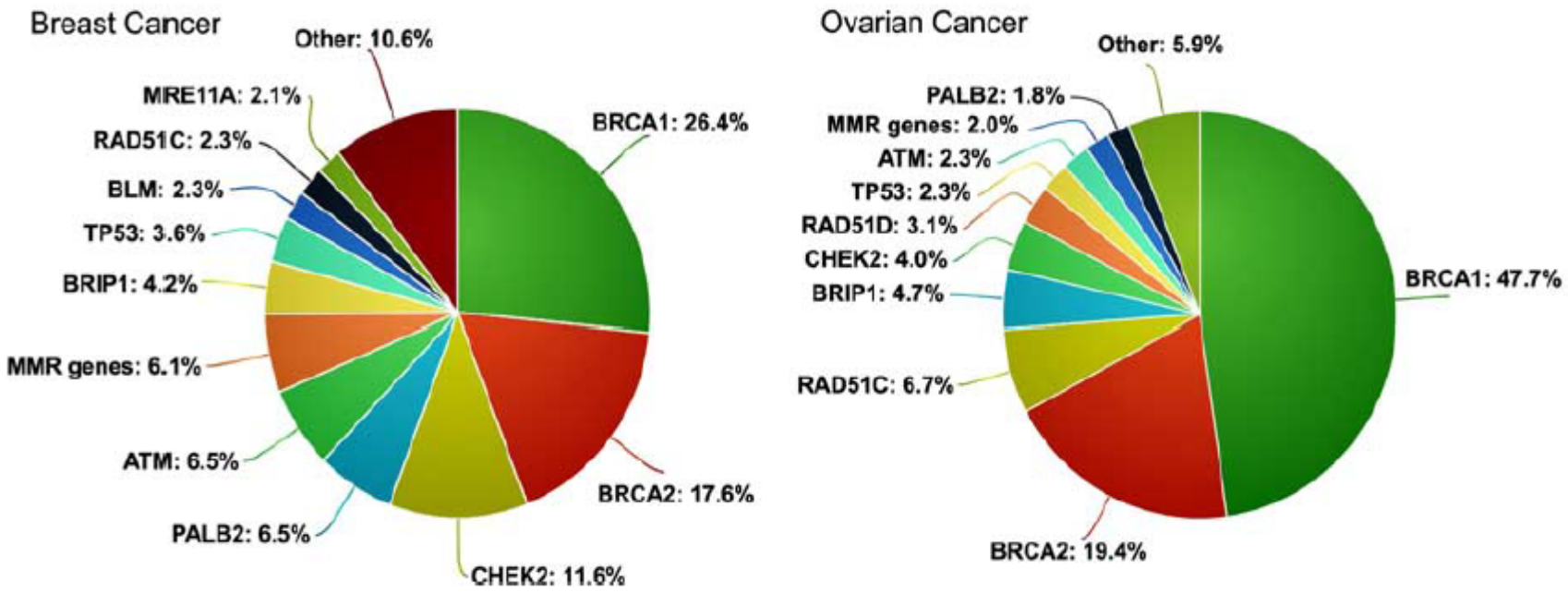
- breast cancer susceptibility is conferred by inheriting a mono-allelic mutation; bi-allelic germline mutations in the same genes cause autosomal recessive syndromes characterised by genomic instability.

- The high risk breast cancer susceptibility genes, *BRCA1* and *BRCA2* are the most common mutational sites predisposing women to breast and other cancers. Inherited autosomal dominant mutations in one or both of these genes account for 15 – 20% of inherited breast cancer, and affect approximately 1% of the population. They confer an increased life-time risk of ~80% for developing the disease

- In the absence of *BRCA* mutations, germline mutations in genes such as *ATM*, *CHEK2*, *RAD51C*, and *PALB2* confer an increased risk (2-, 3- fold) for developing breast/ovarian cancer.

- Approximately 2%-5% of individuals who undergo clinical testing carry pathogenic mutations in these moderately penetrant genes.

