Current Practice for Selecting and Testing Ovarian Cancer Patients for *BRCA* Mutation

Byoung-Gie Kim, M.D., Ph.D.

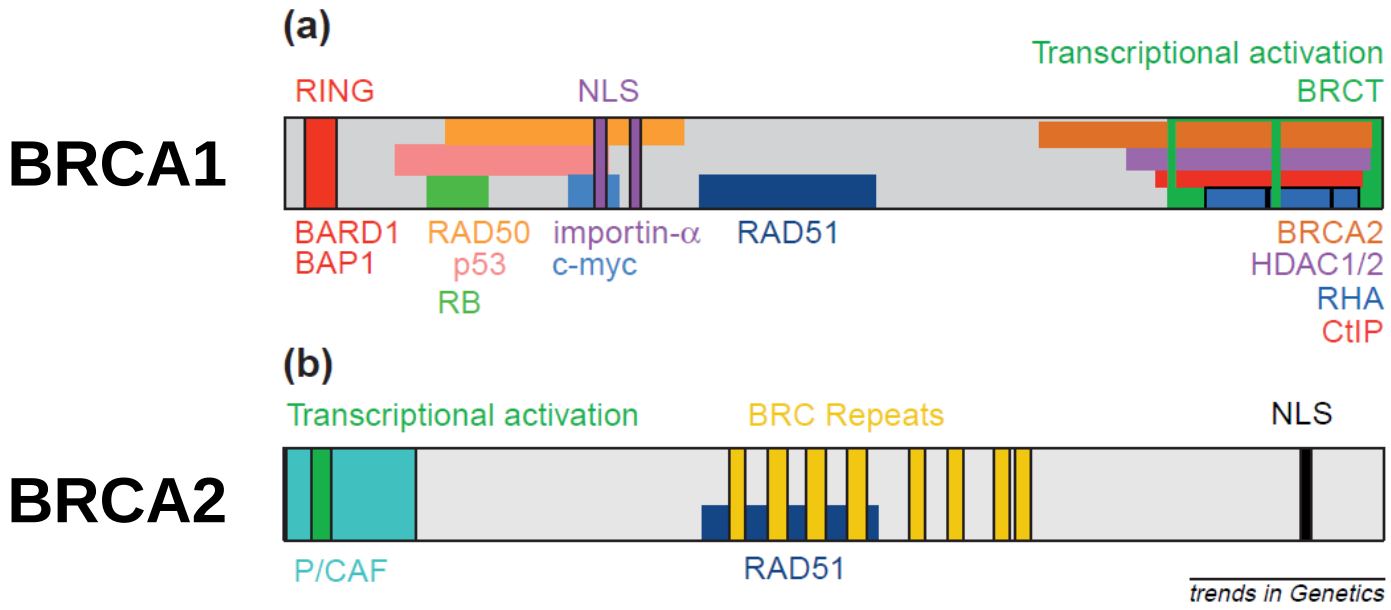
Department of Obstetrics and Gynecology, Samsung Medical Center,
Sungkyunkwan University School of Medicine, Seoul, Korea

Introduction

- Ovarian cancer is the leading cause of death among gynecological cancers.
- Germline mutations of BRCA1/2 are fundamental defects in hereditary ovarian cancer, accounting for about 10% of all cases.
- Recently, PARP inhibitor has been reported effective in patients with recurrent EOC and gBRCA mutation.
- Thus, appropriate personalized medicine for ovarian cancer now needs genetic information including BRCA status of the patients as well as their tumor characteristics.

BRCA1 and BRCA2

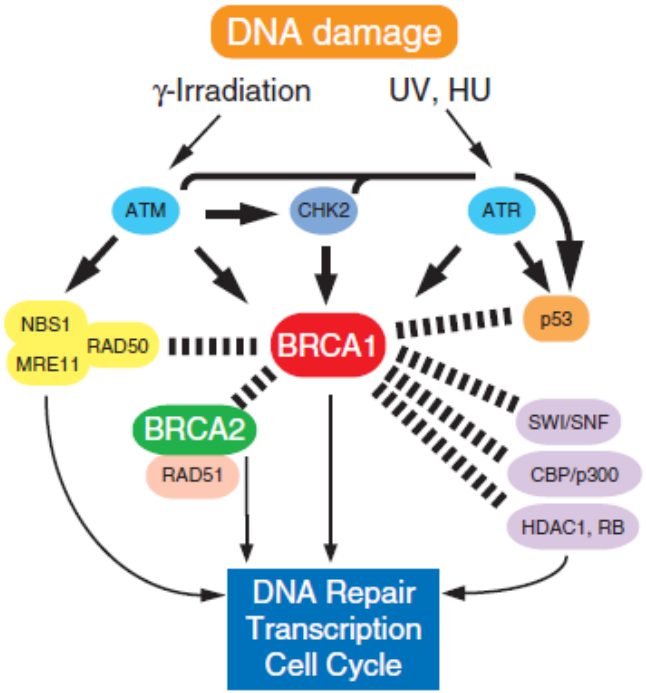
- BRCA1 (17q21) and BRCA2 (13q12-13) genes were first cloned in 1994 (Miki et al.) and 1995 (Wooster et al.) as breast cancer susceptibility genes

