



Genetik des Mamma- und Ovarialkarzinoms: Mehr Hoffnung durch Wissen

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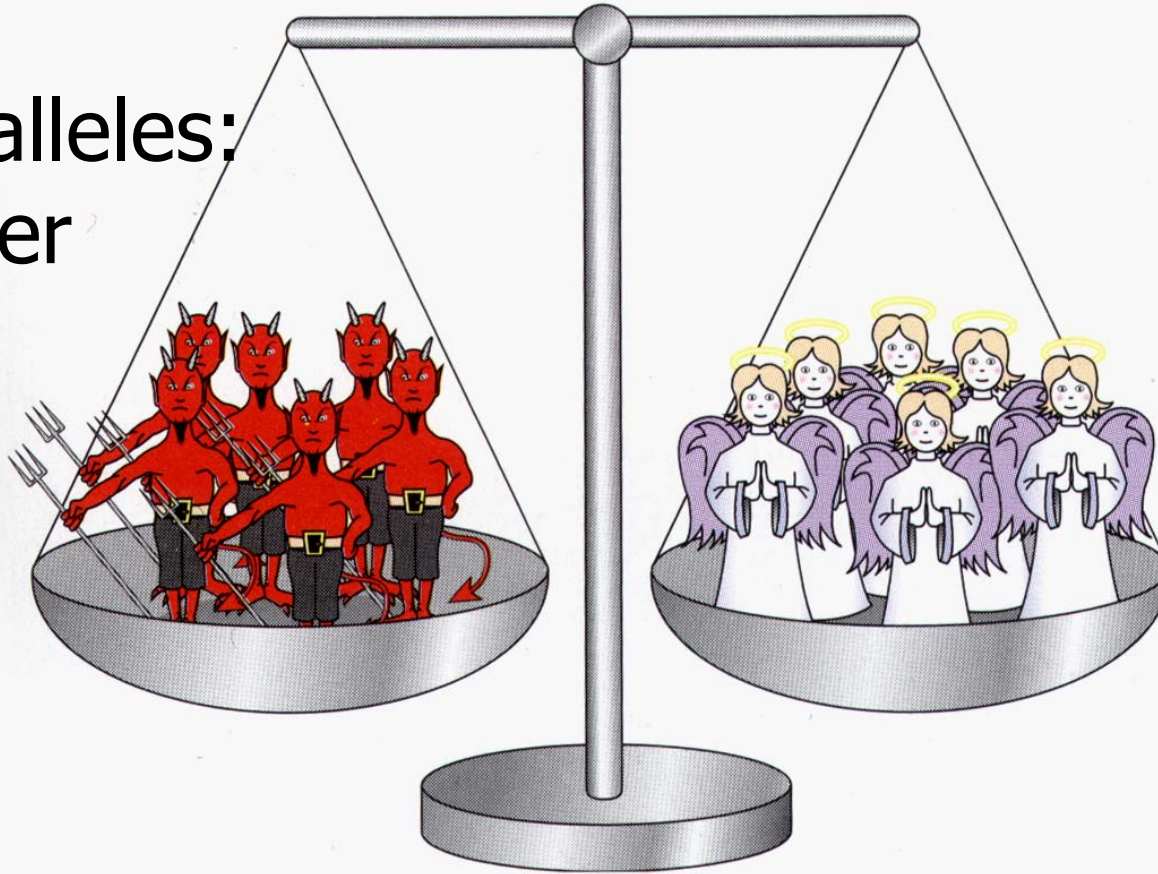


The remainder of excess risk

- The 12% most susceptible individuals may account for 50% of the breast cancers
 - Pharoah et al., 2002
- Multifactorial
 - How many genes?
 - Which environmental factors?
- Complex interactions
 - Additive?
 - Multiplicative?

