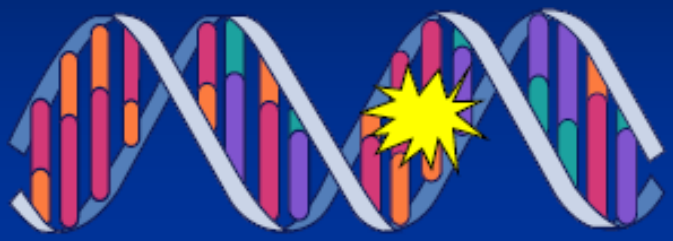
Breast Cancer Genetics

Prof Dr Volkan Baltacı

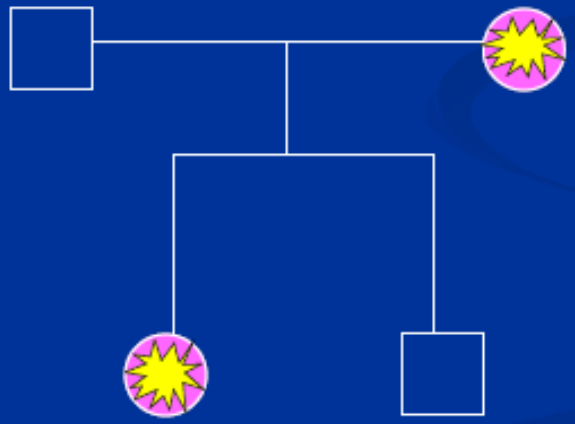
Mikrogen Genetik Hastalıklar
Tanı Merkezi

All cancers are genetic



SPORADIC

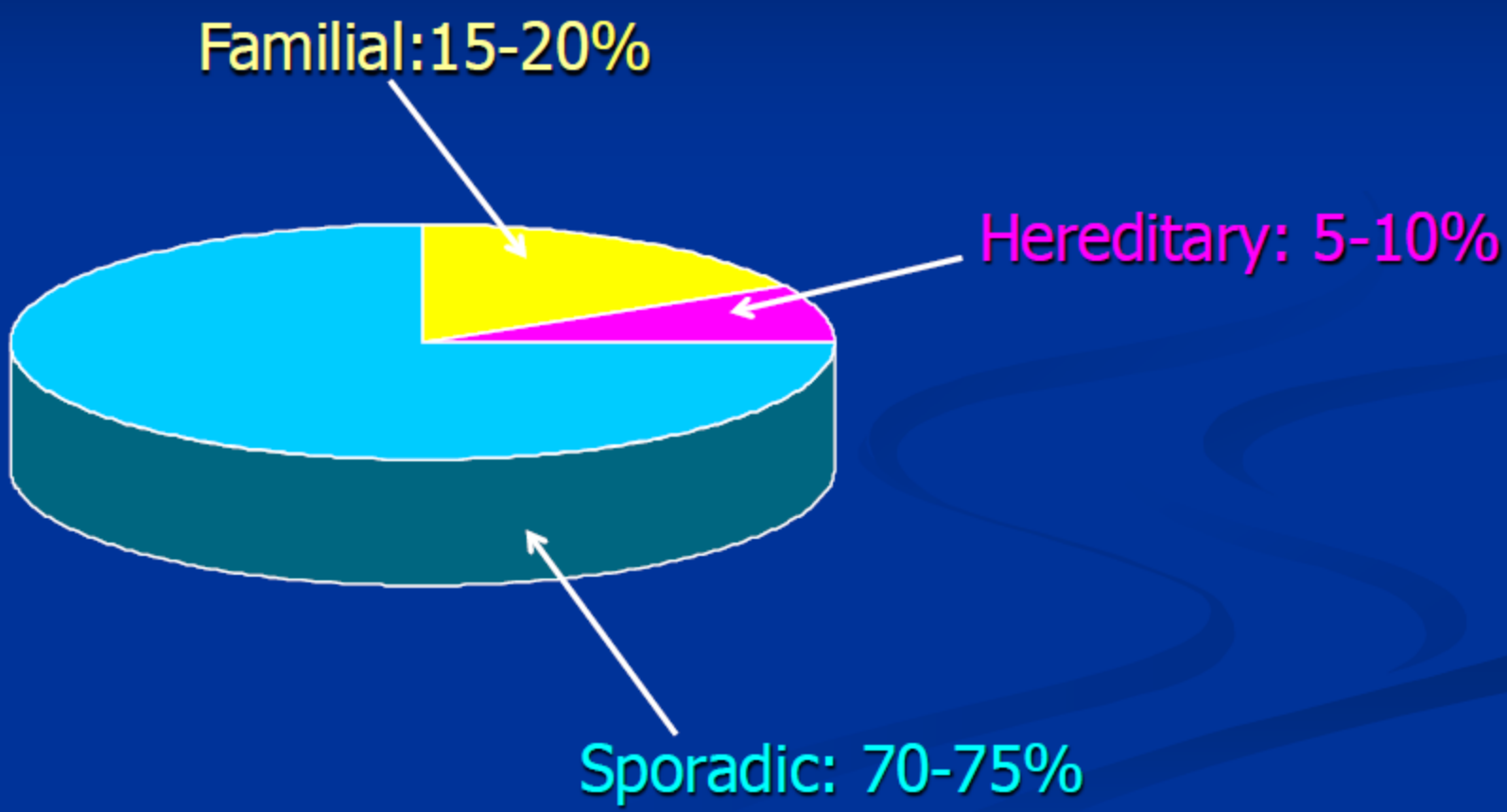
BUT



GERMLINE

Not all cancers are *hereditary*

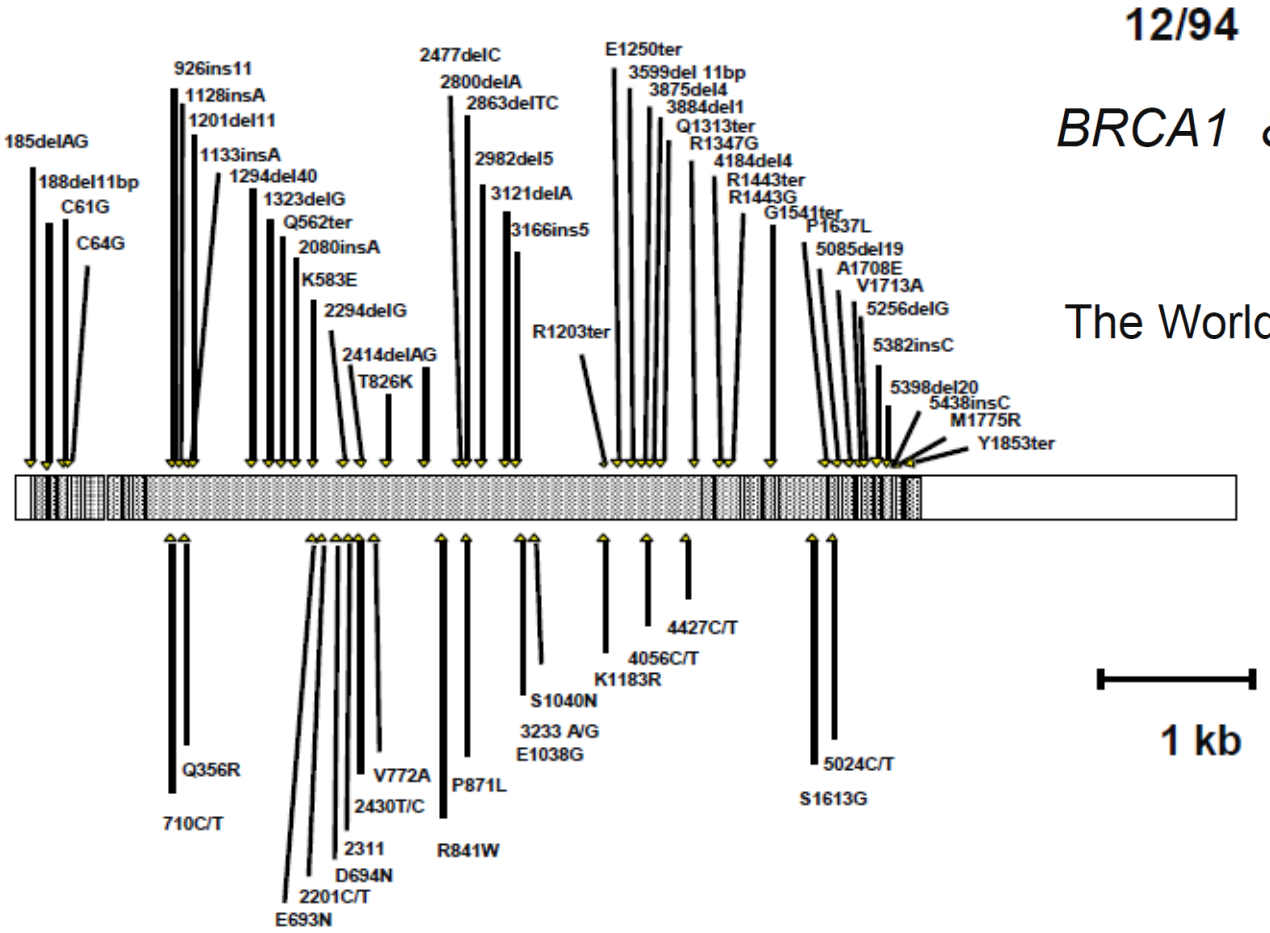
Genetic breakdown of breast cancer



Why Genetics ?