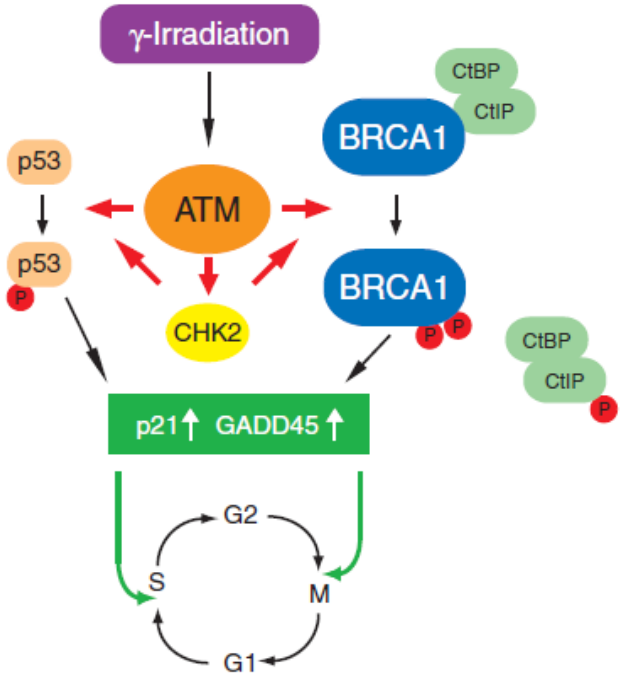


Venkitaraman AR, Cell, 2002; 108:171–182

Broad Functions of BRCA

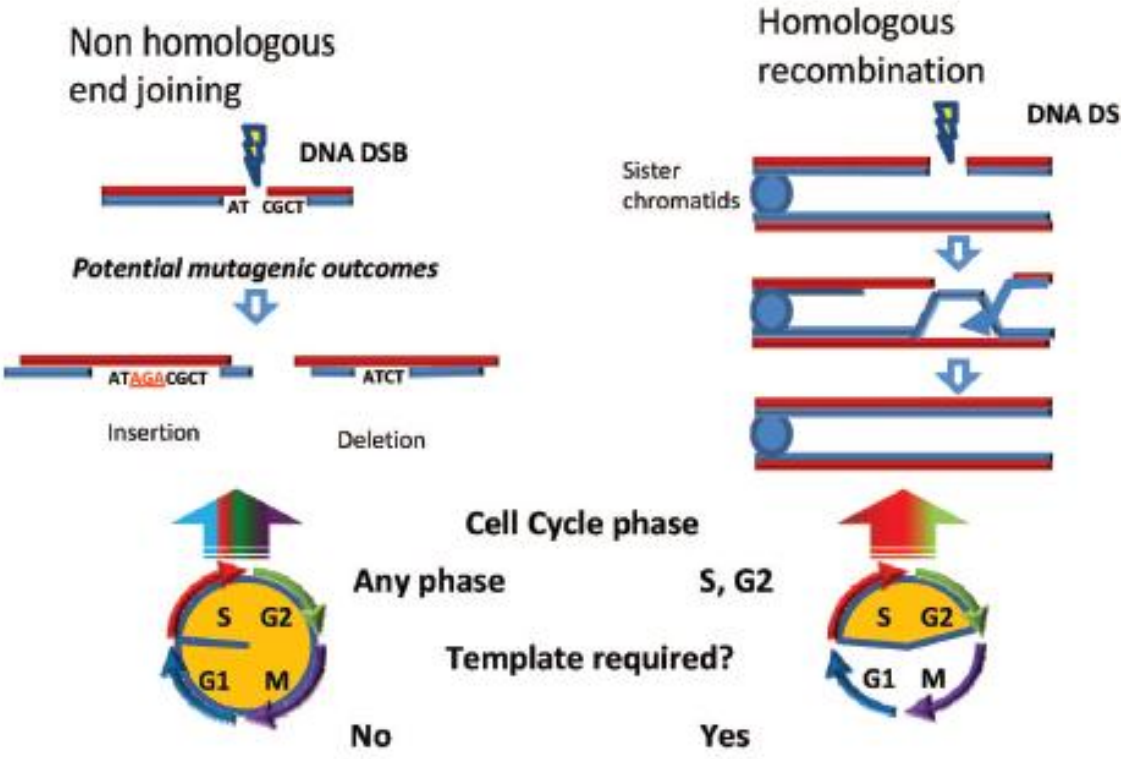
- **BRCA1/2 are involved in DNA repair, cell-cycle, checkpoint control, chromatin remodeling, transcription regulation and mitosis**