Gene	Absolute risk	Estimated relative risk	Comments regarding risks	Associated syndromes / other cancers
ATM	27%	2.8 (2.2-3.7)		Pancreatic cancer, ovarian
TP53	-	105 (62-165)	ascertainment bias	Li-Fraumeni's Syndrome - several cancers
BRCA1/ FANCS	72%	-		Ovarian cancer
BRCA2/ FANCD1	69%	-		ovarian, prostate, pancreatic cancer
PALB2/FANCN	45%	5.3 (3.0-9.4)		Pancreatic cancer
BRIP1/ FANCJ	-	~2.0	Controversial	
RAD51C/FANCO	Unknown	Unknown		Ovarian cancer
RAD51D	Unknown	Unknown		Ovarian cancer
CHEK2	29%	3.0 (2.6-3.5)	Due largely to one founder mutation	Lung cancer
MLH1	~19%	-	controversial	Lynch Syndrome (hereditary non-polyposis colorectal cancer), endometrium
MSH6	~11%	-	controversial	As above
NBS1	23%	2.7 (1.9–3.7)	Primarily one recurrent mutation	
CDH1	53%	6.6 (2.2–19.9)	lobular breast cancer	Hereditary diffuse gastric cancer, colorectal cancer



Hereditary Breast and Ovarian Cancer Syndrome: Moving Beyond BRCA1 and BRCA2
Lien N. Hoang, MD and Blake C. Gilks, MD, FRCPC

- Most breast (and other) cancers – so called sporadic (~70%); accumulation of somatic mutations that occur during a person's lifetime. .

- An inherited predisposition to breast cancer often involves germline mutations in genes which are involved in homologous recombination (HR) – a process that ensures the accurate repair of DNA and maintains genomic integrity.

- Inheriting a deleterious mutation in a breast cancer susceptibility gene promotes genomic instability (protective pathways are compromised), increases the mutation rate, and facilitates earlier tumorigenesis

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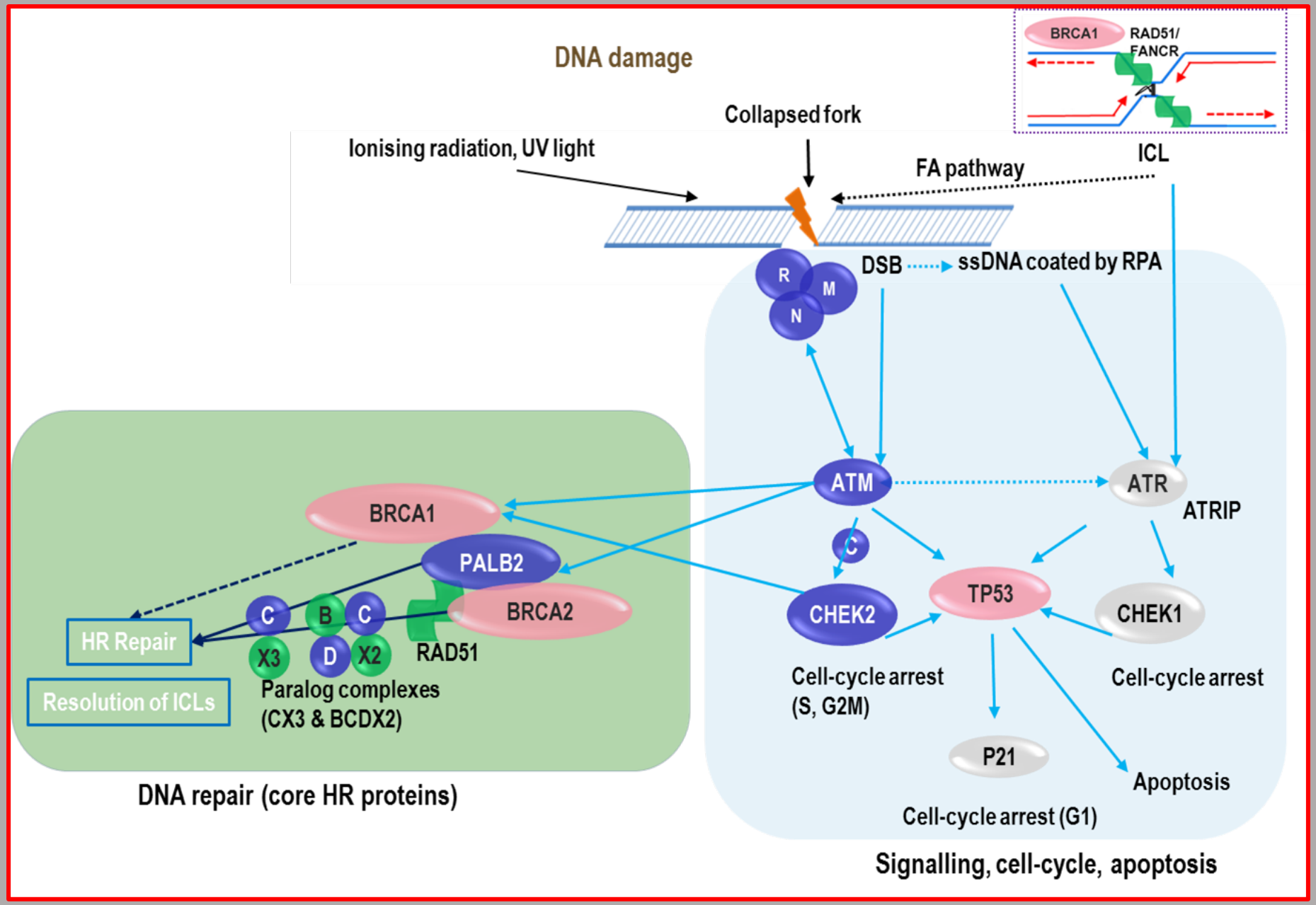