Polygenetic model: tipping the balance

Bad alleles:
Cancer

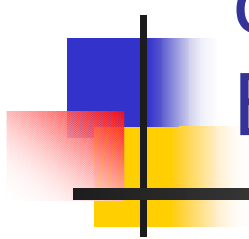


Good alleles:
Alleluia



Finding new genes

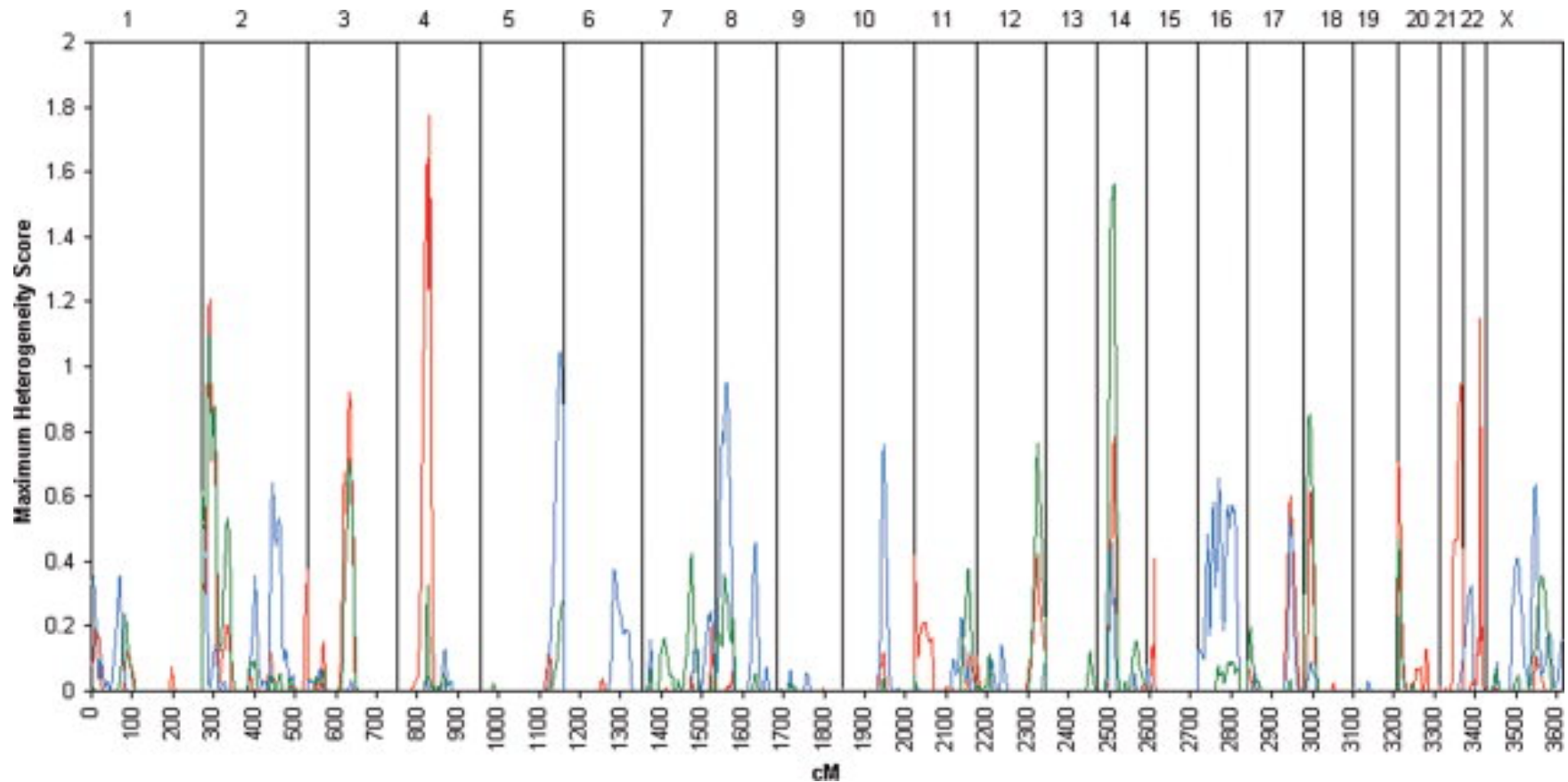
- *Linkage analysis* in families and sib-pairs
 - No new loci since 1996
- *Association studies* in cases and controls
 - Many false-positives
- (Linkage is with loci; Association is with alles)



A genome wide linkage search for breast
cancer susceptibility genes in 149
BRCA1,2-negative high risk families

Smith et al.,
Breast Cancer Linkage Consortium
(Genes, Chromosomes & Cancer, 2006)

After typing nearly 300,000 markers, still
no LOD score >3 , no candidate regions





“Nun weiter denn, nur weiter,
mein treuer Wanderstab”

Lied 21, “Das Wirtshaus”

Die Winterreise

Wilhelm Müller



Association studies: next steps

- Family-based association studies
- Better candidate genes
 - Genomic approaches
 - Animal models
 - Regulatory variation
 - Intermediate phenotypes
- Whole genome scans
- Gene interactions

Genetic variation in the population: Single Nucleotide Polymorphisms (SNPs)



T → A
T → G
C → G
C → A



Whole genome scan: Breast Cancer Association Consortium

- Coordinated by Easton, Pharoah, & Ponder, Cambridge, UK
- Phase I: genome-wide association search
 - 440 familial cases versus 400 controls
- Phase II: validation
 - 4,500 cases versus 4,500 controls
- Phase III: BCAC replication
 - 30,000 cases versus 30,000 controls including
 - 1575 cases from Leiden Prospekt study + 800 controls



Whole genome scan

- First phase: 266,722 tSNP array
- Second phase: 12,000 hits ($p < 0.05$) selected and validated
 - Rank by p-value ($p \ll 10^{-4}$)
- Third phase: BCAC to replicate top 30 of ~ 100 significant candidates



Hoffnung durch Wissen

Results genome-wide association study might help to identify the 12% most susceptible individuals!