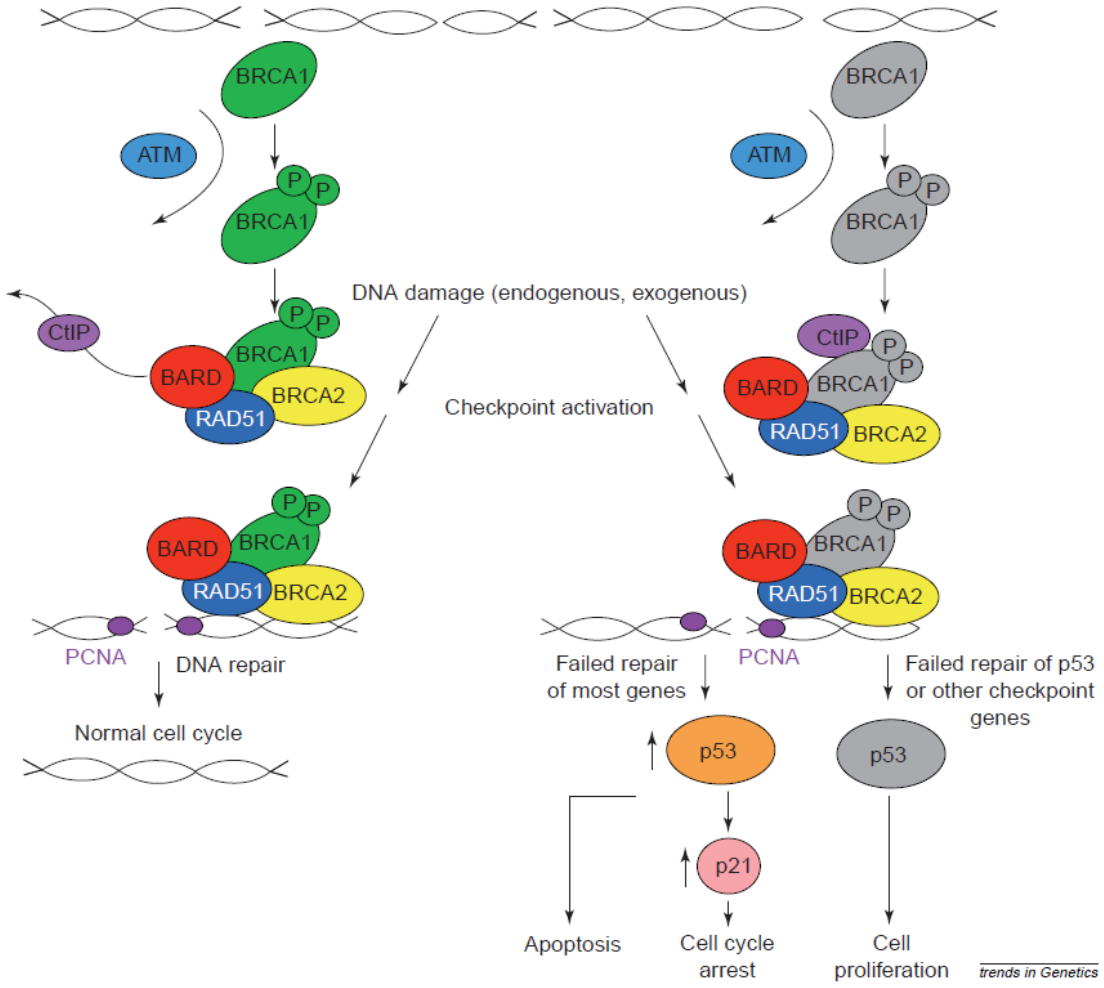
Yoshida K, Cancer Sci 2004; 95: 866–871

Pathways for DNA Double-Strand Break Repair



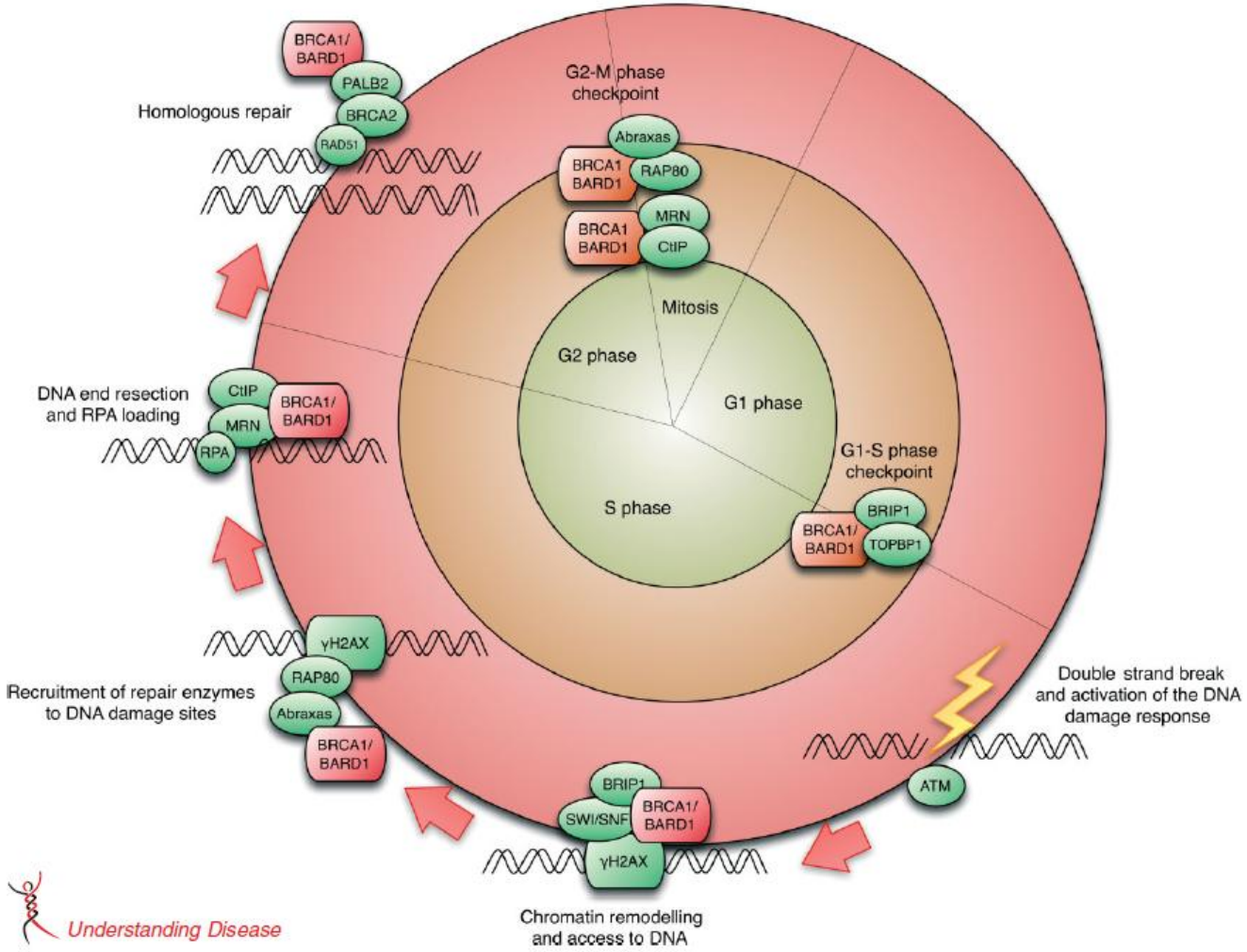
Murphy CG, Cancer J 2010;16: 39–47

BRCA and DNA Repair



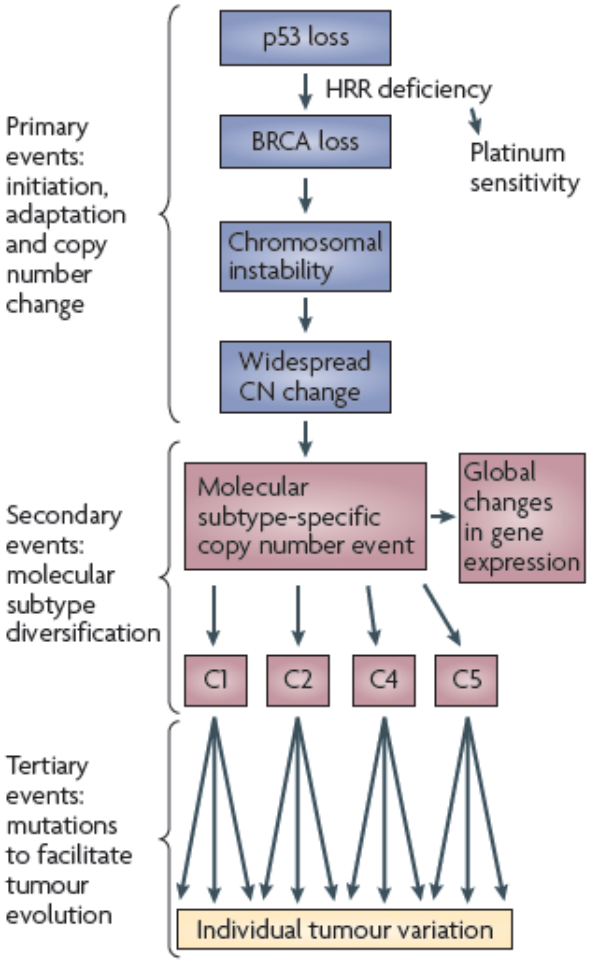
Welcsh PL, TIG 2000;16: 69-74

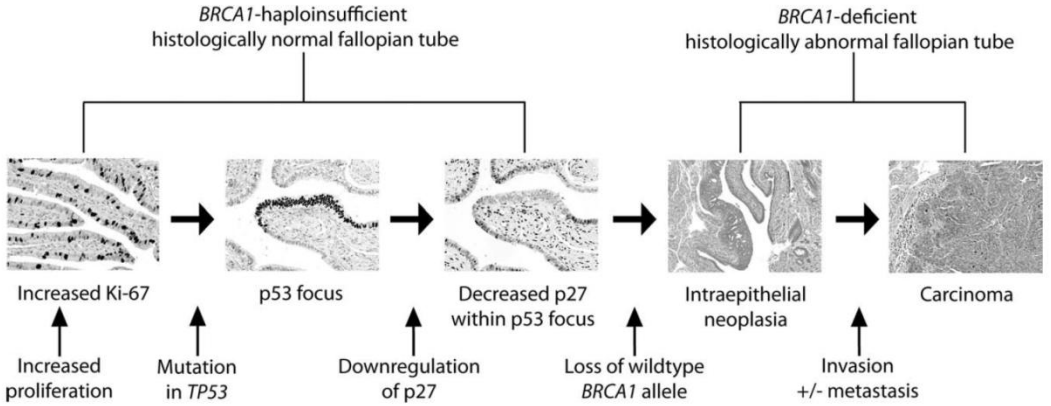
DNA Repair by HR and Cell Cycle



Foulkes WD, J Pathol 2013; 230: 347–349

BRCA Deficient Hereditary Ovarian Cancer

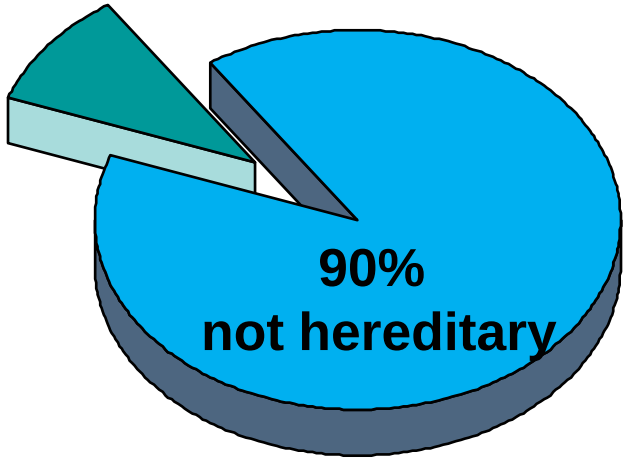




Bowtell DD, Nat Rev Cancer 2010; 10:803-808
Norquist BM, Cancer 2010;116:5261–71

Hereditary Ovarian Cancer

~5–10% ovarian cancers are hereditary