Adapted from (Tavtigian and Chenevix-Trench, 2014) and (Somyajit et al., 2012).

TP53 (Tumour protein 53)

- *TP53* heterozygous germline mutations were found to be the causative factors in Li-Fraumeni's Syndrome; characterised by several types of cancers
- Contribute to ~0.1% of familial breast cancer cases
- For women from Li-Fraumeni families, the BC risk is > 50- fold before the age of 45 years.
- Most studies done on high-risk patients with Li-Fraumeni syndrome features, and the absolute risk is not established
- Outside of Li-Fraumeni families, a diagnosis of breast cancer at a young age (<30 years), may indicate a *TP53* mutation in 4% to 8% of individuals, who do not have a mutation in one of the *BRCA* genes
- TP53 functions in the regulation of the cellular DNA damage response, cell-cycle control, and apoptosis
- ~75% of pathogenic mutations are non-truncating missense mutations

(Easton et al., 2015)

TP53 (Tumour protein 53)

- *TP53* associated breast cancers tend to be hormone-receptor positive and HER2 positive.
- Bilateral mastectomy can be recommended for both healthy carriers and those with breast cancer
- Breast conserving surgery is not recommended; mutation carriers are susceptible to radiation-induced DNA damage
- May develop radiation-associated cancers

BRCA1 and BRCA2 (briefly)

- highlighted the important link between human health, tumour suppression and genomic instability
- normal cell - negatively regulate growth through cell cycle control, and to protect against DNA damage.
- *BRCA1* - gatekeeper of the integrity of the genome
- *BRCA2* - mediation of RAD51 filament formation in homologous recombination
- Both critically associated with homologous recombinational repair (HRR) of DNA double stranded breaks
- Also essential in the Fanconi anaemia-BRCA pathway - functions to resolve particularly deleterious interstrand-crosslinks
- in 2002, it was discovered that the FA gene, *FANCD1* is *BRCA2*.
- Other breast cancer susceptibility genes found by investigating proteins that interact with the BRCA proteins in pathways that protect the integrity of the cell; in this way *PALB2/FANCN* was discovered.