- Prevention
- Early Detection
- Prognosis
- Tailored Therapy

BRCA1 Mutations and Polymorphisms



BRCA1 – 22 exons – 5.5 kb ORF

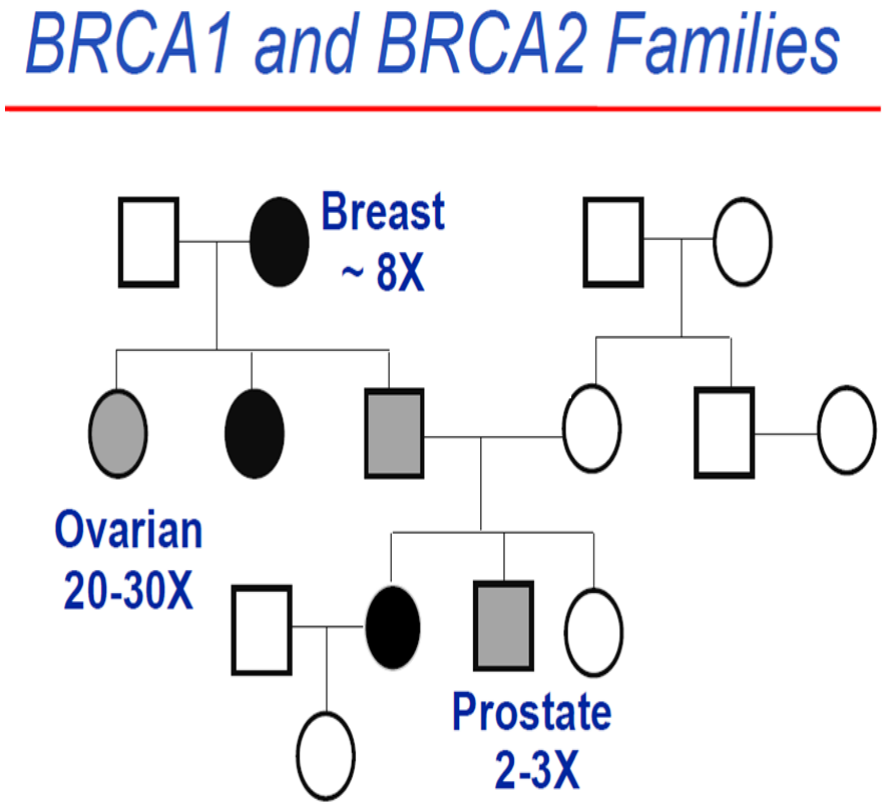
BRCA2 – 28 exons – 10 kb ORF

(Why we need to test BRCA genes?)

Inherited Breast Cancer

- Over 90% of cancer families with breast/over cancer caused by mutations in BRCA1 or BRCA2 genes

- Mutation carrier females have >80% lifetime risk of breast cancer and 20-60% risk of overian cancer



(Why we need to test BRCA genes?)

The Patients with Unilateral Breast Cancer

The mean cumulative risk for contralateral breast cancer by age 70 years were estimated to be; 83% for BRCA1 carriers and

62% for BRCA2 carriers.

Ford D, Am J Hum Genet, 1998
Kuhl CK, Lancet, 2007
Mavaddat N, J Nat Cancer Ins, 2013

(Why we need to test BRCA genes?)

Prostate Cancer Risk (BRCA2)

- BRCA2 mutations were found to ~6-fold increase the risk of prostate cancer.

- No risks were not observed for BRCA1 mutation carriers

- BRCA2 mutation carriers with prostate cancer had more agressive and decreased survival than BRCA1 carriers or non-carriers.

- BRCA2 mutation carriers have also a higher risk for pancreatic cancer and melanoma

Castrol E, J Clin Oncol, 2013
Norad SA, Br J Cancer, 2008
Ferrone CR, J Clin Oncol, 2009

Breast Cancer Genes

- high penetrance / low frequency

BRCA1, BRCA2

- low penetrance / high prevalence

ATM CHECK2
BRIP1 PALB2

TOX3 6q FGFR2
MAO3KI CASP8
LSP1 8q

High Penetrance Breast Cancer Susceptibility Genes

CONDITION	GENE (S)	BREAST CANCER RISK	OTHER ASSOCIATED CANCERS
Hereditary Breast/Overian Cancer (HBOC)	BRCA1 BRCA2	20-35% by age 50, 60 -85 % lifetime	Ovarian, Pancreatic, Prostate, melanoma
Li-Fraumeni Syndrome	TP53	56% by age 45, >90% lifetime	Soft tissue sarcomas, leukemias, brain tumors, osteosarcomas
Cowden Syndrome	PTEN	30-50 % lifetime	Tyroid, endometrial
Peutz-Jeghers Syndrome	STK11	8 % by age 40, 32 % by age 60	Colorectal, gastric, Pancreatic
Hereditary Diffuse Gastric Cancer (HDGC)	CDH1	39 % lifetime (lobular)	Diffuse gastric cancer

Genotypes of BRCA Genes (germline)

- BRCA1 (herited) / Normal (heterozygous)

  high risk heritable cancer (solid cancer via loss of heterozygousity)
- BRCA1 / BRCA1 homozygous not viabl
- BRCA1 / BRCA2 (very low number of documented person in Jewish people)

BRCA1 Mutation Data

	Total Entries	Distinct Alterations	One Family Only
Nonsense	1046	176	84
Frameshift	4780	513	303
Splicing	598	175	100
Missense	2734	489	259

BRCA1 Mutation Data

The Unclassified Variant Problem

(VariationsUnknownSignificance)

Missense	2734	489	259

James Watson (2007)

FIRST GENOME SEQUENCING WITH NGS

Multi-Gene Testing

EXAMPLE 1	EXAMPLE 2
BRCA1, BRCA2, BARD1, BRIP1, CHEK2, MRE11A, MSH6, NBN, PALB2, RAD50, RAD51C, TP53	BRCA1, BRCA2, PALB2, TP53, PTEN, STK11, CDH1

- Next-Generation Sequencing allows for the sequencing of multipl genes simultaneously
- Multigene testing may be most useful when more than one gene can explain an inherited cancer syndrome.
- Multigene testing may also be considered for cases who tested negative but whose personel and family history is strongly suggestive of an inherited susceptibility.
- Multi-gene tests increase the likelihood of detecting VUS
- Large genomic rearrangements are not detectable by a sequencing assay (MLPA)

Bery DA, j Clin Oncol, 2010
Dowsett M, J Clin Oncol, 2008

DNA Testing (bullet points)

- Patients who have received an allogenic bone marrow transplant should not molecular genetic testing via blood or buccal samples due to contamination by donor DNA.
- If available DNA should be extracted from a fibroblast culture.
- If this is not possible, buccal samples can be considered subject to the risk of donor DNA contamination.

DNA Testing (bullet points)

If more than one family member is affected members with the following factors should be considered for testing first:

- Youngest age at diagnosis
- Having bilateral disease or multiple primaries
- Having other associated cancers (eg, ovarian)
- Most closely related to the proband
- If no living family memeber, consider testing first or second degree family members with breast cancer

NCCN Guidelines, V 2.2015

Risk Assessment - Counseling - Management

- Testing is not recommended in childeren less than 18 years of age (since conditions associated with BRCA1 and BRCA2 mutations generally have an adult onset)
- Individuals from a family with a known mutation should be tested for this mutation
- Individuals from a family without a known mutation should be tested with full sequencing (BRCA1-2) and large genomic rearrengements (MLPA)

NCCN Guidelines, V 2.2015

COUNSELING/(TESTING)

- A known mutation in a cancer gene within the family
- Breast cancer primaries in a single individual (> age 50)
- ≥ 1 close blood relative with breast cancer <50 y.
- ≥ 1 close blood relative with overian cancer at any age
- ≥ 1 close blood relative with breast and pancreatic cancer at any age
- Invasive overian cancer

TESTING

- Early onset breast cancer (< age 50)
- Triple negative breast cancers (ER/PR/Her2)
- Multiple cases of breast and/or ovarian cancer in the same family
- Breast and ovarian cancer in the same woman
- Bilateral breast cancer (at least one < age 50)
- Male breast cancer
- At least three relatives with pancreas, prostate, sarcoma, brain, endometrial, thyroid, kidney, dermatologic cancers, GIS polyps and diffuse gastric cancers.

Age Related Breast Cancer Risks for BRCA1 / 2 Mutation Carriers

Chance of Developing Cancer *by* age:	BREAST CANCER RISK		POPULATION RISK (non-carriers)
	BRCA1 carriers	*BRCA2* carriers	
30	1-2%	1-1.5%	0.1%
40	10-14%	6-10%	0.5%
50	24-35%	17-26%	2%
60	37-52%	28-42%	4.5%
70	46-63%	38-53%	8%

What About Men?