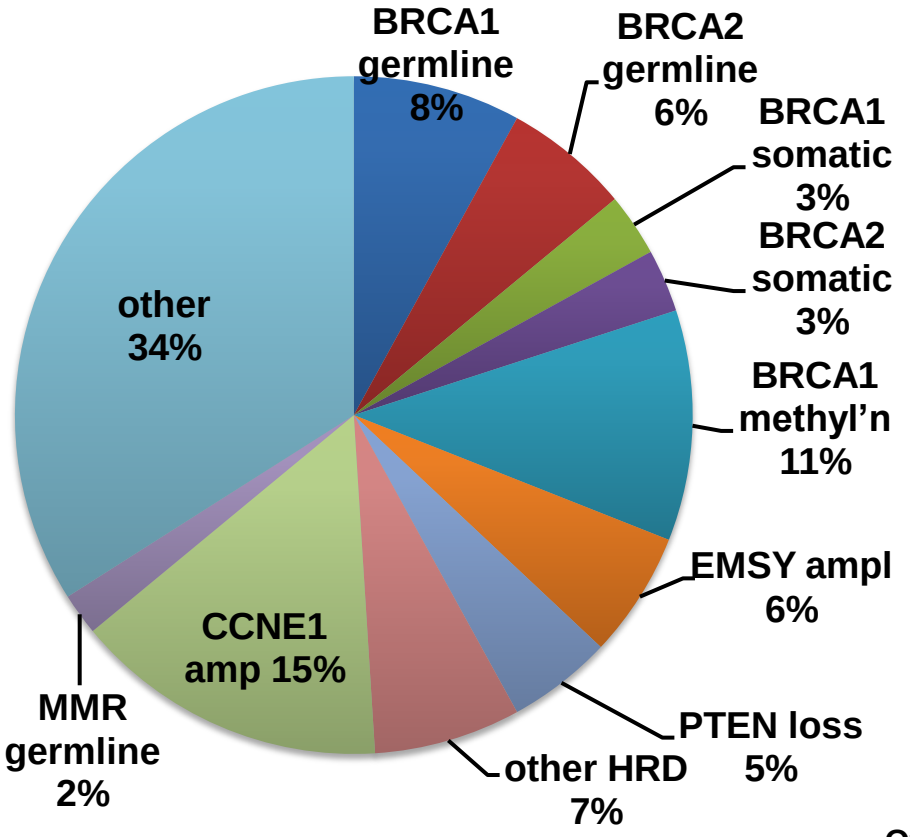


Gene	*BRCA1*	*BRCA2*	*MMR**
Associated lifetime risk	**34–45%**	**13–21%**	**5–25%**

*MMR (mismatch repair, associated with HNPCC)

Chen S, et al. J Clin Oncol 2007: 25:1329–33; Aarnio M, et al. Int J Cancer 1999: 81:214–8;
Garber JE and Offit K. J Clin Oncol 2005; 23:276–292

High Grade Serous Ovarian Cancer



Other HRD 7%: ***TP 53, RAD51C, PALB2, RAD50, MRE11A, BARD1, CHEK2, BRIP1, FANCD2, ATR, ATM***

TCGA, Nature 2011; Swisher et al, PNAS 2011 in press; Turner, et al NatRevCancer 2004; Weberpals, et al JCO 2008; Tan et al, JCO 2008; Mendes-Pereira et al, EMBO Mol. Med. 2009

