Application of Multiple NGS Panels for Germline Variation to Real Practice

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Cancer Predisposition Genes

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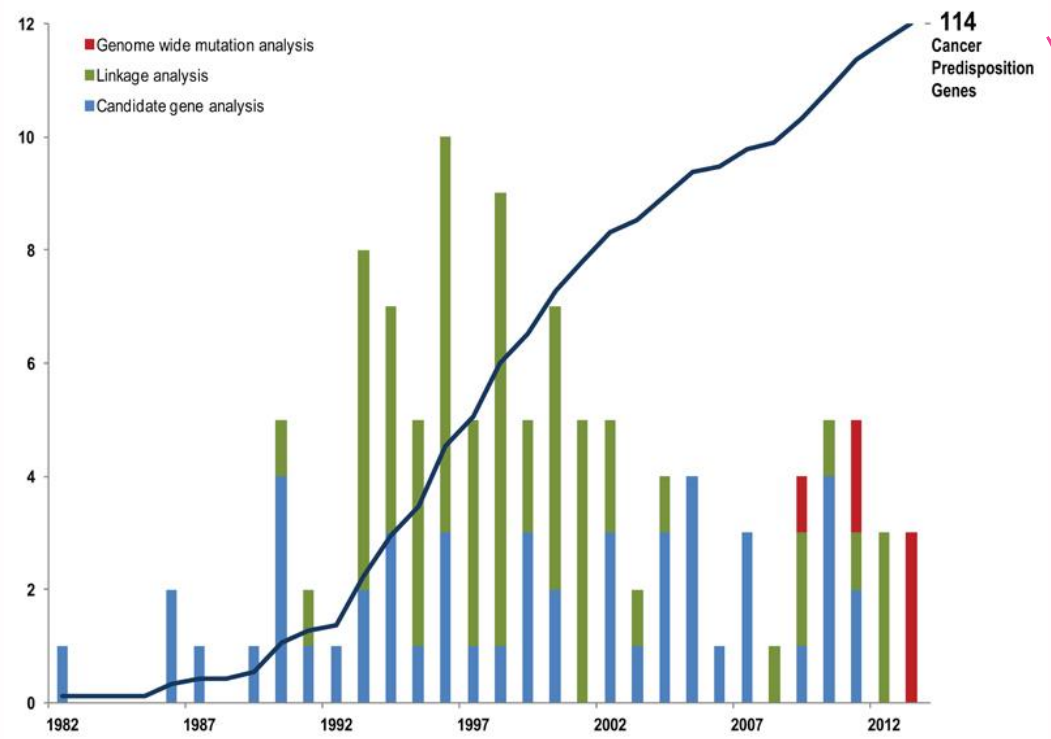

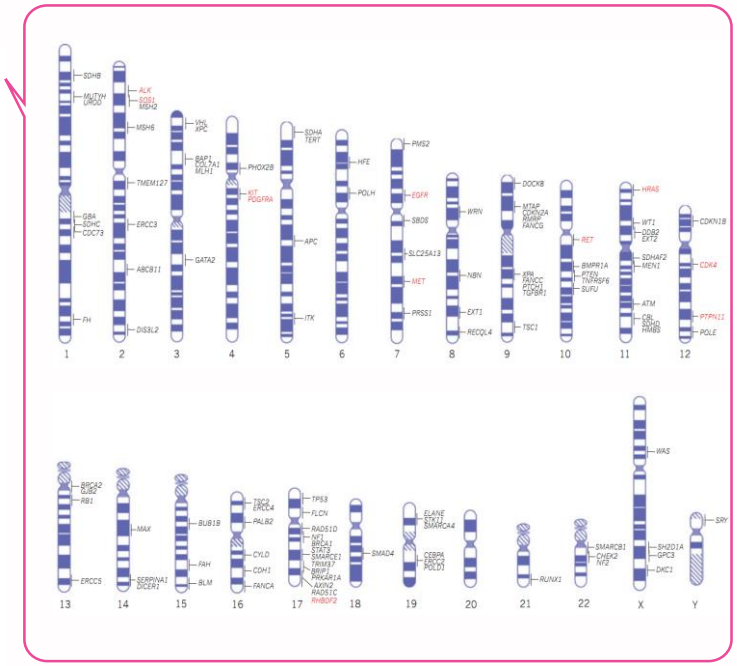

114 Cancer Predisposition Genes (CPGs)

Rahman, Nature 2014

Cancer Gene Truncation Carrier Frequencies across 12 cancer types (rare variants; MAF ≤0.05%)

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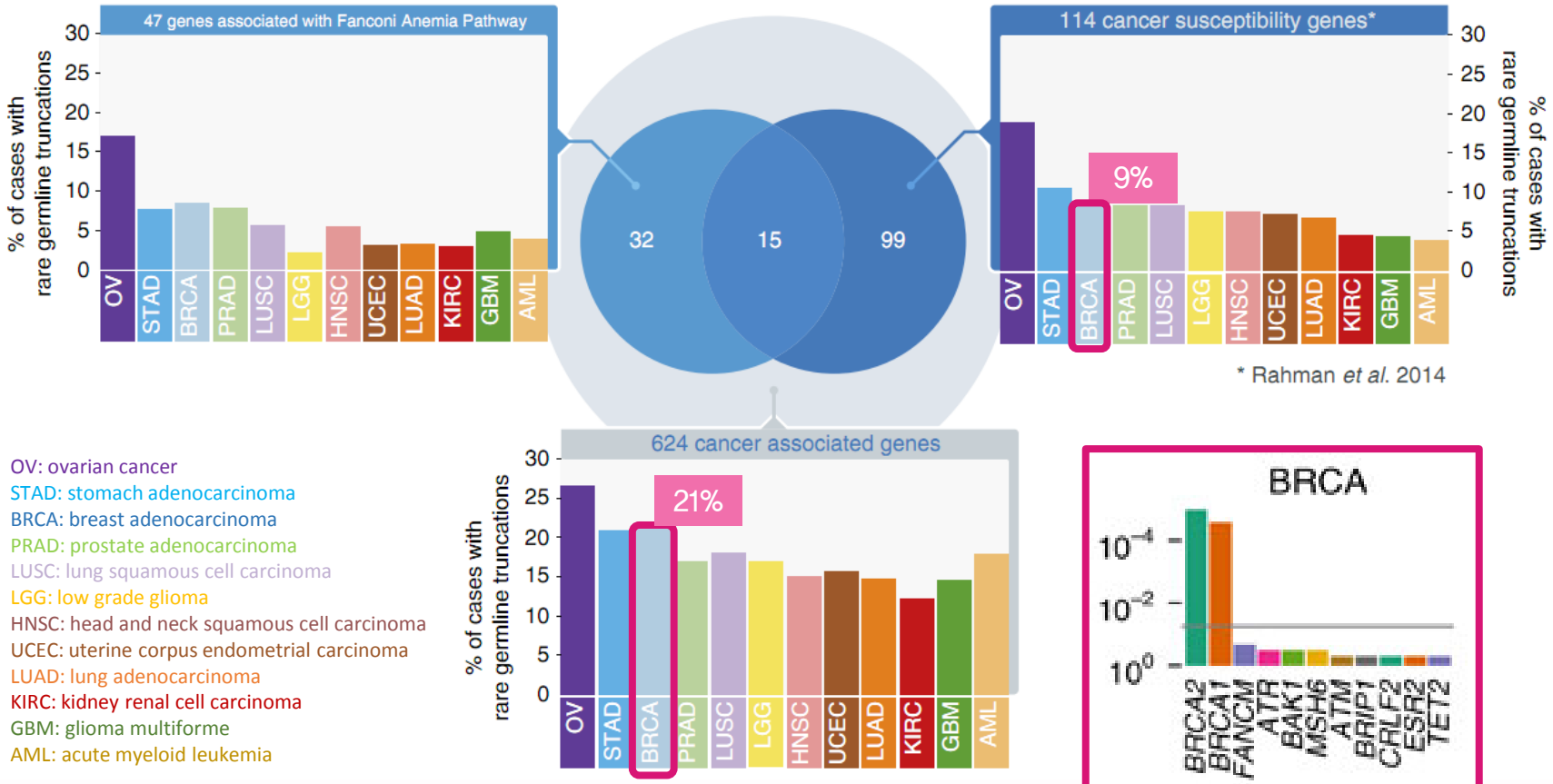

Lu et al., Nat Commun 2015

Cancer susceptibility genes associated with hereditary breast cancer, beyond *BRCA1/2*

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Spectrum of Mutations in *BRCA1*, *BRCA2*, *CHEK2*, and *TP53* in Families at High Risk of Breast Cancer

Results Of the 300 probands, 52 (17%) carried previously undetected mutations, including 35 (12%) with genomic rearrangements of *BRCA1* or *BRCA2*, 14 (5%) with *CHEK2* mutations, and 3 (1%) with *TP53* mutations. At *BRCA1* and *BRCA2*, 22 different genomic rearrangements were found, of sizes less than 1 kb to greater than 170 kb; of these, 14 were not previously described and all were individually rare. At *CHEK2*, a novel 5.6-kb genomic deletion was discovered in 2 families of Czechoslovakian ancestry. This deletion was found in 8 of 631 (1.3%) patients with breast cancer and in none of 367 healthy controls in the Czech and Slovak Republics. For all rearrangements, exact genomic breakpoints were determined and diagnostic primers validated. The 3 families with *TP53* mutations included cases of childhood sarcoma or brain tumors in addition to multiple cases of breast cancer.

Walsh et al., JAMA 2006

Development of sequencing technologies and application

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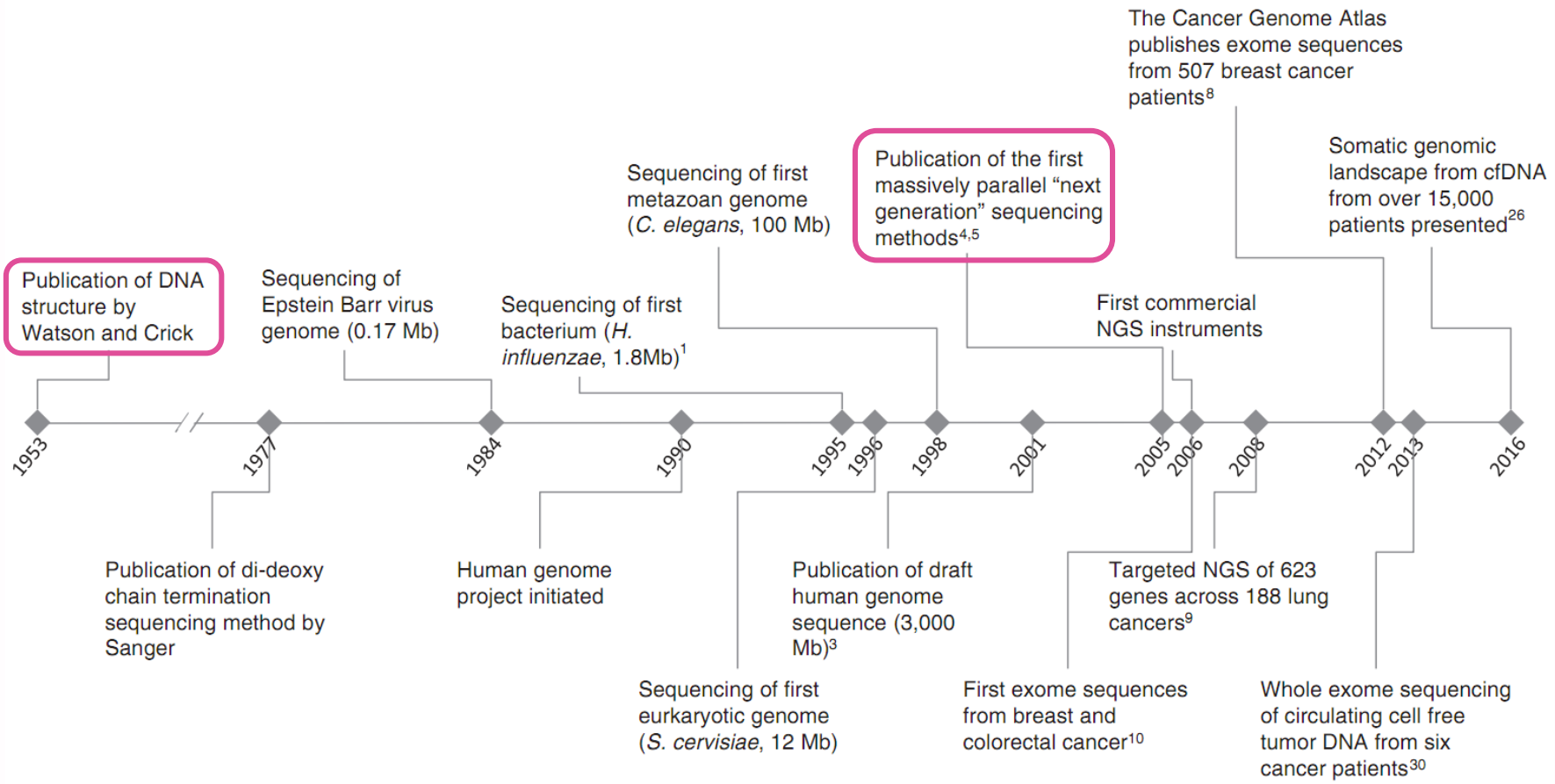

