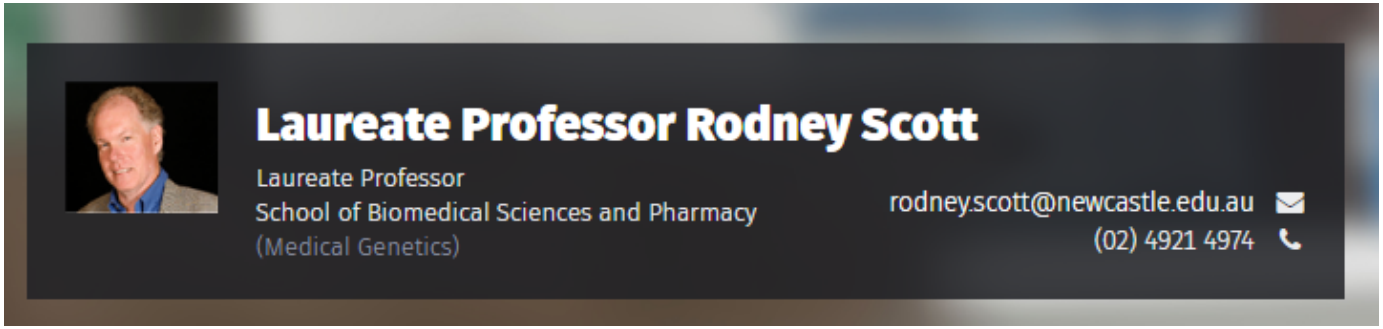
Kathmandu, Bir Hospital visit,
August 2018

Pathology North

Germline Genetic Testing for Breast Cancer Risk

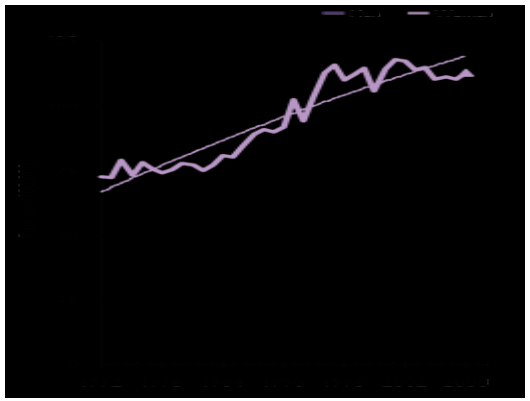
Evidence-based Genetic Screening



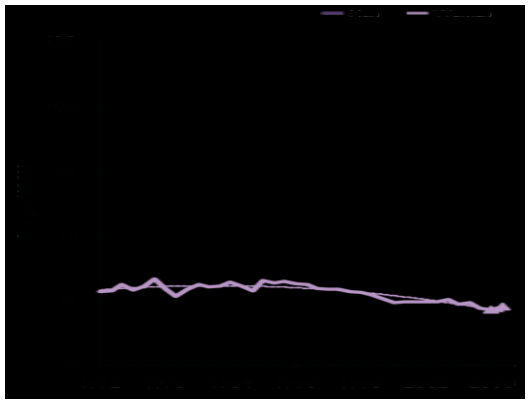
Demography in New South Wales (total population ~ 7,000,000)

Breast Cancer Diagnoses: ~4,400/annum
Breast Cancer Deaths: ~ 900/annum
Relative survival: 88%

The most common malignancy in women



New Cases

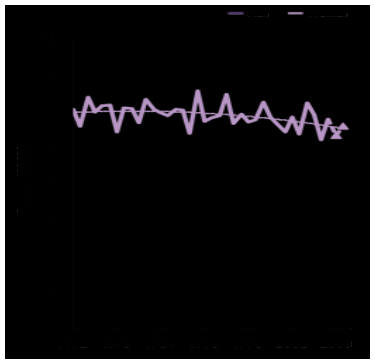


Age-standardised mortality

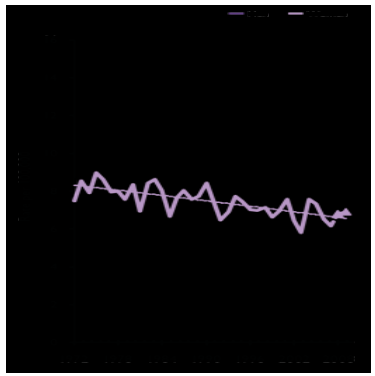
Pathology North

Demography in New South Wales

- Ovarian Cancer Diagnoses: ~430/annum
- Ovarian Cancer Deaths: ~ 298/annum
- Relative survival: 44%
- 1OTH most common malignancy in women



New Cases



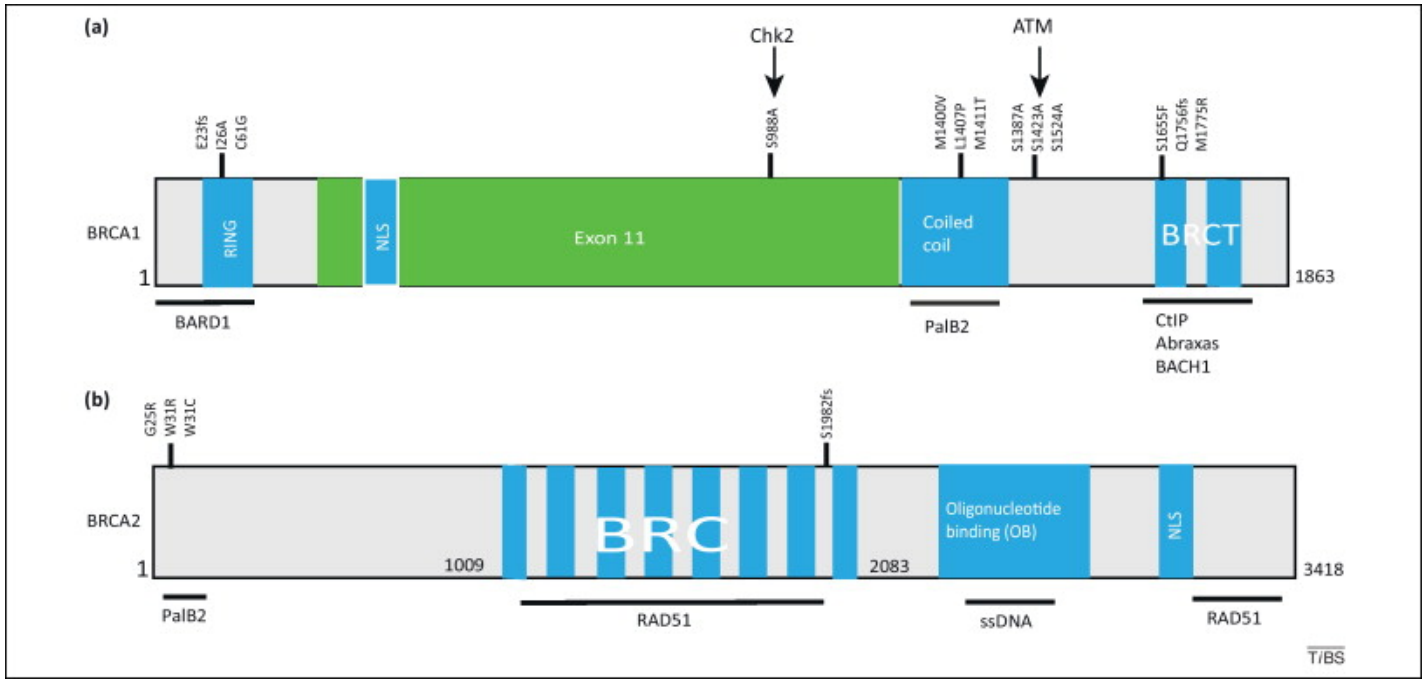
Age-standardised mortality

Pathology North


TIME
THE
ANGELINA
EFFECT
Angelina Jolie's double mastectomy puts genetic testing in the spotlight. What her choice reveals about calculating risk, cost and peace of mind
BY JEFFREY KLUGER & ALICE PARK

1. BRCA1 and BRCA2
2. How many BrCa genes are actionable
3. Genetic Testing – what you should do

BRCA1 and BRCA2 Structure



BRCA1

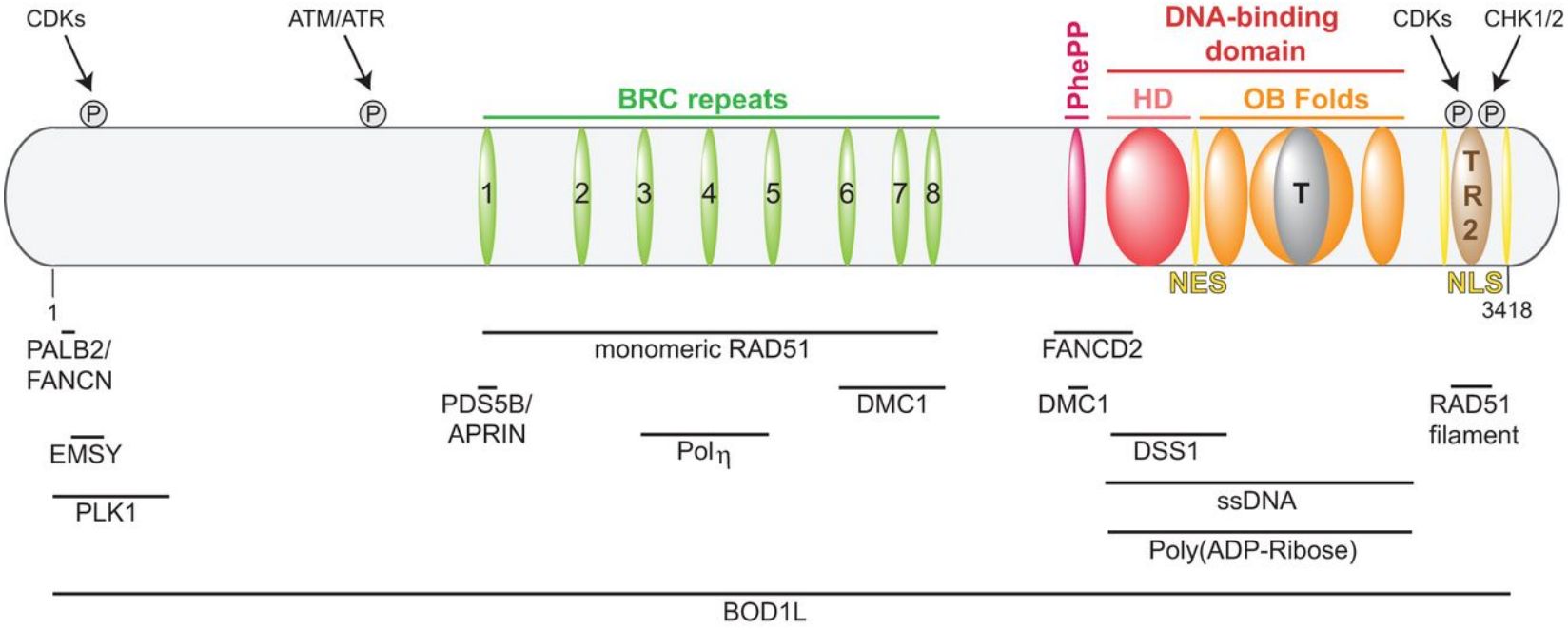
BARD1
BAP1 c-myc p53
E2F
Rb c-myc
BRAP2
Mre11 RAD51
Rad50 ZBRK1
Nbs1
BRCA2, p300/CBP
RAD50
HDAC1/2
AR RNA helicase
RbAp46, RbAp48
RNA Pol II
CtIP