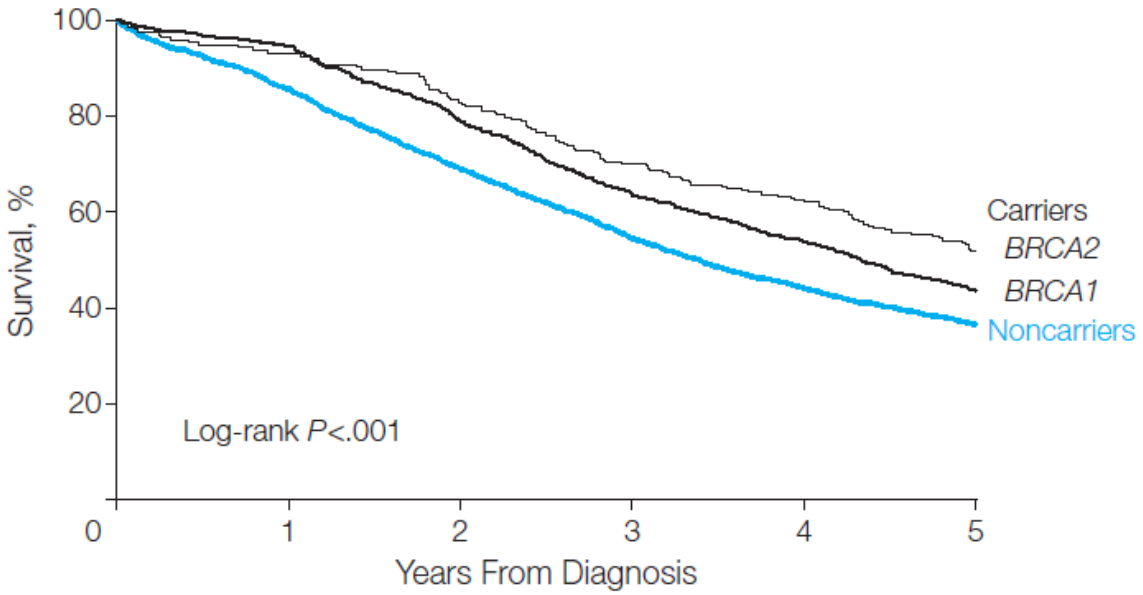
“BRCAness” in EOC

- Relatively uniform phenotype characteristics like tumors with BRCA mutation
  - High grade serous histology
  - High overall response rate to platinum-based chemotherapy
  - Long disease free interval
  - Improved survival rate
  - Higher incidence of visceral metastasis

- Recently, PARP inhibitor has been reported effective in patients with recurrent EOC and gBRCA mutation

Rigakos G, The Oncologist 2012;17:956-962

Survival of Patients with EOC According to BRCA1/2 Status

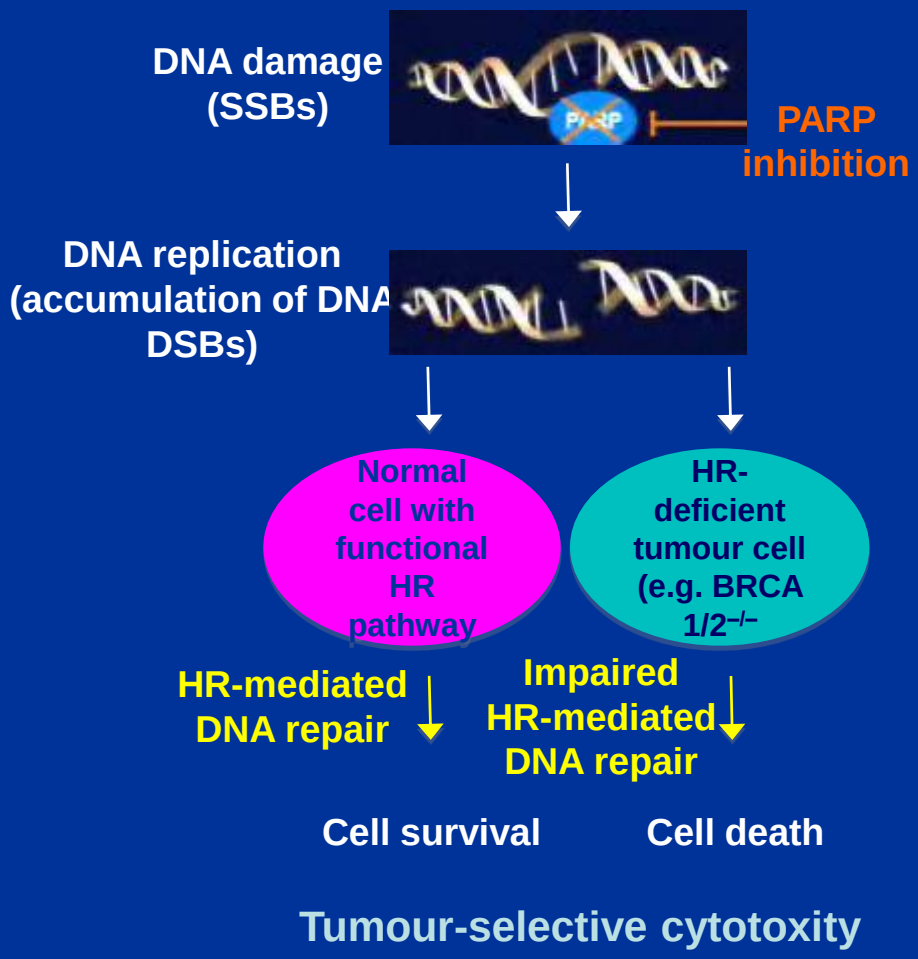


No. at risk						
Noncarriers	1047	1687	1540	1395	1225	1044
Carriers						
BRCA1	327	593	569	490	408	342
BRCA2	117	199	192	179	164	125