Cummings et al., Citation: Clin Transl Sci 2016

Genetic underpinnings of Early-onset familial breast cancer

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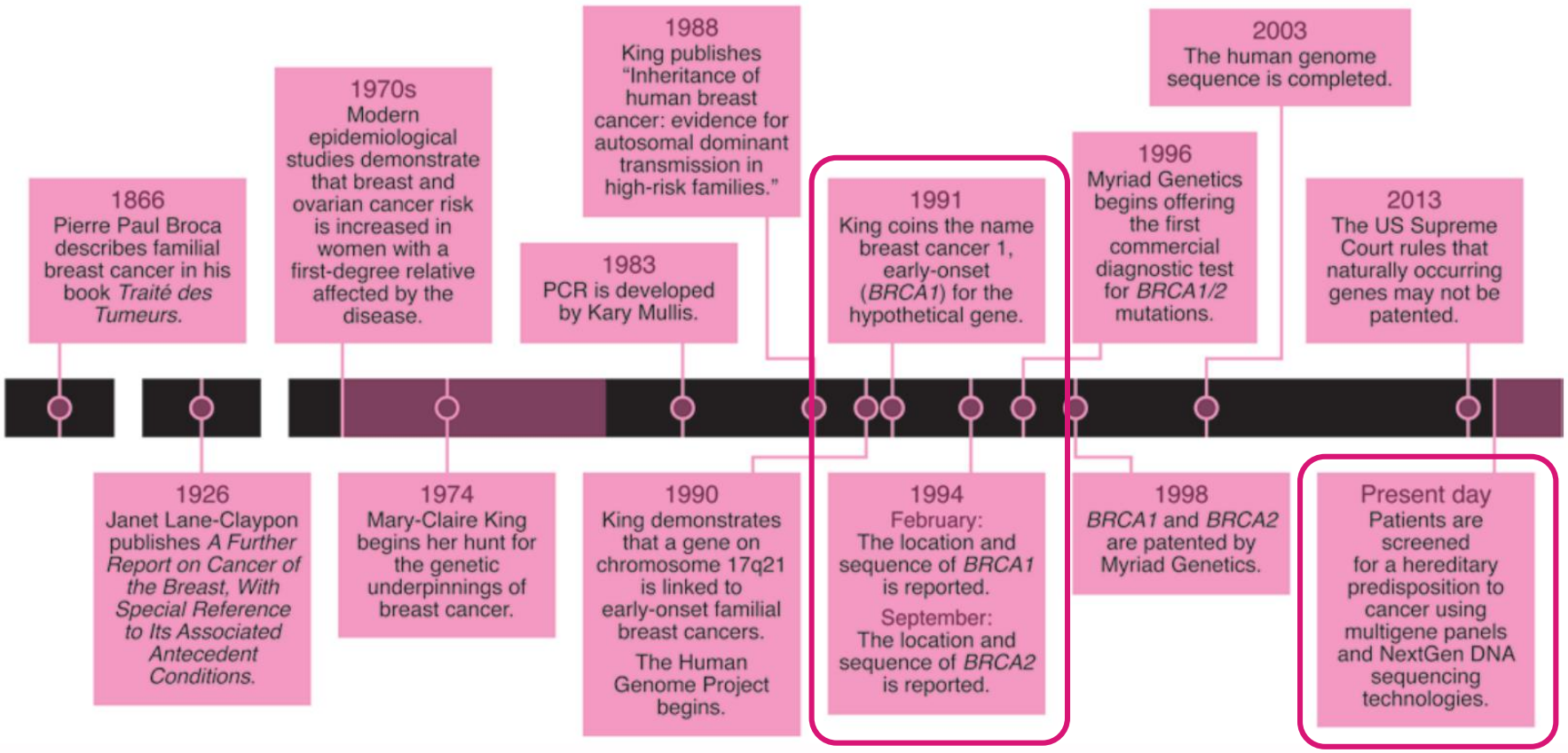

J Clin Invest 2014

Multigene-Panel Sequencing for the Prediction of Breast-Cancer Risk

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Company	Test	Website	Gene included
Ambry Genetics	BreastNext	www.ambrygen.com/tests/breastnext	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, TP53
Breast Health UK	BreastGene	www.breasthealthuk.com/breast-cancer-genetic-testing/breastgene-service	ATM, BRCA1, BRCA2, NBN, CDH1, CHEK2, PALB2, PTEN, TP53, STK11
Centogene	CentoBreast	www.centogene.com/centogene/index.php	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C, STK11, TP53
Eurofins*	High Risk Breast Cancer Panel	www.egl-eurofins.com/?testid=MM201	BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53
Fulgent Diagnostics	Breast Cancer Comprehensive Panel	https://www.fulgentgenetics.com/comprehensivecancer-breast	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MLH1, MRE11, MSH2, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53
GeneDx	Breast/Gyn Cancer Panel	https://www.genedx.com/test-catalog/disorders/breast-cancer/	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MLH1, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, PTEN, RAD51C, RAD51D, RECQL, TP53
Illumina	TruSight Cancer	https://www.illumina.com/products/by-type/clinical-research-products/trusight-cancer.html	94 Genes plus 284 SNPs reported to be associated with risk of breast cancer
Invitae	Invitae Breast Cancer Panel	https://www.invitae.com/en/physician/tests/01202/	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, RAD50, STK11, TP53 (+ AKT1, FAM175A, FANCC, MRE11, MUTYH, PIK3CA, RAD51C, RAD51C, RINT1, SDHB, SDHD, XRCC2)
Myriad Genetics	myRIsk	https://new.myriadpro.com/products/myriad-myrisk/	BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, PALB2, CHEK2, ATM, NBN, BARD1
University of Washington	BROCA-Cancer Risk Panel	web.labmed.washington.edu/tests/genetics/BROCA	AKT1, APC, ATM, ATR, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK1, CHEK2, CTNNA1, FAM175A (Abraxas), FANCM, FH, FLCN, GALNT12, GEN1, GREM1, HOXB13, MEN1, MET, MITF, MLH1, MRE11A, MSH2 (+EPCAM), MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PALLD, PDGFRA, PIK3CA, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTCH1, PTEN, RAD51B, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, RPS20, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCA4, TP53, VHL, XRCC2

**In September 2015,Eurofin scientific acquire a controlling stake in Emory Genetic Laboratory from Emory University's School of Medicine.*

Modified from Easton et al., N Engl J Med 2015 (ver. MAR 2018)

Are *Multi-gene panels* *Useful* for the patients with high risk for hereditary breast cancer?

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Basis of Genetic Tests (ACCE)

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- Analytic Validity
- Clinical Validity
- Clinical Utility
- Ethical, legal, and social issues (ELSI)

established by the Centers for Disease Control and Prevention

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Decrease in error rates of NGS platforms

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Instrument	Purchase cost	Additional instruments[a]	Service contract[b]	Computational resources[c]	Data file sizes (GB)[d]	Primary errors	Error rate (%)[e]
3730xl (capillary)	$376	–	$19.8	Desktop	0.03	Substitution	0.1–1
454 GS Jr. Titanium	$108	$16	$12.6	$5 (desktop)	<3 images, <1 sff	Indel	1
454 FLX Titanium	$500	$30	$50.0	$5 (desktop)	20 images, 4 sff	Indel	1
454 FLX+[f]	$29.5	$30	$50.0	$5 (desktop)	~40 images, 8 sff	Indel	1*
PacBio RS	$695	–	$85	$65 cluster	20 pulsed, 2 Fastq	CG deletions	16
Ion Torrent – 314 chip	$49.5	$18[g]	$7.5	Desktop – $35	0.1Fastq	Indel	~1
Ion Torrent – 316 chip	$49.5	$18[g]	$7.5	Desktop – $35	0.6Fastq	Indel	~1*
Ion Torrent – 318 chip	$49.5	$18[g]	$7.5	Desktop – $35	TBD	Indel	~1*
SOLiD – 4	$475	$54[h]	$38.4	$35 cluster[i]	680[j]	A-T bias	>0.06*
SOLiD – 5500	$349	$54[h]	$29.0	$35 cluster[i]	74[k*]	A-T bias	>0.01*
SOLiD – 5500xl	$595	$54[h]	$38.4	$35 cluster[i]	148[k*]	A-T bias	>0.01*
Illumina MiSeq	$125	–	$12.5	Desktop	1[k*]	~Substitution	>0.1*
Illumina HiScanSQ	$405	$55[l]	$41.5	$222 cluster[m]	50[k*]	Substitution	≥0.1
Illumina GAIIx	$250	$100[n]	$44.5	$222 cluster[m]	600	Substitution	≥0.1
Illumina HiSeq1000	$560[o]	$55[l]	$62.0	$222 cluster[m]	≤300[k*]	Substitution	≥0.1
Illumina HiSeq2000	$690	$55[l]	$75.9	$222 cluster[m]	≤600[k*]	Substitution	≥0.1

2011

Commercial Platform	Most Frequent Error Type	Error Frequency
Capillary sequencing	single nucleotide substitutions	10^{-1}
454 GS Junior	Deletions	10^{-2}
PacBio RS	CG deletions	10^{-2}
Ion Torrent PGM	Short deletions	10^{-2}
Solid	A-T bias	2×10^{-2}
IlluminaMiSeq	single nucleotide substitutions	10^{-3}
Illumina HiSeq2000	single nucleotide substitutions	10^{-3}
Tag-based methods:		
SafeSeq	single nucleotide substitutions	1.4×10^{-5}
CircleSeq	single nucleotide substitutions	7.6×10^{-6}
Duplex Sequencing	Single nucleotide substitutions	5×10^{-8}