Centrosome proteins (p53, Rb, Nm23) H2AX

BASC(ATM, BLM, MSH2, MSH6, MLH1, RCF)

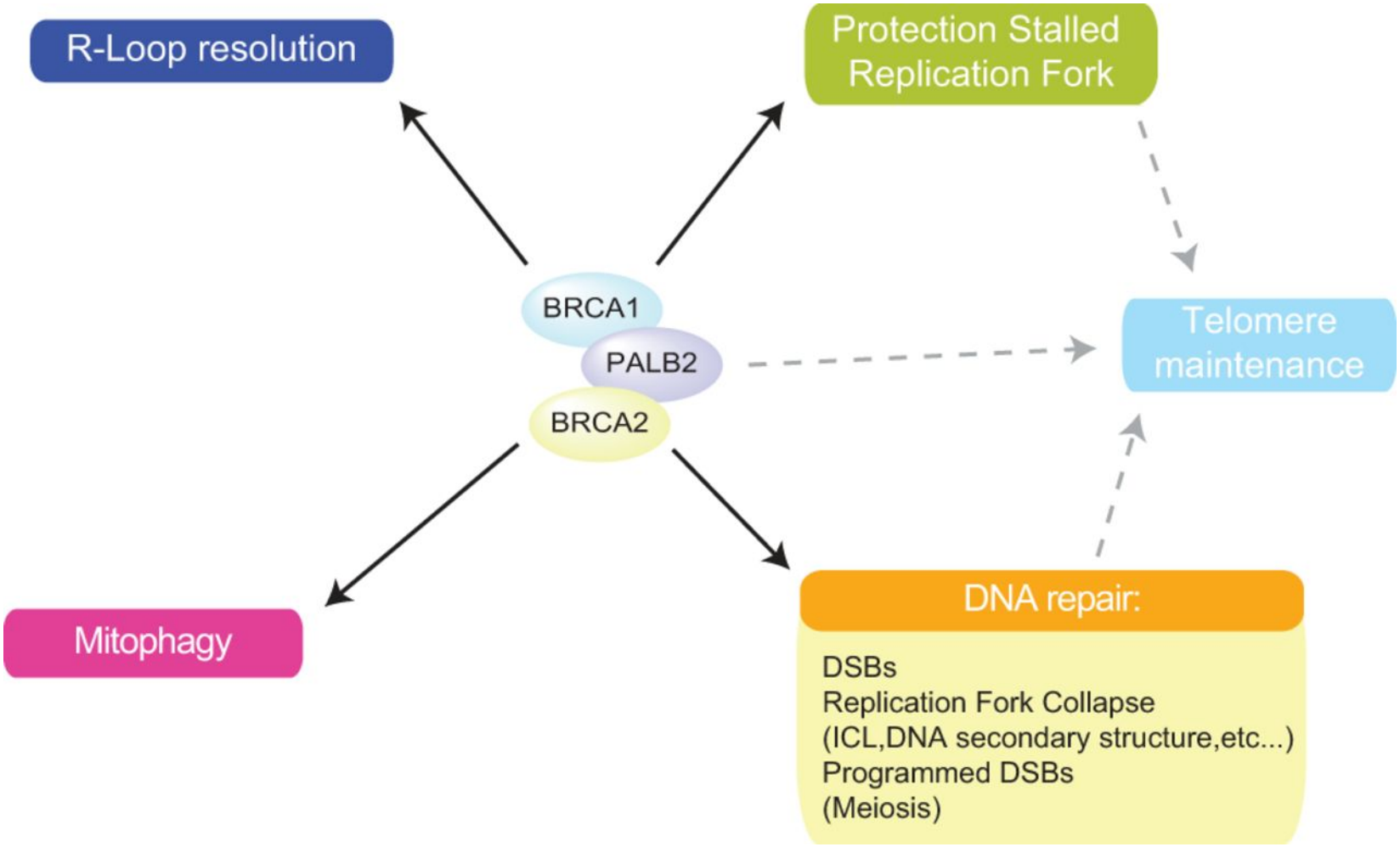
BRCA2 interacting proteins

Structural domains and interaction partners of BRCA2.



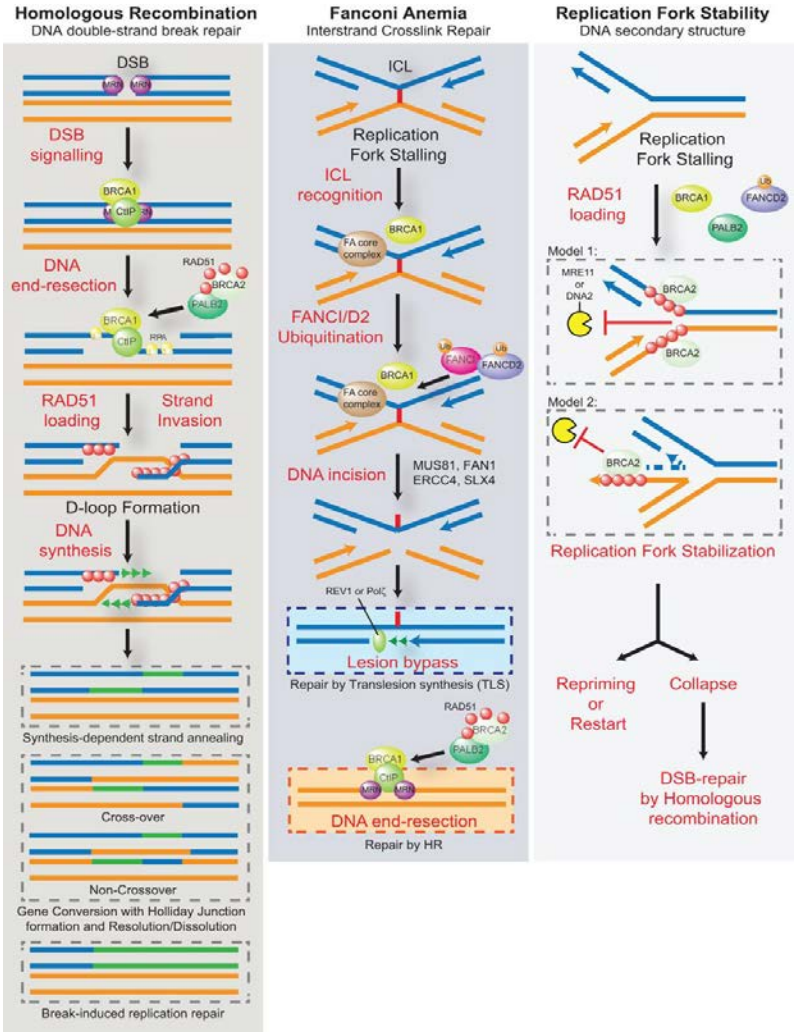
Amélie Fradet-Turcotte et al. Endocr Relat Cancer 2016;23:T1-T17

BRCA2 functions in the maintenance of genome stability.