Bolton KL, JAMA. 2012;307(4):382-390

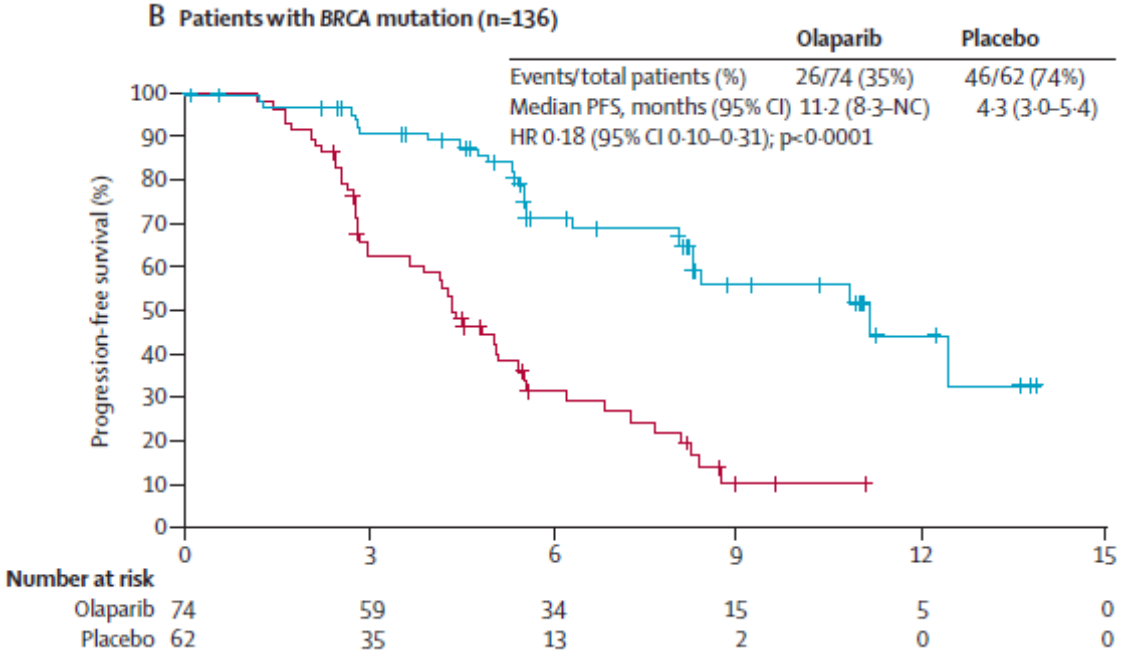
Poly(ADP-ribose) polymerase (PARP) inhibition

- In the absence of PARP activity, DNA single-strand breaks (SSBs) go unrepaired, degenerate and form double-strand breaks (DSBs)
- This accumulation of DSBs, in the absence of an alternative DNA repair mechanism, leads to cell death
- PARP inhibition selectively reduces viability and causes chromosome aberrations in BRCA-deficient tumours



Bryant HE, *et al. Nature* 2005;434:913–7; Farmer H, *et al. Nature* 2005;434:917–21

Progression-free survival in platinum sensitive recurrent patients according to BRCA mutation status after olaparib maintenance therapy



Ledermann J et al., Lancet Oncol 2014; 15: 852–61

Hereditary Breast and/or Ovarian Cancer Syndrome Testing Criteria

- Personal history of breast cancer +
  - Dx ≤ 45y
  - Dx ≤ 50y with (additional primary, 1 close relative breast cancer, unknown FHx)
  - Dx ≤ 60y with triple negative breast cancer
  - Dx with any age (≥1 close relative breast cancer Dx ≤ 50y, ≥2 close relative breast cancer at any age, ≥1 close relative EOC, ≥2 close relative pancreatic cancer and/or prostate cancer, a close male breast cancer, No FHx in higher mutation rate ethnicity)
- Personal history of EOC
- Personal history of male breast cancer
- Personal history of pancreatic cancer or prostate cancer with ≥2 close relative breast and/or ovarian and/or pancreatic or prostate cancer at any age
- Individual from a family with a known deleterious BRCA1/BRCA2 mutation
- FHx only
  - First-or second-degree relative with above criteria
  - Third degree relative with breast cancer and/or ovarian cancer with ≥2 close relative breast cancer (at least one with breast cancer ≤ 50y)

Reimbursement of BRCA Gene Testing in Korea

From 30 April 2012 (Health Insurance Review and Assessment, HIRA),

1. Personal history of breast cancer or ovarian cancer with ≥ one 1st degree or 2nd degree blood relative of breast cancer and/or ovarian cancer
2. Personal history of breast cancer and ovarian cancer
3. Personal history of breast cancer diagnosed ≤ 40y
4. Personal history of bilateral breast cancer
5. Personal history of breast cancer with multiple other primary cancer
6. Personal history of male breast cancer
7. Personal history of epithelial ovarian cancer

gBRCA Mutation in Sporadic EOC in Korea

- Patients:
  - 37 sporadic EOC (21 HGSO)
- Methods:
  - PCR-DHPLC-sequencing
- Results:
  - gBRCA1 mutation (3746insA) was found in one patient (1/37, 2.7%; among HGSO 4.7%, 1/21)
  - gBRCA2 mutation: not found
  - 32 polymorphisms were found without association with clinicopathological parameters

Kim YT, Gynecol Oncol 2005;585-590

gBRCA Mutation in EOC with and without Family History in Korea

- Patients:
  - 40 EOC patients with family history
    - Personal history of ovarian and breast cancer
    - Personal history of ovarian cancer with ≥ one 1st degree blood relative of breast cancer and/or ovarian cancer
  - 23 Patients without family history
- Methods:
  - Direct sequencing
- Results:
  - Patients with family history: gBRCA1 mutation (11/40, 27.5%), BRCA2 mutation (2/40, 5%)
  - Patients without family history: gBRCA1 mutation (2/23, 8.6%)

Lim MC, J Cancer Res Clin Oncol 2009;135:1593-1599

gBRCA Mutation in EOC with Family History

Table 1 Details of patients with one deleterious or unclassified mutation in the *BRCA1* or *BRCA2* gene and a strong family history

Patient	Age	Gene	Site	Mutation	Type	BIC	FH[a]	FH of other cancer
Deleterious mutations								
NCCKOV-00018	58	BRCA1	Exon 11	1041delAGCinsT	Nonsense	NR	Breast (patient)	No
NCCKOV-00035	41	BRCA1	Exon 2	157delATinsGGG	Frameshift	NR	Ovary (mother)	No
NCCKOV-00046	58	BRCA1	Exon 11	1041delAGCinsT	Nonsense	NR	Ovary (sister)	No
NCCKOV-00099	44	BRCA1	Exon 11	2552delC	Frameshift	11	Ovary (mother)	Thyroid (patient)
NCCKOV-00119	51	BRCA1	Exon 11	3746insA	Frameshift	6	Breast (patient, sister)	No
NCCKOV-00186	50	BRCA1	Intron 11	IVS5+1G>A	Splice	5	Breast (patient)	No
NCCKOV-00189	56	BRCA1	Exon 11	2166delA	Frameshift	NR	Breast (patient) Ovary (sister)	No
NCCKOV-00225	54	BRCA2	Exon 15	7708C>T	Nonsense	10	Breast (patient, mother)	No
NCCKOV-00279	71	BRCA1	Exon 11	3746insA	Frameshift	6	Ovary (sister)	Melanoma (Father)
NCCKOV-00287	65	BRCA2	Exon 11	3827delGT	Frameshift	NR	Ovary and Breast (sister)	No
NCCKOV-00295	58	BRCA1	Exon 11	3415delC	Frameshift	2	Breast (sister)	Pancreas (uncle)
NCCKOV-00337	67	BRCA1	Exon 11	2081insT	Frameshift	NR	Ovary (daughter)	No
Sequence variants of unknown significance								
NCCKOV-00030	59	BRCA1	Exon 17	5136delCAC	Missense	1	Ovary (mother)	No
NCCKOV-00047	54	BRCA2	Exon 11	E1812D	Missense	NR	Breast (patient)	Endometrium (aunt)
NCCKOV-00112	56	BRCA2	Exon 18	K2729N	Missense	22	Breast (mother)	No
NCCKOV-00147	71	BRCA2	Exon 11	H1003D	Missense	NR	Breast (sister)	No
NCCKOV-00286	39	BRCA2	Exon 27	I3412V	Missense	>100	Breast (patient)	No
NCCKOV-00330	48	BRCA2	Exon 18	K2729N	Missense	22	Breast (mother)	Stomach (sister) Leukemia (sister)
NCCKOV-00336	44	BRCA2	Exon 24	V3078F	Missense	NR	Breast (patient)	Thyroid (patient)