2014

Glenn TC, Mol Ecol Resour. 2011; Fox et al., Next Gener Seq Appl. 2014

Chemistry, costs, and throughput of NGS platforms

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Platform	Read length (bp)	Throughput	Reads	Runtime	Error profile	Instrument cost (US$)	Cost per Gb (US$, approx.)
Sequencing by ligation							
SOLiD 5500 Wildfire	50 (SE) 75 (SE) 50 (SE)* 50 (SE)	80 Gb 120 Gb 160 Gb* 160 Gb	~700 M*	6 d*	≤0.1%, AT bias‡	NA§	$130‡
SOLiD 5500 xl	75 (SE) 50 (SE)*	240 Gb 320 Gb*	~1.4 B*	10 d*	≤0.1%, AT bias‡	$251,000‡	$70‡
BGISEQ-500 FCS	50–100 (SE/PE)*	8–40 Gb*	NA‖	24 h*	≤0.1%, AT bias‡	$250	NA‖
BGISEQ-500 FCL	50–100 (SE/PE)¶	40–200 Gb*	NA‖	24 h¶	≤0.1%, AT bias‡	$250,000	NA‖
Sequencing by synthesis: CRT							
Illumina MiniSeq Mid output	150 (SE)*	2.1–2.4 Gb*	14–16 M*	17 h*	<1%, substitution‡	$50,000	$200–300
Illumina MiniSeq High output	75 (SE) 75 (PE) 150 (PE)*	1.6–1.8 Gb 3.3–3.7 Gb 6.6–7.5 Gb*	22–25 M (SE)* 44–50 M (PE)*	7 h 13 h 24 h*	<1%, substitution‡	$50,000	$200–300
Illumina MiSeq v2	36 (SE) 25 (PE) 150 (PE) 250 (PE)*	540–610 Mb 750–850 Mb 4.5–5.1 Gb 7.5–8.5 Gb*	12–15 M (SE) 24–30 M (PE)*	4 h 5.5 h 24 h 39 h*	0.1%, substitution‡	$99,000‡	~$1,000 $996 $212 $142‡
Illumina MiSeq v3	75 (PE) 300 (PE)*	3.3–3.8 Gb 13.2–15 Gb*	44–50 M (PE)*	21–56 h*	0.1%, substitution‡	$99,000‡	$250 $110‡
Illumina NextSeq 500/550 Mid output	75 (PE) 150 (PE)*	16–20 Gb 32–40 Gb*	Up to 260 M (PE)*	15 h 26 h*	<1%, substitution‡	$250‡	$42 $40‡
Illumina NextSeq 500/550 High output	75 (SE) 75 (PE) 150 (PE)*	25–30 Gb 50–60 Gb 100–120 Gb*	400 M (SE)* 800 M (PE)*	11 h 18 h 29 h*	<1%, substitution‡	$250‡	$43 $41 $33‡
Illumina HiSeq2500 v2 Rapid run	36 (SE) 50 (PE) 100 (PE) 150 (PE) 250 (PE)*	9–11Gb 25–30 Gb 50–60 Gb 75–90 Gb 125–150 Gb*	300 M (SE)* 600 M (PE)*	7 h 16 h 27 h 40 h 60 h*	0.1%, substitution‡	$690‡	$230 $90 $52 $45 $40‡
Illumina HiSeq2500 v3	36 (SE) 50 (PE) 100 (PE)*	47–52 Gb 135–150 Gb 270–300 Gb	1.5 B (SE) 3 B (PE)*	2 d 5.5 d 11 d*	0.1%, substitution‡	$690‡	$180 $78 $45‡
Illumina HiSeq2500 v4	36 (SE) 50 (PE) 100 (PE) 125 (PE)* 50 (SE)	64–72 Gb 180–200 Gb 360–400 Gb 450–500 Gb* 105–125 Gb	2 B (SE) 4 B (PE)*	29 h 2.5 d 5 d 6 d*	0.1%, substitution‡	$690‡	$150 $58 $45 $30‡ $50
Illumina HiSeq3000/4000	75 (PE) 150 (PE)*	325–375 Gb 650–750 Gb*	2.5 B (SE)*	1–3.5 d*	0.1%, substitution‡	$740/$900	$31 $22
Illumina HiSeq X	150 (PE)¶	800–900 Gb per flow cell*	2.6–3 B (PE)*	<3 d*	0.1%, substitution‡	$1,000‡,¶	$7.0‡
Qiagen GeneReader	NA‖	12 genes; 1,250 mutations	NA‖	Several days	Similar to other SBS systems	NA‖	$400–$600
Sequencing by synthesis: SNA							
454 GS Junior	Up to 600; 400 average (SE, PE)*	35 Mb*	~0.1 M*	10 h*	1%, indel‡	NA§	$40,000‡
454 GS Junior+	Up to 1,000; 700 average (SE, PE)*	70 Mb*	~0.1 M*	18 h*	1%, indel‡	$108,000‡	$19,500‡
454 GS FLX Titanium XLR70	Up to 600; 450 mode (SE, PE)*	450 Mb*	~1 M*	10 h*	1%, indel‡	NA§	$15,500‡
454 GS FLX Titanium XL+	Up to 1,000; 700 mode (SE, PE)*	700 Mb¶	~1 M¶	23 h¶	1%, indel‡	$450,000‡	$9,500‡
Ion PGM 314	200 (SE) 400 (SE)	30–50 60–100 Mb*	400,000–550,000*	23 h 3.7 h*	1%, indel‡	$49‡	$25–3,500‡
Ion PGM 316	200 (SE) 400 (SE)*	300–500 Mb 600 Mb–1 Gb*	2–3 M*	3 h 4.9 h*	1%, indel‡	$49‡	$700–1,000‡
Ion PGM 318	200 (SE) 400 (SE)*	600 Mb–1 Gb 1–2 Gb*	4–5.5 M*	4 h 7.3 h*	1%, indel‡	$49‡	$450–800‡
Ion Proton	Up to 200 (SE)	Up to 10 Gb*	60–80 M*	2–4 h*	1%, indel‡	$224‡	$80‡
Ion S5 520	200 (SE) 400 (SE)*	600 Mb–1 Gb 1.2–2 Gb*	3–5 M*	2.5 h 4 h*	1%, indel‡	$65	$2,400* $1,200¶
Ion S5 530	200 (SE) 400 (SE)*	3–4 Gb 6–8 Gb*	15–20 M*	2.5 h 4 h*	1%, indel‡	$65	$950* $475¶
Ion S5 540	200 (SE)¶	10–15 Gb*	60–80 M*	2.5 h*	1%, indel‡	$65	$300¶
Single-molecule real-time long reads							
Pacific BioSciences RS II	~20 Kb	500 Mb–1 Gb*	~55,000*	4 h*	13% single pass, ≤1% circular consensus read, indel‡	$695‡	$1,000‡
Pacific Biosciences Sequel	8–12 Kb	3.5–7 Gb*	~350,000*	0.5–6 h*	NA‖	$350	NA‖
Oxford Nanopore MK 1 MinION	Up to 200 Kb	Up to 1.5 Gb	>100,000	Up to 48 h	~12%, indel	$1,000*	$750*
Oxford Nanopore PromethION	NA‖	Up to 4 Tb*	NA	NA	NA‖	$75*	NA‖

Goodwin et al., Nat Rev Genet 2016

Basis of Genetic Tests (ACCE)

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- Analytic Validity
- Clinical Validity
- Clinical Utility
- Ethical, legal, and social issues (ELSI)

established by the Centers for Disease Control and Prevention

Multigene-Panel Sequencing for the Prediction of Breast-Cancer Risk

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		Gene lists																											
Company	**Test**	**BRCA1**	**BRCA2**	**TP53**	**CDH1**	**PTEN**	**STK11**	**PALB2**	**ATM**	**BRIP1**	**CHEK2**	**BARD1**	**MRE11A**	**NBN**	**RAD50**	**RAD51C**	**RAD51D**	**XRCC2**	**MLH1**	**MSH2**	**MSH6**	**MUTYH**	**NF1**	**MEN1**	**PMS1**	**PMS2**	**POLD1**	**RECQL**	**EPCAM**	**FANCC**
Ambry Genetics	BreastNext	o	o	o	o	o		o	o	o	o	o	o	o	o	o	o					o	o							
Breast HealthUK	BreastGene	o	o	o	o	o	o	o	o	o	o			o																
Centogene	CentoBreast	o	o		o			o	o	o	o	o	o	o	o	o			o	o	o	o		o	o	o				
Eurofins	HighRiskBreast Cancer Panel	o	o	o	o	o	o	o																						
Fulgent Diagnostics	Breast Cancer Comprehensive Panel	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o			o							
GeneDx	Breast/Gyn Cancer Panel	o	o	o	o	o	o	o	o	o	o	o		o		o	o	o	o	o	o	o	o			o	o	o	o	o
Invitae	Invitae Breast Cancer Panel	o	o	o	o	o	o	o	o	o	o			o	o								o							
Myriad Genetics	myRisk	o	o	o	o	o	o	o	o		o	o		o																

Modified from Easton et al., N Engl J Med 2015 (ver. MAR 2018)

Germline mutations that confer susceptibility

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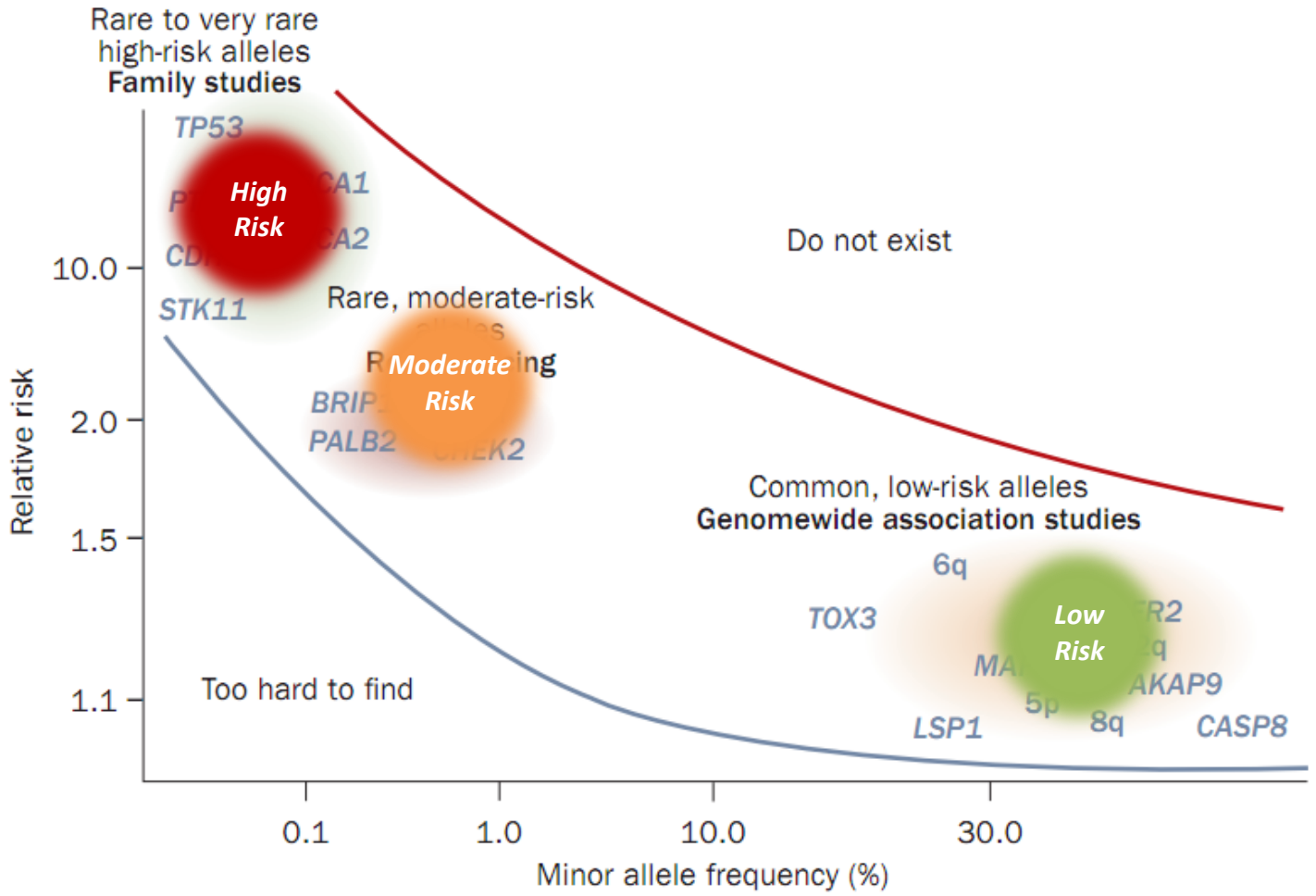

Foulkes, N Engl J Med 2008

Multigene-Panel Sequencing for the Prediction of Breast-Cancer Risk

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		High Risk							Moderate Risk										Low Risk / Equivocal (for breast cancer)											
Company	**Test**	**BRCA1**	**BRCA2**	**TP53**	**CDH1**	**PTEN**	**STK11**	**PALB2**	**ATM**	**BRIP1**	**CHEK2**	**BARD1**	**MRE11A**	**NBN**	**RAD50**	**RAD51C**	**RAD51D**	**XRCC2**	**MLH1**	**MSH2**	**MSH6**	**MUTYH**	**NF1**	**MEN1**	**PMS1**	**PMS2**	**POLD1**	**RECQL**	**EPCAM**	**FANCC**
Ambry Genetics	BreastNext	o	o	o	o	o		o	o	o	o	o	o	o	o	o	o					o	o							
Breast HealthUK	BreastGene	o	o	o	o	o	o	o	o	o	o			o																
Centogene	CentoBreast	o	o		o			o	o	o	o	o	o	o	o	o			o	o	o	o		o	o	o				
Eurofins	HighRiskBreast Cancer Panel	o	o	o	o	o	o	o																						
Fulgent Diagnostics	Breast Cancer Comprehensive Panel	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o			o							
GeneDx	Breast/Gyn Cancer Panel	o	o	o	o	o	o	o	o	o	o	o		o		o	o	o	o	o	o	o	o			o	o	o	o	o
Invitae	Invitae Breast Cancer Panel	o	o	o	o	o	o	o	o	o	o			o	o								o							
Myriad Genetics	myRisk	o	o	o	o	o	o	o	o		o	o		o																