- Man can also carry BRCA mutations and can pass them on to his doughter or son

- Men with a BRCA2 mutation have an
  - increased risk of breast cancer
  - increased risk of prostate cancer

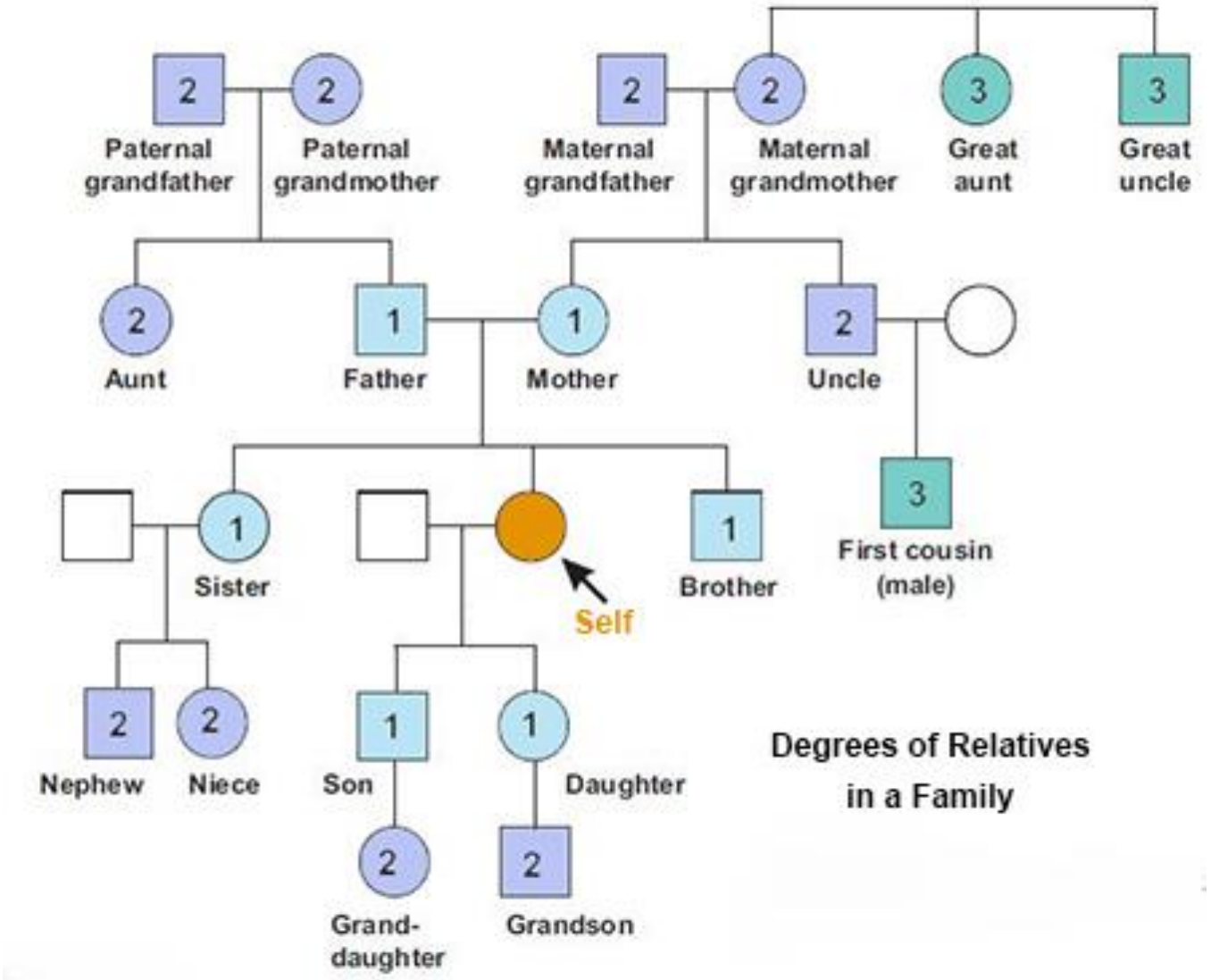
Survival Outcomes

- Survival outcomes between BRCA1 and BRCA2 mutation carrier patients with overian cancer was reported to be longer than non-carrier patients.

- BRCA mutation carriers appeared to be more responsive to chemotherapy compared with non-carrier patients.

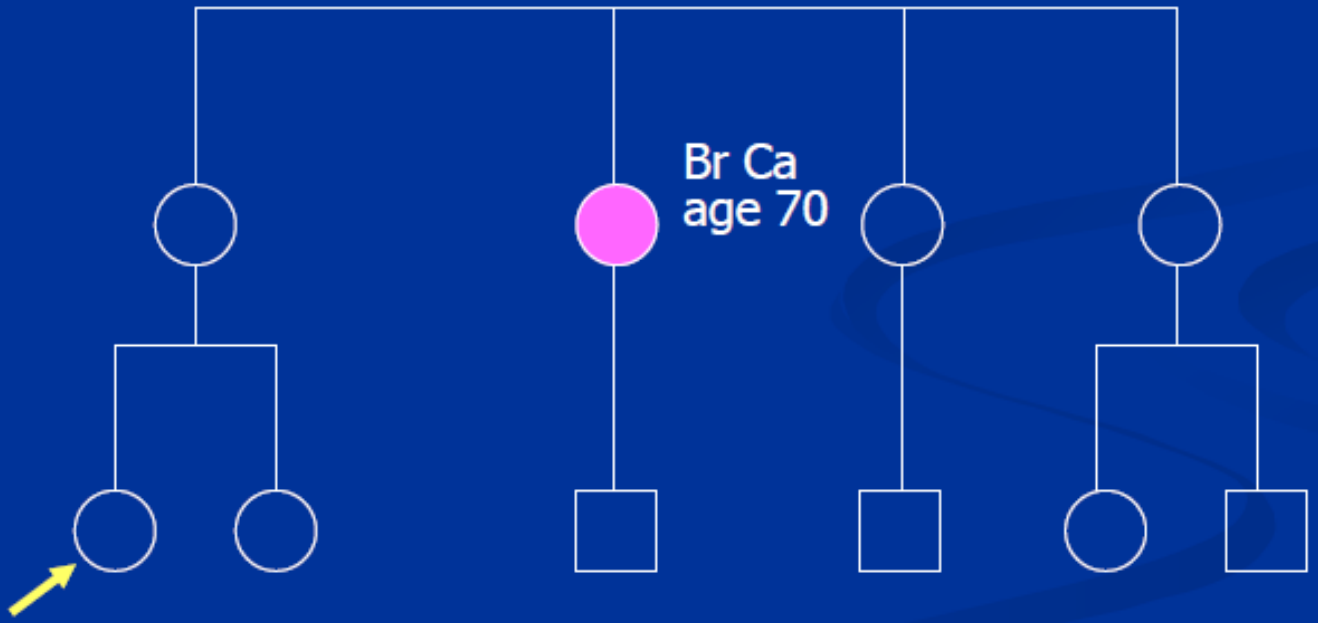
Alsop K, J Jin Oncol, 2012
Yang D, Jama, 2012
Bolton KL, Jama, 2012

PEDIGREE: FIRST-SECOND and THIRD DEGREE RELATIVES of PROBAND



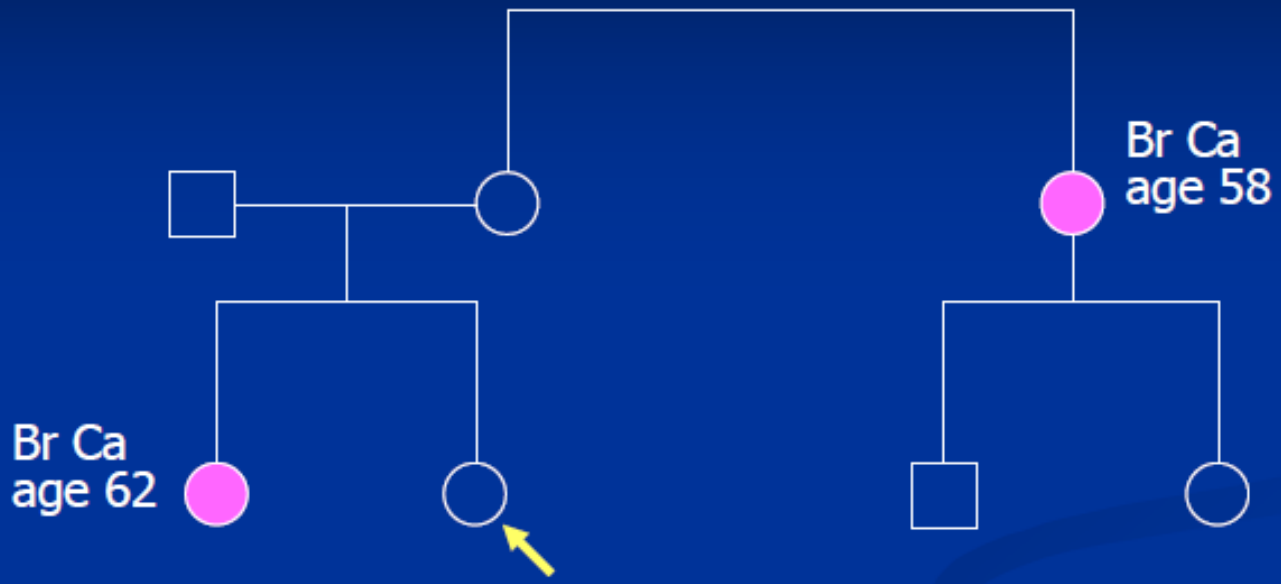
Sporadic Cancer = Single occurrence of cancer in family