Age age at diagnosis (years), *BIC* breast cancer information core, *FH* familial history, *NR* not reported

[a] FH family history of breast cancer or ovarian cancer

Lim MC, J Cancer Res Clin Oncol 2009;135:1593-1599

gBRCA Mutation in EOC without Family History

Table 4 Details of patients with one deleterious or unclassified mutation in the *BRCA1* or *BRCA2* gene and without a strong family history

Patient	Age	Gene	Site	Mutation	Type	BIC	FH[a]	FH of other cancer
Deleterious mutations								
NCCKOV-00201	38	BRCA1	Exon 11	2081insT	Frameshift	NR	No	No
NCCKOV-00278	47	BRCA1	Exon 11	3276delG	Frameshift	NR	No	Endometrium (aunt)
Variants of unknown significance								
NCCKOV-00230	51	BRCA2	Exon11	I1929V	Missense	NR	No	Stomach (father)
NCCKOV-00271	50	BRCA2	Exon 27	I3412 V	Missense	>100	No	Colon (mother) Stomach (grandmother)
NCCKOV-00296	52	BRCA1	Exon 16	M1628T	Missense	94	No	No
NCCKOV-00297	58	BRCA2	Exon 27	I3412V	Missense	>100	No	No

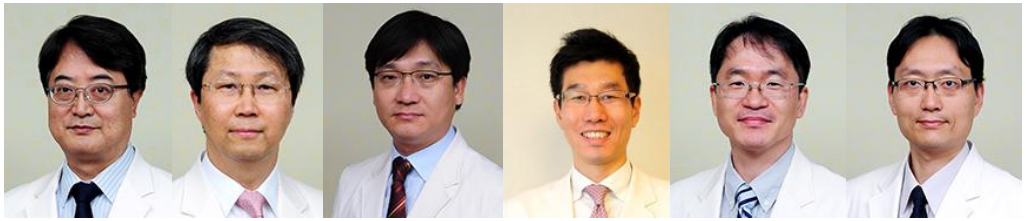
Age age at diagnosis (year), *BIC* breast cancer information core, *FH* family history, *NR* not reported

[a] FH family history of breast cancer or ovarian cancer

Lim MC, J Cancer Res Clin Oncol 2009;135:1593-1599

Cancer Genetics Program in Samsung Medical Center

Gynecologic Cancer Genetics Clinic



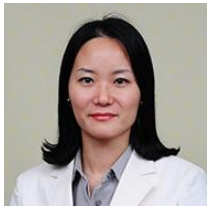
Screening, Risk assessment, Medical and physical examination, Disease F/U

Breast Cancer Genetics Clinic

Gastrointestinal Cancer Genetics Clinic

Cancer Genetics Center

Dr. Kim JW



Dr. Park SK

Medical, Familial Hx Review, Risk Assessment, Counseling, Gene Testing, Interpretation, Reporting, Registration, Education

BRCA1/2 Testing: Direct whole gene sequencing

gBRCA Mutation Testing in SMC Gyn. Cancer Genetics Clinic

- Patients with EOC and family history of ≥ 1 breast and/or ovarian cancer
- PCR-based direct sequencing of BRCA1/2
- 9/35 (25.7%) positive, 9/35(25.7%) equivocal

Patient	Age	Gene	Site	Mutation
S-107	41	BRCA1	Exon 10	c.2433delC(p.Lys812Argfs*3)
S-169	47	BRCA1	Exon 10	c.1823delA (p.Lys608Argfs*4)
S-174	51	BRCA1	Exon 13	c.4335_4338dupAGAA (p.Gln1447ArgfsX16)
S-184	54	BRCA2	Exon 15	c.7480C>T (p.Arg2494*)
S-200	56	BRCA2	Exon 15	c.7480C>T (p.Arg2494X)
S-231	58	BRCA1	Exon 10	c.3296delC(p.Pro1099Leufs*10)
S-286	48	BRCA1	IVS17	c.5074+1G>T
S-312	49	BRCA1	Exon 10	c.3627dupA (p.Glu1210Argfs*9)
S-322	47	BRCA1	Exon 10	c.922_924delinsT (p.Ser308*)

From SMC Cancer Genetics Clinic, Aug. 2014

gBRCA Mutation with Family History in Korea

- Patients:
  - 134 patients with breast cancer and/or ovarian cancer
    - family history of ≥ 1 breast and/or ovarian cancer
    - Breast cancer diagnosed ≤ 35y
    - Bilateral breast cancer
    - Multiple organ cancer
- Methods:
  - PCR-Direct whole gene sequencing
- Results:
  - gBRCA1/2 mutation (31/134, 23.1%),
  - gBRCA1 sequence variation(13/134, 9.7%)

Jang JH, J Human Genetics 2012;57:212-215

gBRCA Mutation with Family History in Korea

Table 1 ***BRCA1*** and ***BRCA2*** mutations in Korean patients with breast cancer