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Germline mutations and breast cancer risks

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Gene	Magnitude of Relative Risk associated with Truncating Variants*		Risk associated with Missense Variants†	Estimated Relative Risk (90% CI)	P Value	Cancer risk OR (95% CI)	P value	Absolute risk by 80 years of Age	Other Associated Cancers
	Moderate (2-4 times)	High (>4 times)						%	
BRCA1	●	●	●	**11.4**				**75**	Ovary
BRCA2	●	●	●	**11.7**				**76**	Ovary, prostate, pancreas
TP53	●	●	●	**105 (62-165)**					Childhood sarcoma, adreno-cortical carcinoma, brain tumors
PTEN	Unknown	Unknown	●						Thyroid, endometrial cancer
CDH1	**Likely**	Unknown	Unknown	**6.6 (2.2-19.9)**	0.004			**53**	Diffuse gastric cancer
STK11	Unknown	Unknown	Unknown					45-50	Colon, pancreas, ovarian sex cord–stromal tumors
NF1	**Likely**	Unlikely	Unknown	**2.6 (2.1-3.2)**	2.3×10^{-13}			**26**	Malignant tumors of peripheral nerve sheath, brain, central nervous system
PALB2	**Likely**	Unknown	Unknown	**5.3 (9.0-9.4)**	4×10^{-10}	**7.46 (5.12-11.19)**	4.31×10^{-38}	**45**	Pancreas
ATM	**Likely**	Unknown	●	**2.8 (2.2-3.7)**	5×10^{-11}	**2.78 (2.22-3.62)**	2.42×10^{-19}	**27**	Pancreas
CHEK2	**Likely**	Unlikely	●	**3.0 (2.6-3.5)**	8×10^{-37}	**2.26 (1.89-2.72)**	1.75×10^{-20}	**29**	Lung, although p.Ile1577Thr is associated with reduced risk
NBN	**Likely**	Unlikely	Unknown	**2.7 (1.9-3.7)**	5×10^{-7}			**23**	Unknown
BARD1						**2.16 (1.31-3.63)**	2.26×10^{-3}		

Modified from Easton et al., N Engl J Med 2015; Couch et al., JAMA Oncology 2017

Germline mutations and breast cancer risks

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	Moderate (2-4 times)	High (>4 times)						%	
BRCA1	●	●	●	**11.4**				**75**	Ovary
BRCA2	●	●	●	**11.7**				**76**	Ovary, prostate, pancreas
TP53	●	●	●	**105 (62-165)**					Childhood sarcoma, adreno-cortical carcinoma, brain tumors
PTEN	Unknown	Unknown	●						Thyroid, endometrial cancer
CDH1	**Likely**	Unknown	Unknown	**6.6 (2.2-19.9)**	0.004			**53**	Diffuse gastric cancer
STK11	Unknown	Unknown	Unknown					45-50	Colon, pancreas, ovarian sex cord–stromal tumors
NF1	**Likely**	Unlikely	Unknown	**2.6 (2.1-3.2)**	$2.3x10^{-13}$			**26**	Malignant tumors of peripheral nerve sheath, brain, central nervous system
PALB2	**Likely**	Unknown	Unknown	**5.3 (9.0-9.4)**	$4x10^{-10}$	**7.46 (5.12-11.19)**	$4.31x10^{-38}$	**45**	Pancreas
ATM	**Likely**	Unknown	●	**2.8 (2.2-3.7)**	$5x10^{-11}$	**2.78 (2.22-3.62)**	$2.42x10^{-19}$	**27**	Pancreas
CHEK2	**Likely**	Unlikely	●	**3.0 (2.6-3.5)**	$8x10^{-37}$	**2.26 (1.89-2.72)**	$1.75x10^{-20}$	**29**	Lung, although p.Ile1577Thr is associated with reduced risk
NBN	**Likely**	Unlikely	Unknown	**2.7 (1.9-3.7)**	$5x10^{-7}$			**23**	Unknown
BARD1						**2.16 (1.31-3.63)**	$2.26x10^{-3}$		

Modified from Easton et al., N Engl J Med 2015; Couch et al., JAMA Oncology 2017

Germline mutations, breast cancer risks, and preventive strategies

GBCC2018

Gene	Magnitude of Relative Risk associated with Truncating Variants*		Risk associated with Missense Variants†	Estimated Relative Risk (90% CI)	Absolute risk by 80 years of Age	Other Associated Cancers	Prevention option for breast cancer in NCCN guidelines
	Moderate (2-4 times)	High (>4 times)			%		
BRCA1	●	●	●	11.4	75	Ovary	▪ 18y-, Breast awareness ▪ 25y-, Clinical Breast Exam ▪ 25-29y, annual Breast MRI ▪ 30-75y, Annual mammography, consider tomosynthesis and MRI ▪ Discuss about RRM; Recommend RRSO, 35-40y
BRCA2	●	●	●	11.7	76	Ovary, prostate, pancreas	
TP53	●	●	●	105 (62-165)		**Childhood sarcoma, adreno-cortical carcinoma, brain tumors**	▪ 20y-, Clinical Breast Exam ▪ 20-29y, Annual Breast MRI ▪ 30-75y, Annual Breast MRI + mammography, consider tomosynthesis ▪ **Discuss about RRM**
PTEN	Unknown	Unknown	●			follicular > papillary thyroid endometrial cancer, harmatoma syndrome	▪ 25y-, Clinical Breast Exam ▪ 30-35y ~ 75y or 5-10y before the earliest known BC family, annual mammography, consider tomosynthesis and breast MRI ▪ **Discuss about RRM**
CDH1	Likely	Unknown	Unknown	6.6 (2.2-19.9)	53	Diffuse gastric cancer	▪ 30y- Annual mammogram, consider breast MRI ▪ RRM: evidence insufficient, manage based on family history
PALB2	Likely	Unknown	Unknown	5.3 (9.0-9.4)	45	Pancreas	▪ 40y- Annual mammogram, consider tomosynthesis, breast MRI ▪ RRM: evidence insufficient, manage based on family history

Modified from Easton et al., N Engl J Med 2015; NCCN guideline version 1.2018

Germline mutations, breast cancer risks, and preventive strategies

GBCC2018

Gene	Magnitude of Relative Risk associated with Truncating Variants*		Risk associated with Missense Variants†	Estimated Relative Risk (90% CI)	Absolute risk by 80 years of Age	Other Associated Cancers	Prevention option for breast cancer in NCCN guidelines
	Moderate (2-4 times)	High (>4 times)			%		
BRCA1	●	●	●	11.4	75	Ovary	▪ 18y-, Breast awareness ▪ 25y-, Clinical Breast Exam ▪ 25-29y, annual Breast MRI ▪ 30-75y, Annual mammography, consider tomosynthesis and MRI ▪ Discuss about RRM; Recommend RRSO, 35-40y
BRCA2	●	●	●	11.7	76	Ovary, prostate, pancreas	
TP53	●	●	●	105 (62-165)		**Childhood sarcoma, adreno-cortical carcinoma, brain tumors**	▪ 20y-, Clinical Breast Exam ▪ 20-29y, Annual Breast MRI ▪ 30-75y, Annual Breast MRI + mammography, consider tomosynthesis ▪ **Discuss about RRM**
PTEN	Unknown	Unknown	●			follicular > papillary thyroid endometrial cancer, harmatoma syndrome	▪ 25y-, Clinical Breast Exam ▪ 30-35y ~ 75y or 5-10y before the earliest known BC family, annual mammography, consider tomosynthesis and breast MRI ▪ **Discuss about RRM**
CDH1	Likely	Unknown	Unknown	6.6 (2.2-19.9)	53	Diffuse gastric cancer	▪ 30y- Annual mammogram, consider breast MRI ▪ RRM: evidence insufficient, manage based on family history
PALB2	Likely	Unknown	Unknown	5.3 (9.0-9.4)	45	Pancreas	▪ 40y- Annual mammogram, consider tomosynthesis, breast MRI ▪ RRM: evidence insufficient, manage based on family history

Modified from Easton et al., N Engl J Med 2015; NCCN guideline version 1.2018

Multigene-Panel Sequencing for the Prediction of Breast-Cancer Risk

GBCC2018

		High Risk							Moderate Risk										Low Risk / Equivocal (for breast cancer)											
Company	**Test**	BRCA1	BRCA2	TP53	CDH1	PTEN	STK11	PALB2	ATM	BRIP1	CHEK2	BARD1	MRE11A	NBN	RAD50	RAD51C	RAD51D	XRCC2	MLH1	MSH2	MSH6	MUTYH	NF1	MEN1	PMS1	PMS2	POLD1	RECQL	EPCAM	FANCC
Ambry Genetics	BreastNext	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0					0	0							
Breast HealthUK	BreastGene	0	0	0	0	0	0	0	0	0	0			0																
Centogene	CentoBreast	0	0		0			0	0	0	0	0	0	0	0	0			0	0	0	0		0	0	0				
Eurofins	HighRiskBreast Cancer Panel	0	0	0	0	0	0	0																						
Fulgent Diagnostics	Breast Cancer Comprehensive Panel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0							
GeneDx	Breast/Gyn Cancer Panel	0	0	0	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0	0	0			0	0	0	0	0
Invitae	Invitae Breast Cancer Panel	0	0	0	0	0	0	0	0	0	0			0	0								0							
Myriad Genetics	myRisk	0	0	0	0	0	0	0	0		0	0		0																

How about Moderate- or Low- risk genetic mutations?

Modified from Easton et al., N Engl J Med 2015 (ver. MAR 2018)

Counselling for the carriers with germline mutations in moderate-risk cancer-susceptibility genes

GBCC2018

Germline mutations and breast cancer risks

GBCC2018

Gene	Magnitude of Relative Risk associated with Truncating Variants*		Risk associated with Missense Variants†	Estimated Relative Risk (90% CI)	P Value	Cancer risk OR (95% CI)	P value	Absolute risk by 80 years of Age	Other Associated Cancers
	Moderate (2-4 times)	High (>4 times)						%	
BRCA1	●	●	●	11.4				75	Ovary
BRCA2	●	●	●	11.7				76	Ovary, prostate, pancreas
TP53	●	●	●	105 (62-165)					Childhood sarcoma, adreno-cortical carcinoma, brain tumors
PTEN	Unknown	Unknown	●						Thyroid, endometrial cancer
CDH1	Likely	Unknown	Unknown	6.6 (2.2-19.9)	0.004			53	Diffuse gastric cancer
STK11	Unknown	Unknown	Unknown					45-50	Colon, pancreas, ovarian sex cord–stromal tumors
NF1	Likely	Unlikely	Unknown	2.6 (2.1-3.2)	2.3×10^{-13}			26	Malignant tumors of peripheral nerve sheath, brain, central nervous system
PALB2	Likely	Unknown	Unknown	5.3 (9.0-9.4)	4×10^{-10}	7.46 (5.12-11.19)	4.31×10^{-38}	45	Pancreas
ATM	Likely	Unknown	●	2.8 (2.2-3.7)	5×10^{-11}	2.78 (2.22-3.62)	2.42×10^{-19}	27	Pancreas
CHEK2	Likely	Unlikely	●	3.0 (2.6-3.5)	8×10^{-37}	2.26 (1.89-2.72)	1.75×10^{-20}	29	Lung, although p.Ile1577Thr is associated with reduced risk
NBN	Likely	Unlikely	Unknown	2.7 (1.9-3.7)	5×10^{-7}			23	Unknown
BARD1						2.16 (1.31-3.63)	2.26×10^{-3}		

