ULB Center of Human Genetics

NGS for oncogenetics

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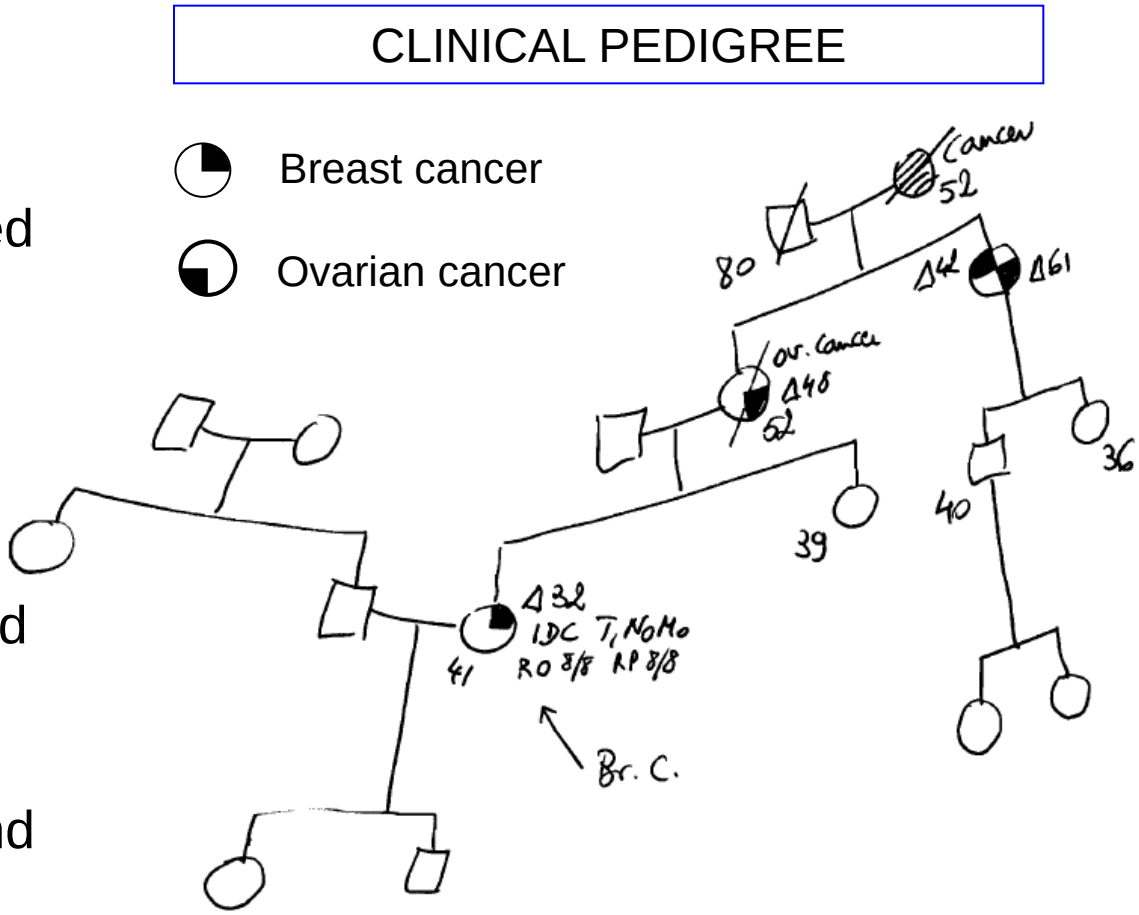
Hereditary cancer

- 5-10% of patients with breast, or colon cancer have an inherited predisposition = inherited cancer syndrome

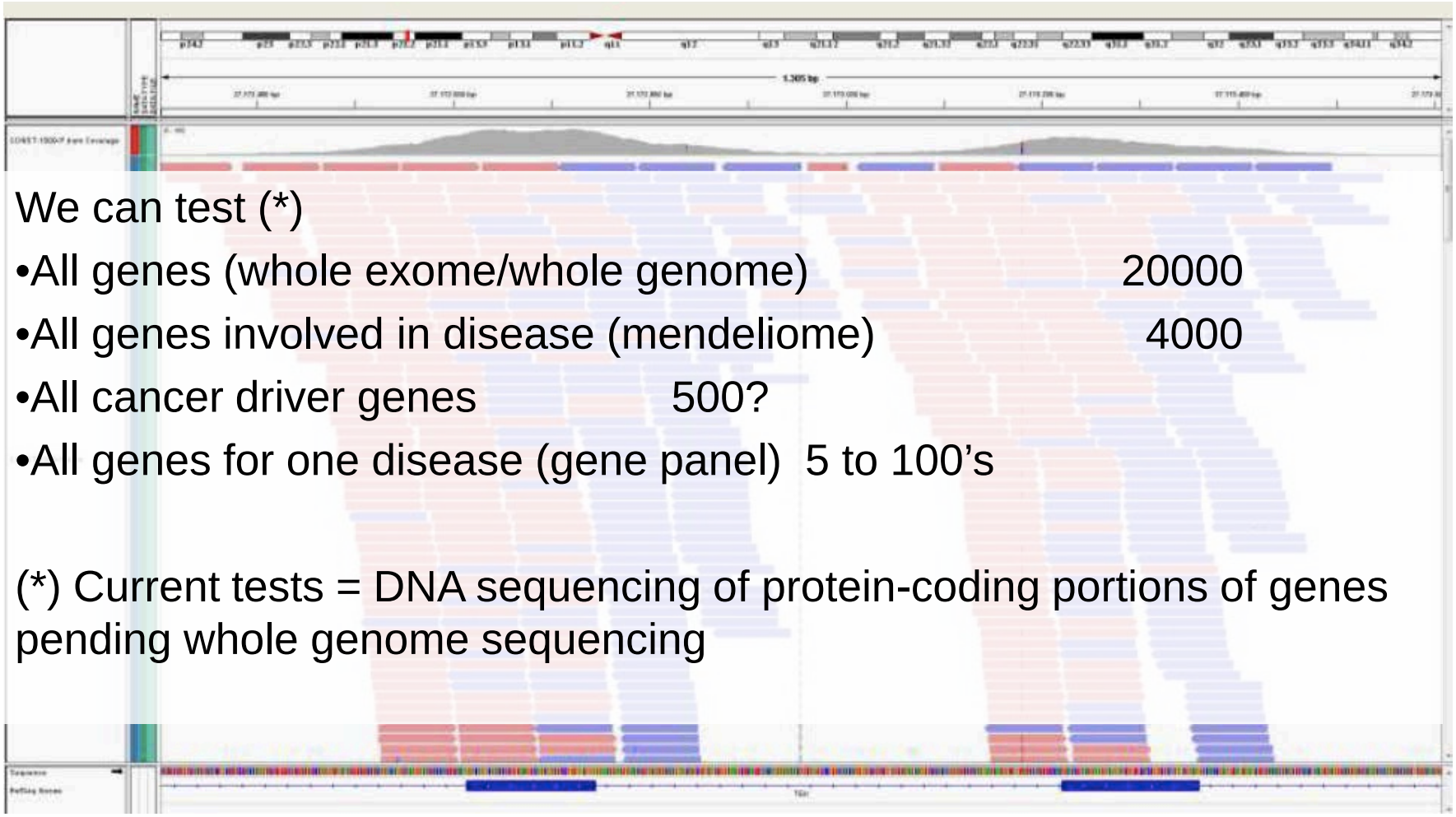
  20% of patients with (epithelial) ovarian cancer
- Predisposition < mutated gene
- Predisposition may be passed via germ-line and inherited in family