- Majority of cases
- Not usually inherited
- Onset later in life



Low or no increased risk to family members beyond general population risk

Familial Cancer = Cluster of Cancer within Families

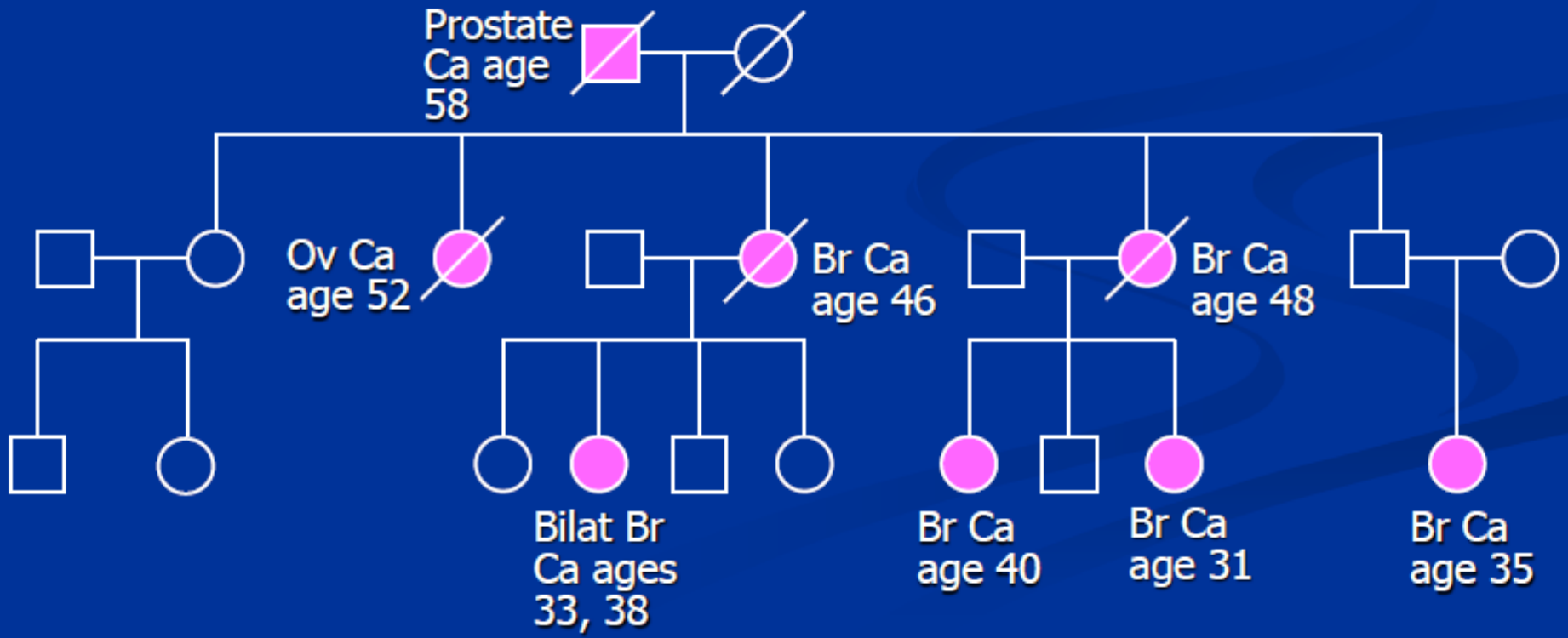


- 2 or more affected 1st or 2nd degree relatives
- Later onset
- Unilateral (one breast)
- Unclear inheritance pattern:
  - Chance alone
  - Common environment
  - Genetic factors (minor)

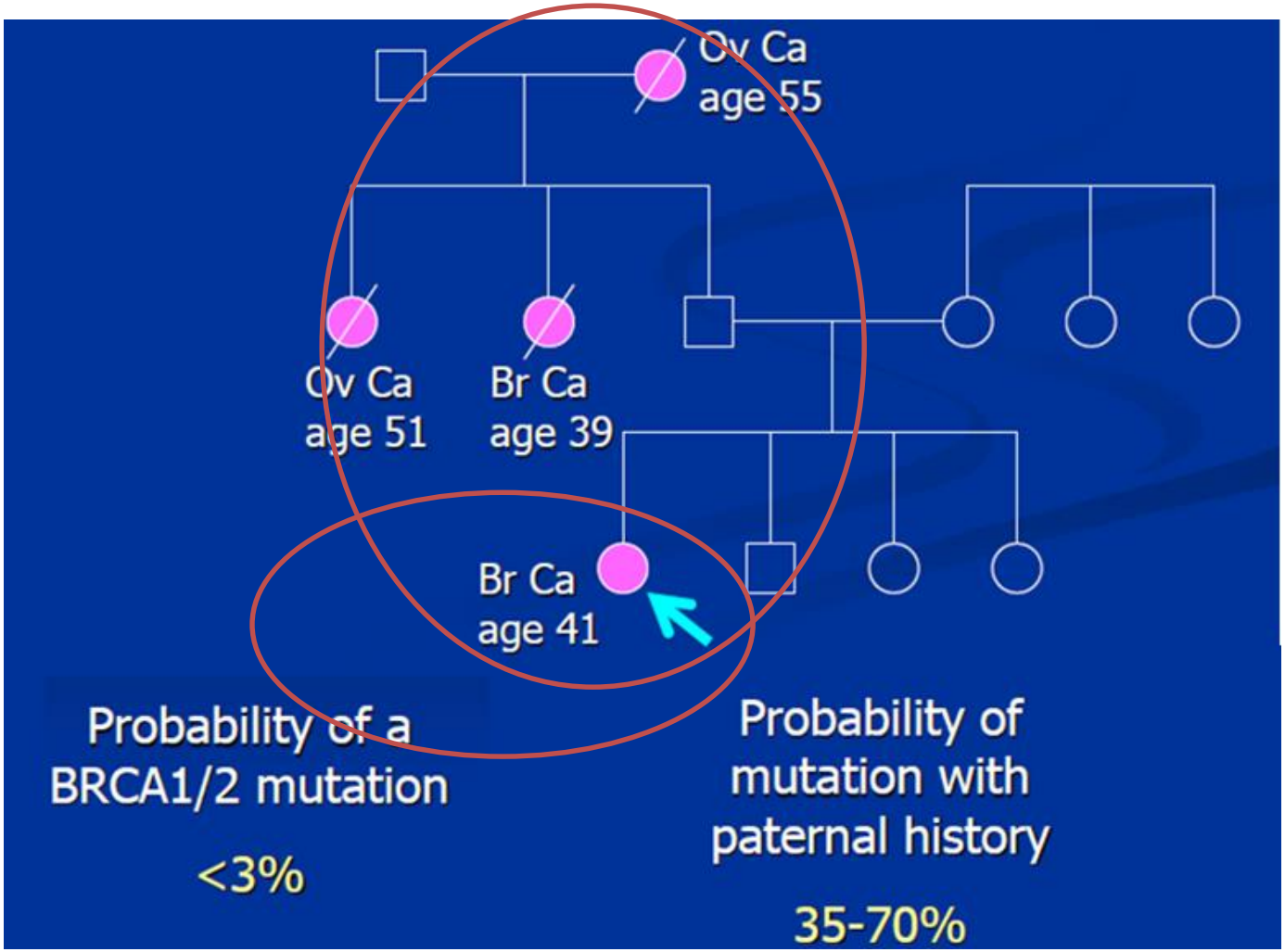
"Modest" increase in risk to family members ~ 2 fold general population

Hereditary Cancer

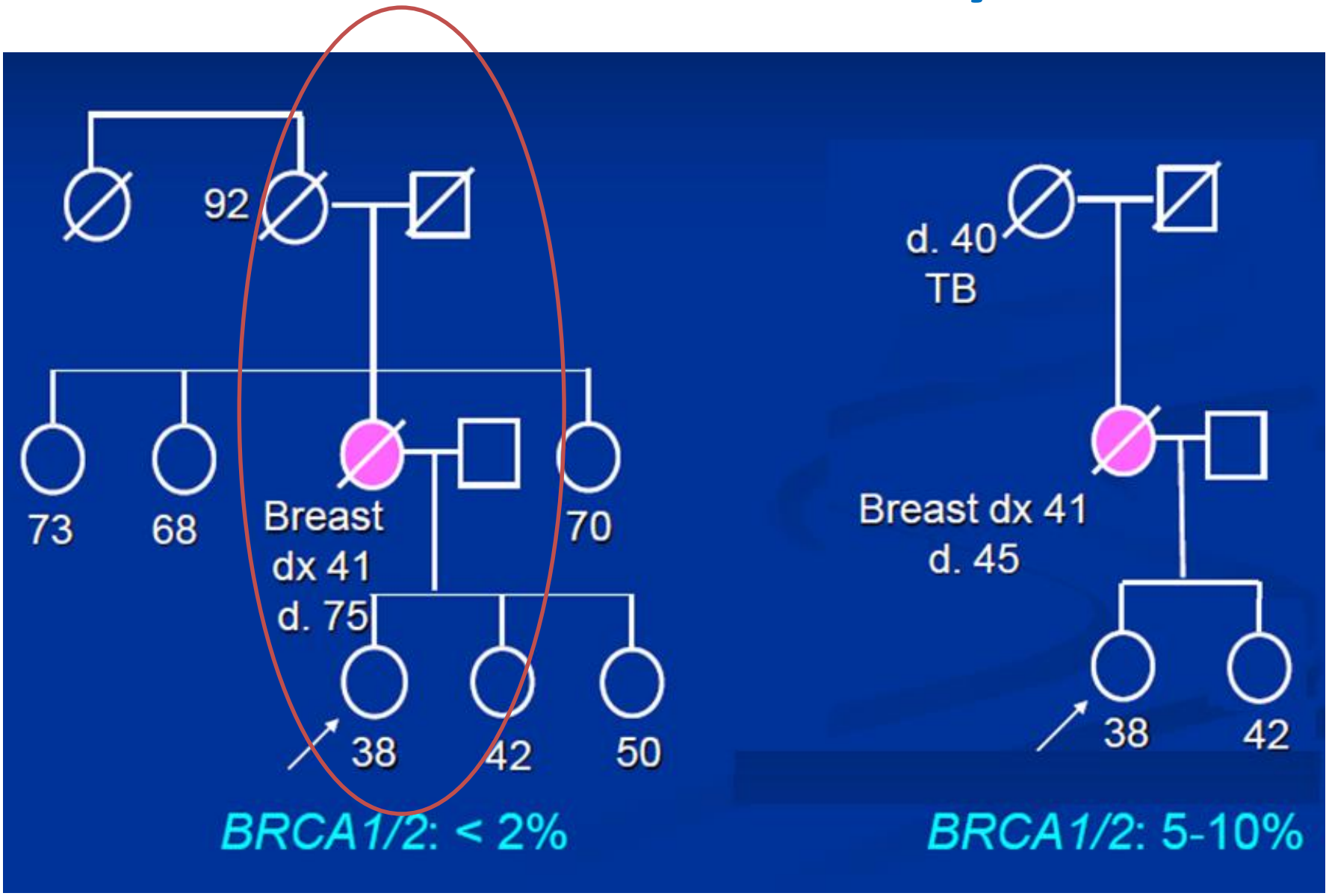
- Multiple affected individuals in multiple generations
- Early age of onset
- Multiple primary tumors
- Dominant inheritance
- Specific cancer clusters