Acknowledgments

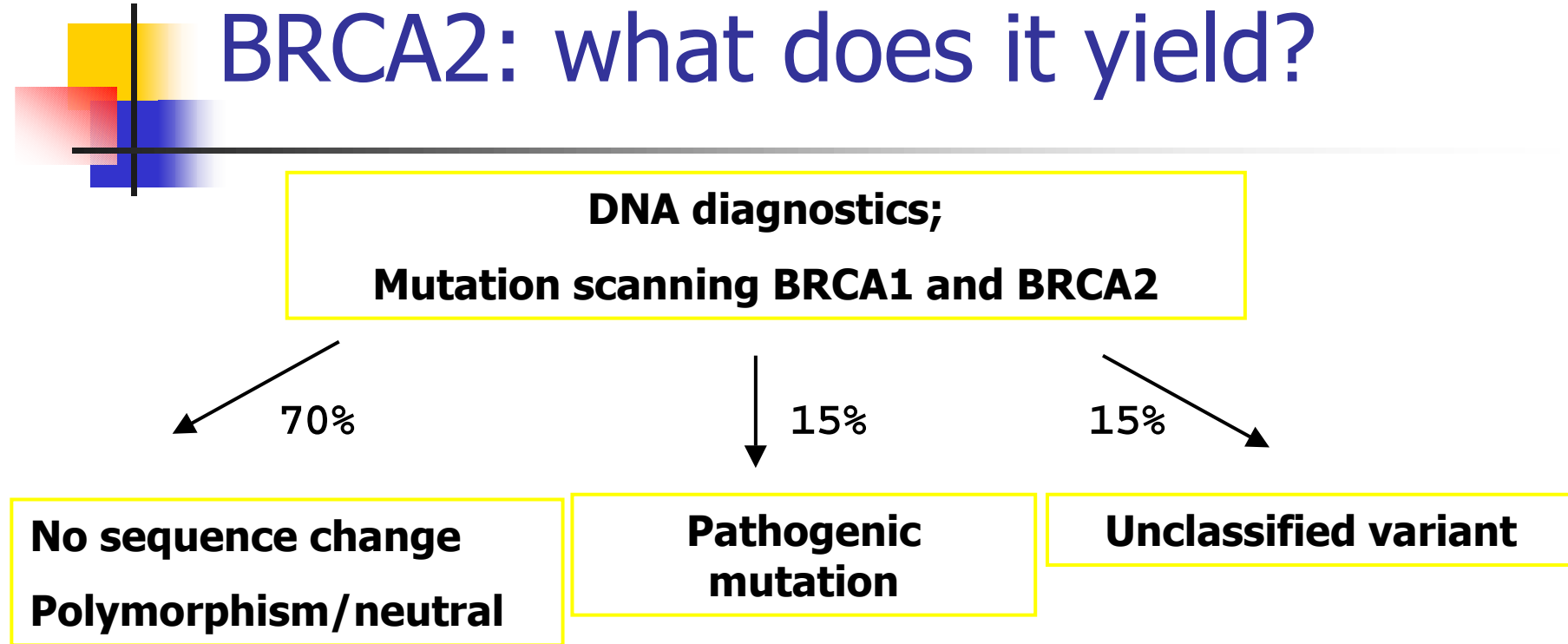
- Rogier Oldenburg, M.D. Dr. Maaïke Vreeswijk, Prof. Peter Devilee,
 - Depts. of Pathology and Human Genetics
- ORIGO/PROSPEKT Breast Cancer Study Group,
 - Leiden University Medical Center
- Dutch Cancer Society



Counselors with a higher than 10%
familial risk are offered a DNA test

Guidelines Am. Soc. Clin. Oncol.

Mutation scanning BRCA1 and BRCA2: what does it yield?



Predicting carriership of BRCA1,2
germline mutations?

Predicted BRCA1/BRCA2 carrier probabilities with the BOADICEA vs BRCAPRO model

Table 3 Predicted BRCA1/2 carrier probabilities for a breast cancer case whose mother developed breast cancer

Index age at onset (years)	Age at onset of breast cancer for mother (years)				
	30	40	50	60	70
30					
BRCA1	0.400 (0.566)	0.223 (0.415)	0.075 (0.244)	0.045 (0.103)	0.042 (0.049)
BRCA2	0.318 (0.036)	0.221 (0.034)	0.196 (0.037)	0.153 (0.030)	0.102 (0.020)
40					
BRCA1	0.212 (0.407)	0.087 (0.270)	0.025 (0.144)	0.014 (0.056)	0.013 (0.025)
BRCA2	0.211 (0.033)	0.107 (0.028)	0.082 (0.027)	0.062 (0.021)	0.041 (0.013)
50					
BRCA1	0.056 (0.228)	0.020 (0.137)	0.006 (0.067)	0.003 (0.024)	0.003 (0.011)
BRCA2	0.180 (0.035)	0.080 (0.027)	0.061 (0.024)	0.047 (0.018)	0.032 (0.011)
60					
BRCA1	0.023 (0.079)	0.008 (0.043)	0.002 (0.020)	0.001 (0.007)	0.001 (0.003)
BRCA2	0.134 (0.028)	0.058 (0.020)	0.045 (0.017)	0.036 (0.012)	0.025 (0.007)
70					
BRCA1	0.019 (0.022)	0.006 (0.011)	0.002 (0.005)	0.001 (0.001)	0.001 (0.000)
BRCA2	0.080 (0.016)	0.034 (0.011)	0.028 (0.009)	0.024 (0.006)	0.017 (0.004)

Corresponding probabilities using BRCAPRO within parentheses.

Predicting BRCA1 carriership by immuno-phenotyping

ER	CK1 4	CK5/ 6	BRCA1 (%)	Control (%)	Adj OR (95% CI)
Pos			9.6	67.2	1
Neg	Neg	Neg	20.9	24.0	4.15 (1.76-9.82)
Neg	Neg	Pos	13.4	2.4	26.9 (7.18-101.1)
Neg	Pos	Neg	12.4	4.8	14.01 (3.41-57.5)
Neg	Pos	Pos	43.8	1.6	147.9 (35.2-621.3)

ER = estrogen receptor α , CK, cytokeratin; adj OR: adjusted OR



Unclassified variants: pathogenic or neutral?