Modified from Easton et al., N Engl J Med 2015; Couch et al., JAMA Oncology 2017

Germline mutations and breast cancer risks

GBCC2018

Gene	Magnitude of Relative Risk associated with Truncating Variants*		Risk associated with Missense Variants†	Estimated Relative Risk (90% CI)	P Value	Cancer risk OR (95% CI)	P value	Absolute risk by 80 years of Age	Other Associated Cancers
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BRCA2	●	●	●	11.7				76	Ovary, prostate, pancreas
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PTEN	Unknown	Unknown	●						Thyroid, endometrial cancer
CDH1	Likely	Unknown	Unknown	6.6 (2.2-19.9)	0.004			53	Diffuse gastric cancer
STK11	Unknown	Unknown	Unknown					45-50	Colon, pancreas, ovarian sex cord–stromal tumors
NF1	Likely	Unlikely	Unknown	2.6 (2.1-3.2)	$2.3x10^{-13}$			26	Malignant tumors of peripheral nerve sheath, brain, central nervous system
PALB2	Likely	Unknown	Unknown	5.3 (9.0-9.4)	$4x10^{-10}$	7.46 (5.12-11.19)	$4.31x10^{-38}$	45	Pancreas
ATM	Likely	Unknown	●	2.8 (2.2-3.7)	$5x10^{-11}$	2.78 (2.22-3.62)	$2.42x10^{-19}$	27	Pancreas
CHEK2	Likely	Unlikely	●	3.0 (2.6-3.5)	$8x10^{-37}$	2.26 (1.89-2.72)	$1.75x10^{-20}$	29	Lung, although p.Ile1577Thr is associated with reduced risk
NBN	Likely	Unlikely	Unknown	2.7 (1.9-3.7)	$5x10^{-7}$			23	Unknown
BARD1						2.16 (1.31-3.63)	$2.26x10^{-3}$		

Modified from Easton et al., N Engl J Med 2015; Couch et al., JAMA Oncology 2017

Germline mutations and breast cancer risks

GBCC2018

Gene	Magnitude of Relative Risk associated with Truncating Variants*		Risk associated with Missense Variants†	Estimated Relative Risk (90% CI)	P Value	Cancer risk OR (95% CI)	P value	Absolute risk by 80 years of Age	Other Associated Cancers
	Moderate (2-4 times)	High (>4 times)						%	
BRCA1	●	●	●	11.4				75	Ovary
BRCA2	●	●	●	11.7				76	Ovary, prostate, pancreas
TP53	●	●	●	105 (62-165)					Childhood sarcoma, adreno-cortical carcinoma, brain tumors
PTEN	Unknown	Unknown	●						Thyroid, endometrial cancer
CDH1	Likely	Unknown	Unknown	6.6 (2.2-19.9)	0.004			53	Diffuse gastric cancer
STK11	Unknown	Unknown	Unknown					45-50	Colon, pancreas, ovarian sex cord–stromal tumors
NF1	Likely	Unlikely	Unknown	2.6 (2.1-3.2)	$2.3x10^{-13}$			26	Malignant tumors of peripheral nerve sheath, brain, central nervous system
PALB2	Likely	Unknown	Unknown	5.3 (9.0-9.4)	$4x10^{-10}$	7.46 (5.12-11.19)	$4.31x10^{-38}$	45	Pancreas
ATM	Likely	Unknown	●	2.8 (2.2-3.7)	$5x10^{-11}$	2.78 (2.22-3.62)	$2.42x10^{-19}$	27	Pancreas
CHEK2	Likely	Unlikely	●	3.0 (2.6-3.5)	$8x10^{-37}$	2.26 (1.89-2.72)	$1.75x10^{-20}$	29	Lung, although p.Ile1577Thr is associated with reduced risk
NBN	Likely	Unlikely	Unknown	2.7 (1.9-3.7)	$5x10^{-7}$			23	Unknown
BARD1						2.16 (1.31-3.63)	$2.26x10^{-3}$		

Modified from Easton et al., N Engl J Med 2015; Couch et al., JAMA Oncology 2017

Odds ratio of pathogenic or likely pathogenic variants beyond BRCA

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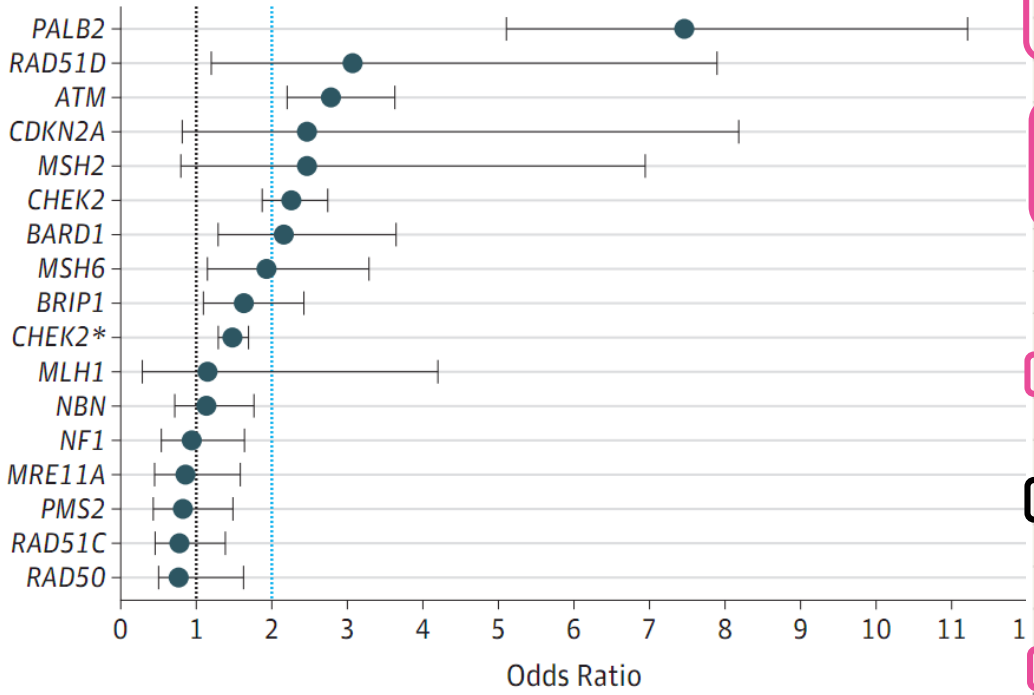
Figure. Odds Ratio Between Combined Pathogenic Variants in Each Gene and Breast Cancer Among White Women With Breast Cancer and Reference Controls



Gene	Ambr Muta Allele	Cancer Risk OR (95% CI)	*P* Value
ATM	274	2.78 (2.22-3.62)	2.42×10^{-19}
BARD1	52	2.16 (1.31-3.63)	2.26×10^{-3}
BRIP1	71	1.63 (1.11-2.41)	.01
CDKN2A	6	2.47 (0.83-8.16)	.11
CHEK2	424	2.26 (1.89-2.72)	1.75×10^{-20}
CHEK2 1100delC	338	2.31 (1.88-2.85)	3.04×10^{-17}
*CHEK2**	721	1.48 (1.31-1.67)	1.11×10^{-10}
MLH1	4	1.15 (0.30-4.19)	>.99
MRE11A	21	0.86 (0.46-1.57)	.65
MSH2	9	2.46 (0.81-6.93)	.11
MSH6	32	1.93 (1.16-3.27)	.01
NBN	48	1.13 (0.73-1.75)	.59
NF1	27	0.94 (0.55-1.62)	.89
PALB2	241	7.46 (5.12-11.19)	4.31×10^{-38}
PMS2	17	0.82 (0.44-1.47)	.56
RAD50	45	0.77 (0.52-1.61)	.23
RAD51C	26	0.78 (0.47-1.37)	.43
RAD51D	18	3.07 (1.21-7.88)	.01

Couch et al., JAMA Oncology 2017; Obeid et al., JAMA Oncol 2017 (editorial)

Moderate penetrance genes and preventive strategies for breast and ovarian cancers

GBCC2018

Gene	BC Risk OR (95% CI)*	Absolute risk by 80 years of age	Breast cancer risk management		Ovarian cancer risk management	Other cancer risk management
			Screening	RRM		
ATM	2.78 (2.22-3.62)	27	Annual mammogram, starting at **40y** (consider tomosynthesis, MRI)	Evidence insufficient	NO increased risk of OC	Unknown or insufficient evidence for pancreas or prostate cancer
BARD1	2.16 (1.31-3.63)		Unknown or insufficient evidence for BC risk		Unknown or insufficient evidence for OC risk	
BRIP1	1.63 (1.11-2.41)		NO increased risk of BC		**Consider RRSO at 40-50y**	N/A
CHEK2	2.26 (1.89-2.72)	29	Annual mammogram, starting at **40y** (consider tomosynthesis, MRI)	Evidence insufficient	NO increased risk of OC	Colon (no established preventive strategies)
NBN	1.13 (0.73-1.75)	23	Annual mammogram, starting at **40y** (consider tomosynthesis, MRI)	Evidence insufficient	Unknown or insufficient evidence for OC risk	Unknown or insufficient evidence
NF1	0.94 (0.55-1.62)	26	Annual mammogram, consider tomosynthesis starting at **30y** (consider MRI from 30-50y)	Evidence insufficient	NO increased risk of OC	Recommend referral to NF specialist for evaluation and management of MPNST, GIST, and others
RAD51C	0.78 (0.47-1.37)		Unknown or insufficient evidence for BC risk		**Consider RRSO at 45-50y**	N/A
RAD51D	3.07 (1.21-7.88)		Unknown or insufficient evidence for BC risk		**Consider RRSO at 45-50y**	N/A

**Risks are according to Cough et al., JAMA Oncol 2017*

Modified from Easton et al., N Engl J Med 2015; Couch et al., JAMA Oncol 2017; NCCN guideline version 1.2018

Moderate penetrance genes and preventive strategies for breast and ovarian cancers

GBCC2018

EDITORIAL

Multigene Panel Testing and Breast Cancer Risk
Is It Time to Scale Down?

Elias I. Obeid, MD, MPH; Michael J. Hall, MD, MS; Mary B. Daly, MD, PhD

- *However, the comprehensive genetic risk information provide **preventive strategies for the carriers** with moderate-risk genes.*
- *We are in immediate need of well-designed studies to provide **further clarification of risk estimates** for low- and moderate-risk genes, as well as expanded guidelines on **how to best manage these risks** over the lifetime of the patient.*

Obeid et al., JAMA Oncol 2017 (editorial)

Incidental findings
detected on multi-gene panels

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Multigene cancer panels and associated cancers

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GENES	BREAST	OVARIAN	COLORECTAL	UTERINE	MELANOMA	PANCREATIC	GASTRIC	PROSTATE	OTHERS
BRCA1	●	●				●		●	
BRCA2	●	●			●	●		●	
MLH1		●	●	●		●	●	●	●
MSH2		●	●	●		●	●	●	●
MSH6		●	●	●		●	●	●	●
PMS2		●	●	●		●	●	●	●
EPCAM		●	●	●		●	●	●	●
APC			●			●	●		●
MUTYH bialleric			●						●
MUTYH monoallelic			●						
CDKN2A (p16INK4a)					●	●			
CDKN2A (p14ARF)					●	●			
CDK4					●	●			
TP53	●	●	●	●	●	●	●	●	●
PTEN	●		●	●	●				●
STK11	●	●	●	●		●	●		●
CDH1	●		●				●		
BMPR1A			●			●	●		●
SMAD4			●			●	●		●
PALB2	●					●			
CHEK2	●		●						
ATM	●					●			
NBN	●							●	
BARD1	●								
BRIP1		●							
RAD51C		●							
RAD51D		●							
POLD1			●						
POLE			●						
GREMI			●						

Myriad laboratories, https://new.myriadpro.com/products/myriad-myrisk/myrisk-gene-table/ (visited in Mar 2018)

Recommendations for incidental findings

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ACMG POLICY STATEMENT | Genetics inMedicine | © American College of Medical Genetics and Genomics