PALB2 (Partner and localizer of BRCA2)

- 2006 - PALB2 interacts with BRCA2, and is required for its effective functioning in HR; 2007 - five heterozygous germline mutations were identified in familial BC cases, accounting for a carrier frequency of 1.1%
- truncating mutations in *PALB2* confer an increased lifetime risk for breast cancer (2.3-fold)
- Not as high as mutations in *BRCA2*, despite the functional relationship between the two proteins
- classified as a moderate penetrance breast cancer susceptibility gene
- Since 2007, studies in several populations have identified rare, truncating *PALB2* mutations.

PALB2 (Partner and localizer of BRCA2)

- In 2012, the relative risk for developing breast cancer was estimated to be 5.3
- Association between *PALB2* mutations and contralateral breast cancer was demonstrated (Tischkowitz et al., 2012)
- difficult to accurately estimate the risk of developing breast cancer for carriers of these mutations compared with non-carriers in the general population.
- *PALB2* founder mutations, identified at relatively high frequencies in breast cancer patients with no family history of cancer, and in unaffected individuals, can be used in population based studies to assess the overall breast cancer risk
- Risk estimates can also be inferred from family-based studies (Foulkes et al., 2016, Southey et al., 2016).

PALB2 (Partner and localizer of BRCA2)

- In 2014, the PALB2 interest group established that *PALB2* mutations can confer a high risk for breast cancer, approximating the risk conferred by BRCA2 germline mutations (Antoniou et al., 2014)

- Risk estimate depends on family histories and possibly *PALB2* genotype (Antoniou et al., 2014, Southey et al., 2016).

- For carriers of *PALB2* mutations, the absolute risk is 33% for women who have no family history of cancer and increases to 58% for women with a family history of breast cancer – including more than one first degree relative with breast cancer at 50 years old. The risk estimate also varies depending on environment, and lifestyle – any factors that affect the incidence rate.

- It is stated by Antoniou et al., 2014 that “No single set of penetrance estimates applies to all PALB2 mutation carriers” (Antoniou et al., 2014).

PALB2 (Partner and localizer of BRCA2)

Relative risk for *PALB2* mutation carriers by age	
<40 years	8 - 9
40 – 60 years	6 – 8
>60 years	~5
All	9.47
Mean cumulative risk of breast cancer – female carriers	
By age 50 years	14%
By age 70 years	35%
Absolute risk by 70 years of age – female carriers	
Family history	58%
No family history	33%

PALB2 (Partner and localizer of BRCA2)

- Importantly, for women who carry *PALB2* pathogenic mutations, the disease risk at 30 years of age, indicates that increased screening should be recommended – even if the carrier has no family history of cancer.

- The association of PALB2 with FA, was also discovered in 2007

Lee A.J., et al., Incorporating truncating variants in PALB2, CHEK2, and ATM into the BOADICEA breast cancer risk model. Genet Med. 2016 Apr 14

PALB2 (Partner and localizer of BRCA2)

- PALB2 bridges BRCA1 and BRCA2, forming the BRCA-complex, allowing an indirect interaction between the two caretakers of genomic stability
- The functions of PALB2 extend beyond assisting BRCA2; the two proteins share several functions

PALB2 (Partner and localizer of BRCA2)

- PALB2 : Breast Cancer Prevention and Screening
- annual clinical breast examination
- Annual mammogram with consideration of tomosynthesis starting at age 30
- Magnetic Resonance Imaging (MRI) starting at age 20 to 30 (absolute risk by age 30 years is ~0.47%)
- Mastectomy – insufficient data and depends on family history and clinical factors
- After *PALB2*, another gene, *RAD51C*, was discovered to be involved with HBOC and Fanconi anaemia.