Unclassified sequence Variants

- Missense mutations
 - Amino acid change
- Silent mutations
 - No amino acid change
- **Mutation in intron sequence**
 - Project of Dr. Maaïke Vreeswijk & Prof. Dr. Peter Devilee

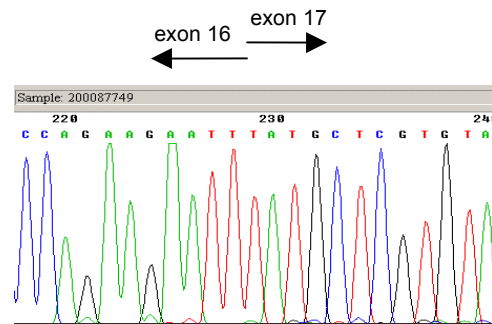
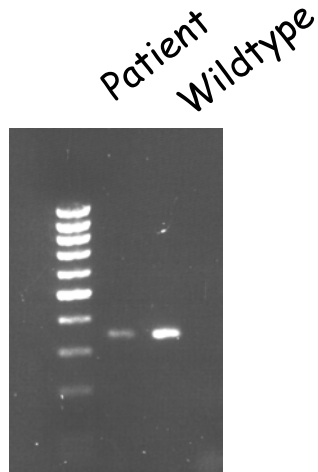
BRCA1 IVS16+5 G>T

exon 16

intron 16

CCCCAGAAGA ATTTgtgagt gtatccatat gtatctccct

RT-PCR from exon 16-exon 17 shows wildtype sequence.



RNA patient

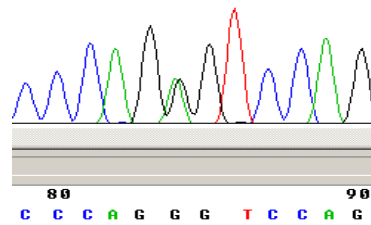
Are both alleles expressed ?

BRCA1 IVS16+5 G>T

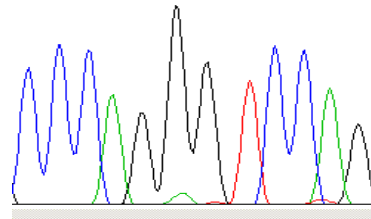
↓
70
C C C A G G G T C C A G

DNA

S1613G
(4956 A>G)
polymorphism
exon 16



RNA



A single nucleotide polymorphism enables discrimination between the two alleles

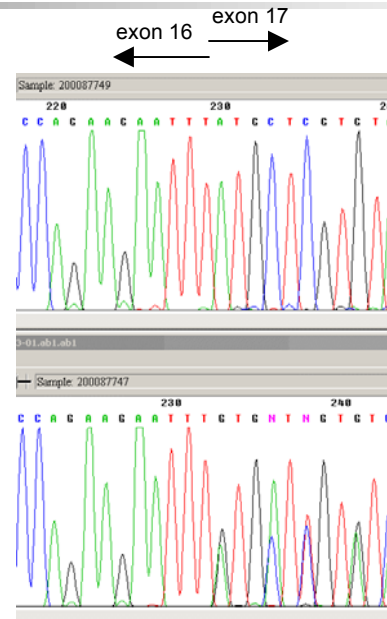
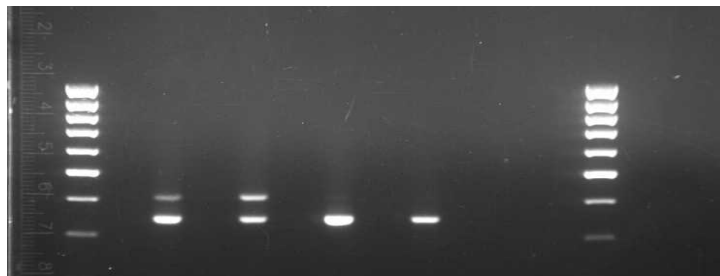
Monoallelic expression

Is mRNA of variant allele

degraded by nonsense-mediated decay?

BRCA1 IVS16+5 G>T

Patient		Control		cyclo
-	+	-	+	



RNA from wildtype
+ cycloheximide

RNA from patient
+ cycloheximide

In the presence of cycloheximide (inhibition of NMD)
a 65 nucleotide insertion, derived from intron 16, could be detected.

BRCA1 IVS16+5 G>T


Conclusion

- Only expression of the wildtype allele,
- No expression of the aberrant allele
- IVS16+5 G>T in BRCA1 is a **pathogenic mutation** and carriers have a high lifetime risk for breast and ovarian cancer



CHEK2, a risk modifier

- *CHEK2*1100delC* truncating mutation
 - First detected in Li-Fraumeni families
 - (*Bell et al.*)
 - 5% prevalence in patients with familiar breast cancer, 1% in controls
 - (*Meyers-Heijboer et al.,*)
 - Twofold increased breast cancer risk
 - (*CHEK2 Breast Cancer Case-Control Consortium, 10,000 cases*)
 - Excess risk for contra-lateral breast cancer:
 - (*De Bock, et al., RR= 5.7*) *Broeks et al., OR =6.5, Schmidt et al., HR =2.1*)
 - Risk modifier in non-BRCA1/BRCA2 multiple-case families
 - (*Oldenburg et al.,*)

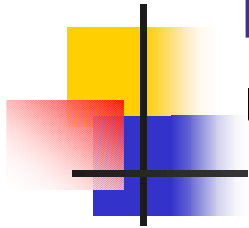


*CHEK2*1100delC* prevalence in 71 non
BRCA1/BRCA2 families with early onset disease
(Oldenburg et al., 2003)