ACMG STATEMENT | Genetics inMedicine

ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing

Phenotype	MIM-disorder	PMID-Gene Reviews entry	Typical age of onset	Gene	MIM-gene	Inheritance[a]	Variants to report[b]
Hereditary breast and ovarian cancer	604370 612555	20301425	Adult	*BRCA1* *BRCA2*	113705 600185	AD	KP and EP
Li–Fraumeni syndrome	151623	20301488	Child/adult	*TP53*	191170	AD	KP and EP
Peutz–Jeghers syndrome	175200	20301443	Child/adult	*STK11*	602216	AD	KP and EP
Lynch syndrome	120435	20301390	Adult	*MLH1* *MSH2* *MSH6* *PMS2*	120436 609309 600678 600259	AD	KP and EP
Familial adenomatous polyposis	175100	20301519	Child/adult	*APC*	611731	AD	KP and EP
MYH-associated polyposis; adenomas, multiple colorectal, *FAP* type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas	608456 132600	23035301	Adult	*MUTYH*	604933	AR[c]	KP and EP
Von Hippel–Lindau syndrome	193300	20301636	Child/adult	*VHL*	608537	AD	KP and EP
Multiple endocrine neoplasia type 1	131100	20301710	Child/adult	*MEN1*	613733	AD	KP and EP
Multiple endocrine neoplasia type 2	171400 162300	20301434	Child/adult	*RET*	164761	AD	KP
Familial medullary thyroid cancer[d]	1552401	20301434	Child/adult	*RET*	164761	AD	KP
PTEN hamartoma tumor syndrome	153480	20301661	Child/adult	*PTEN*	601728	AD	KP and EP
Retinoblastoma	180200	20301625	Child	*RB1*	614041	AD	KP and EP
Hereditary paraganglioma–pheochromocytoma syndrome	168000 (PGL1)	20301715	Child/adult	*SDHD*	602690	AD	KP and EP
	601650 (PGL2)			*SDHAF2*	613019		KP
Tuberous scler							
WT1-related W							
Neurofibroma							

Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics

Phenotype	MIM disorder	PMID Gene Reviews entry	Typical age of onset	Gene	MIM gene	Inheritance[a]	Variants to report[b]
Hereditary breast and ovarian cancer	604370 612555	20301425	Adult	*BRCA1* *BRCA2*	113705 600185	AD	KP and EP
Li-Fraumeni syndrome	151623	20301488	Child/adult	*TP53*	191170	AD	KP and EP
Peutz-Jeghers syndrome	175200	20301443	Child/adult	*STK11*	602216	AD	KP and EP
Lynch syndrome	120435	20301390	Adult	*MLH1* *MSH2* *MSH6* *PMS2*	120436 609309 600678 600259	AD	KP and EP
Familial adenomatous polyposis	175100	20301519	Child/adult	*APC*	611731	AD	KP and EP
MYH-associated polyposis; adenomas, multiple colorectal, *FAP* type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas	608456 132600	23035301	Adult	*MUTYH*	604933	AR[c]	KP and EP
Juvenile polyposis	174900	20301642	Child/adult	*BMPR1A* *SMAD4*	601299 600993	AD	KP and EP
Von Hippel–Lindau syndrome	193300	20301636	Child/adult	*VHL*	608537	AD	KP and EP
Multiple endocrine neoplasia type 1	131100	20301710	Child/adult	*MEN1*	613733	AD	KP and EP
Multiple endocrine neoplasia type 2	171400 162300	20301434	Child/adult	*RET*	164761	AD	KP
Familial medullary thyroid cancer[d]	1552401	20301434	Child/adult	*RET*	164761	AD	KP
PTEN hamartoma tumor syndrome	153480	20301661	Child/adult	*PTEN*	601728	AD	KP and EP
Retinoblastoma	180200	20301625	Child	*RB1*	614041	AD	KP and EP
Hereditary paraganglioma-pheochromocytoma syndrome	168000 (PGL1) 601650 (PGL2) 605373 (PGL3)	20301715	Child/adult	*SDHD* *SDHAF2* *SDHC*	602690 613019 602413	AD	KP and EP KP KP and EP

Genes with high risk for breast cancer	*BRCA1, BRCA2, TP53, STK11, PTEN*
Other genes to be recommended of report, even when they are incidental findings	*MLH1, MSH2, MSH6, PMS2, APC, MUTYH(AR), VHL, MEN1, RET, NTRK1, RB1, SDHAF2, SDHB, SDHC, SDHD, TSC1, TSC2, ST1, NF2, BMPR1A, SMAD4*

Green et al., Genet Med 2013; Kalia SS et al., Genet Med 2017

Application of NGS multiple gene panels in clinical practice

GBCC2018

JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility

- *Quality assurance in genetic testing*
- *Clinical implication of germline mutations*

Components of Informed Consent and Pretest Education in Clinical Cancer Genetics

- Discussion of specific genes may need to be batched, because it may not be feasible to review each gene individually; high-penetrance syndromes being evaluated should be described (eg, hereditary breast-ovary, Lynch, hereditary diffuse gastric, Li-Fraumeni); patients should be aware of possible detection of high-penetrance mutations not suggested by personal of family history; genes of uncertain clinical utility may need to be described more generally
- Particular attention should be paid to implications of positive results in less well-understood or lesser penetrance genes and in findings of mutations in genes associated with syndromes not suggested by personal or family history
- Attention should be paid to current high rate of variants of uncertain significance
- Highlight potential reproductive implications to family of mutations in genes linked to recessive disorders (eg, *ATM*, Fanconi's (*BRCA2*, *PALB2*), *NBN*, *BLM*)

Robson et al., J Clin Oncol 2015

Clinical application of multigene panels in Real Practice

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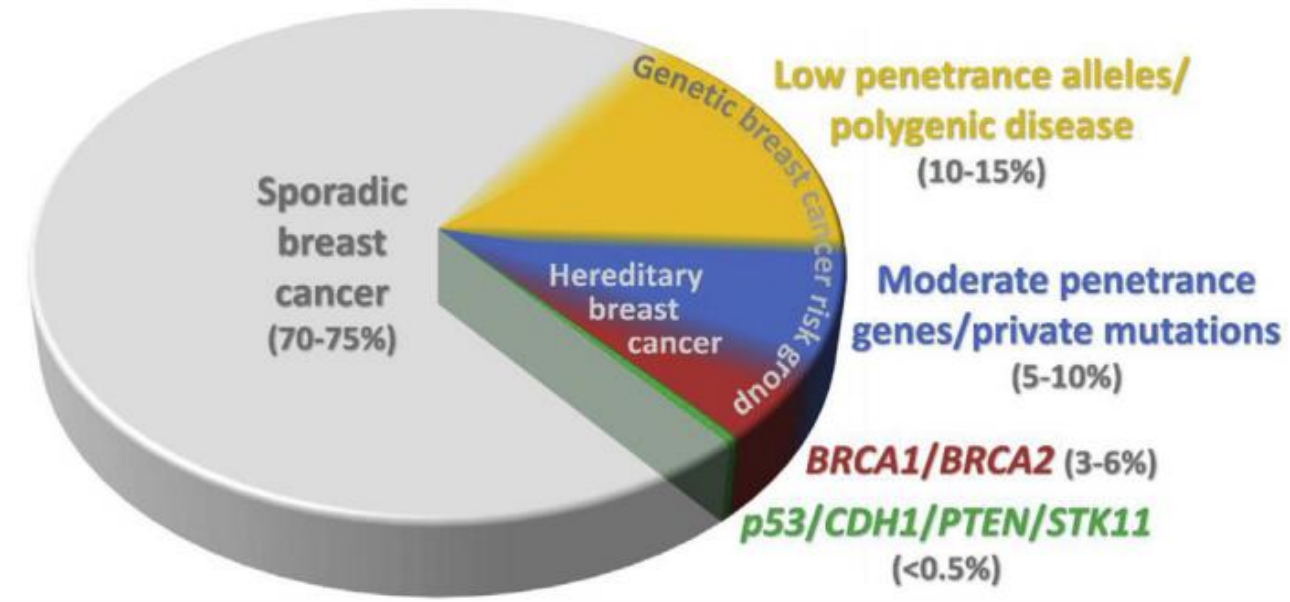
Distribution of breast cancer according to genetic risk



Kleibl et al., The Breast 2016

Frequency of pathogenic variants beyond *BRCA1/2* among patients without *BRCA1/2* mutation

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References	Study populations	N of genes included in the panel	Frequency among BRCA1/2 mut negative	Genes (number) with pathogenic variants
Castéra et al (2014)	BRCA1/2 mut positive + negative (N=708)	27	36/639 (5.6%)	ATM(5), BARD1(1), CDH1(1), CHEK2(5), MLH3(1), MRE11A(3), MSH2(3), NBS1(3), PALB2(7), PMS1(1), PMS2(2), RAD50(1), RAD51C(3)
Kurian et al (2014)	Negative for BRCA1/2 mutation (N=198)	42	16/198 (11.4%)	ATM(2), BLM(1), CDH1(1), CDKN2A(1), MLH1(1), MUTYH(5), NBN(2), PRSS1(1), SLX4(2)
Hirotsu et al (2015)	BRCA1/2 mut positive + negative (N=155)	25	9/144 (6.3%)	ATM(1), MSH6(4), MRE11A(1), MUTYH(4)
Tung et al (2015)	BRCA1/2 mut positive + negative (cohort 1, n=1781; cohort2, n=377)	25	14/377 (3.7%)	APC(1), ATM(1), BARD1(1), CDH1(2), CDKN2A(1), CHEK2(5), MUTYH(1), NBN(1), PALB2(1)
Tung et al (2016)	BRCA1/2 mut positive + negative (N=488)	25	25/458 (5.5%)	ATM(4), BRIP1(4), CHEK2(10), MSH6(1), NBN(1), PALB2(1), PMS2(1), PTEN(1), RAD51C(1), RAD51D(1)
Tedaldi et al (2017)	BRCA1/2 mut positive + negative (N=255)	94	17/198 (8.6%)	ATM(2), BRIP1(1), ERCC3(1), FANCI(1), FANCL(2), FANCM(1), MSH6(1), PALB2(6), PPM1D(1), RAD51D(1), RECQL4(1), SLX4(1), TSC2(1)
Couch et al (2017)	41,611 white women with breast cancer, negative for BRCA1/2 mutation	21	10.2%	ATM(274), BARD1(52), BRIP1(71), CDKN2A(6), CHEK2(424), MLH1(4), MRE11A(21), MSH2(6), MSH6(32), NBN(48), NF1(27), PALB2(241), PMS2(17), RAD50(45), RAD51C(26), RAD51D(18)

3.7 - 11.4%

Castera et al., Eur J Human Genet 2014; Kurian et al., J Clin Oncol 2014; Hirotsu et al., Mol Genet & Genomic Med 2015; Tung et al., Cancer 2015; Tung et al., J Clin Oncol 2016; Tedaldi et al., Oncotarget 2017; Couch et al., JAMA Oncology 2017

Our experiences

NGS multigene panel for the patients without *BRCA1/2* mutation

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High risk features

- *Family history:* At least one case of breast or ovarian cancer in the first- or second-degree relatives
- *Young age:* Diagnosis of first breast cancer before age 40
- *Laterality:* Bilateral breast cancer
- *Ovarian cancer:* Diagnosis with breast cancer and ovarian cancer in the same patient

BRCA1/2 mutation not detected by Sanger sequencing

NGS with 35-gene panel

Genes	Breast	Ovarian	Colorectal	Endometrial	Pancreatic	Gastric	Prostate	Other
BRCA1, BRCA2	O	O			O		O	
MLH1, MSH2, MSH6, PMS2, EPCAM		O	O	O	O	O		O
STK11	O	O	O	O	O	O		O
APC, BMPR1A, SMAD4			O		O	O		O
MUTYH			O					O
CDKN2A, CDK4					O			
TP53	O	O	O	O	O	O	O	O
PTEN	O		O	O				O
CDH1	O		O			O		
PALB2, ATM	O							
CHEK2	O		O		O		O	
NBN	O						O	
BARD1	O							
BRIP1, RAD51C, RAD51D, RAD50, SLX4	O	O						
BLM								O
MEN1, RET								O
MRE11A	O							
POLE			O					
PRSS1					O			
VHL, WT1								O