Managing Patient with Mutations in PALB2, CHEK2, or ATM
Francisco Acevedo & Zhengyi Deng & Victor D. Armengol & Kevin Hughes

The RAD51 paralogs emphasizing RAD51C

- The RAD51 recombinase has five paralogs in eukaryotic cells
- In 2010 - six heterozygous germline mutations were identified in 480 breast and ovarian cancer families from Germany, (frequency = 1.3%), (Meindl et al., 2010).
- Complete segregation in families suggested a high penetrance level similar to *BRCA1* and *BRCA2* mutations (RR of 1.5 to 7.2).
- mean age of breast cancer diagnosis was 53 years; ovarian cancer, 60 years.
- *RAD51C* was purported to be a moderate to high risk breast cancer susceptibility gene (Meindl et al., 2010).

The RAD51 paralogs emphasizing RAD51C

- All families included ovarian cancer (480 families); no pathogenic mutations were identified in breast cancer only families.
- the overall prevalence of *RAD51C* mutations actually 0.5%.

- Also in 2010, contrasting result was reported: no pathogenic *RAD51C* mutations were observed in 454 HBOC patients
- suggesting that *RAD51C* mutations were not as common as previously described
- *RAD51C* truncating mutations are rarely seen in BC only families or in BC patients with no family history of cancer
- Researchers began to concentrate on *RAD51C* as an ovarian cancer susceptibility gene

The RAD51 paralogs emphasizing RAD51C

- It is accepted that *RAD51C* is an ovarian cancer susceptibility gene LR = 5.2 (95% C.I. 1.1–24)
- the debate about the breast cancer risk conferred by *RAD51C* mutations is ongoing
- Missense mutations are relatively common (found in ~1% of HBOC families); such mutations may have a relatively low penetrance, and not cluster in families.
- Implicitly, *RAD51C* mutations carriers may not be identified by high-risk criteria, the basis for the selection of participants in most hereditary breast/ovarian cancer studies.

The RAD51 paralogs emphasizing RAD51C

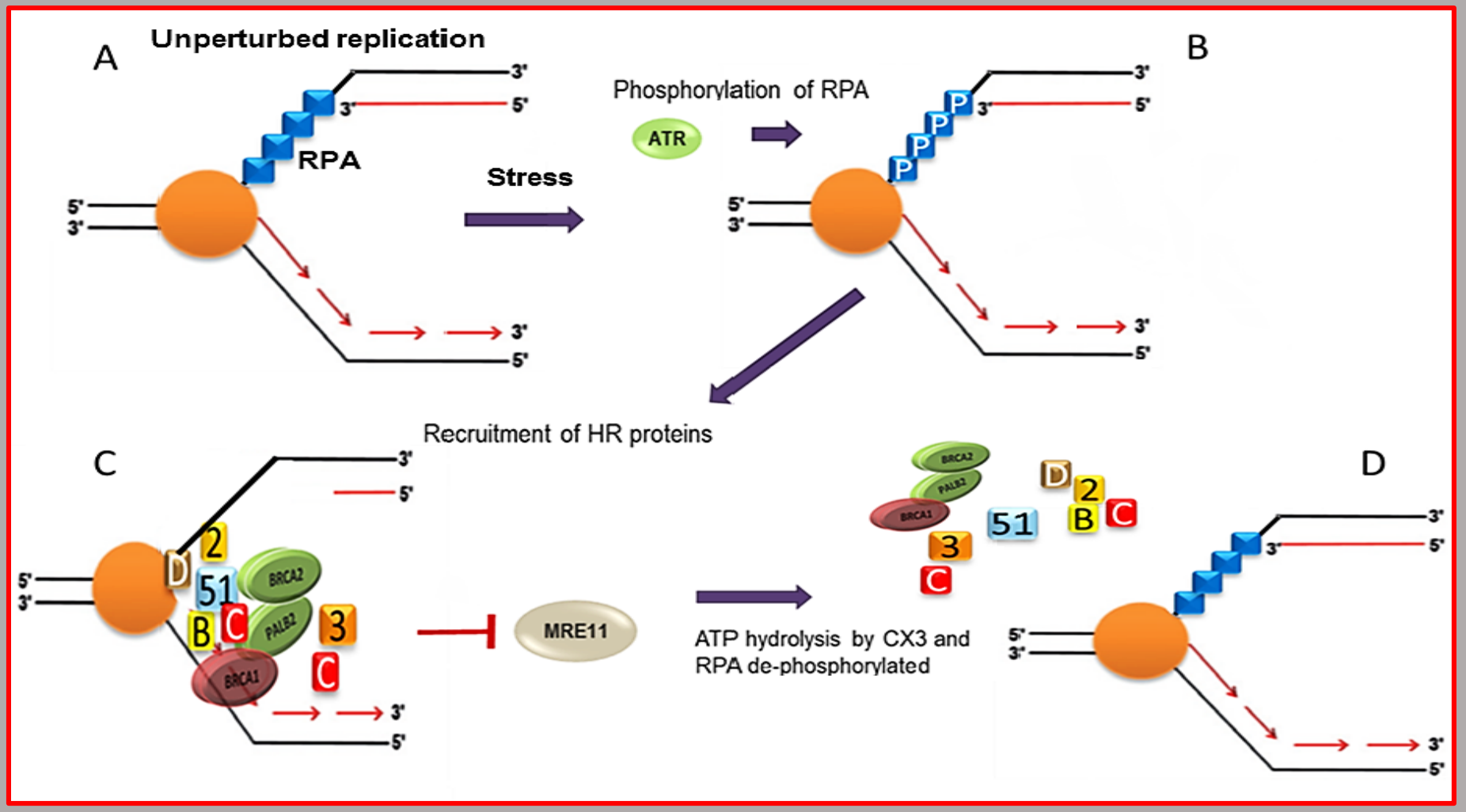
- RAD51 filament formation and the subsequent invasion of an undamaged homologous template is the definitive factor of HRR.
- The RAD51 paralogs regulate the function of RAD51, and all are essential for homologous recombination, in meiosis, in replication, and in the repair of dsDNA lesions.
- Most of the understanding of the RAD51 paralogs has come from research focusing on RAD51C due to its clinical importance in HBOC.
- RAD51C combines with other RAD51 paralogs as part of the two complexes, BCDX2 and CX3, which function upstream and downstream of RAD51 recruitment to nuclei foci