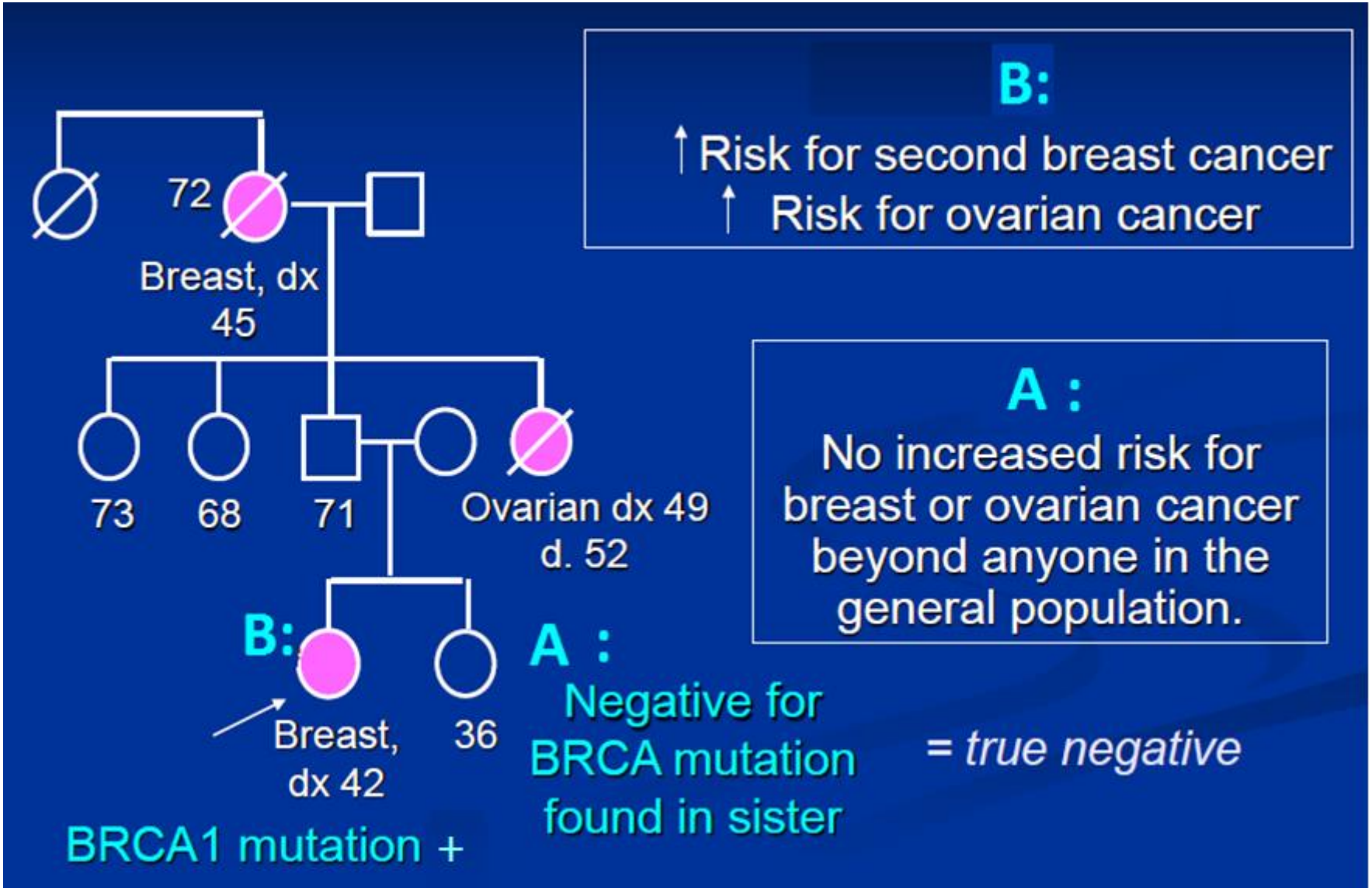
Importance of Parental Family History
(probability of being BRCA mut. carrier)



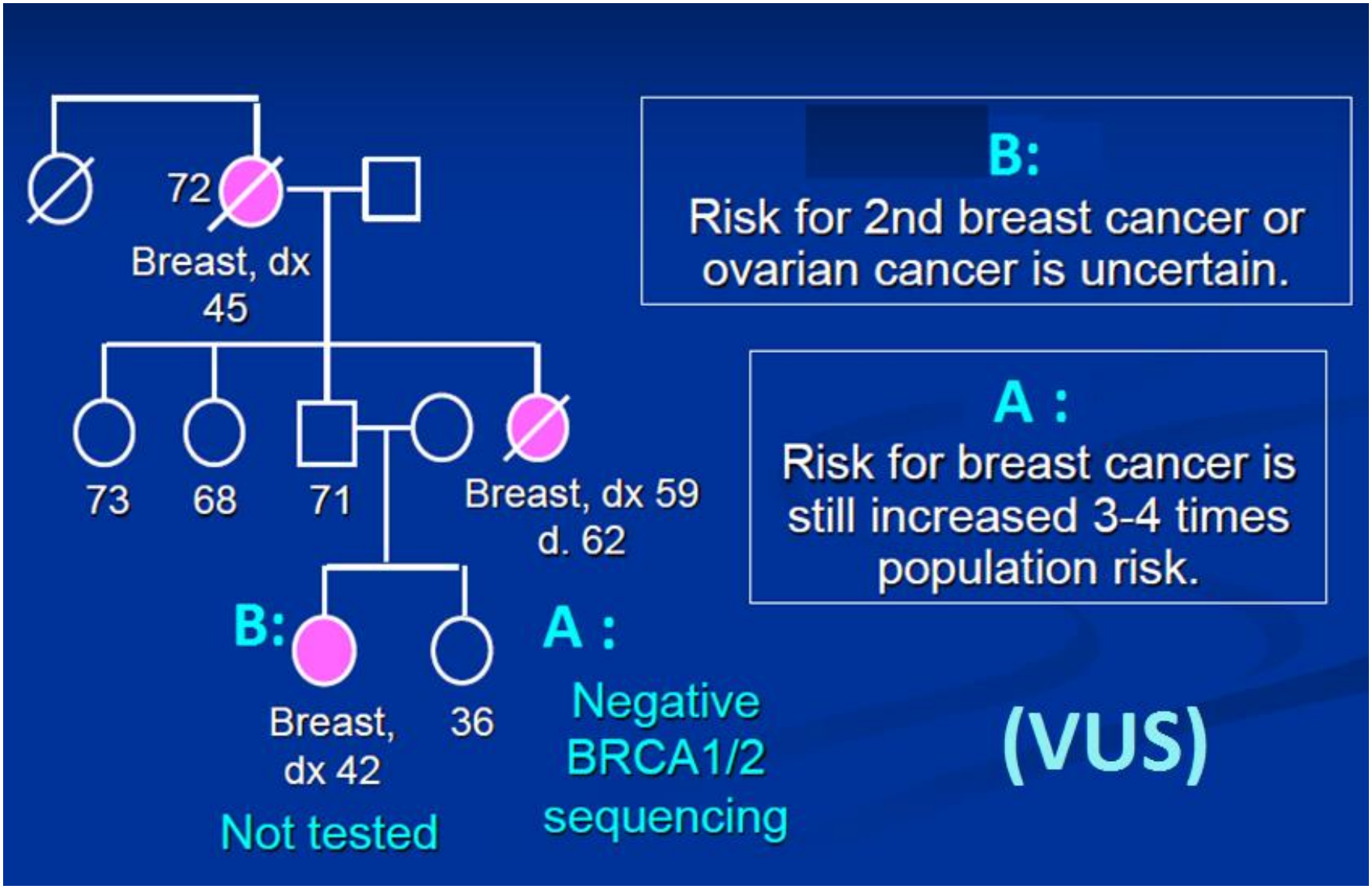
The Problem of Limited Family Structure



Impact of Genetic Test Results



Impact of Genetic Test Results



Risk Reduction Surgery

- **Bilateral Total Mastectomy**

  Bilateral risk-reduction mastectomy (RRM) decreased the risk of developing breast cancer by at least **90%** in BRCA1 and BRCA2 mutation carriers women.