NGS for oncogenetics

- Large, multiple gene panels available for testing
  - Tumor DNA (somatic cells) => Precision therapy
  - Patient DNA (constitutive/germ line cells) => 2ary prevention in patient
    => prevention in family relatives
- Areas of uncertainty
  - Gene level : penetrance
  - Genetic variant/mutation level : VUS
- Tumor driving, somatic mutation may be present in patient's germ-line
  => need strategy for prevention in patient's family relatives

Large, multiple gene panels

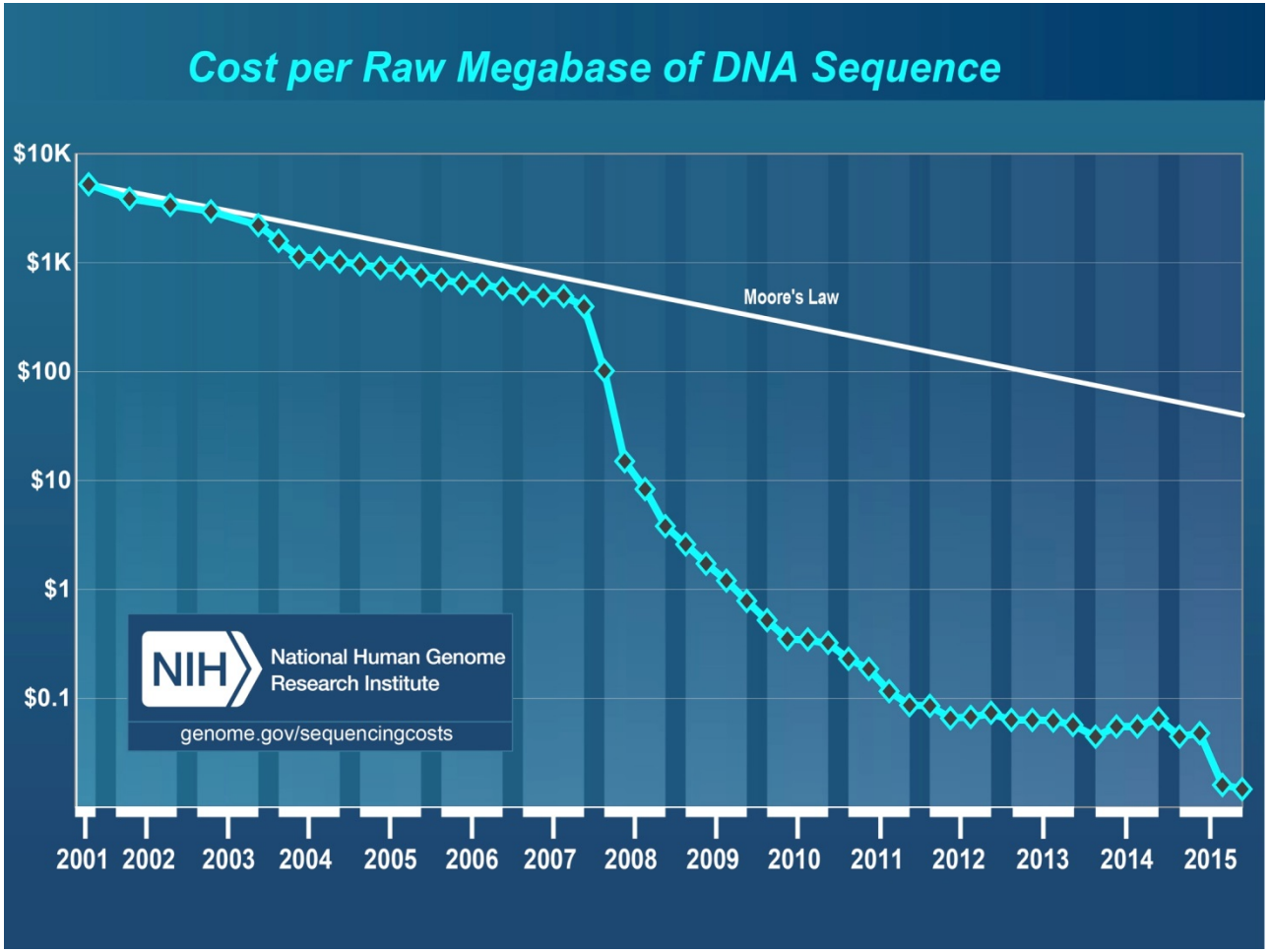


We can test (*)

- All genes (whole exome/whole genome) 20000
- All genes involved in disease (mendeliome) 4000
- All cancer driver genes 500?
- All genes for one disease (gene panel) 5 to 100’s

(*) Current tests = DNA sequencing of protein-coding portions of genes pending whole genome sequencing