The RAD51 paralogs emphasizing RAD51C

- part of the HR Complex, formed by PALB2.
- The BCDX2 complex preferentially binds ssDNA created by the resection of DSBs
- It has a high affinity for branched DNA allowing for its function in the repair of replication fork damage, by forming or stabilizing RAD51 filaments.
- The RAD51 paralog complex, CX3 is associated with several processes in HR and replication; it is linked to Holliday junction resolution in the later stages of HRR, and in the maintenance of stalled replication forks

The RAD51 paralogs emphasizing RAD51C

- The RAD51 paralogs have an important role in protecting replication forks and in replication restart



The RAD51 paralogs emphasizing RAD51C

- There are no specific guidelines for managing patients who carry a *RAD51C* mutation
- Suggested that risk-reducing salpingo-oophorectomy (RRSO) could be considered depending on family history (50 – 55 years)

Tung et al. 2016. Counselling framework for moderate-penetrance cancer-susceptibility mutations. Nature reviews. Vol 13: 581-8.

ATM (Ataxia Telangiectasia)

- ATM senses and responds to DNA lesions, generating a signalling cascade
- it phosphorylates several downstream proteins involved in DNA repair (core HR proteins), checkpoint regulation (CHK1 and CHK2), apoptosis (TP53), and damage recognition (genes of the MRN complex); it also interacts with ATR
- Monoallelic mutations lead to higher risk of breast cancer and biallelic mutations are associated with the development of ataxia-telangiectasia
- Pathogenic mutations confer a lifetime risk of developing breast cancer at ages 70 and 85 of 21.6 and 39.6%, respectively (compared to 8.8 and 13.7% in general population)

ATM (Ataxia Telangiectasia)