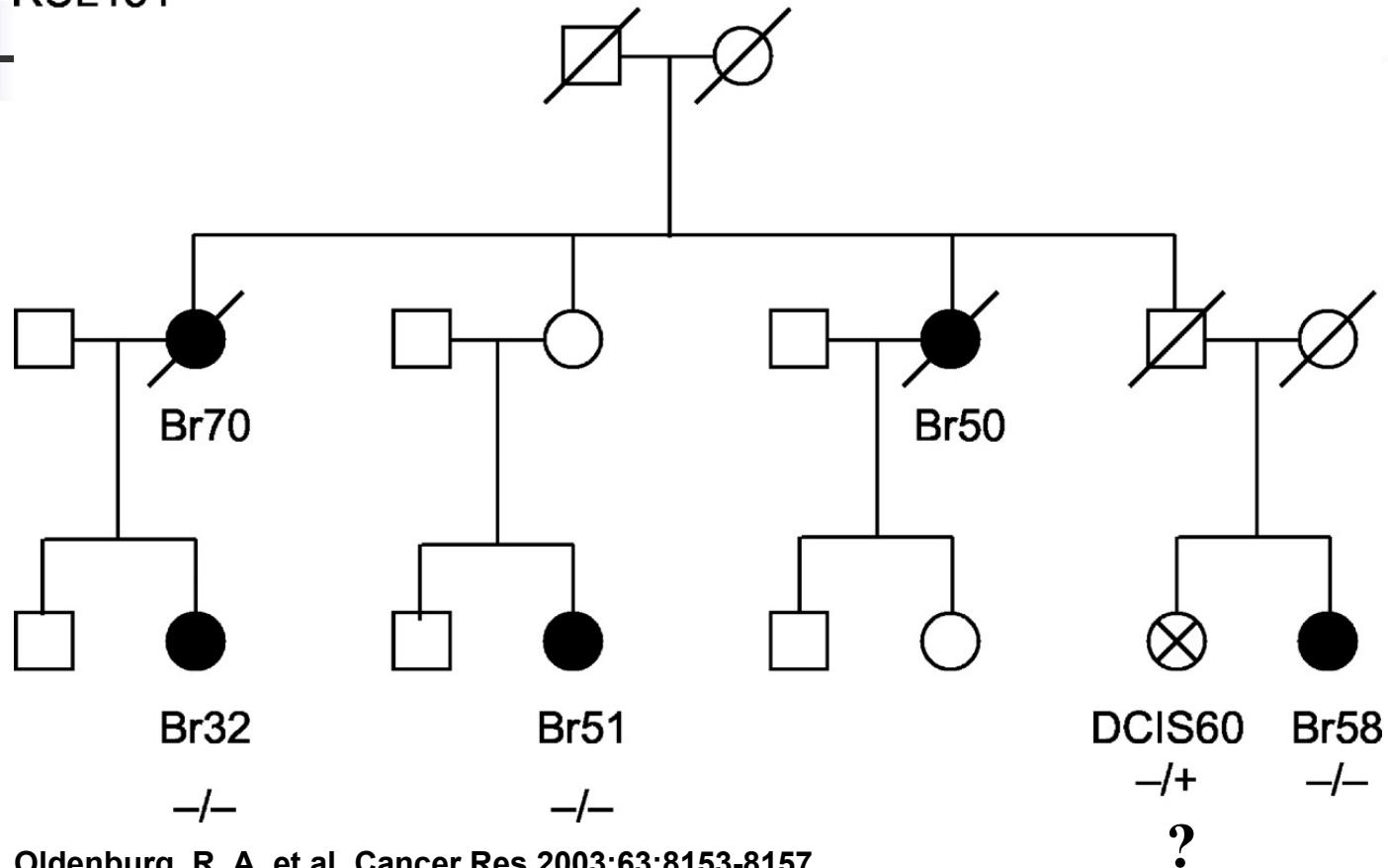
- All families
 - 21.1% (15/71)
- 3 cases < 60
 - 13.3% (4/30)
- 4 cases < 60
 - 21.1% (4/19)
- 5 = cases > 60
 - 31.8% (7/22)

Pedigree of non-BRCA1,2 breast cancer family: mutation does not segregate with the disease!

Fig. 1



RUL154

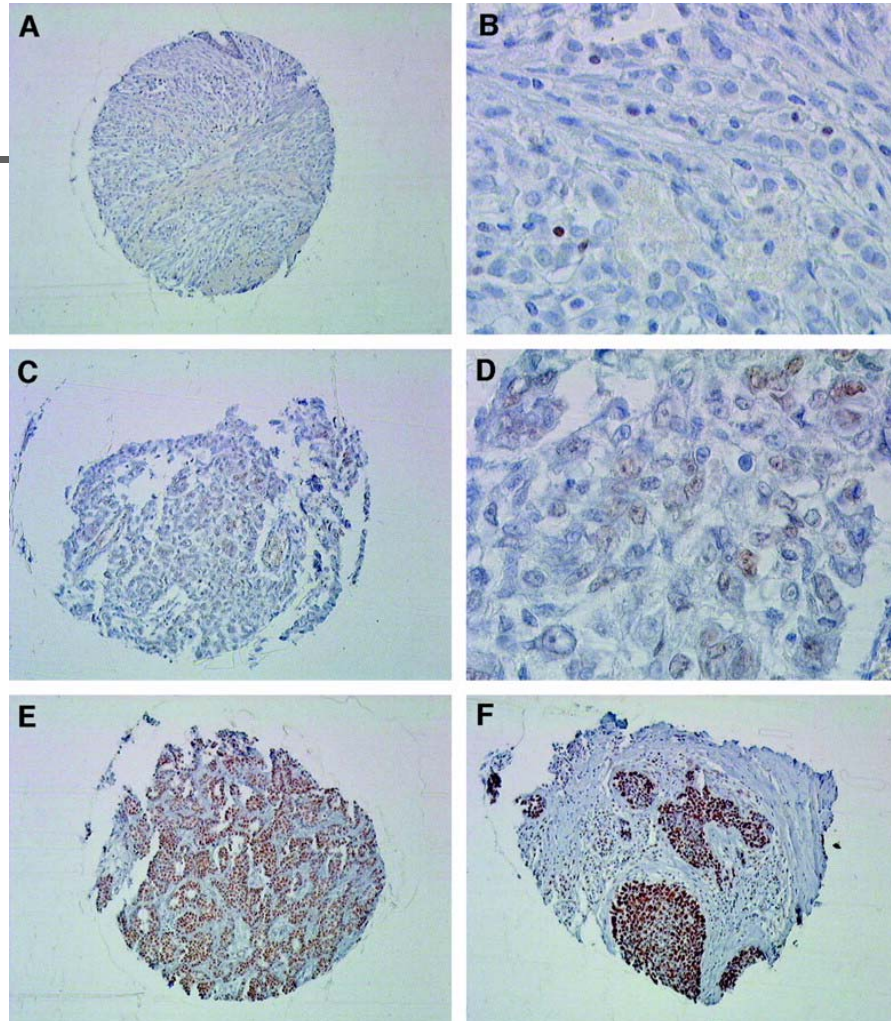
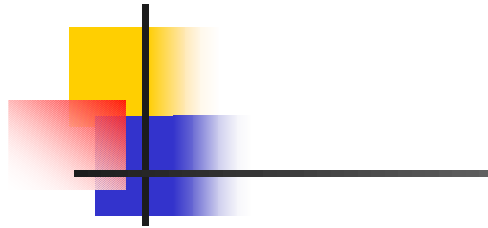