Our experiences

NGS multigene panel for the patients without BRCA1/2 mutation

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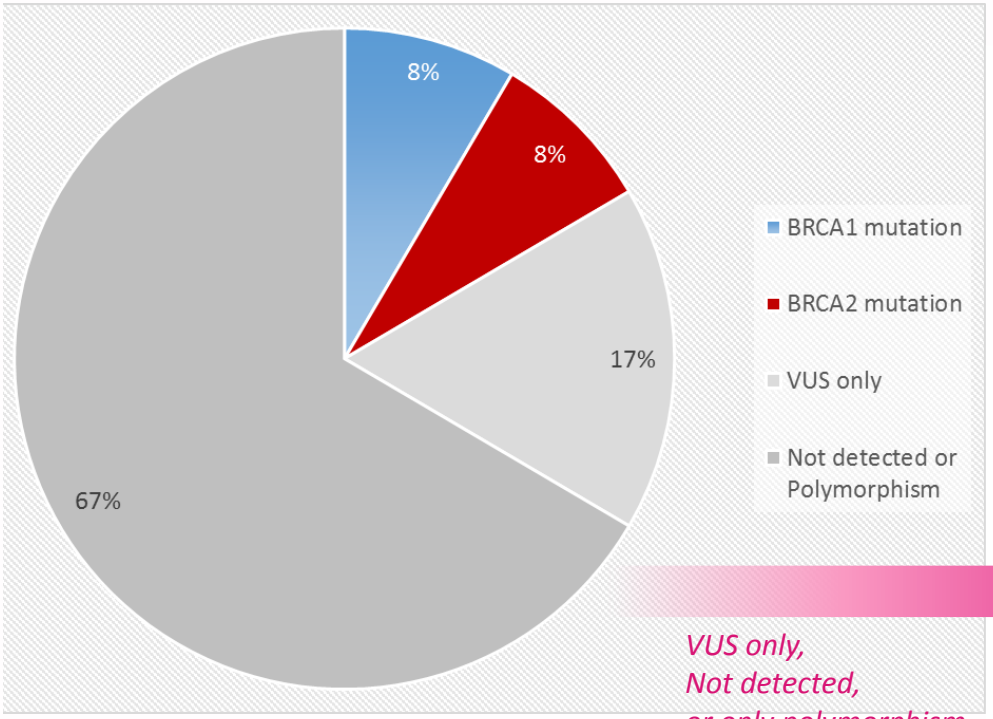
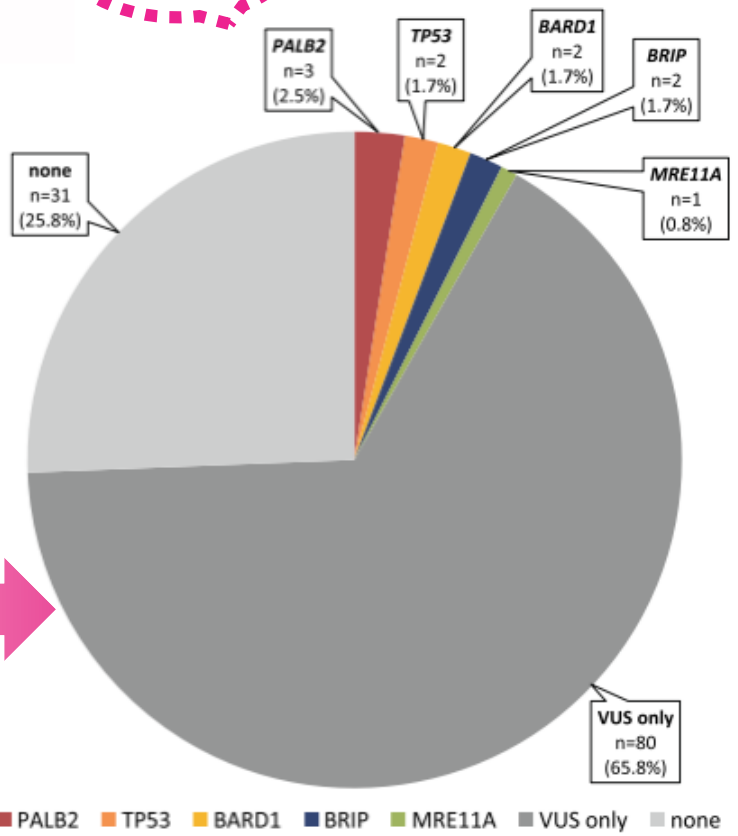
BRCA1/2 mutation test and result database in Yonsei Cancer Center (n=1510; 2009.1-2017.3)



VUS only,
Not detected,
or only polymorphism

7.5%

Pilot study (N=120)
4.2% with high-penetrance



Park JS, Park HS, Nam EJ et al., Clinical Breast Cancer 2018 (accepted);
Park JS, Lee ST, Nam EJ, Han JW, Lee JY, Kim J, Kim TI, and Park HS., BMC cancer 2018

Our experiences: characteristics of patients with pathogenic or likely pathogenic variants beyond *BRCA1/2*

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Case number	Site/ histology of breast cancer	Breast cancer subtype	Breast cancer stage (AJCC 7th ed)	Concomitant cancers	Affected gene	Nucleotide change	Amino acid change	dbSNP	Variant effect	Family cancer history (family member, age)	MAF by ExAC (n=60,704)	MAF by ExAC Asian (n=12,583)	MAF by KRGDB (n=622)	Confirmation method	Pathogenicity
1	L/IDC	ER+/PR+/HER2-	IIA	–	TP53	exon2-9 deletion	N/A	–	Large deletion	Breast ca (mother, 32)	N/A	N/A	N/A	MLPA	Pathogenic
2	B/IDC	ER+/PR+/HER2-	IIA	–	PALB2	c.3267_3268delGT	p.Phe1090SerfsTer6	rs587781890	Frameshift	Breast ca (aunt, 47), Colon ca (GF, 60), Stomach ca (GM, 60)	–**	–**	–**	Sanger sequencing	Likely pathogenic
3	R/IDC	ER+/PR+/HER2-	IIB	AoV	PALB2	c.2257C>T	p.Arg753Ter	rs180177110	Nonsense	Breast ca (sister, 53)	3.29×10^{-5}	–**	–**	Sanger sequencing	Pathogenic
4*	L/poorly differentiated	TNBC	IA	Stomach	PALB2	c.695delG	p.Gly232ValfsTer6	–	Frameshift	Stomach ca (GF, 90), Liver ca (uncle, 60)	–**	–**	–**	Sanger sequencing	Likely pathogenic
4*	L/poorly differentiated	TNBC	IA	Stomach	MRE11A	c.1773_1774delAA	p.Gly593LysfsTer4	–	Frameshift	Stomach ca (GF, 90), Liver ca (uncle, 60)	–**	–**	–**	Sanger sequencing	Likely pathogenic
5†	L/mucinous	TNBC	IA	–	BARD1	c.1345C>T	p.Gln449Ter	–	Nonsense	Breast ca (sister1, 67; sister2, 47)	–**	–**	–**	Sanger sequencing	Likely pathogenic
6†	L/IDC	ER+/PR-/HER2-	IIA	–	BARD1	c.1345C>T	p.Gln449Ter	–	Nonsense	Breast ca (sister1, 67; sister2, 58)	–**	–**	–**	Sanger sequencing	Likely pathogenic
7	L/IDC	ER-/PR-/HER2+	IA	–	BRIP1	exon5-6 deletion	N/A	–	Large deletion	Ovarian ca (mother, 35)	N/A	N/A	N/A	MLPA	Pathogenic
8	R/IDC	ER-/PR-/HER2+	IA	Cervix uteri	BRIP1	c.1066C>T	p.Arg356Ter	rs730881633	Nonsense	Breast ca (sister, 40)	–**	–**	–**	Sanger sequencing	Likely pathogenic
9	B/IDC	ER-/PR-/HER2+	IIA	–	TP53	c.733G>A	p.Gly245Ser	rs28934575	Missense	Stomach ca (father, 56); Pancreatic ca (father, 73)	8.24×10^{-6}	–**	–**	Sanger sequencing	Likely pathogenic

High penetrance genes: TP53(2), PALB2(3); 4.5%
Moderate penetrance genes: BARD1(2), BRIP1(2), MRE11A(1); 4.5%

Park JS, Lee ST, Nam EJ, Han JW, Lee JY, Kim J, Kim TI, and Park HS., BMC cancer 2018

Clinicopathological feature of the carriers with pathogenic or likely pathogenic variants beyond BRCA1/2

GBCC2018

Characteristics of patients with pathogenic or likely pathogenic variants beyond *BRCA1/2* (1)

GBCC2018

Table 6. Clinical and Pathologic Predictors of Germline Mutations in *BRCA1/2* and Other Breast Cancer Predisposition Genes*

Variable	No Mutation (n = 436) No.	%	*BRCA1/2* Mutation (n = 30)† No.	%	Other BC Mutation (n = 19)†* No.	%	P: No Mutation v *BRCA1/2* Mutation	P: No Mutation v Other BC Mutation
Patient characteristic								
Age at BC diagnosis, years							< .01	.72
Mean ± SD	50.7 ± 11.2		42.6 ± 9.7		51.6 ± 10.9			
Median	49		40		53			
Range	28-88		31-66		34-68			
≤ 45	150	34.4	22	73.3	7	36.8	< .01	.96
46-60	184	42.2	6	20.0	8	42.1		
> 60	102	23.4	2	6.7	4	21.1		
Ashkenazi Jewish heritage								
Yes	29	6.7	7	23.3	2	10.5	< .01	.51
No	407	93.3	23	76.7	17	89.5		
History of cancer‡								
Yes	37	8.5	1	3.3	3	15.8	.32	.27
No	399	91.5	29	96.7	16	84.2		
BC characteristic								
Subtype								
TNBC	72	16.5	12	40.0	2	10.5	.01	.11
HR-positive/HER2-negative	275	63.1	15	50.0	9	47.4		
HR-negative/HER2-positive	33	7.6	2	6.7	2	10.5		
HR-positive/HER2-positive	56	12.8	1	3.3	6	31.6		
Histology								
Ductal	325	74.5	22	73.3	10	52.6	.50	.08
Lobular	33	7.6	1	3.3	2	10.5		
Ductal and lobular	58	13.3	4	13.3	4	21.1		
Other	20	4.6	3	10.0	3	15.8		
Histologic grade§								
1	57	13.1	0	0.0	3	15.8	< .01	.94
2	167	38.4	4	13.3	7	36.8		
3	211	48.5	26	86.7	9	47.4		
Stage								
I	169	38.8	12	40.0	4	21.1	.03	.12
II	198	45.4	8	26.7	9	47.4		
III	69	15.8	10	33.3	6	31.6		
Bilateral disease								
Yes	8	1.8	0	0.0	1	5.3	.45	.29
No	428	98.2	30	100.0	18	94.7		
Family history of cancer and prior genetic testing								
First-degree relative with any cancer‡‖								
Yes	242	56.8	15	50.0	12	63.2	.47	.58
No	184	43.2	15	50.0	7	36.8		
First- or second-degree relative with any cancer‡‖								
Yes	356	83.6	30	100.0	15	78.9	.02	.60
No	70	16.4	0	0.0	4	21.1		
First- or second-degree relative with BC or ovarian cancer‡‖								
Yes	202	47.4	22	73.3	9	47.4	.01	1.0
No	224	52.6	8	26.7	10	52.6		
First- or second-degree relative < 50 years of age with BC, ovarian cancer, or male BC‡‖								
Yes	71	16.7	12	40.0	5	26.3	< .01	.27
No	355	83.3	18	60.0	14	73.7		

- *No significant difference in clinical and pathologic predictors between the patients with no mutation vs. other mutations beyond BRCA1/2*

Tung et al., J Clin Oncol 2016

Characteristics of patients with pathogenic or likely pathogenic variants beyond *BRCA1/2* (2)

GBCC2018

41,611 white or Ashkenazi Jewish women with breast cancer, negative for BRCA1/2 mutation (tested by Ambry genetics)

Phenotypic associations	Genes	OR (95% CI)
Bilateral breast cancer	*CHEK2*	1.35 (1.12-1.63)
	PALB2	1.51 (1.09-2.05)
	TP53	2.46 (1.26-4.65)
Personal history of ovarian cancer	*BRIP1*	5.22 (1.99-12.67)
	MSH2	18.44 (3.98-77.80)
Family history (1st- or 2nd-degree relatives) of breast cancer	*PALB2*	1.59 (1.15-2.19)
Family history of ovarian cancer	*BRIP1*	2.42 (1.41-4.13)
	RAD51C	2.89 (1.26-6.45)
	TP53	14.58 (3.02-103.47)
Younger age	*CHEK2*	47.7 years of age (vs 49.7)
	TP53	37.1 years of age (vs. 49.4)

Known high risk genes or odds ratio (OR) above 5 in the study; OR, 2-4.99 in the study; OR, 1-1.99 in the study

Couch et al., JAMA Oncology 2017

Characteristics of patients with pathogenic or likely pathogenic variants beyond *BRCA1/2* (3)

GBCC2018

Table 2 Association between the clinicopathological features of suspected hereditary breast cancer and the pathogenic or likely pathogenic variants of non-*BRCA* cancer predisposition genes ($n = 120$ patients)

Clinicopathological features		High-penetrance mutations		Moderate-penetrance mutations		None or VUS		
		Number ofpatients	%	Number ofpatients	%	Number ofpatients	%	*p*-value
Breast cancer site								
	Bilateral	2	18.2	0	0	9	81.8	0.106*
	Unilateral	3	2.8	4	3.7	102	93.5	
Breast cancer subtype ($n = 117$, excluding patients with unknown breast cancer subtypes)								
	TNBC	0	0	1	4.5	21	95.5	>0.99*
	hormone + and/or HER2+	4	4.2	3	3.2	88	92.6	
Concomitant diagnosis with ovarian cancer								
	Yes	0	0	0	0	3	100	>0.99*
	No	5	4.3	4	3.4	108	92.3	
Age at first diagnosis of breast cancer								
	< 35 years	4	21.1	0	0	15	78.9	0.003*
	≥ 35 years	1	1.0	4	4.0	96	95.0	
Family history of young (< 50 years old at diagnosis) breast and/or ovarian cancer patients within 2nd degree family								
	Yes	2	6.3	3	9.			
	No	3	3.4	1	1.			