- **Bilateral Salphingo-oophorectomy**

  Risk Reduction Salphingo-oophorectomy (RRSO) after completion of childbearing in women reduce the risk of overian, fallopian tube and primary peritoneal cancer by **80%.**

Dowsett M, J clin Oncol, 2010

Risk Reduction Surgery

- RRSO works for BRCA1 cases especially for 40 years old or under ones. Not enough data for BRCA2 carriers

Dowsett M, J clin Oncol, 2010

- A 1% to 4.3% residual risk for a primary peritoneal carcinoma has been reported in some studies

Van de Valde CJ, Lancet, 2011
Goss PE, N Eng J Med 2003
Perez EA, J Clin Oncol, 2006

- RRSO after age 50 is not associate d with a substantial decrease breast/over /peritoneal cancer risk

Davies C, Lancet, 2012

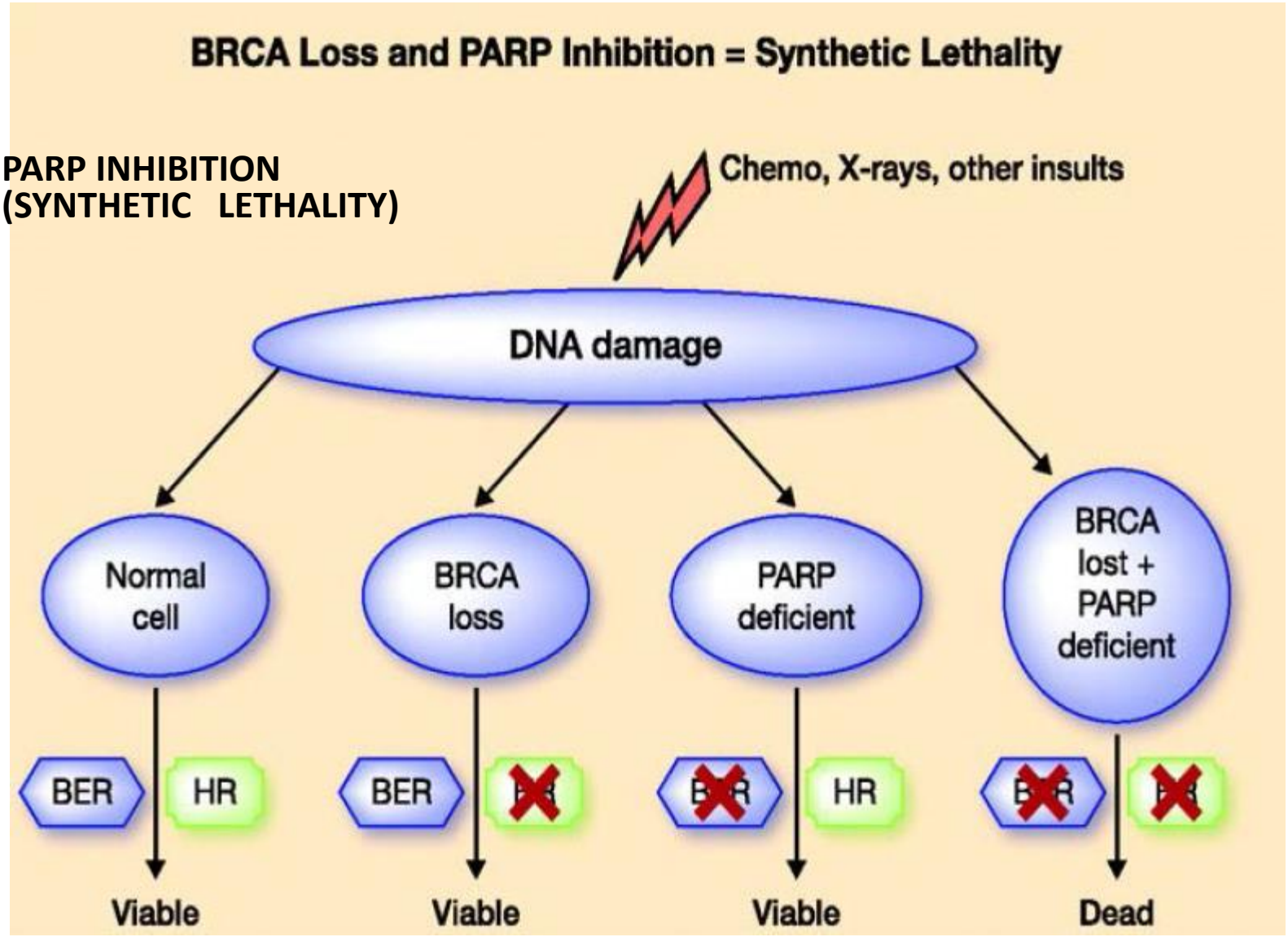
BREAST and OVERIAN MANAGEMENT BASED ON GENETIC TEST RESULTS

	Recommend MRI	Recommend RRSO	Discuss Option of RRM
Intervention Warranted based on gene and/or risk level	ATM BRCA1 BRCA2 CDH1 CHEK2 PALB2 PTEN STK11 TP53	BRCA1 BRCA2 Lynch syndrome	BRCA1 BRCA2 CDH1 PTEN TP53
Insufficient evidence for intervention	BARD1 BRIP1	BARD1 BRIP1 PALB2 RAD51C RAD51D	ATM BARD1 CHEK2 PALB2 STK11

Why Genetics?

- Prevention
- Early Detection
- Prognosis
- Tailored Therapy

PARP INHIBITION
(SYNTHETIC LETHALITY)



PARP : poly ADP ribose polymerase
BER : base exision repair
HR : homologue repair (for double strand break)

PARP Inhibitor Drugs

Table 1 Current Status of the Clinical Development of PARP Inhibitors in the United States

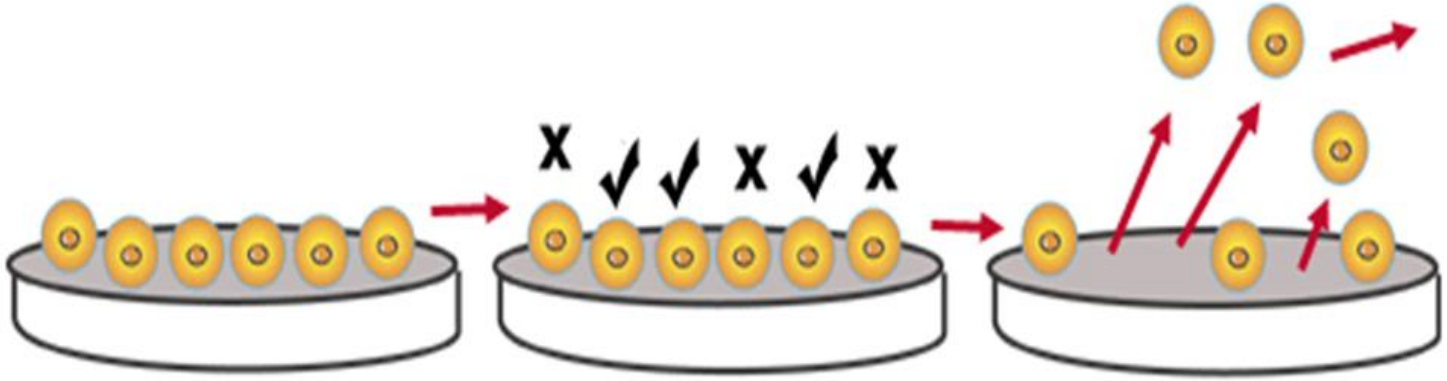
Agent	Sponsor	Route	Clinical Status
AG014699/PF-01367338	Pfizer	IV (oral)	Phase I/II
KU59436/AZD2281/Olaparib	AstraZeneca/Kudos	Oral	Phase II/III
Veliparib/ABT888	Abbott	Oral	Phase I/II
Iniparib/BSI-201	BiPar/Sanofi-Aventis	IV	Phase II/III
INO-1001	Inotek	IV	Phase I
GP121016	MGI Pharma/Eisai	Oral	Phase I
CEP-9722	Cephalon	Oral	Phase I/II
MK4827	Merck & Co.	Oral	Phase I
BMN-673	Biomarin/Lead Pharmaceuticals	Oral	Phase I/II

- Fong et al reported the first phase I study using the oral PARP inhibitor **olaparib** (AZD2281; KU-0059436)