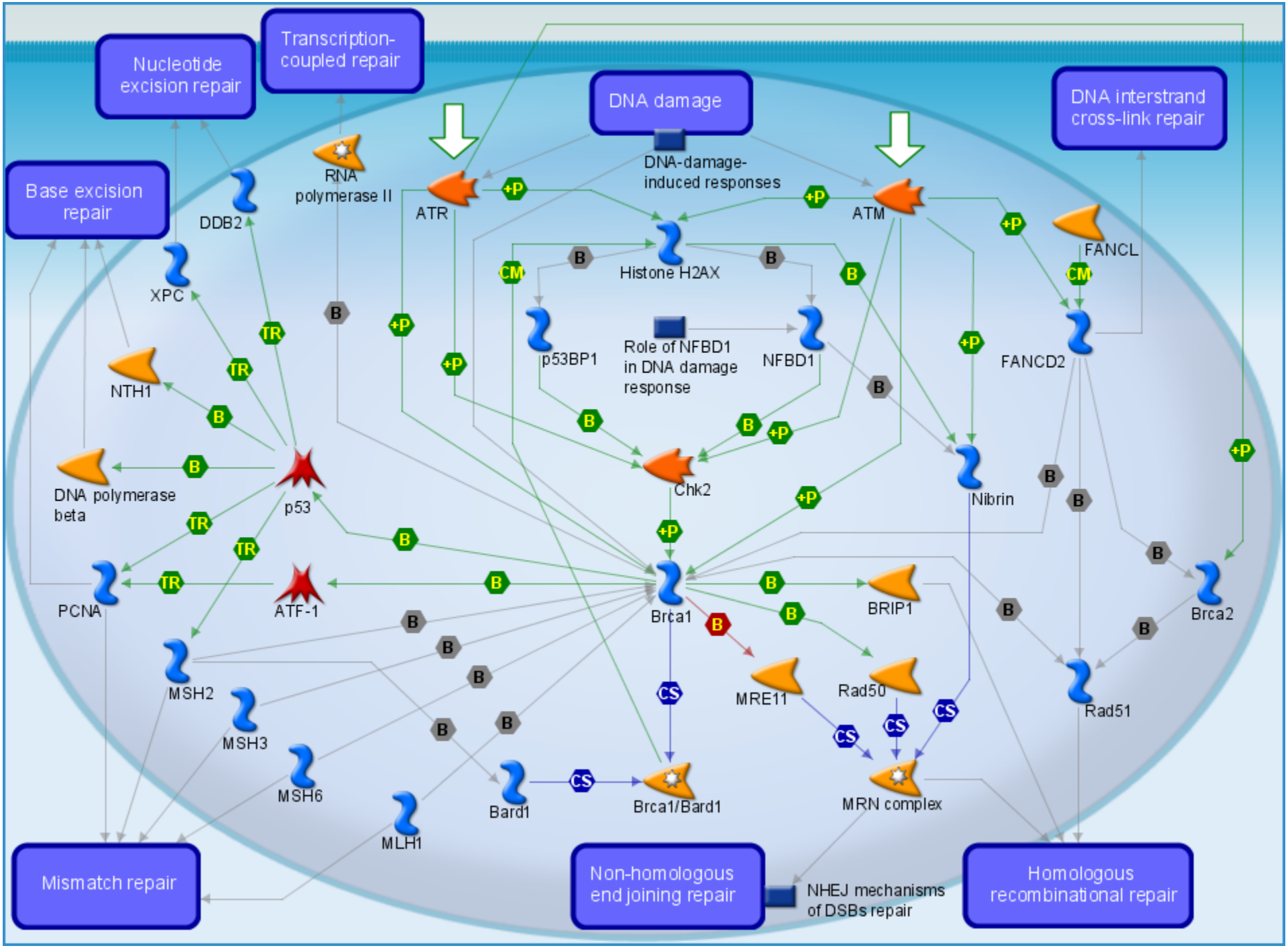


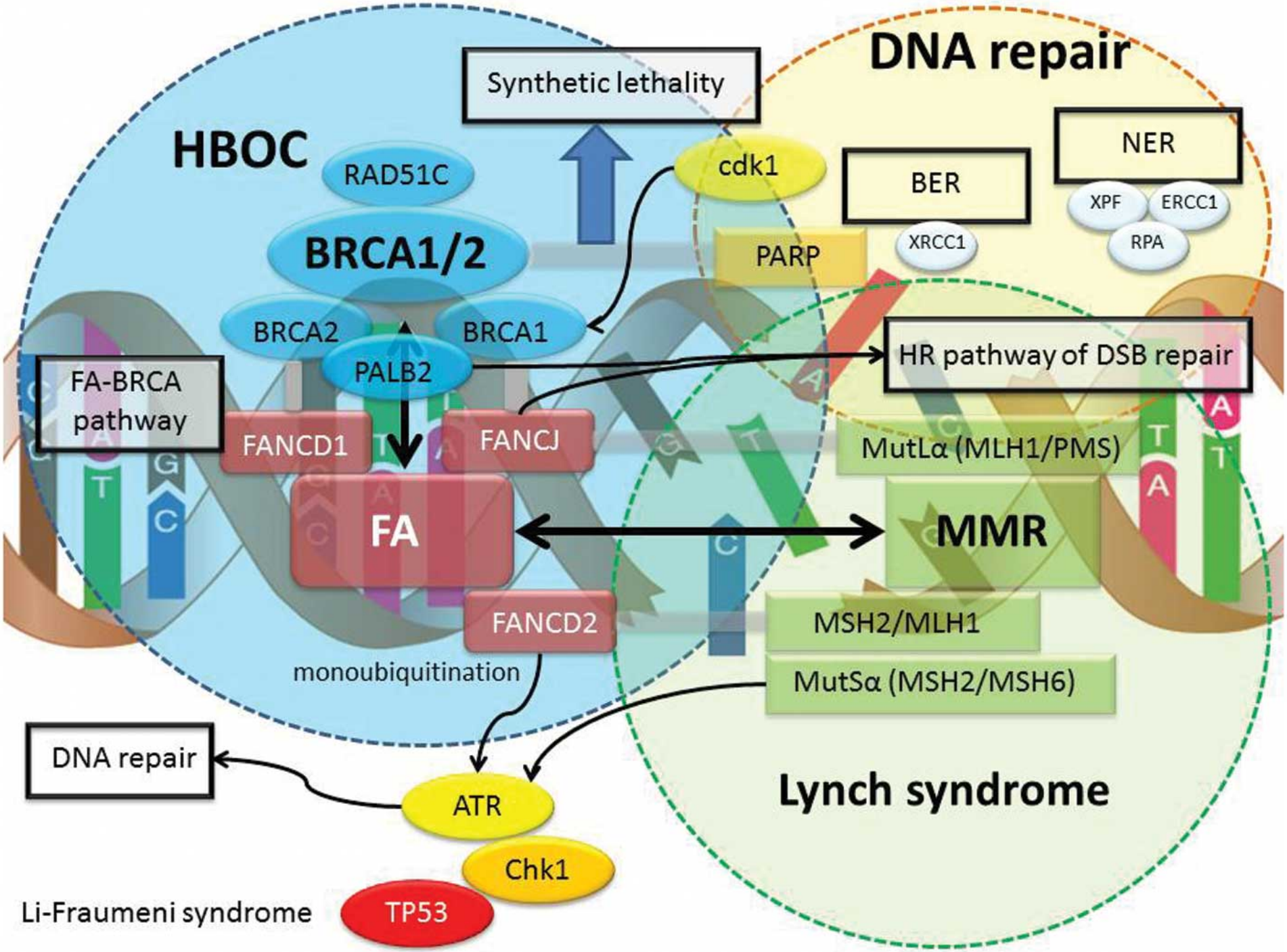
Amélie Fradet-Turcotte et al. Endocr Relat Cancer 2016;23:T1-T17

Role of BRCA2 during DSB repair, ICL repair and stabilization of stalled replication forks.



Amélie Fradet-Turcotte et al. Endocr Relat Cancer 2016;23:T1-T17


Nucleotide excision repair
Transcription-coupled repair
DNA damage
DNA interstrand cross-link repair
Base excision repair
RNA polymerase II
DDB2
ATR
DNA-damage-induced responses
ATM
FANCL
XPC
Histone H2AX
p53BP1
Role of NFBD1 in DNA damage response
NFBD1
FANCD2
NTH1
DNA polymerase beta
p53
Chk2
Nibrin
PCNA
ATF-1
Brca1
BRIP1
Brca2
MSH2
MSH3
MSH6
MLH1
Bard1
Brca1/Bard1
MRE11
Rad50
Rad51
MRN complex
Mismatch repair
Non-homologous end joining repair
NHEJ mechanisms of DSBs repair
Homologous recombinational repair

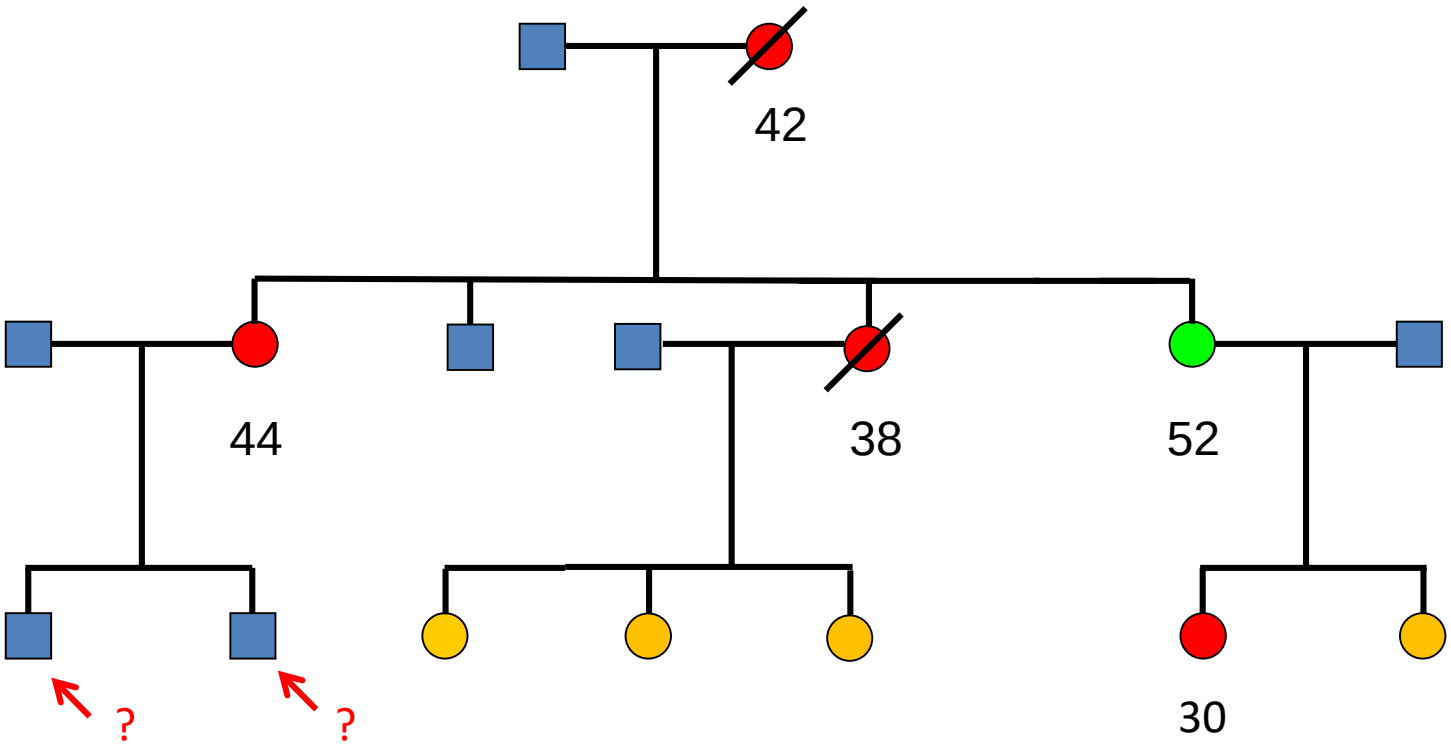

DNA repair
Synthetic lethality
HBOC
RAD51C
cdk1
BER
NER
XPF
ERCC1
RPA
XRCC1
PARP
BRCA1/2
BRCA2
BRCA1
PALB2
HR pathway of DSB repair
FA-BRCA pathway
FANCD1
FANCJ
MutLα (MLH1/PMS)
FA
MMR
FANCD2
MSH2/MLH1
MutSα (MSH2/MSH6)
monoubiquitination
DNA repair
ATR
Lynch syndrome
Chk1
Li-Fraumeni syndrome
TP53

The identification of patients with genetic a predisposition

1. Family Studies
2. Tumour Pathology

FAMILY STUDIES

HBOC Family



= unaffected

= at risk of BrCa or Ovca

= BrCa affected

= OvCa affected

Pathology North

Figure 1. Breast–ovarian cancer families positive for BRCA1-Lys505ter.