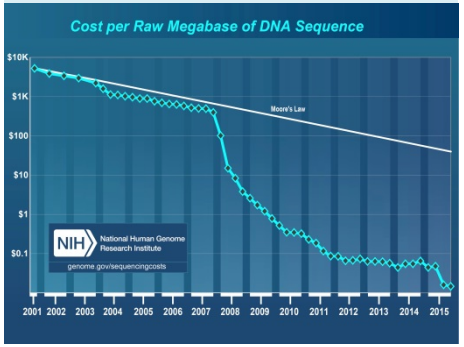
Dropping cost of DNA sequencing



Increasing costs of DNA tests

Preparing patient's DNA (library)

DNA sequencing



- Bioinformatics analyses for relevant DNA variants
- Medical interpretation
- ISO15189 certification/ accreditation

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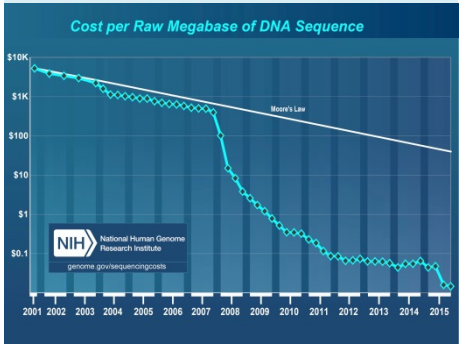

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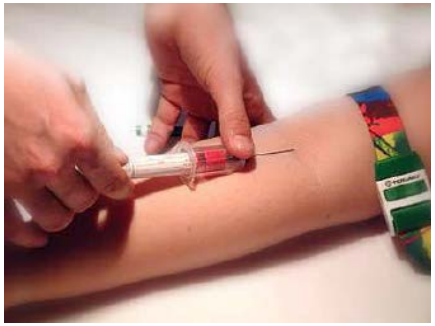
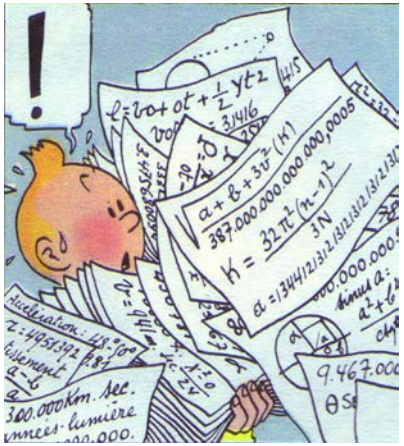
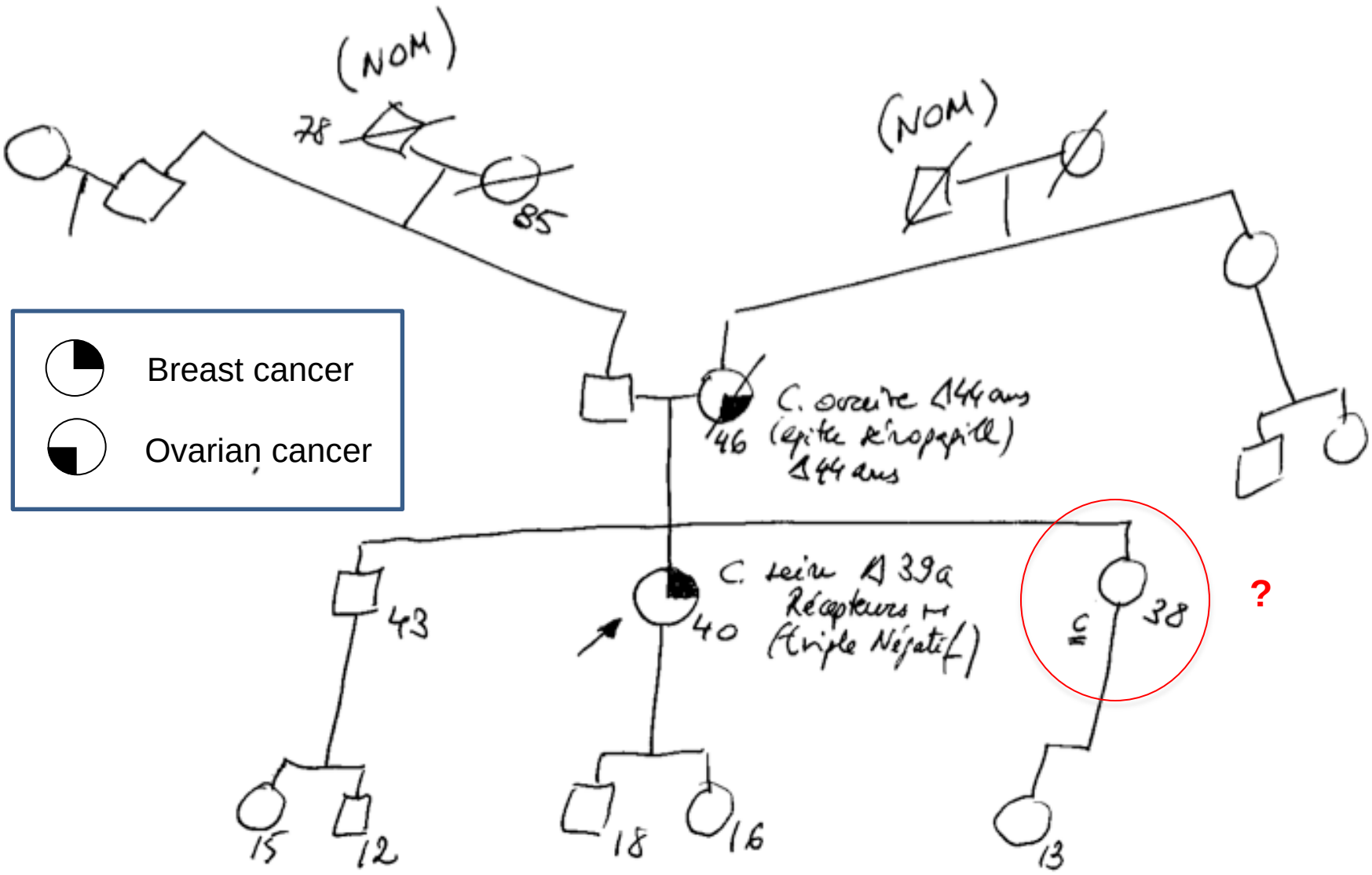




Testing cancer patients for an inherited predisposition

- Blood sample => germ line DNA analysis
- If inherited predisposition is suspected
  - Breast / ovarian cancer
  - Colorectal / endometrial cancer
  - Multiple Endocrine Neoplasia
  - others

Clinical pedigree



To test or not to test ?