Oldenburg, R. A. et al. Cancer Res 2003;63:8153-8157

*CHEK2*1100delC* carrier

Immunohistochemical staining of CHEK2 protein in tissue micro-array

Fig. 2



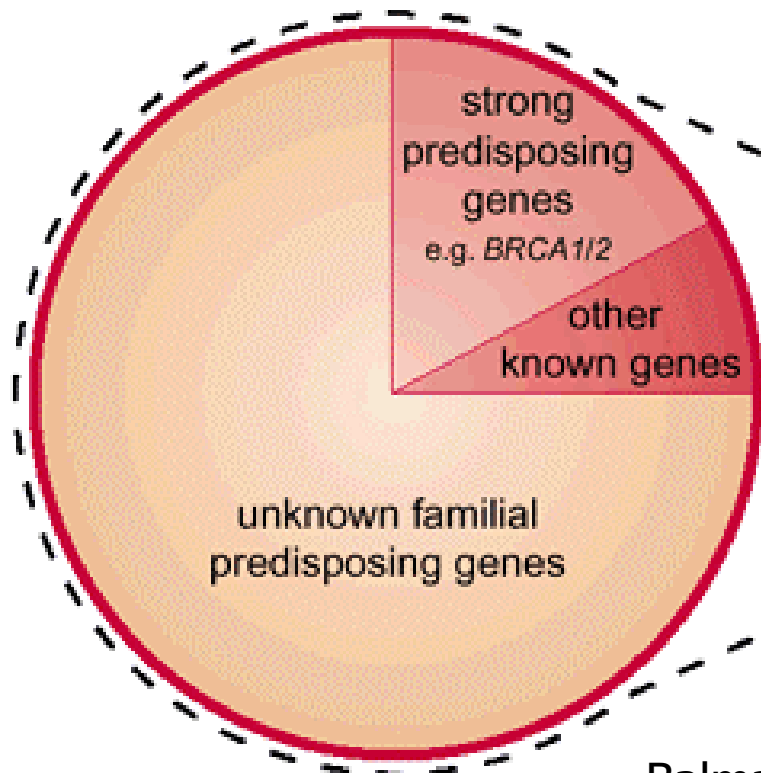
Negative in 12/15
tumors from
CHEK*1100delC
carriers

Positive in 73/76
tumors from
non-carriers

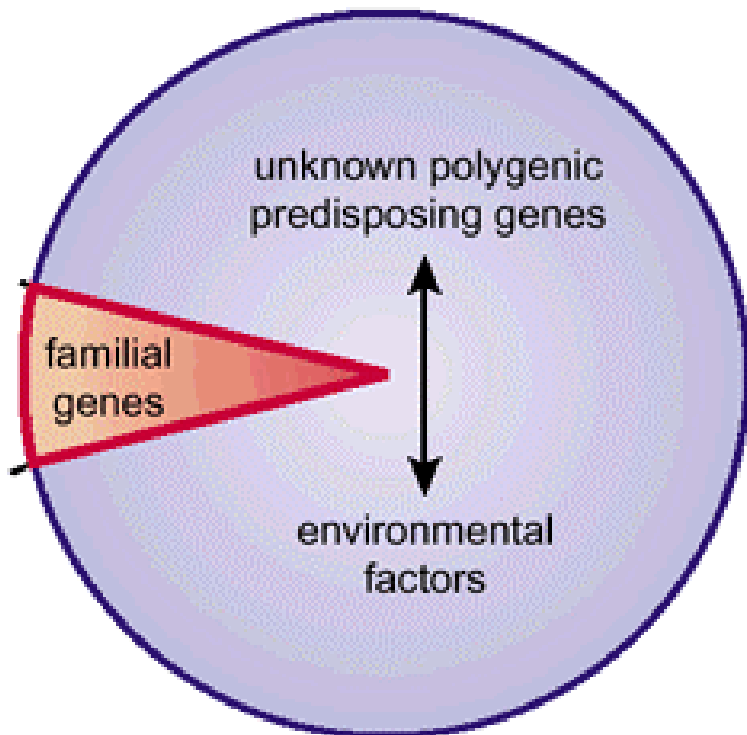
Oldenburg, R. A. et al. *Cancer Res* 2003;63:8153-8157