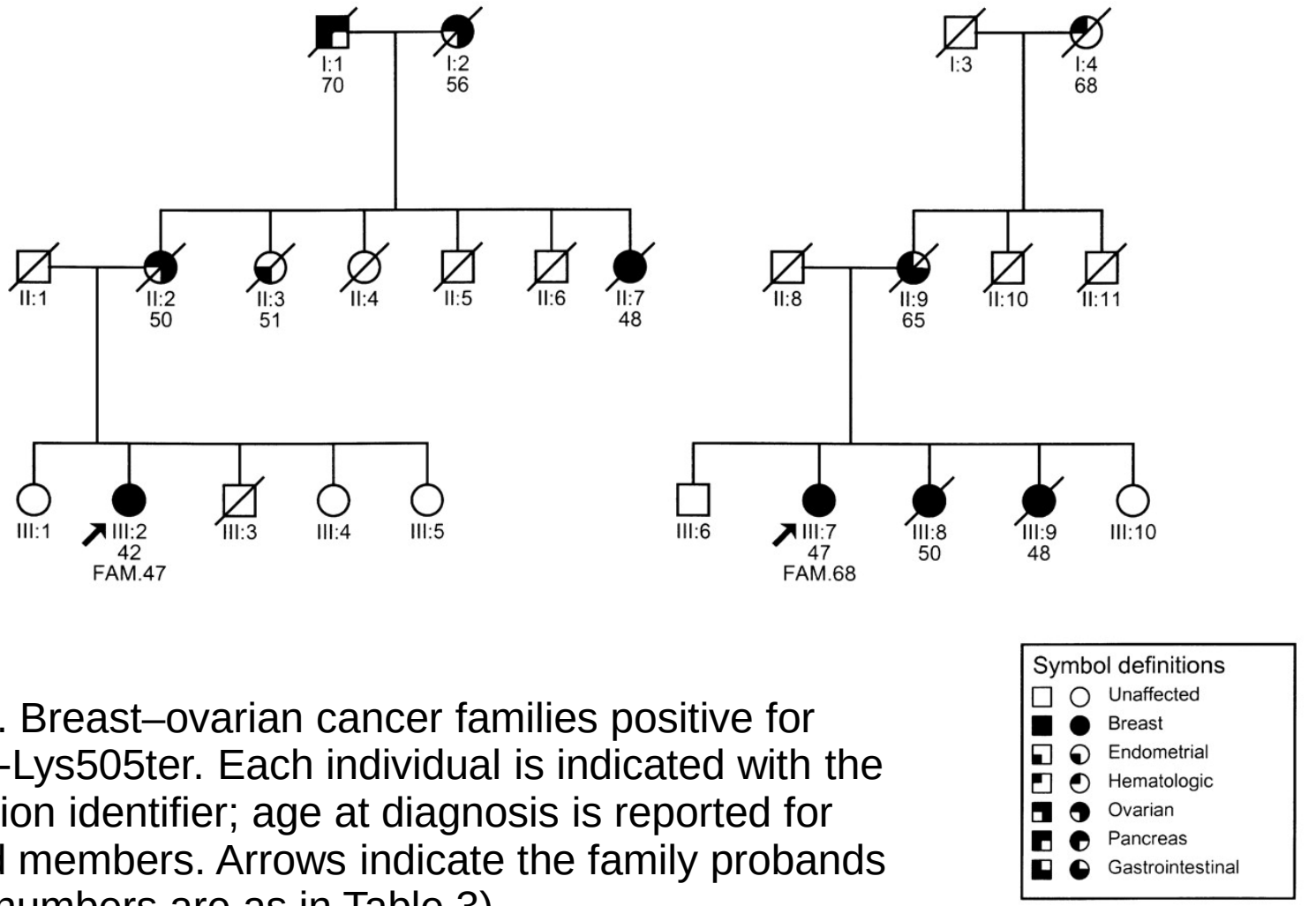


Figure 1. Breast–ovarian cancer families positive for BRCA1-Lys505ter. Each individual is indicated with the generation identifier; age at diagnosis is reported for affected members. Arrows indicate the family probands (family numbers are as in Table 3).

Palmieri G et al. Ann Oncol 2002;13:1899-1907

Figure 3. Pedigree of the breast cancer family from Thiesi.

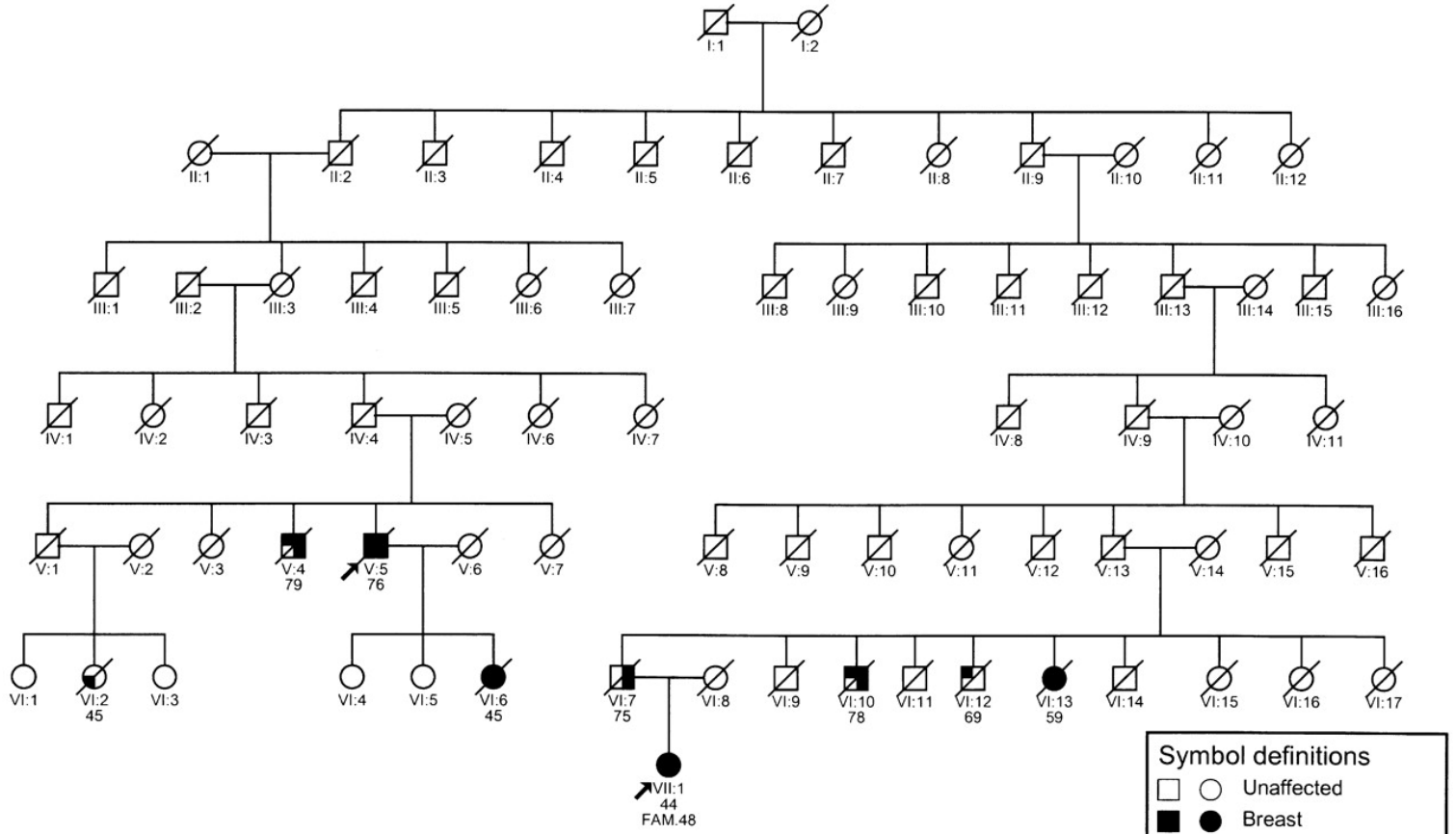


Figure 3. Pedigree of the breast cancer family from Thiesi. For each individual, a generation identifier is indicated; age at diagnosis is reported for affected members. Arrows indicate the two patients positive for BRCA2-8765delAG and independently identified during the breast cancer population screening (family number is as in Table 3).