Guidelines : see www.BeSHG.be > guidelines

- HBOC (Hereditary Breast and Ovarian Cancer Syndrome)
- HNPCC Lynch syndrome (pending)
- Cystic fibrosis
- others

Benefits of mutation identification

- Identify risk of cancer in other organ (ovary) for secondary prevention (BRCA ; PTEN, and all syndromic BC genes)
- Refine risk of recurrence (CHEK2, ATM)
- Precision drugs (olaparib in BRCA carriers)
- Individualized therapy (avoid radiotherapy in TP53)
- Trace risk in relatives > 1ary / 2ary prevention

Testing cancer patients for an inherited predisposition : how large should a gene panel be?

AREAS OF UNCERTAINTY :

- **Penetrance** of the gene defect

  Penetrance = probability of disease if mutation present

- **Clinical significance** of the patient's variant in the gene

  Disease causing mutation >< normal variant, harmless (polymorphism)

Large multigene panels include genes with unknown penetrance, and identify variants of unknown significance (**VUS**)

GENETIC **VARIANTS**

VARIANT	Frequency	Penetrance (fonctional effect)
Mutation	Rare	High
VUS	Rare	? ?
Polymorphism	Frequent	Low or null
« Rare polymorphism »	Rare	Low or null

VUS = variant of uncertain clinical significance : currently impossible to tell if high penetrance (phenotype-causing, mutation) or low/null penetrance (« rare polymorphism »)

VUS classification will require time:

- epidemiology of mutation and/or
- functional data (bioinformatics, machine learning approach)

ACCE criteria for genetic tests

Center of Disease Control, USA

✓ **A**nalytical validity	You bet ! CLIA and ISO15189
✓ **C**linical validity	Which gene variants (which alleles) cause disease risks?
✓ **C**linical utility	How much risk ?
✓ **E**thical, Legal & Social issues	

CLINICAL VALIDITY :
Which variants cause disease risk ?

- We will eventually need epidemiological evidence of causality for ***each mutation*** i.e., each genetic variant , each allele

- Class of variant may indicate functional effect on gene
  - Truncating variant (premature stop codon) >< missense variant

CLINICAL VALIDITY :
Which variants cause disease risk ?

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Current functional interpretation of variants :

✓ Disease-causing (mutation)

✓ Non-disease causing (polymorphisms, frequent or rare in population)

✓ VUS (variants of uncertain clinical significance)

CLINICAL UTILITY

magnitude of the risk caused by gene variant

Limitations in clinical utility of test :

- VUS

- RR of various alleles in one gene may differ

- RR is higher in patients with strong family histories
  => overestimated in individual patients (Evans et al 2014 JMG)

  Strong family histories select for :
  - Modifyer genes
  - Lifestyle, hormonal factors, reproductive history

CLINICAL UTILITY

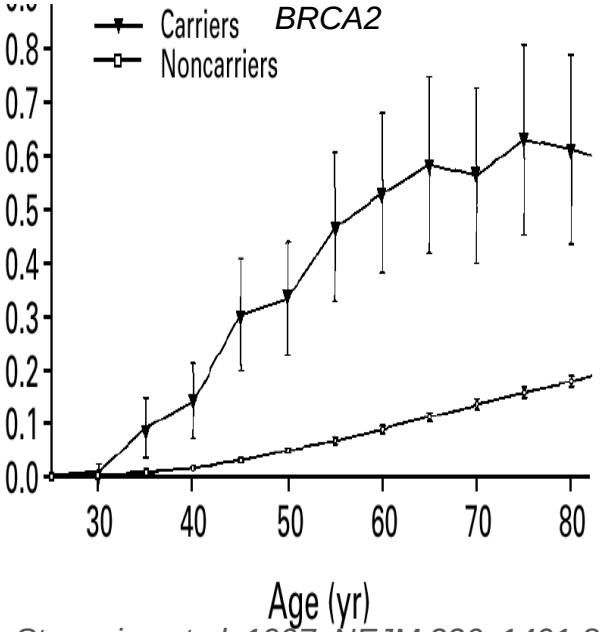
magnitude of the risk caused by gene variant

	Cumulative risk, lifetime *
• High penetrance genes / alleles Disease incidence > 4 x normal	> 32%
• Moderate penetrance genes / alleles Disease incidence 2 – 4 x normal	18 – 32 %
• Low penetrance genes / alleles Disease incidence <2 x normal	< 18 %

* Baseline = 9%

Easton et al 2015 NEJM

Clinical utility depends on allele penetrance

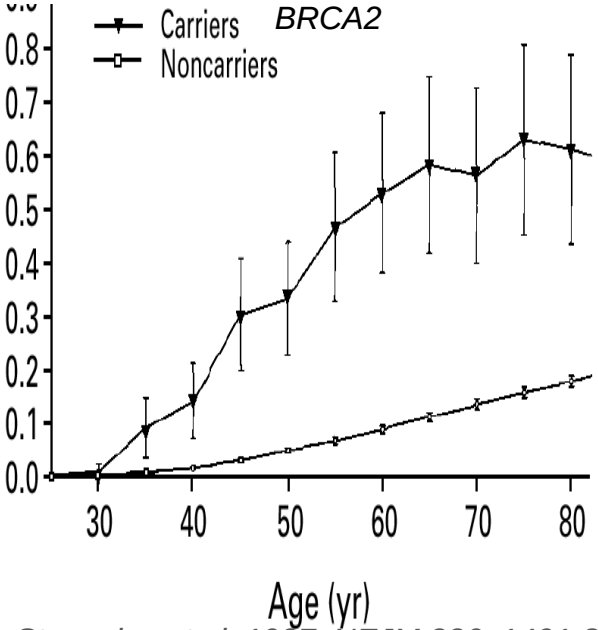


Struewing et al. 1997, NEJM 336: 1401-8.