Cancer Network ARS 2016

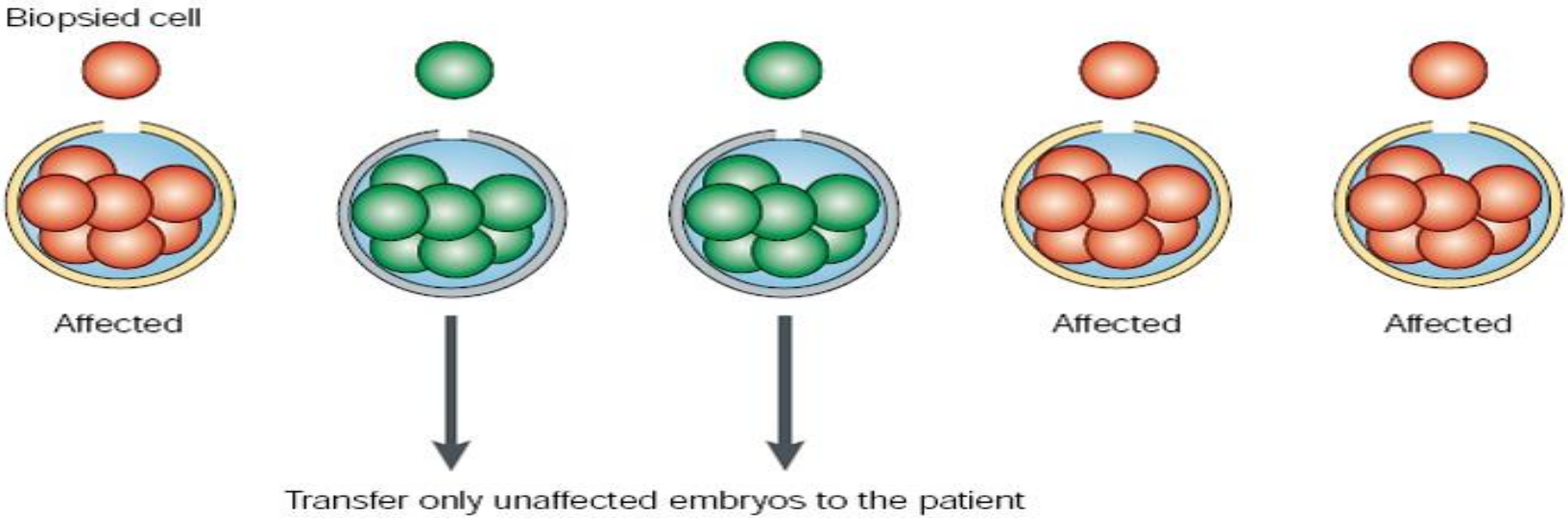
PGD

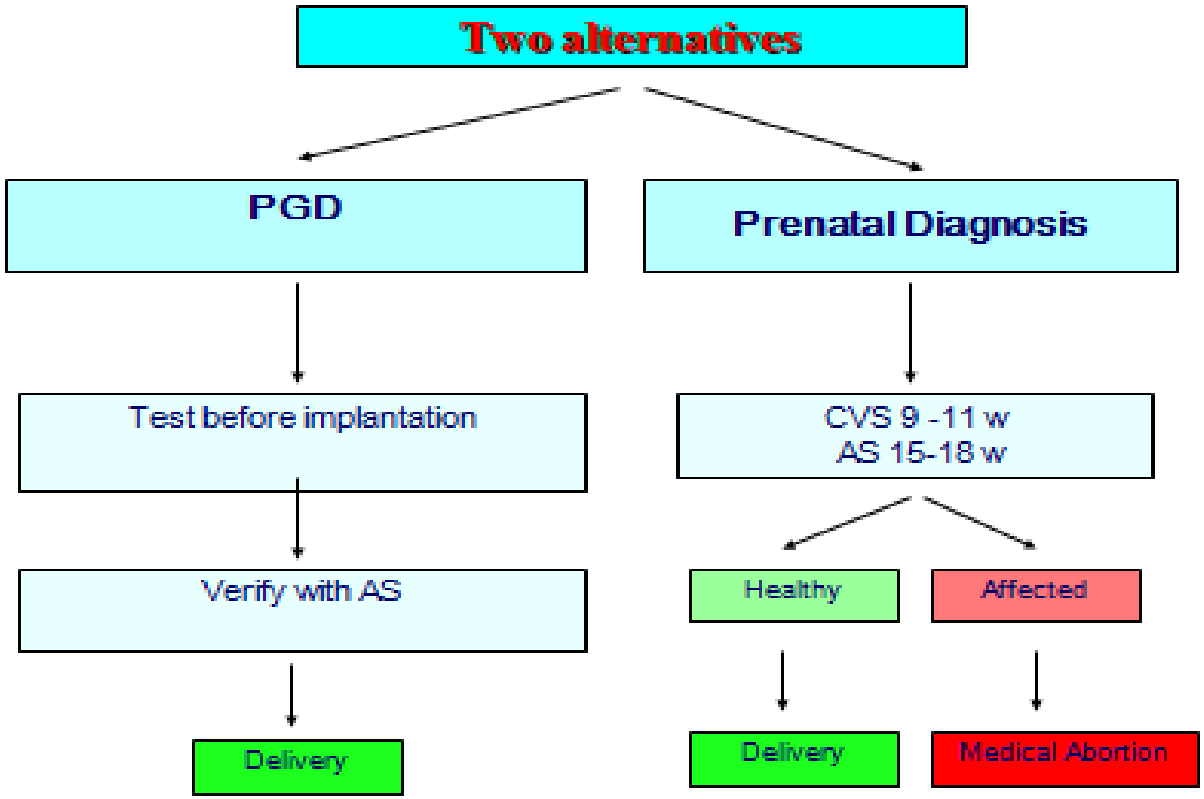
Preimplantation Genetic Analysis



OBJECTIVE :

The goal of PGD is to prevent certain diseases or disorders or conditions from being passed on to the child.


Biopsied cell
Affected
Affected
Affected
Transfer only unaffected embryos to the patient


Two alternatives
PGD
Prenatal Diagnosis
Test before implantation
CVS 9 -11 w
AS 15-18 w
Verify with AS
Healthy
Affected
Delivery
Delivery
Medical Abortion

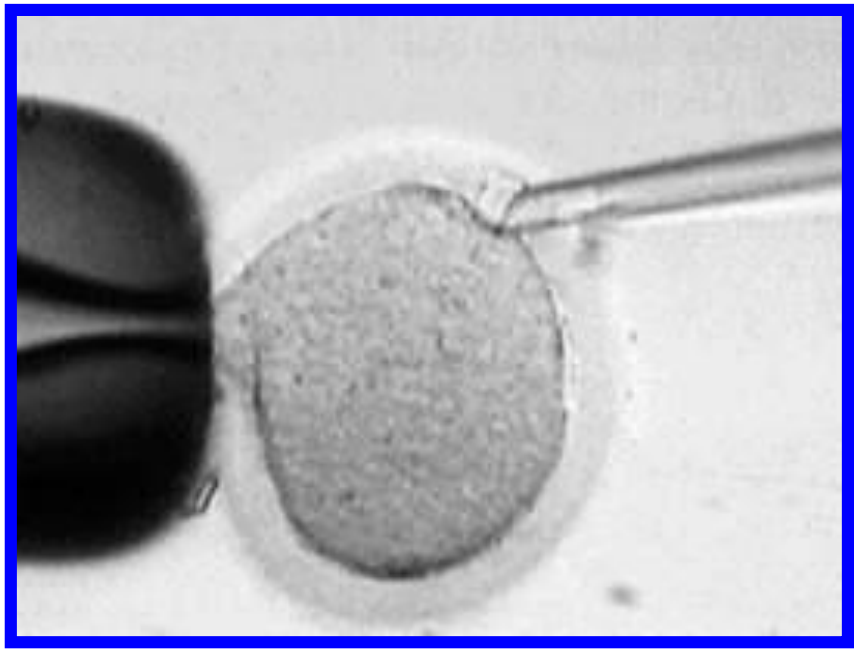
Preimplantation Genetic Diagnosis (PGD)

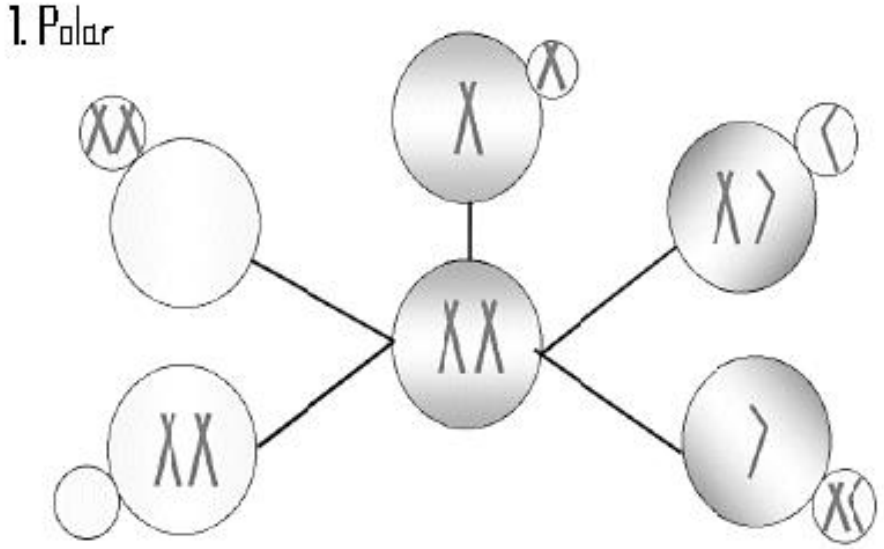
- For certain prospective parents with a known genetic mutation, PGD is a perfect tool to protect unborn baby from potential cancer

- The test can determine if the embryo has a known genetic mutation that may predispose it to increased risk for cancer later in life.

Polar body Biopsy

- Ethical and logical
- Only maternal information
- Certified oocytes





Preimplantation Genetic Diagnosis (PGD)

- Prenatal diagnosis is not logical and ethical application for breast cancer mutations (one exception is Fanconi anemia (AR) due to BRCA2 mutation)

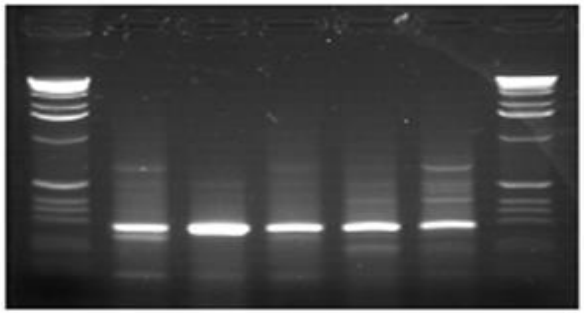
- PGD provide both healthy and HLA matched baby (sibling) for childeren with Fanconi anemia of family.