Pathology North

Palmieri G et al. Ann Oncol 2002;13:1899-1907

Hereditary Breast and Ovarian Cancer

BRCA1 (17q11.2-q23)
BRCA2 (13q12-13)

Carrier frequency: 1:433

No other evidence to suggest any other major autosomal dominant predisposition to breast cancer

Evidence to suggest that early onset breast cancer is a result of complex disease inheritance

Pathology North

Family History Summary

How to identify familial breast/ovarian cancer

1. Early age of disease onset (< 40 y.o.a)
2. Multiple affected family members (usually with one or more under 55 y.o.a.)
3. Family history of breast and ovarian cancer
4. Family history of breast cancer and other cancers
5. Need to be aware of several familial cancer syndromes

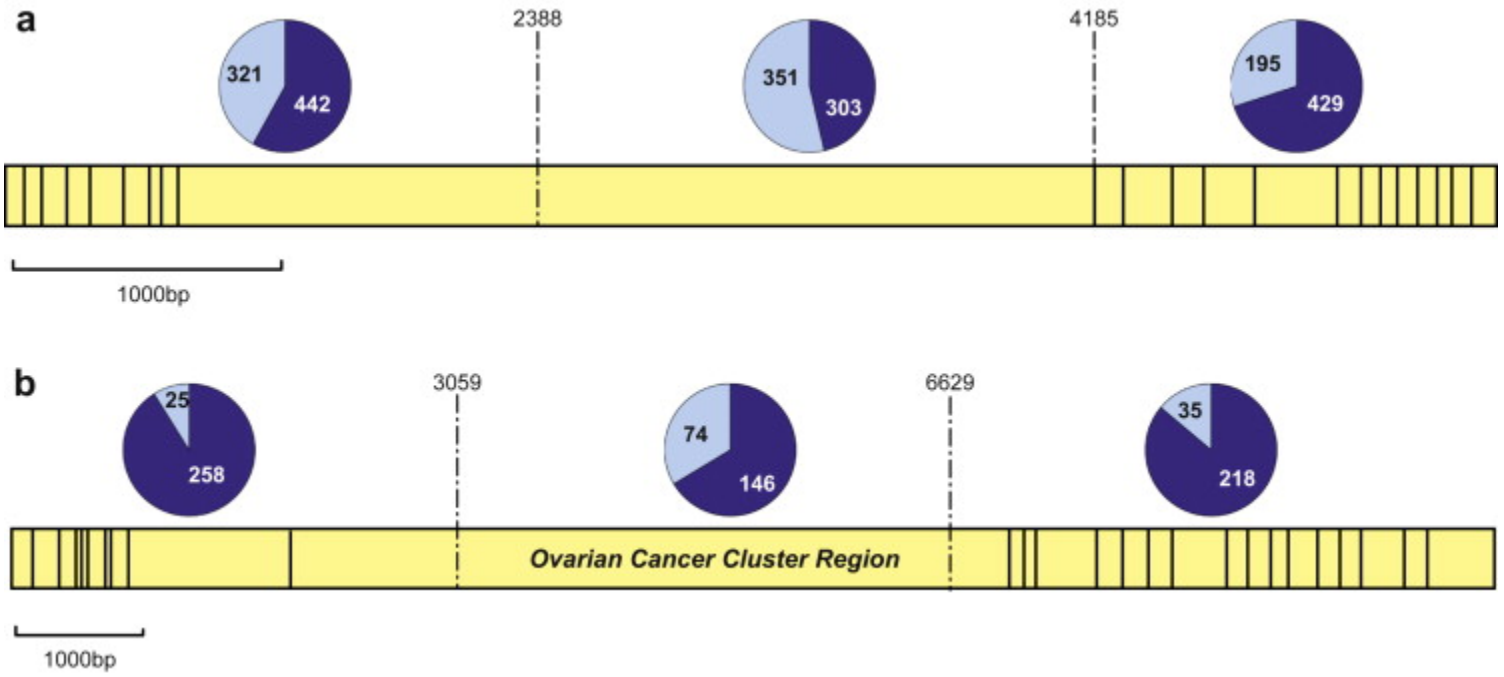
Familial Breast Cancer

BRCA1 associated with:

- Breast Cancer
- **HIGH GRADE SEROUS** Ovarian Cancer (accounts for ALL OvCa families)
- Pancreatic cancer, RR 2.26 (95%CI)
- Uterine body and cervix, RR 2.65 (95%CI)
- Prostate cancer (<65 y.o.a.) RR 1.82 (95%CI)
- Prostate cancer (>65 y.o.a.) RR 0.78
- Cancer incidence outside of Br or OvCa

  increased in women RR 2.30
- **No overall change in RR in men**

Pathology North



Ramus and Gayther 2009

Familial Breast Cancer

BRCA2 associated with:

- prostate cancer (rr 4.65)

  (2% of all early onset (<55 y.o.a.) - harbour BRCA2 mutations)
- pancreatic cancer (rr 3.51)
- gall bladder cancer (rr 4.97)
- buccal cavity & pharynx (rr 2.26)
- stomach cancer (rr 2.59)
- malignant melanoma (rr 2.58)

Pathology North

TUMOUR PATHOLOGY

Pathology North

The Pathology of BRCA1 and BRCA2 breast tumours

- *BRCA1* & *BRCA2* mut +ve tumours - higher grade
- *BRCA1* mut +ve tumours: basal-like, more pleomorphic, higher mitotic content, medullary & atypical medullary carcinoma more frequent, less ductal carcinoma *in situ*
- *BRCA2* mut +ve tumours: less tubule formation, no difference in pleomorphism or mitotic content compared to sporadic breast cancer
- **The ER-ve, PR-ve and HER2-ve tumour phenotype significantly over-represented**

Pathology North

TNBC population

- Australian Cohort: n = 439
  - Average age at diagnosis 57 ± 15 years
    - » ≤ 50 years n = 153 (34.9%)
    - » > 50 years n = 286 (65.1%)

- Polish Cohort: n = 335
  - Average age of diagnosis 59 ± 10 years
    - » < 50 years 49 (14.6%)
    - » > 50 years 286 (85.4%)

- Type of primary tumour:

	Aus.	Pol.
» Ductal	93.2%	67.8%
» Papillary	0.7%	0.6%
» Medullary	2.3%	12.5%
» Other	3.8%	19.1%

TNBC Characteristics

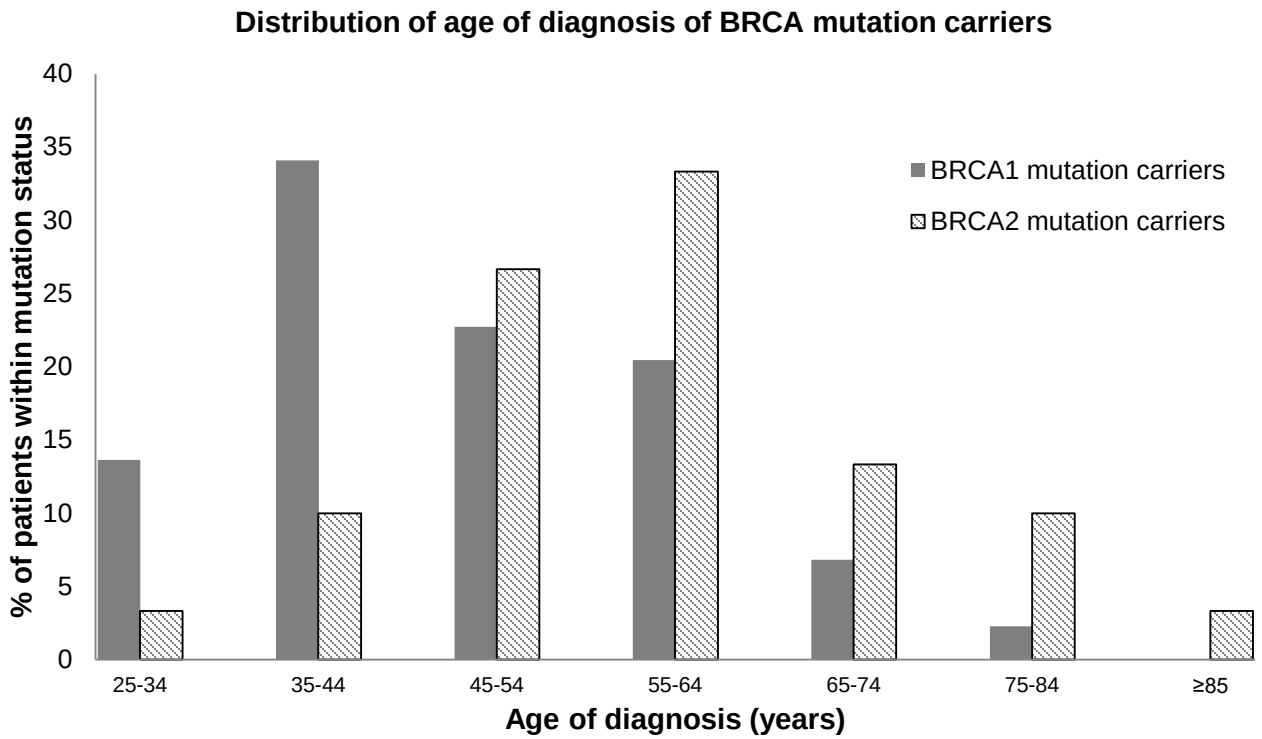
- 36 patients **had a** family history of disease
- 38 had **NO** family history

- Mean age of disease onset for **BRCA** mutation carriers 52 .1 ± 13.3 years years for non-carriers 58.7 ± 17.7 years

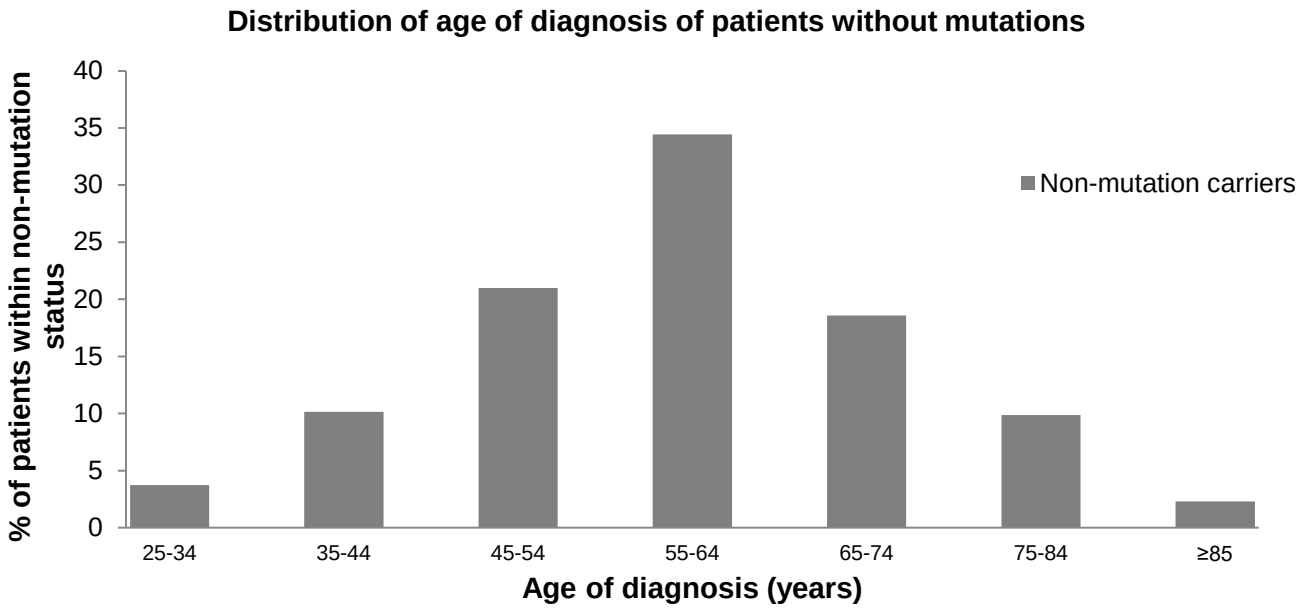
- BRCA1 mutation carriers average age of disease diagnosis 47.2 ± 11.8 years
- BRCA2 mutation carriers average age of disease diagnosis 58.8 ± 13.2 years