Terra incognita of breast cancer susceptibility

familial breast cancer



all breast cancer



Balmain et al., Nat. Genet, 2003

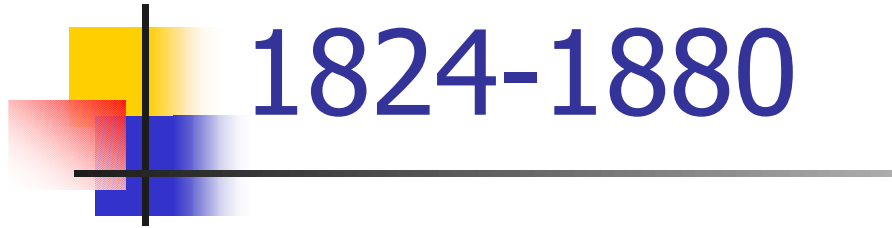


Familial risk to breast cancer

<i>Family history</i>	<i>Relative risk¹</i>
A first-degree relative	1.9 (1.7-2.0)
Mother	2.0 (1.8-2.1)
Sister	2.3 (2.1-2.4)
Daughter	1.8 (1.6-2.0)
Mother and sister	3.6 (2.5-5.0)
Second-degree relative	1.5 (1.4-1.6)

¹ Pooled estimate from a meta-analysis of 74 studies.
Pharoah et al. (1997) Int J Cancer 71, 800.

Paul Broca, 1824-1880



In 1866, this famous French surgeon published a pedigree of a breast cancer family (probably of his wife)



The first breast cancer pedigree, reported by Broca (1866)

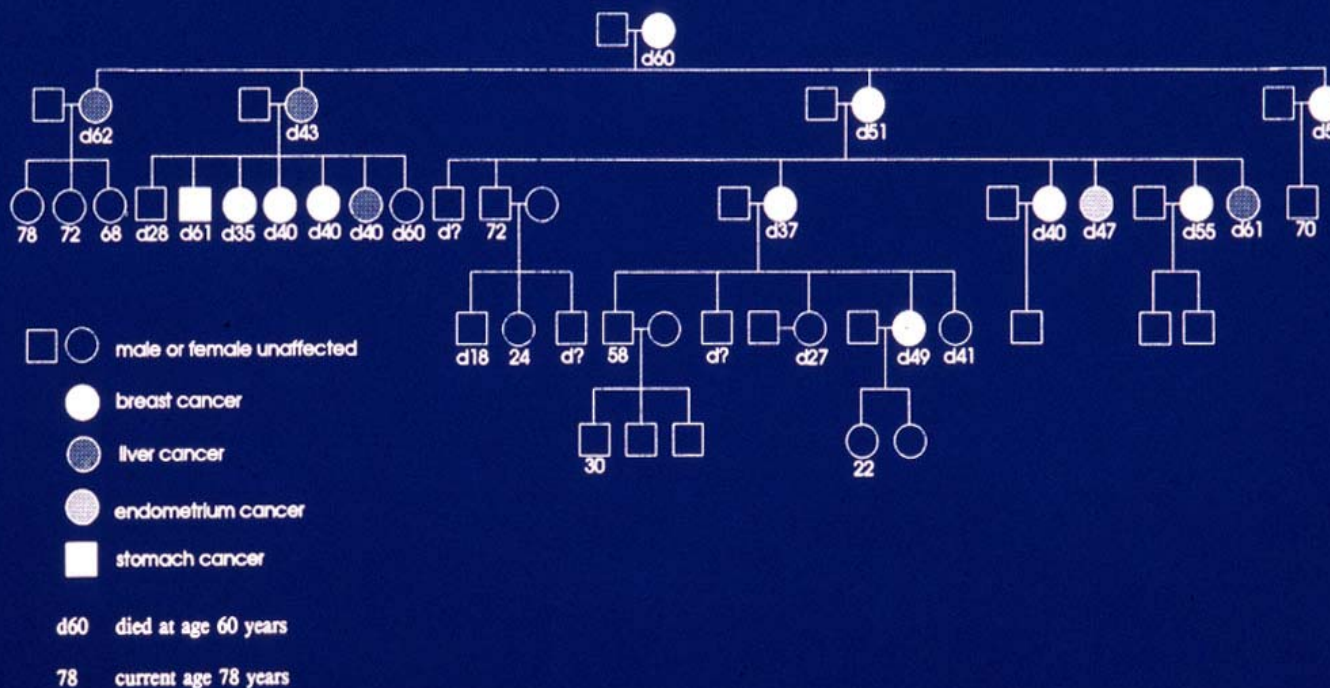
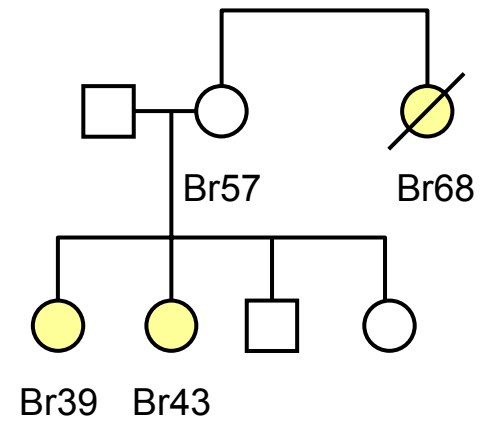
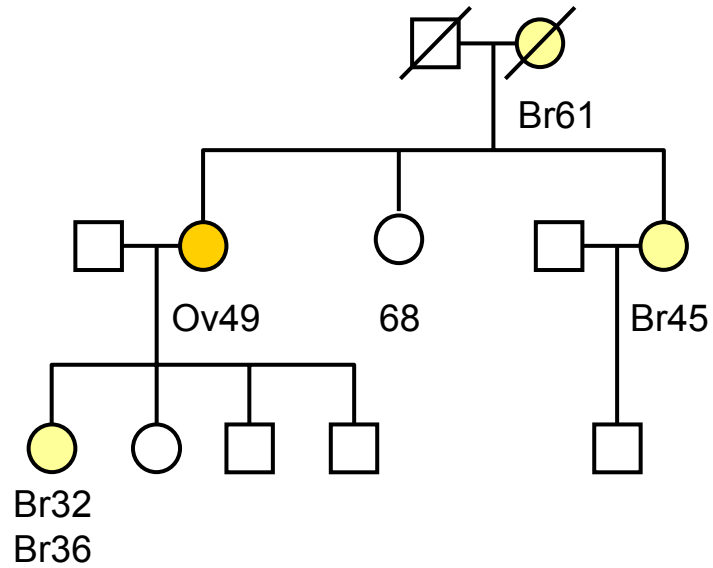