If mutation explains risk only partially, caveat:

- Undue alarm in mutation carrier
- False reassurance in non-carrier

Clinical utility depends on allele penetrance



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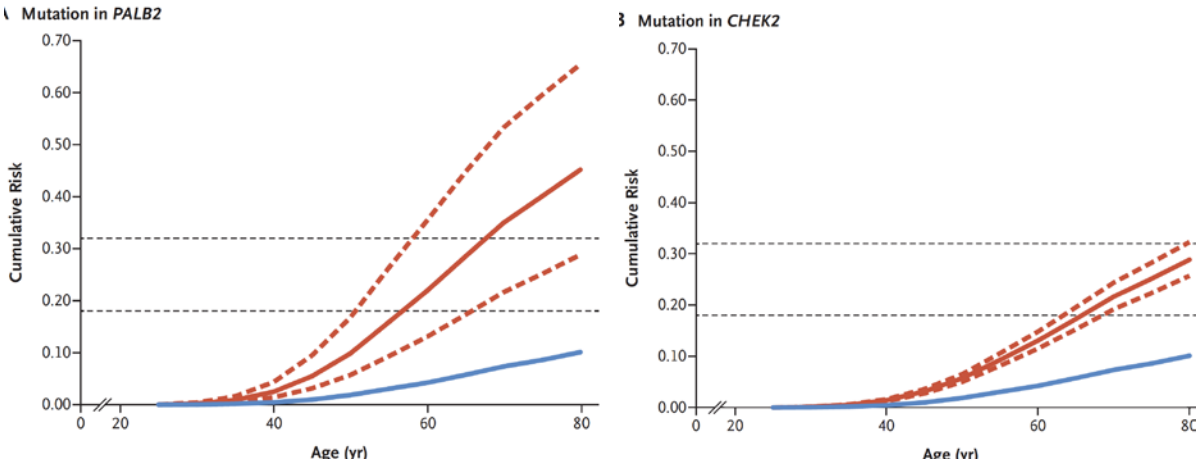

Easton et al. 2015, NEJM

If mutation explains risk only partially, caveat:

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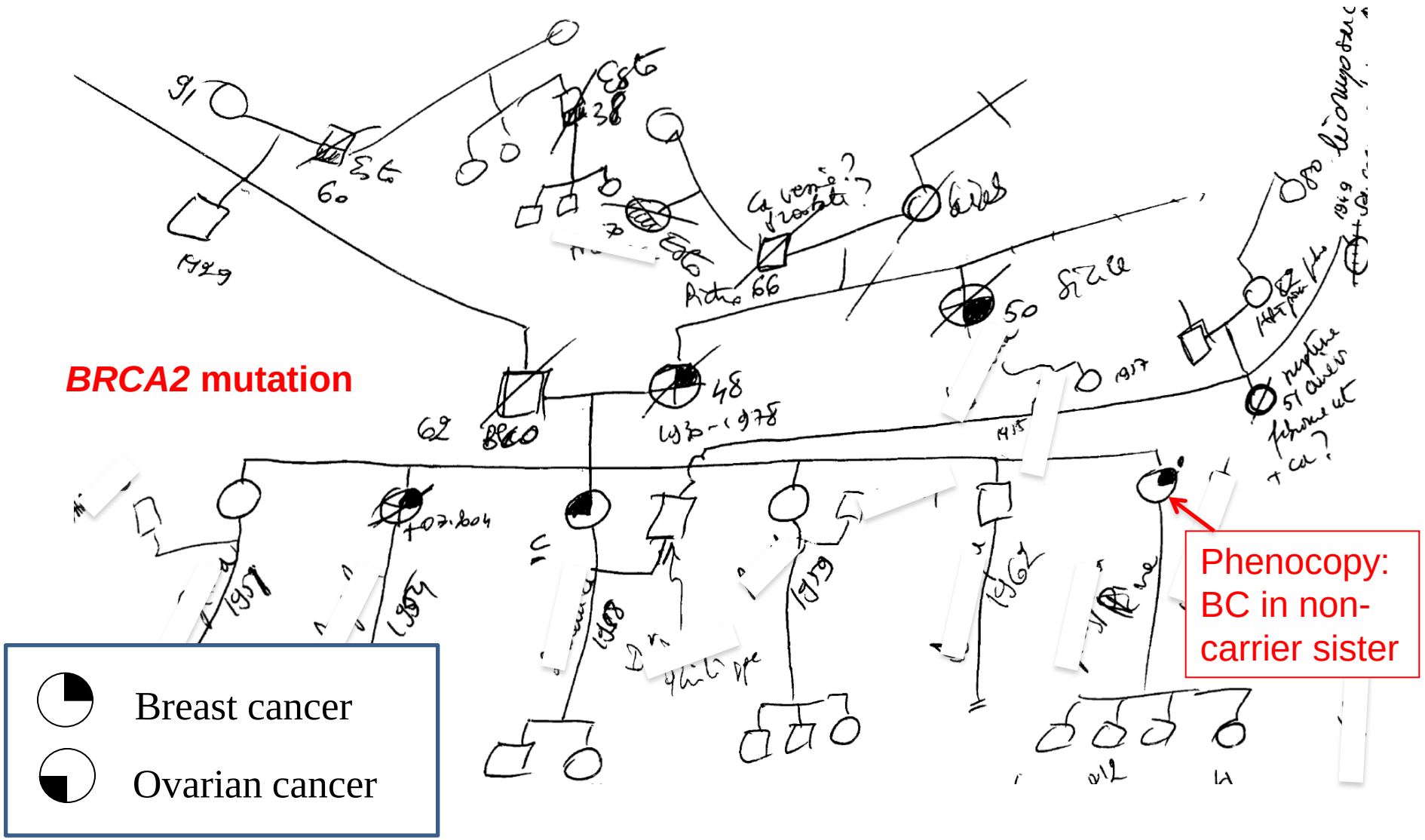
Phenocopies

chance occurrence of BC in non-carrier



Recommendations of the Belgian College of Human Genetics

Clinically useful, 2016

Non syndromic BC

- **BRCA1**
- **BRCA2**
- **PALB2**
- **TP53**
- **CHEK2** (mutation c.1100delC)