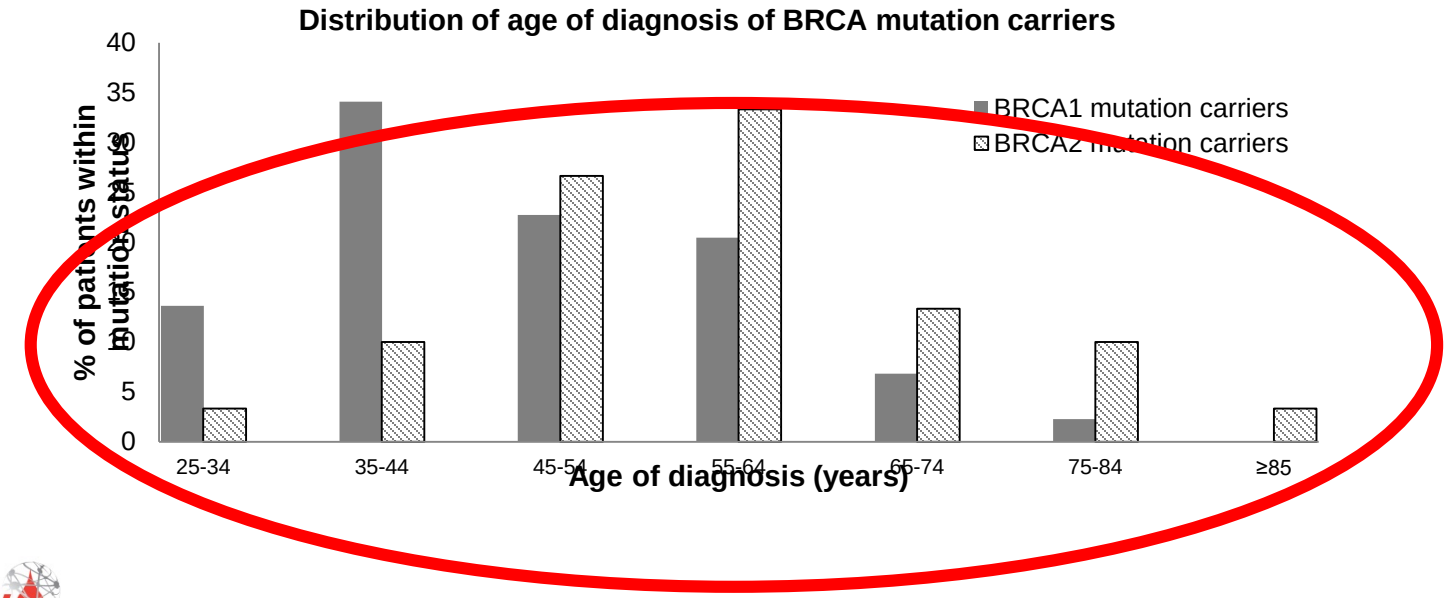
- **NO difference in the average age of disease diagnosis of BRCA2 carriers and non-carriers**

Pathology North



Pathology North





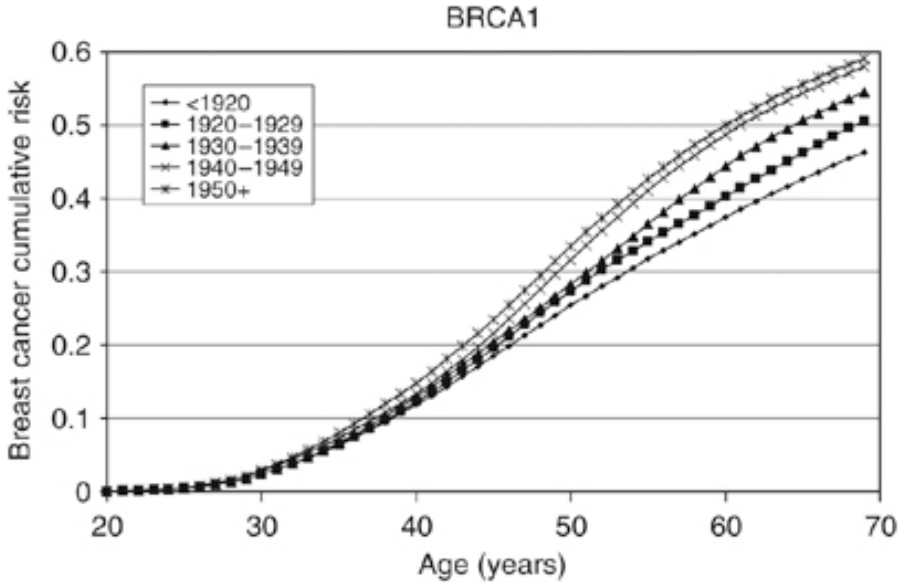
Pathology North

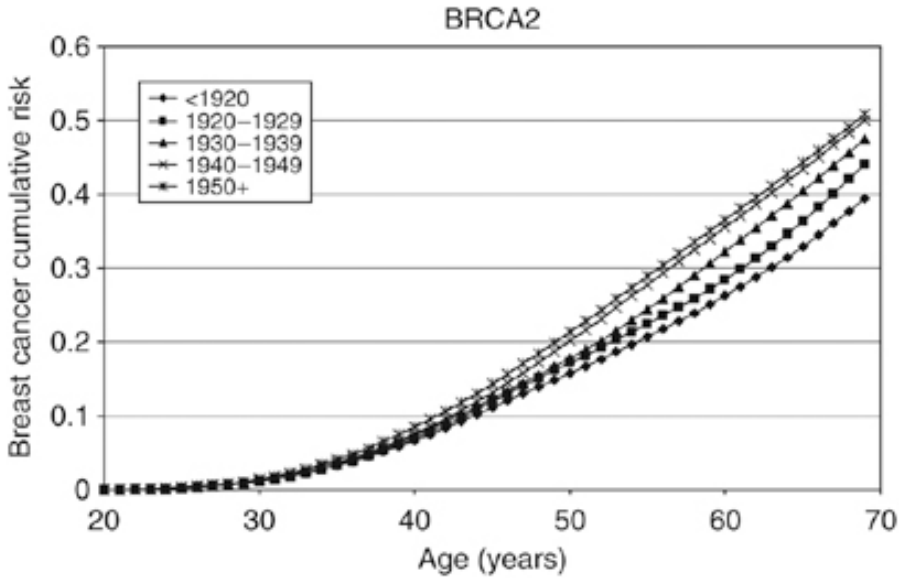
CLINICAL IMPLICATIONS

- ~10% of women with TNBC may have a *BRCA1* or *BRCA2* mutation
- Patients with TNBCs should be considered as candidates for genetic screening
- Should not restrict screening to women without a family history
- Age restrictions for BRCA testing should be relaxed (BRCA2…)
- A sub-population of TNBCs could receive better targeted therapy

Pathology North

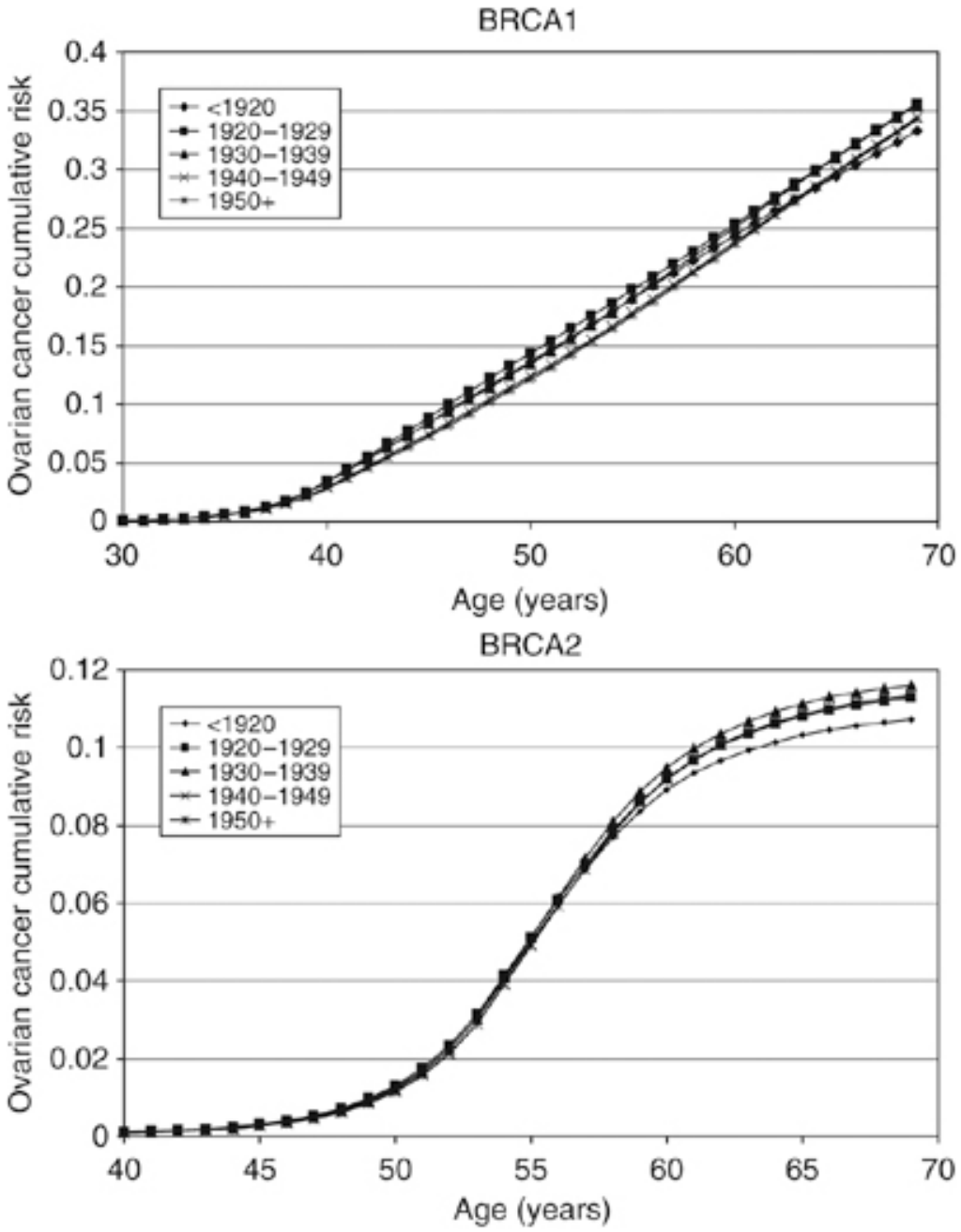
Population based risks
of BrCa for BRCA1 and BRCA2
mutation carriers