Figure 1 Pedigree reported by Broca in 1866, of his wife's family with familial breast cancer and associated malignant neoplasms.

Typical breast cancer families





Excess familial risk

- How much is genetic?
- How are the risks distributed?
- How do we find the genes?



A few high-risk genes and a few low risk genes explain 20-25% of excess familial risk

Which genes?

BRCA1 (1994) and BRCA2 (1995) identified!

A Strong Candidate for the Breast and Ovarian Cancer Susceptibility Gene *BRCA1*

Yoshio Miki, Jeff Swensen, Donna Shattuck-Eidens, P. Andrew Futreal, Keith Harshman, Sean Tavtigian, Qingyun Liu, Charles Cochran, L. Michelle Bennett, Wei Ding, Russell Bell, Judith Rosenthal, Charles Hussey, Thanh Tran, Melody McClure, Cheryl Frye, Tom Hattier, Robert Phelps, Astrid Haugen-Strano, Harold Katcher, Kazuko Yakumo, Zahra Gholami, Daniel Shaffer, Steven Stone, Steven Bayer, Christian Wray, Robert Bogden, Priya Dayananth, John Ward, Patricia Tonin, Steven Narod, Pam K. Bristow, Frank H. Norris, Leah Helvering, Paul Morrison, Paul Rosteck, Mei Lai, J. Carl Barrett, Cathryn Lewis, Susan Neuhausen, Lisa Cannon-Albright, David Goldgar, Roger Wiseman, Alexander Kamb, Mark H. Skolnick*

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LETTERS TO NATURE

Identification of the breast cancer susceptibility gene *BRCA2*

Richard Wooster*, Graham Bignell*, Jonathan Lancaster†, Sally Swift†, Sheila Seal*, Jonathan Mangion*, Nadine Collins*, Simon Gregory§, Curtis Gumbs||, Gos Micklem§, Rita Barfoot*, Rifat Hamoudi*, Sandeep Patel*, Catherine Rice§, Patrick Biggs*, Yasmin Hashim*, Amanda Smith†, Frances Connor†, Adalgeir Arason¶, Julius Gudmundsson¶, David Ficenec¶***, David Kelsell#, Deborah Ford*, Patricia Tonin**, D. Timothy Bishop††, Nigel K. Spurr#, Bruce A. J. Ponder†††, Rosalind Eeles*, Julian Peto*, Peter Devilee§§, Cees Cornelisse§§, Henry Lynch|||, Steven Narod** ***, Gilbert Lenoir¶¶, Valdgardur Egilsson¶, Rosa Bjork Barkadottir¶, Douglas F. Easton##, David R. Bentley§, P. Andrew Futreal||, Alan Ashworth† & Michael R. Stratton*



Breast cancer susceptibility genes (modified after Thompson & Easton, 2004)

Gene	Location	Carrier frequency	Risk at age 70
<i>BRCA1</i>	17q	1 in 860	44-78%
<i>BRCA2</i>	13q	1 in 740	31-56%
<i>CHEK2</i>	22q	1 in 90	9-14%
<i>ATM</i>	11q	1 in 100	13-39%
<i>TP53</i>	17p	1 in 5,000	50-60% (45yr)
<i>PTEN</i>	10q	1 in 250,000	30-50%
<i>STK11/ LKB1</i>	19p13		31% (60yrs)

Red=
High
risk



Higher prevalence of BRCA1,2 mutation carriers among unselected young breast cancer patients

- Papelard et al., *Leiden* n=624
 - 2.1% all patients
 - **9.5% < 40 yr**
- Anglian Breast Cancer Study Group., *UK*, n=1220
 - 2 % all patients
 - **12.4% < 35 yr**
- Bonadona et al., *Lyon*, n= 232 < 46 yr
 - 9.1%
 - **12.8% <41 yr**
- Lubinski et al., *Szczecin*, n = 3472 < 51 yr (**3 BRCA1 founder mutations, only**)
 - 5.7%
 - **9% < 40 yr**
- Lalloo et al., *Manchester*, n= 100,
 - **18% < 30 yr**
 - 4% TP53



Prevalence and clinical correlations of BRCA1/BRCA2 mutations in 205 unselected ovarian cancer patients

A Gdansk-Leiden collaboration
Majdak et al., Eur J. Cancer 41, 2005
Majdak et al., Cancer 104, 2005



Results

- *BRCA1*

- 18 (9%) pathogenic mutations*
 - 15% in patients < 50
- 16 (8%) unclassified variants
- 13 (6%) polymorphic mutations

- *BRCA2*

- no pathogenic mutations
- 8 (4%) unclassified variants
- 3 (1.5%) polymorphic mutations

Genetic counseling