Syndromic BC

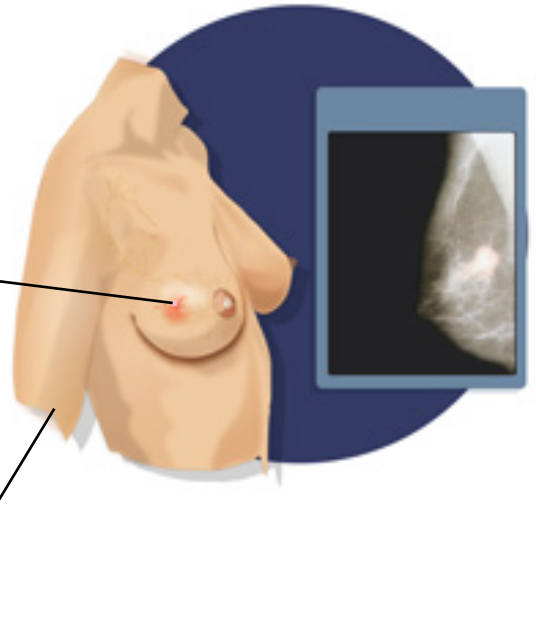
- STK11 (Peutz Jeghers)
- NF1 (neurofibromatosis)
- CDH1 (lobular BC + linitis plastica)
- PTEN (Cowden)

Research Only

- BARD1
- BRIP1
- RAD51C
- RAD51D
- MRE11A
- RAD50
- NBN
- FAM175A
- ATM
- CHEK2 (other mutations)
- XRCC2
- MEN1

Testing tumor DNA for pathway analysis

Cancer Genetics



- **In tumors** (tumor DNA = somatic DNA) :

  Molecular pathology; tumorigenesis routes.

  – Genetic
  – Epigenetic

- **In patients** (constitutional DNA = germ-line DNA) :

  Cancer risk profiling, from inherited mutations/polymorphisms

Somatic mutation may be present in the germ line

Driver mutations in tumors of some cancer patients are also present in all other cells

- e.g., BRCA1/2 mutations in ~20% epithelial ovarian cancer
  = 15% constitutive + 5% tumor only

•These patients are at higher risk for other tumors

- e.g., breast cancers and/or ovarian cancers

•Constitutive change is present in germ line
=> at-risk relatives need genetic counseling in view of presymptomatic genetic testing

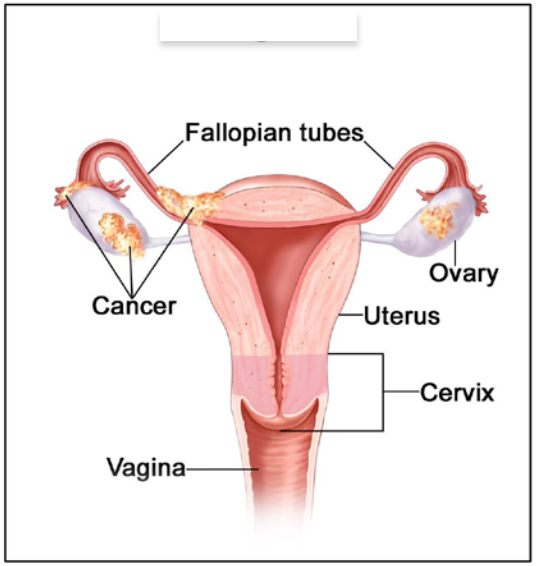
Germline BRCA mutations are frequent in ovarian carcinoma

Germ line

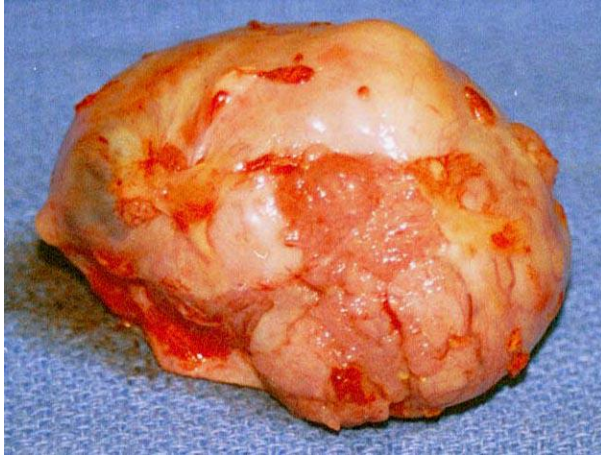
Frequency of mutations in cases of ovarian cancer by tumor histology.

Category	No. (%) positive for mutations in				Total no. of cases
	BRCA1	BRCA2	BRCA1 and BRCA2	Total	
Tumor histology					
Serous	81	52	2	135 (18.0%)	751
Endometrioid	18	8	0	26 (9.1%)	287
Mucinous	0	0	0	0 (0%)	112
Clear cell	1	1	0	2 (2.2%)	91
Carcinosarcoma	1	0	0	1 (7.1%)	14
Brenner	0	0	0	0 (0%)	4
Other	0	0	0	0 (0%)	4
Not specified	6	6	0	12 (15.2%)	79