Pathology North

Population based risks of OvCa for BRCA1 and BRCA2 mutation carriers



Pathology North

TYPES OF MUTATION

- THE BIG RED DOG BIT THE CAT - reference sequence

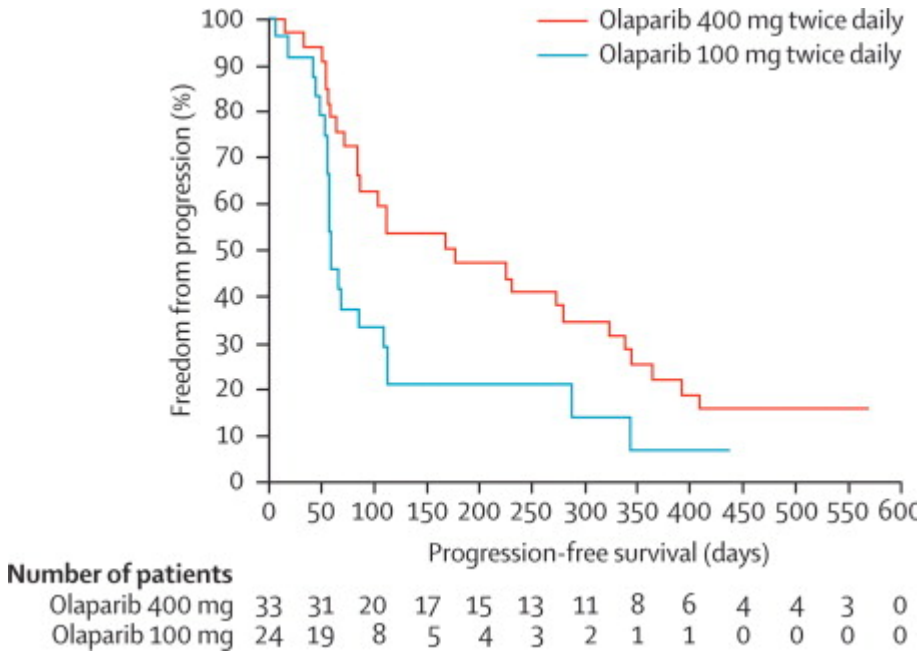
- THE BIG RED DOB BIT THE CAT – missense variant
- THE BIG RED OGB ITT HEC AT – nonsense variant
- THE BIG RED THE CAT – deletion
- THE BIG RED DOG RED DOG BIT THE CAT – insertion
- THE BIG GOD DER BIT THE CAT – inversion
- THE BIG RED DOg BIT THE CAT – “silent” variant

- Others include splice variants, cryptic splice sites, altered epigenetic marks, altered expression controlling elements…

BRCA Disease Phenotypes

- Breast Cancer
  - Triple Negative Breast Cancer – Over-represented
- Ovarian Cancer
  - High Grade Serous Ovarian Cancer

PARP Inhibitors
A proof of concept trial for advanced ovarian cancer

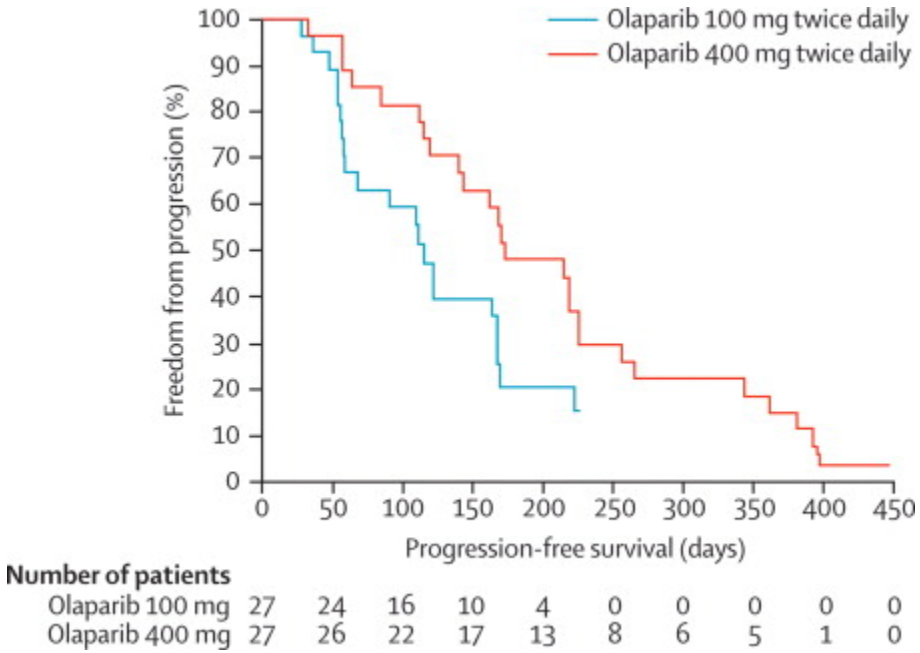


Kaplan-Meier curves of progression-free survival for the intention-to-treat population

Pathology North

Tutt A et al. The Lancet 376 245-251

PARP Inhibitors
A proof of concept trial for advanced breast cancer



Kaplan-Meier curves of progression-free survival for the intention-to-treat population

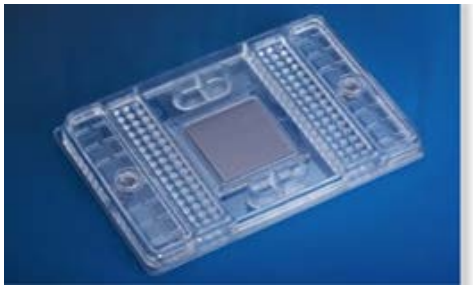
Pathology North

Tutt A et al. The Lancet 376 235-244

How many BrCa genes are actionable

Breast Cancer and Personal Genome Sequencing

- Technology of Mutation Detection has improved
  - Microfluidics
  - Massively Paralleled Sequencing (Next Generation Sequencing



Pathology North

Mutation Detection

- Next Generation DNA sequencing has revolutionised mutation detection
- More genes, less cost and decreased turn-around times
- Commercial entities driving genetic testing
- **Availability of "Gene Panels" that include a wide variety of breast cancer susceptibility genes**
- How many are clinically actionable???

Pathology North

Genetic Predispositions to Breast Cancer

- Risk genes defined by the presence of Loss of Function (LoF) variants
- Many have not had population control data assessed
- Most do not have any disease penetrance estimates – therefore difficult to assign causality

Genes associated with inherited breast cancer

BRCA1	*BRIP1*	*PALB2*
BRCA2	*CHEK2*	*PTEN*
ATM	*MRE11A*	*RAD50*
ATR	*NBN*	*STK11*
BARD1	*NF1*	***TP53***
BLM	*CDH1*	*XRCC2*

Pathology North

What is required for a gene to be used for clinical purposes

1. Transmission of phenotype is obvious (often at younger than normal ages)
2. High to very high disease penetrance (>50%)
3. Mutations are unequivocally pathogenic
4. Mutations are absent (or present at very low rates) in a control population
5. Population carrier frequencies estimated

Pathology North

Genes associated with inherited breast cancer

BRCA1

BRCA2

TP53

PALB2

Pathology North

Options for someone with a BRCA1 or BRCA2 mutation

- Surveillance
  - Mammography, clinical breast examination, MRI, transvaginal ultrasound, CA-125 antigen detection (for OvCa it is not known if this reduces the chance of dying of disease)
- Prophylactic Surgery
  - Bilateral prophylactic mastectomy and salpingo-oophorectomy (protection may differ between BRCA1 and BRCA2 carriers). Residual risk of disease!!!
- Chemoprevention
  - Tamoxifen reduces BrCa risk ~50% and reduces recurrence. Raloxifen may also reduce BrCa risk (no direct studies to date)

Genes that have been associated with breast cancer risk – but where information is lacking

ATM
ATR
BARD1
BRIP1
BLM
CHEK2
CDH1
MRE11A
NBN
NF1
PTEN
RAD50
STK11
XRCC2

Pathology North

What is the problem

- No or little information about disease penetrance i.e. age dependant risk
- Very small numbers of patients identified to date with causative mutations
- Little, if any, information about what diseases are associated with causative mutations
  - Is it just BrCa or are other cancers over-represented
- No or little information about the presence of genetic variants in a healthy population
- No information about environmental risk factors

Pathology North

Addressing the shortfall in knowledge

- Examine the frequency of mutations in case/control populations
- Try to define disease penetrance
- Determine if mutations in specific genes alter risk of a specific malignancy
- Assess whether single gene associated with multiple tumour types (i.e. PaCa and BrCa)