Abbreviations: HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; VUS, variant of unl
Fisher's exact test

Young age (age at first diagnosis of breast cancer, < 35years)

Park JS, Lee ST, Nam EJ, Han JW, Lee JY, Kim J, Kim TI, and Park HS., BMC cancer 2018

Concerns about variants of unknown significance (VUS) in multigene panels

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Possible outcomes of genetic testing

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Deleterious True positive

Associated with a significantly increased cancer risk

VUS (variant of unknown significance)

a detected genetic change without a good description of any correlating clinical risk.

Suspected Deleterious

Available evidence strongly suggests association with significantly increased cancer risk

Favor Polymorphism

Evidence strongly indicates the variant is harmless and not associated with an increased cancer risk

True negative

i.e. an individual in a family with a k

Needs for Reclassification

Uninformative

i.e. a negative test in a family where a mutation has yet to be identified

https://www.myriad.com/; Shiovitz and Korde, Ann Oncol 2015

Reclassification of VUS

Multifactorial likelihood prediction models

GBCC2018

Indirect evidences

- Structural features of the gene or protein
- *In vitro* assays
- Occurrence of LOH in tumor DNA
- Conservation across species

Direct (genetic) evidences

- Frequency of the variant in cases and controls
- Co-segregation with the disease in families
- Co-occurrence with a deleterious mutation in the same gene
- Pathology profile, personal and family history of cancer of the carriers of the variant

Prior probability = *in silico* method using functional study, evolutionary conservation, …

Likelihood of pathogenicity = LR Co-occurrence × LR Pathology × LR Segregation × LR cancer history

Posterior Odds = Likelihood ratio × [prior probability/(1-prior probability)]

Posterior Probability of Pathogenicity = Posterior Odds / (Posterior Odds + 1)

Goldgar et al., Hum Mutat 2008; Lindor et al., Hum Mutat 2012

Reclassification of VUS

Evidence framework by ACMG guidelines

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	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affected statistically increased over controls PS4	
Computational and predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an Established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data			Missense in gene with low rate of benign missense variants and path. Missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data			Cosegregation with disease in multiple affected family members PP1	→	→	
***De novo* data**				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in trans with a dominant variant BP2 Observed in cis with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other Database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Others		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

S Richard, ACMG laboratory quality assurance committee, Genet Med 2015

Variant of Unknown Significance (VUS) of cancer susceptibility genes beyond *BRCA1/2*

GBCC2018

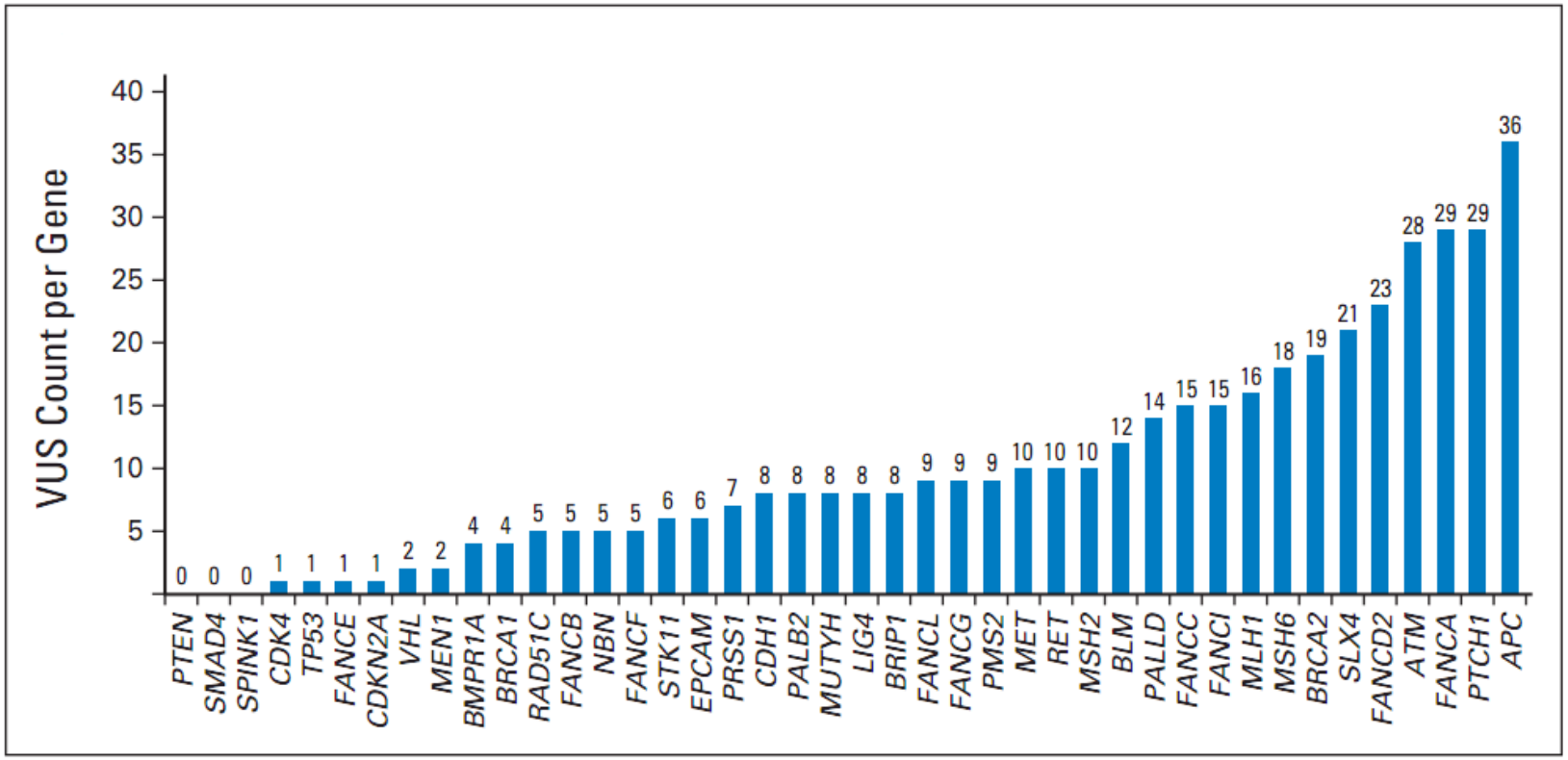

Fig 1. (B) Variants of uncertain significance (VUS) count, per gene, across 198 participants.

Kurian et al., J Clin Oncol 2014

Our experiences

VUS of cancer susceptibility genes beyond *BRCA1/2*

GBCC2018

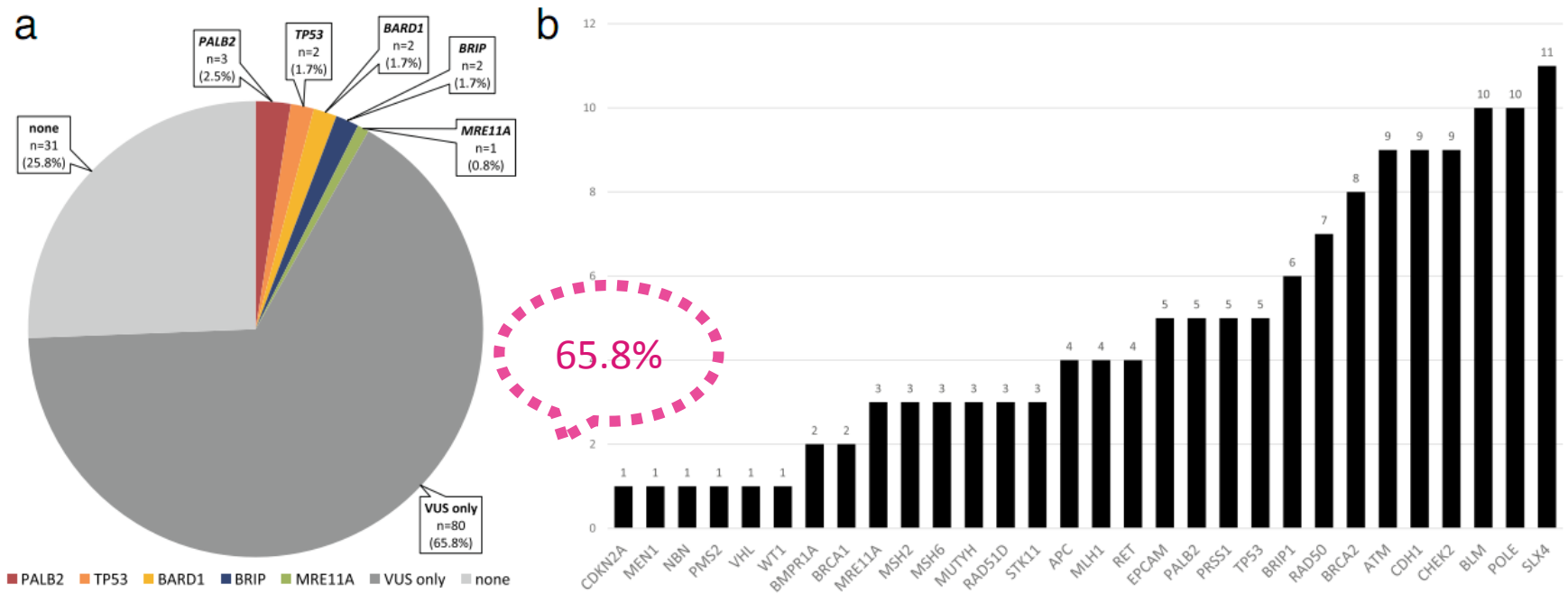

Fig. 1 **a** Percentage of patients with pathogenic or likely pathogenic mutations corresponding with each gene. **b** Number of patients with variants of uncertain significance (VUS) for each gene (*n* = 120 patients total)

Needs for clinical database and laboratory studies

Park JS, Lee ST, Nam EJ, Han JW, Lee JY, Kim J, Kim TI, and Park HS., BMC cancer 2018

Conclusions

GBCC2018

Despite many limitations,

- Considering advances in performance capacity, accuracy, and economic benefit, application of NGS panel will continue to expand.

There is a growing need to introduce:

- Guidelines for selection of candidates with high risk for hereditary cancer
- Interpretation of each genetic variants
- Reclassification of VUS
- Providing of psychosocial support
- Establishment of preventive strategies to the mutation carriers

We are trying to establish a well-organized population-based database, and conduct clinical trials to help to improve knowledge and quality of life of the carriers with germline mutations of cancer susceptibility genes.


Thank you for your attention!
GBCC2018