ATM - Breast Cancer Prevention and Screening

- annually clinical breast examination starting at age 40 years
- Mammography - annual mammograms with consideration of tomosynthesis starting at age 40 (depending on family history)
- Patients with an ATM mutation may be considered radiation sensitive
- There are no data regarding the benefit of prophylactic mastectomy in patients with ATM mutation; should be based on family history

CHEK2 (Checkpoint kinase gene/protein 2)

- CHEK2 is involved in the regulation of cell division in the unperturbed cell.
- In response to DNA damage, it is activated by ATM
- it then activates (through phosphorylation) several proteins, including p53 and BRCA1, to promote DNA repair or apoptosis
- it delays the progression of the cell cycle by regulating the G2/M cell-cycle checkpoints, allowing time for HRR to take place
- Several *CHEK2* variants have been identified
- Most common mutation on which estimates are based is c.1100delC
- This mutation confers a moderate breast cancer risk – life time risk of 21.6% at age 70 and 39.6% at age 85 (whereas risk in the general population is 8.8 and 13.6%, respectively)
- Other variants confer a lower risk OR = 1.67 [1.18-2.27]

CHEK2 (Checkpoint kinase gene/protein 2)

- CHEK2 : ESMO recommends clinical breast examination starting at age 20–25 but others recommend age 40 years (30 years if patient is homozygous for a mutation)
- Mammogram: annual from age 40 years.
- MRI – high risk mutation then beginning at age 40 years
- RRM - no data regarding the benefit of prophylactic mastectomy

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BREAST AND OVARIAN MANAGEMENT BASED ON GENETIC TEST RESULTS[a]

	Recommend Breast MRI[d] (>20% risk of breast cancer[e])	Discuss Option of RRM	Recommend/Consider RRSO
Intervention warranted based on gene and/or risk level	*ATM* *BRCA1* *BRCA2* *CDH1* *CHEK2* *PALB2* *PTEN* *STK11* *TP53*	*BRCA1* *BRCA2* *CDH1* *PTEN* *TP53* *PALB2*	*BRCA1* *BRCA2* Lynch syndrome[f] *BRIP1* *RAD51C* *RAD51D*
Insufficient evidence for intervention[b,c]	*BRIP1*	*ATM* *CHEK2* *STK11*	*PALB2*

Managing Moderate Penetrance

Thomas Slavin, MD, FACMG

Recommend carriers check back or set-up follow-ups

NCCN Guidelines Version 1 (2016), Genetic/Familial High-Risk Assessment: Breast and Ovarian

- Essentially, in hereditary breast cancer, the cellular response to external and internal damage is altered. A defect in any of the HR proteins means that the accurate repair of DNA by HR is compromised, and DSBs are instead repaired by the more error-prone process of NHEJ (Somyajit et al., 2014).

- Compromised HRR (or the abrogation of the pathway), potentially as a result of inheriting a germline mutation in one of the core HR genes, promotes genomic instability and the development of breast cancer.

Summary and conclusion

- Most research has been conducted on women from high-risk breast cancer families and/or on women with breast cancer diagnosed at a young age < 40 years).

- increases the likelihood of identifying a mutation in a breast cancer susceptibility gene but could exclude a large proportion of carriers in a population

- This is significant in South Africa where family history may be unknown or inaccurate.

- Identifying mutations that confer a predisposition to breast cancer has implications for affected and unaffected carriers, and for their families - breast cancer can be managed appropriately, in terms of risk reducing options, and treatment programs.

Summary and conclusion

- conferred breast cancer risks must be known
- Difficult to estimate the risk conferred by moderate penetrance genes
- Guidelines for managing patients harbouring mutations in *PALB2* and other moderate penetrant genes are being established, but depend on the further accumulation and evaluation of data
- Molecular data is essential for the design of preventive, diagnostic, and treatment strategies. Importantly, tumours with mutated homologous recombination repair pathway genes are sensitive to the Poly (ADP-ribose) polymerase (PARP) inhibiting class of drugs, through ‘synthetic lethality’. This sensitivity, and future targeted therapies may be utilised in the treatment, of patients with tumours resulting from inherited mutations in *PALB2* and *RAD51C* (Lord and Ashworth, 2016).