Laboratory Workflow

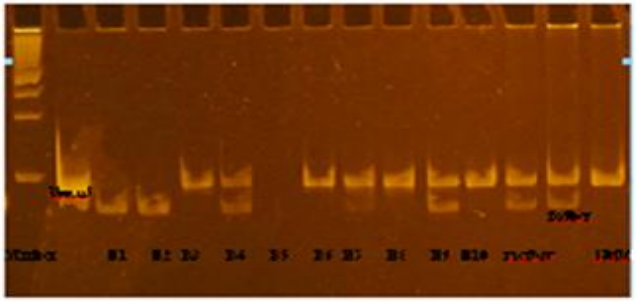
Cell Lysis (45min)

First Round PCR (3,5h)

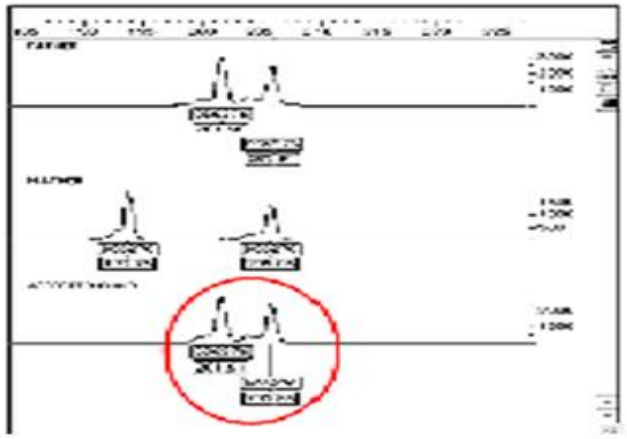
Second Round PCR (1,5h)



PCR Control

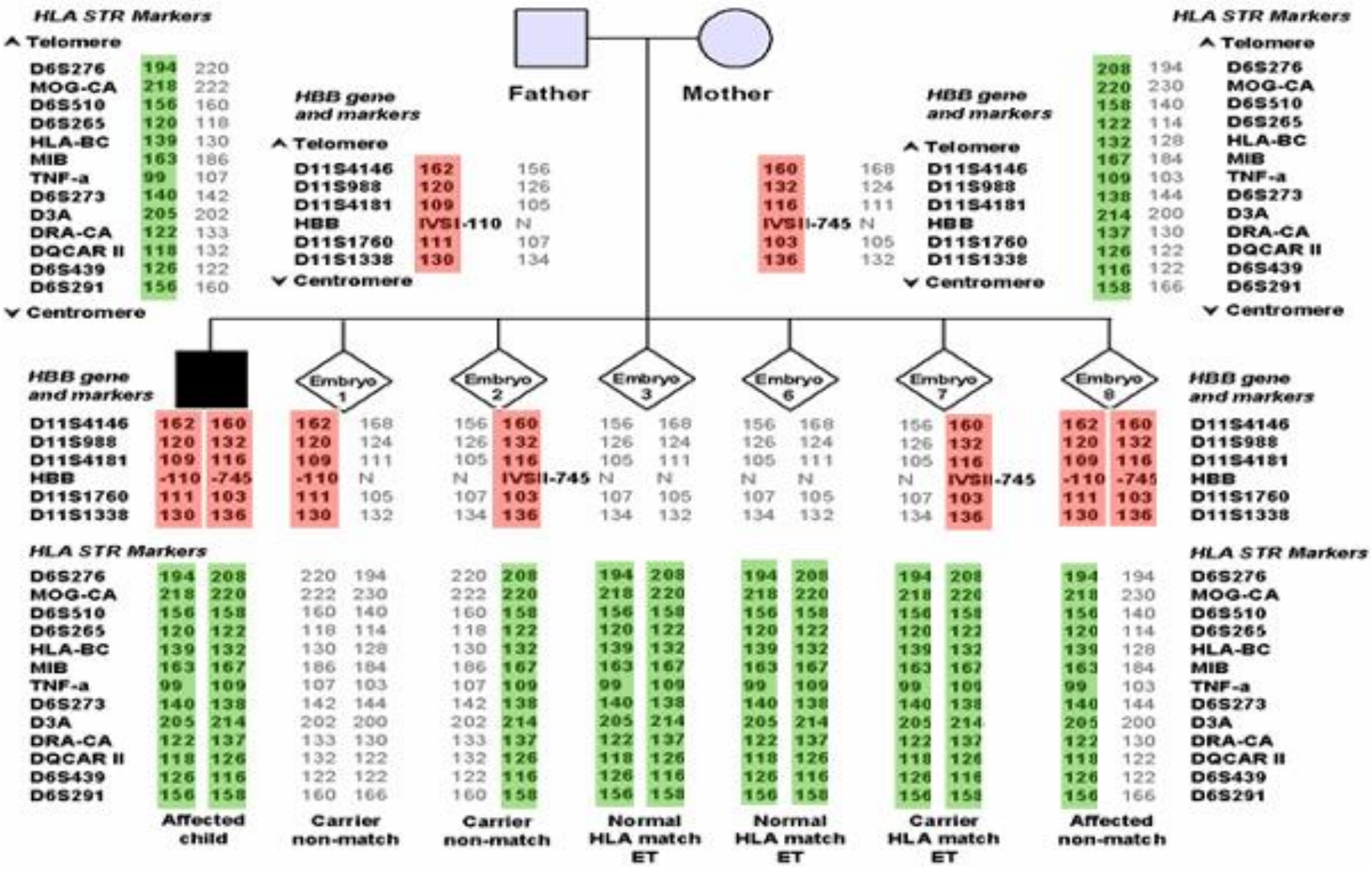


RFLP - Mutation Analysis

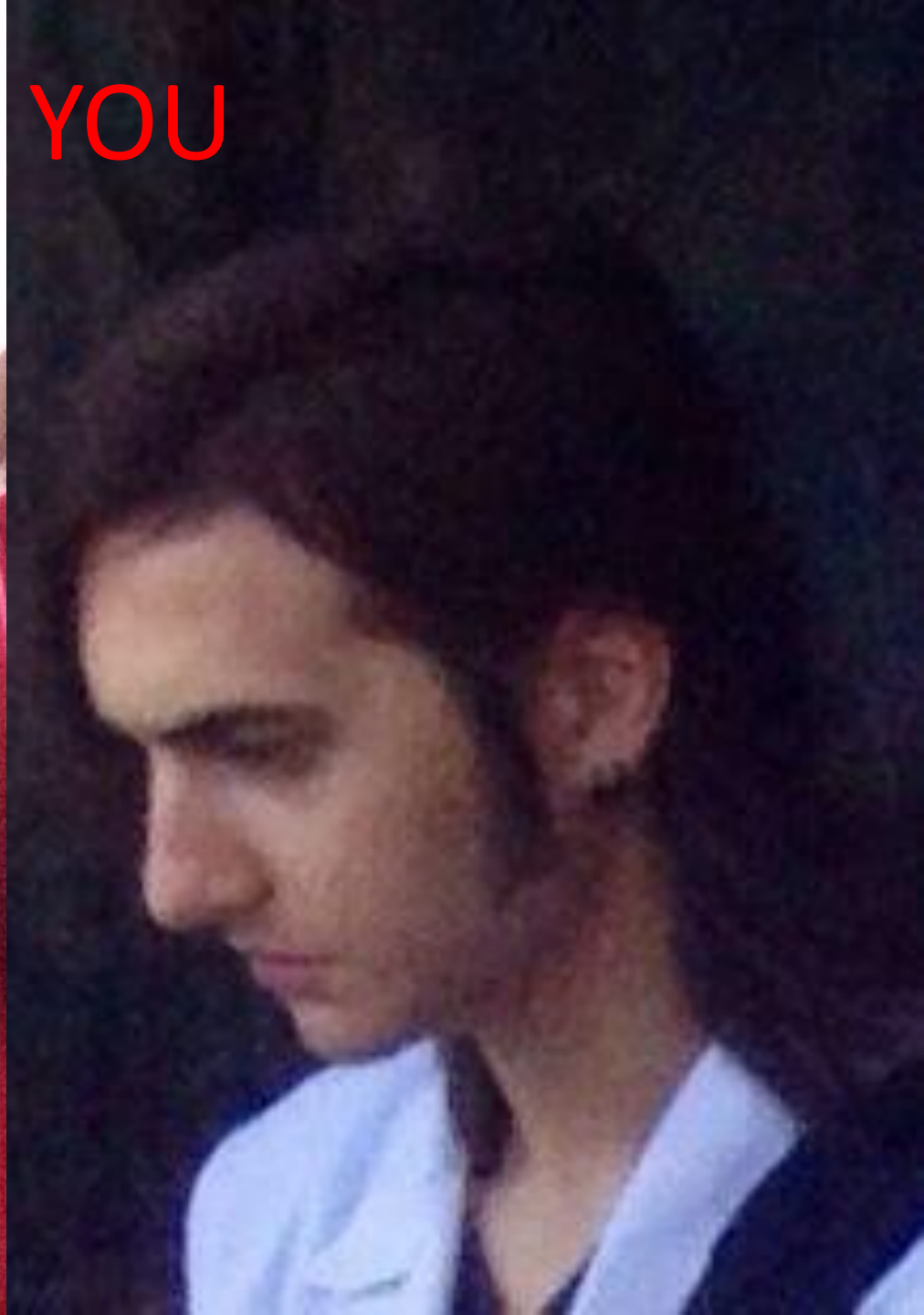


HLA matched embryo selection with fragment analysis (3-4h)

STR marker typing for the mutation + HLA



THANK YOU



L.E.S. MİKROGEN
GENETİK HASTALIKLAR TANI MERKEZİ

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Bio. Elif Tunç YAYLALI

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Bio. Adem ÖĞÜTVEREN

Kurumsal İlişkiler

Orhan DOĞAN

MSc. İlkiz DURUKAN

Muhasebe - Satınalma

Murat ŞİŞGİNOĞLU

Hesna Kalaycı

Sekreterya

Burcu DOĞAN

Zehra ÇATAL

Nazife ASLAN

Teknik Personel

Samet ASLAN

Gülten MUTLUER

THANKS