S. Zhang et al. / Gynecologic Oncology 121 (2011) 353–357



BRCA mutations (germ line + somatic) in 15%-25% ovarian cancer tissue

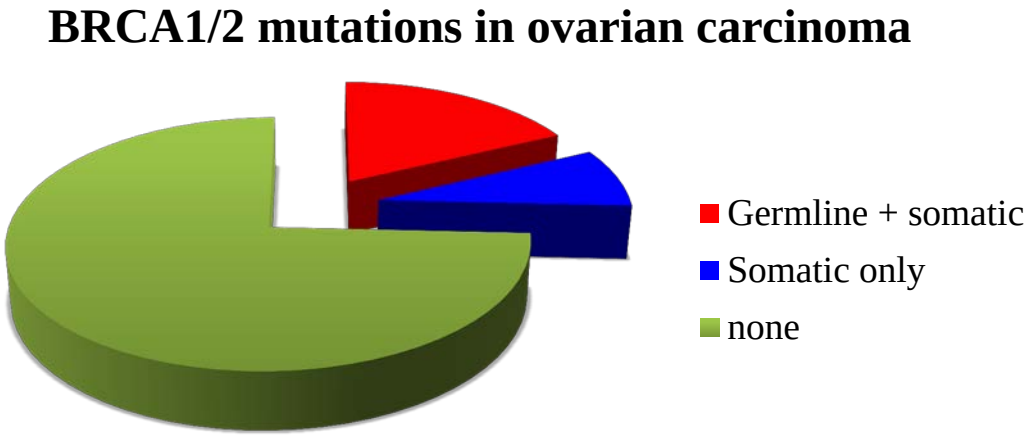


- Somatic BRCA1/2 mutation in 19% of ovarian cancers unselected for type (n=235; Hennessy et al 2012 JCO)
  - 23% in high-grade serous tumors
  - Among tumors with mutations, 20-40% are somatic only

- Germline BRCA1/2 mutations in 15% of (non-mucinous) ovarian carcinoma (n=1001; Alsop et al. 2012 JCO)
  - Better progression-free and better overall survival
  - 44% had no reported family history of breast or ovarian cancer

- Beyond BRCA1/2, homologuous recombination defect in up to 50% high-grade serous ovarian adenocarcinomas (TCGA research network 2011 Nature)

Most BRCA1/2 mutations found in ovarian carcinomas are also present in germline

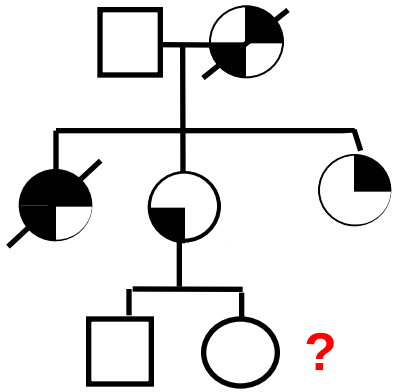


☐☐ If mutation is present in germ line, it may be inherited and present in other family members !

=> Need for integrated clinical work up and patient trajectories from oncology to pathology to genetics

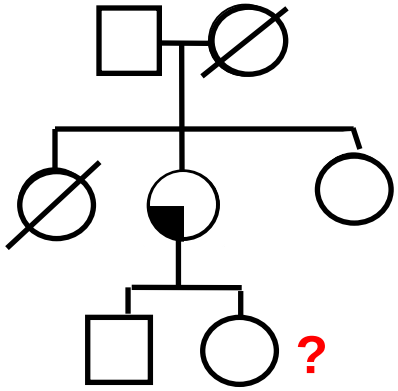
If BRCA1/2 mutation present in germline

- Genetic counseling is indicated in FAMILY MEMBERS
- Presymptomatic genetic testing in young adult relatives at-risk
  - Asymptomatic 20-25 years old
  - With psychological support and coaching
  - Pre-test and post-test counseling
  - If mutation present, start prevention of BREAST and OVARIAN cancer

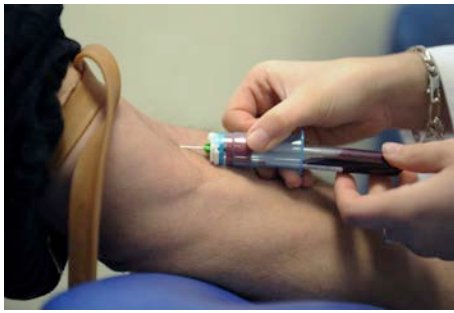


Ovarian cancer patients with germline BRCA mutation: **44% had no reported family history** of breast or ovarian cancer