How to address the problem

Can’t answer all questions at once – it requires time and resources

Can construct an approach to begin to address these issues

The Study

- 2000 index patients (>95% BrCa; <5% OvCa) all pre-screened for *BRCA1* or *BRCA2* mutations
- All patients were <50 y.o.a OR had a strong family history of disease
- All patients collected between 1997 - 2014
- 1997 population controls (*LifePool* Study) cancer free censored Jan. 2015. Average age 59.9 (range 40 – 92)
- Institutional review board approved study

Pathology North

What did we observe

- 3994 samples sequenced – 94% of the coding regions of all genes covered
- 6 actionable mutations identified in *BRCA1* and *BRCA2*: frequency in patient group 0.4 % and control group 0.2%
- *BRCA1* and *BRCA2* mutation carriers not included in further analysis

Pathology North

Gene	Cases n=2,000			Controls n= 1,997			P value[a]	OR (95% CI)
	LoF	Pathogenic missense	Total (carrier frequency %)	LoF	Pathogenic missense	Total (carrier frequency %)		
BRCA1	2	2	4	4	0	4 (0.2%)	na	na
BRCA2	1	1	2	8	0	8 (0.4%)	na	na
ATM	7	1	8 (0.4%)	4	0	4 (0.20%)	P=0.14	2.67 (95% CI, 0.71 to 10.1)
ATR	3	0	3 (0.15%)	1	0	1 (0.05%)	P=0.37	3.03 (95% CI, 0.31 to 29.1)
BARD1	3	0	3 (0.15%)	0	0	0	P=0.12	7 (95% CI, 0.36 to 136)
BLM	3	0	3 (0.15%)	3	0	3 (0.15%)	P=1.00	1.00 (95% CI, 0.20 to 5.0)
BRIP1*	7	0	7 (0.35%)	1	0	1 (0.05%)	P=0.04	7.05 (95% CI, 0.87 to 57.4)
CDH1	1	0	1 (0.05%)	0	0	0	P=0.50	3.01 (95% CI, 0.12 to 74.1)
CHEK2	1	6	7 (0.35%)	0	6	6 (0.3%)	P=0.99	1.17 (95% CI, 0.39 to 3.49)
MRE11A	3	0	3 (0.15%)	0	0	0	P=0.12	7.0 (95% CI, 0.36 to 136)
NBN	2	0	2 (0.1%)	3	0	3 (0.15%)	P=1.00	0.67 (95% CI, 0.11 to 4.0)
NF1	1	0	1 (0.05%)	1	0	1 (0.05%)	P=1.00	1.0 (95% CI, 0.06 to 16)
PALB2	22	0	22 (1.1%)	3	0	3 (0.15%)	P= <0.0001	7.43 (95% CI, 2.22-24.9)
PTEN	0	0	0	0	0	0	na	na
RAD50	2	0	2 (0.1%)	4	0	4 (0.2%)	P=0.69	0.50 (95% CI, 0.09-2.74)
STK11	0	0	0	0	0	0	na	na
TP53	1	4	5 (0.25%)	0	0	0	P=0.03	11 (95% CI, 0.61 to 201)
XRCC2	2	0	2 (0.1%)	0	0	0	P=0.25	5.05 (95% CI, 0.24-105)

Pathology North

From Thompson, Campbell, Scott et al. JCO, 2016

- In 1994 patients 69 variants identified; 1985 controls 26 variants identified
- *PALB2* contributed 22 causative changes in the patient group and 3 in the control population*
- 5 patients harboured a pathogenic *TP53* mutation (none had a family history of disease)
- **BrCa** is not a criteria to select for *PTEN* or *STK11* testing (Cowden's Syndrome and Peutz-Jeghers Syndrome)

*see later

Pathology North

- Mutation detection rate stratified by age did NOT differ between the two groups

- Presence of a significant number of mutations in the CONTROL group => Positive predictive value **0.73** (95% CI 0.62-0.81)

- Population attributable risk (PAR) = 2.1% - just over half of the PAR due to *PALB2*

Pathology North

- Aside from *TP53* and *PALB2* the contribution of the remaining 12 genes was modest
- 2.1% (42 individuals) patients harboured Loss of Function change vs 1.15% (23 individuals) in the control population
- Consistent with a modest risk OR 1.83 (95%CI 1.32-2.34) in patient group

Pathology North

- *NBN* & *RAD50* more mutations in controls!
- *CHEK2* and *ATM* similar frequencies
- *PALB2**, *TP53* & *BRIP1** variants: Patients > Controls

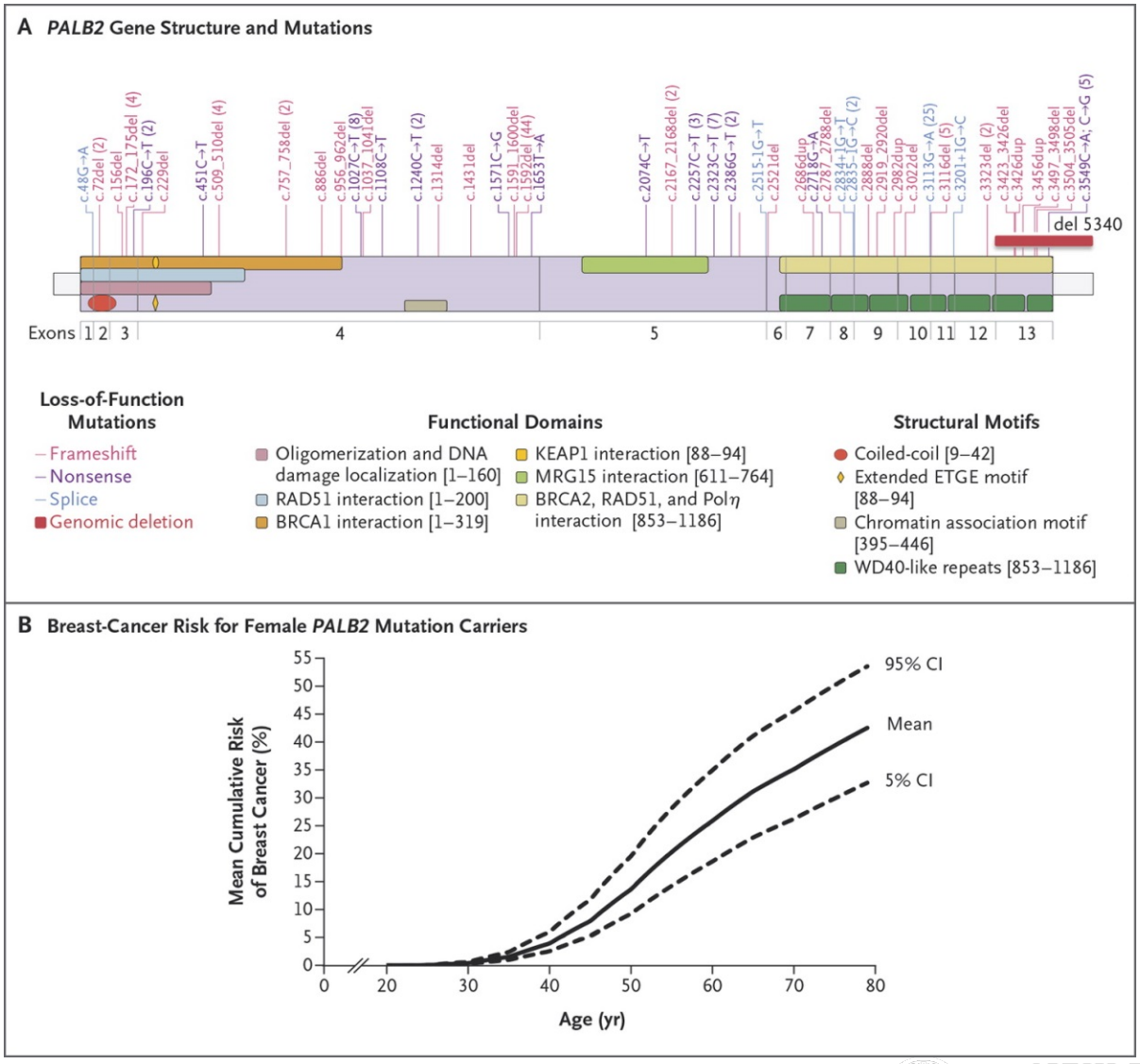
* see later

Pathology North

PALB2

- Compared to UK population based data
  - *PALB2* mutation carriers have **9.47** fold increase in BrCa risk
  - i.e. 47.5% risk of BrCa by 70 y.o.a.
  - Suggestion that BrCa risk was greater for younger women (RR = 17.6 for women 20 – 39 y.o.a. & 8.7 for women 40 – 70 y.o.a.
  - Best fitting model (taking family history into account) suggests the RR between 20 - 24 y.o.a. is 9.01 decreasing to 4.56 at >75 y.o.a

Loss-of-Function *PALB2* Germline Mutations in Relation to Functional Domains and Structural Motifs of the PALB2 Protein, and Cumulative Breast-Cancer Risk for Female Mutation Carriers



Pathology North

The NEW ENGLAND JOURNAL of MEDICINE

Antoniou AC et al. N Engl J Med 2014;371:497-506.

PALB2

- Ovarian Cancer risk increased relative to population figures
- Overall risk 2.31
- Birth cohort seemed to be associated with risk of BrCa in *PALB2* carriers, with greatest risk in women born after 1960 compared to those born between 1940 and 1959 or before 1940.
- <1940: RR = 1.00
- 1940-1959: RR 2.84
- ≥ 1960: RR 6.29

Pathology North

PALB2

- What we do not know
  - Mastectomy or oophorectomy and ↓risk
  - Epidemiological risk factors
  - Accurate population frequency information
  - Treatment effects (i.e. Cisplatin for *BRCA1& BRCA2* carriers is v. effective)
  - Accurate disease penetrance estimates

BRIP1

- >64,000 cases and 51,500 controls

- Causative changes found in:

  77 cases: 42 controls

  OR 0.99 (95% CI 0.61-1.61, P=0.98)

NOT ASSOCIATED WITH BREAST CANCER RISK BUT IS LINKED TO OVARIAN CANCER RISK

Pathology North

Summary

- Family history (FH) is the simplest method to identify women at risk
- Tumour pathology reveals the same percentage of *BRCA* variant carriers compared to FH
- New genes associated with breast cancer being identified
- Many of the new genes require more supportive evidence of causality