Identification of a germline deleterious mutation with a known risk

↓ mainly BRCA1 and BRCA2 mutation carriers)

Counselling
Assists affected or unaffected carriers in making informed decisions.

Increased breast cancer screening:
mammography or magnetic resonance imaging is recommended, particularly if preventative surgery is not performed.

Chemoprevention: effective against oestrogen receptor-positive breast cancer. Tamoxifen reduces the incidence of cancer among women who have a high-risk for developing the disease. Aromatase inhibitors decrease breast cancer incidence among high-risk, post-menopausal women.

Surgery
Prophylactic bilateral mastectomy - reduces the incidence of breast cancer, and prevents recurrence. Bi-lateral salpingo-oophorectomy is effective for prevention and survival, and is recommended for women with a high risk of developing breast and ovarian cancer.

Chemotherapies/radiation:
HRR deficient tumours are vulnerable to cytotoxic chemotherapies - particularly to the platinum analogues that cause ICLs.

PARP inhibitors: used in combination with platinum drugs (or alone) if tumours are HRR deficient. PARP inhibitors cause 'synthetic lethality'- cell death which occurs if two independently viable processes are abrogated and DNA cannot be repaired. In cancer cells with an already defective HR pathway, an accumulation of SSBs and an inability to accurately repair resultant dsDNA breaks, creates genomic instability, and cell death.

OR

No indication of an increased breast cancer susceptibility (no family history; age dx >40 years of age)

↓

Routine: Tumour biopsy and molecular analysis (histopathological classification). Not basal like and tumour is not HRR deficient

↓

Conventional treatment

Southey, M. C., Goldgar, D. E., et al. 2016a. PALB2, CHEK2 and ATM rare variants and cancer risk: data from COGS. Journal of Medical Genetics, 10.1136/jmedgenet-2016-103839.

Summary and conclusion

- It must be noted that prevention and treatment strategies are developed based on research focused (predominantly) on women of European descent.
- provided invaluable data on the prevalence, penetrance, and effects of deleterious mutations which cause a predisposition to breast cancer.
- However, the limited studies on women from other population groups is a recognized shortcoming in current research

“The ultimate aim will be reached when all genes involved in cancer predisposition will be determined, all pathogenic variants in these genes identified and their contribution to cancer predisposition evaluated in each possible combination for each tissue.” (Teugels and De Brakeleer, 2017)

Summary and conclusion

- There is great potential to mitigate the cancer risks (incidence and mortality) for women harbouring deleterious germline mutations in the moderate penetrance genes.
- South African women, even given historical and current disparities, benefit from the data obtained through on-going research in other countries.
- Future studies must be extended to include African (and other) population groups
- The genetic diversity in Africa, where human beings originated, necessitates the use of a so-called African reference genomic dataset; the current basis for determining pathogenicity is skewed by the use of primarily European, healthy (assumed) controls against which comparisons are made (Hayden, 2016).
- There is an evident bias when defining the reference allele

Summary and conclusion

- Currently it is possible to test for mutations in multiple candidate genes through NGS, but such panel tests are opening up new issues and mutations are being found for which strategies for management are not yet developed.

- Massive parallel sequencing is dependent on technology, predictive bioinformatic tools, and on the accurate interpretation of genetic variants in relation to disease. The detection of variants through NGS has outpaced the ability of researchers to accurately analyse phenotypic consequences of the data

- it is evident that even physicians and researchers do not yet fully understand the implications of most mutations.

- Clinical action is often not based on any factors other than a family history of cancer.

Summary and conclusion

The final aim of all breast cancer research is risk reduction and adequate treatment for affected women. Much more research is required in South Africa to realise this aim in a cost effective manner. The hope is that all South African women will eventually benefit from affordable, quality health care, and unnecessary deaths as a result of breast cancer will be prevented.