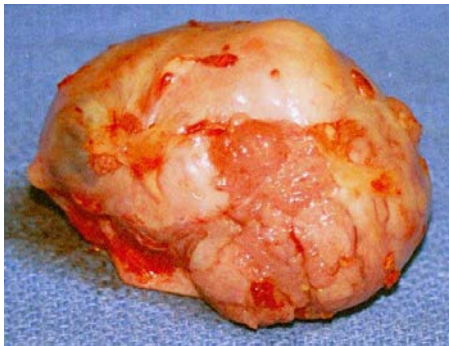
Alsop et al. 2012 JCO



Test ovarian carcinoma DNA and blood DNA (= germ line) for BRCA1/2 mutation

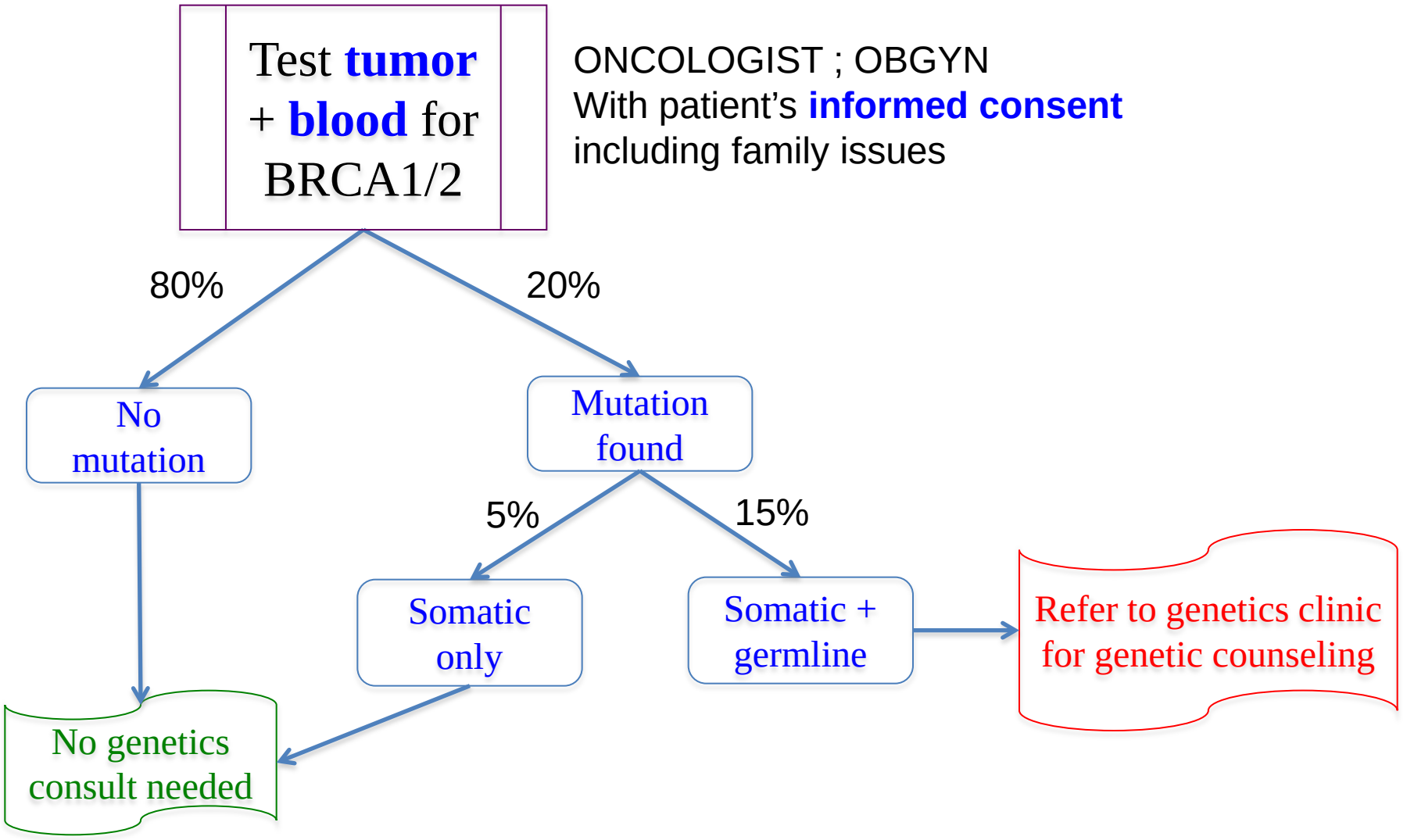


- In every patient with high grade serous epithelial ovarian carcinoma
- Blood DNA analysis allows for exon duplication / deletion analysis (10-15% of all BRCA1/2 mutations)
- Blood DNA analysis goes beyond BRCA1/2
  - Multigene panel of 26 genes, including 5 validated, high penetrance genes



- Some mutations are somatic only
  - Test tumor DNA + blood DNA in parallel

Patients with epithelial ovarian carcinoma, serous, high grade



NGS for oncogenetics 2016-17: conclusions

- Variants of Uncertain Clinical Significance (VUS) limit clinical validity of test
  - Genetic evidence must be collected over the coming years
- Penetrance limit clinical utility of test
  Lower penetrance means higher unexplained risk in patient
  - Undue alarm, false reassurance
  - Education of lay public, genetic counseling (pretest)
- Hence too large multi gene panels are not recommended
- Sequencing is cheap but medical genetic testing is expensive
- Tumor DNA tests allow for precision therapy: eg, BRCA1/2 in ovarian
- Many cancer-driving mutations in tumors are also present in germ line => need for integrated patients trajectory onco/pathol/genetics

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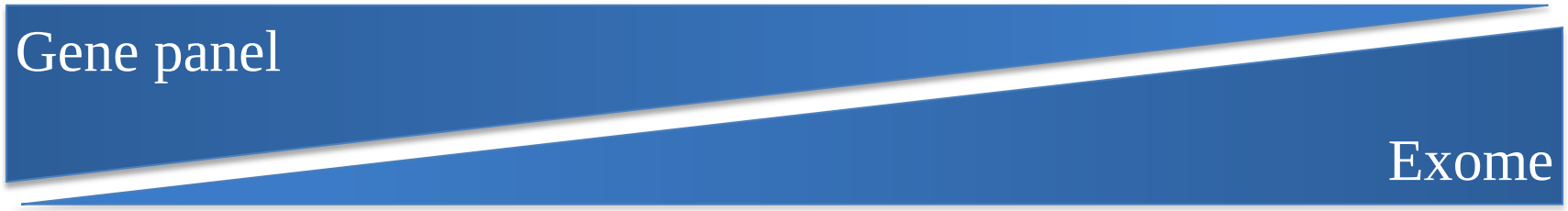
Testing strategy, riziv inami coverage

Specific syndrome	Semi-specific	Non-specific syndrome
Gene panel, sequenced with 100% accuracy. => Confirm / ***exclude*** known diagnosis (NPV!)	**Mendeliome + CORE GENES**, for which sequencing completed to 100% accuracy	**All genes (exome),** whatever their individual sequencing accuracy. => ***Include*** any diagnosis
Covered by riziv inami		***Not covered by riziv***



Sequencing efficiency

Sequencing width

Remboursement INAMI echelonné des analyses génétiques

76 EUR	152 EUR		350 EUR	547 EUR	1350 EUR
Simple test One or a few mutations, analysed in one or 2 PCR reactions or with (simple) kits	Simple test One or a set mutations, analysed in one or 2 PCR reactions and/or sequencing reactions, or with (more elaborate) kits (Default category for simple tests (O and P categories), until BELMOLGEN advises to move them to O)	Exceptional category, for simple testing, not related to a specific disease.	Complex test - Less than 10 amplicons OR - Deletion/ duplication analysis (as the only test for a specific diagnosis) OR - Simple PCR-based test which are in some cases complemented with Southern blot OR - Dynamic mutation OR - Diagnostic confirmation by sequencing (specific cases, e.g. very rare conditions, parental confirmation) (Default category for complex tests (Q, R and S categories), until BELMOLGEN advises to move item to R or S)	Complex test - Between 10 and 39 amplicons, or between 1.5 and 9kb coding sequence per gene or per package of genes (equals per diagnosis)* OR - Sets of dynamic mutations *Testing includes mutation analysis plus deletion/ duplication analysis	Complex test - 40 or more amplicons per gene or more than 9 kb coding sequence per gene or per package of genes (equals per diagnosis)* OR - Sequence capture results *Testing includes mutation analysis plus deletion/ duplication analysis

+ liste limitative des tests remboursables, mise à jour 1x/an