Exon/intron	*Nucleotide change*[a]	*BIC nomenclature*	*Amino-acid change*[a]	*Citation*	*Risk factors*
BRCA1					
IVS6	c.301-2A>C	420-2A>C	—	BIC	M (1)
7	c.390C>A	509 C>A	p.Y130X	BIC	F (1)
11	c.922_924delAGCinsT	1041_10423delAGCinsT	p.S308X	[b]	FM (1)
11	**c.1511dupA**	**1610dupG**	**p.K505X**	**Novel**	**MF (1)**
11	**c.1936delA**	**2055delA**	**p.S646AfsX5**	**Novel**	**F (1)**
11	c.3627dupA	3746dupA	p.E1210RfsX9	BIC	FM (1)/EF (1)
11	**c.3814dupT**	**3933dupT**	**p.N1272X**	**Novel**	**FB (1)**
IVS18	c.5152+1G>C	5271+1G>C	—	BIC	EF (1)
IVS21	**c.5278-2A>T**	**5397-2A>T**	**—**	**Novel**	**F (1)**
23	c.5445G>A	5564G>A	p.W1815X	BIC	F (2)
24	c.5496_5506del11insA	5615_5625del11insA	p.V1833SfsX7	[b]	F(1)/EF(1)
BRCA2					
3	c.97G>T	325G>T	p.E33X	[c,d]	EF (1)/FB (1)
3	**c.196C>T**	**424C>T**	**p.Q66X**	**Novel**	**F (1)**
10	c.1310_1313delAAGA	1538_1541delAAGA	p.K437IfsX22	BIC	EF (1)
10	**c.1514delT**	**1742delT**	**p.I505NfsX4**	**Novel**	**F (1)**
11	**c.3018delA**	**3246delA**	**p.G1007VfsX36**	**Novel**	**EF (1)**
11	c.3744_3747delTGAG	3792_3975delTGAG	p.S1248RfsX10	BIC	BF (1)
11	c.4766delC	4994delC	p.P1589QfsX28	[b]	BEF (1)
11	**c.5116_5119delAATA**	**5344_5347delAATA**	**p.N1706LfsX5**	**Novel**	**FM (1)**
11	**c.5574_5577delAATT**	**5802_5805delAATT**	**p.I1859KfsX3**	**Novel**	**F (1)**
11	**c.6723_6724delAG**	**6951_6952delAG**	**p.D2242FfsX2**	**Novel**	**BE (1)**
13	c.6952C>T	7180C>T	p.R2318X	BIC	F (1)/EF (1)
14	**c.7258G>T**	**7486G>T**	**p.E2420X**	**Novel**	**F (1)**
15	c.7480C>T	7708G>T	p.R2494X	BIC	F (1)
IVS20	**c.8633-2A>T**	**8861-2A>T**	**—**	**Novel**	**EF (1)**
23	c.9117G>A	9345G>A	Aberrant splicing	[e]	F (1)

Jang JH, J Human Genetics 2012;57:212-215

Methods for gBRCA Mutation Screening

Technique	Principles for mutations detection	Advantages	Disadvantages
SSCA	Altered electrophoretic mobility of single stranded DNA (non-denaturing gels)	Rapid and easy to carry out	Low detection rate (70-80%) Scans short fragments
HAD	Altered electrophoretic mobility of heteroduplex (non-denaturing gels)	Rapid and easy to carry out Detects insertion/deletion mutations in large fragments	Low detection rate (80%) Poor sensitivity for point mutations
DGGE	Altered electrophoretic mobility of heteroduplex based on their melting behaviour (denaturing gradient gels)	Rapid and easy after the initial laborious planning	Low detection rate unless well established conditions (95%) Effort required to set up the technique
CCM/FAMA	Detection of heteroduplex through chemical cleavage at the site of DNA mismatch	Good sensitivity (>95%) Scans large fragments Provides an approximate location of DNA alterations	Time consuming and labour intensive Hazardous chemicals are required
PTT	Detection of pre-terminal in vitro synthetized protein products	Good sensitivity in identifying pathogenetic mutations in large fragments (98%) Provides their approximate location	Only detects sequence alterations responsible of truncated proteins Unsuitable to analyze small exons using genomic DNA as template
DHPLC	Detection of heteroduplex through their chromatographic elution profile	High sensitivity (≤100%) Extremely rapid and easy after initial setting up Provides an almost precise location of alterations	Efforts required to establish the technique Initial investment in equipment (but low cost for single analysis)
DS	Direct sequencing of DNA fragments	Best sensitivity (~100%) Defines the exactly location and the nature of alterations	Still labour intensive despite automated steps Expensive if few mutations are expected

Palma M, Crit Rev Oncol Hematol 2006; 57: 1-23

gBRCA1/2 Mutation in Patient with High Grade Serous Ovarian Cancer in Korea

- **Patients**
  - **Screening for SOLO-1 trial**
  - **Random selection but several known mutation carriers included**

- **gBRCA1 mutation: 26/96 (27.0%)**
  - **3746insA (4)**
  - **3415delC (3)**
  - **1041del3insT (3)**

- **gBRCA2 mutation: 7/96 (7.2%)**

- **gBRCA1/2 mutation variant of uncertain significance: 9/96 (9.3%)**

Courtesy of Myriad

Prevalence of gBRCA1/2 Mutation in Patient with EOC in Korea

Author	No. of pts	Inclusion criteria	Method	gBRCA1 mutation	gBRCA2 mutation	Variants found
Kim YT (2005)	37	Sporadic	PCR-DHPLC-Seq	1 (2.7%)	None	32 polymorphism
Kim TJ (2005)	13	Familial Hx	PCR-SSCP-Seq	None	Not done	3
Lim MC (2009)	40	Familial Hx	PCR-Direct Seq	11/40 (27.5%)	3/40(7.5%)	7
	23	Sporadic	PCR-Direct Seq	2/23 (8.6%)	None	4
SOLO-1* (2014)	96	Familial Hx and sporadic HGSO	Myriad Integrated BRC analysis	26/96 (27.0%)	7/96 (7.2%)	9
SMC GCCC (2014)*	35	Familial Hx	PCR-Direct Seq	7/35 (20.0%)	2/35 (5.7%)	9

Kim YT, Gynecol Oncol 2005;585-590, Kim TJ, Oncol Rep 2006; 15:565-569, Lim MC, J Cancer Res Clin Oncol 2009;135:1593-1599
* Not published

Challenges for BRCA Gene Testing

- Selection of the patient
  - Family Hx focused gene testing
  - High-risk feature targeted gene testing
  - Routine gene testing
- Sensitivity of the test
- Cost and time frame
- Interpretation (uncertain significance variants)

Summary

- BRCA1/2 play a central role in HR dsDNA repair pathway and BRCA-defective cells are lethal to dsDNA damaging agents.

- BRCA1/2 mutations are the most common defect in hereditary ovarian cancer and frequently observed in high-grade serous ovarian cancer (**14% germline and 6% somatic in TCGA data**).

- Prevalence of gBRCA1/2 mutation in Korean patients with epithelial ovarian cancer is estimated as **about 25-33% with family history and about 10% without family history**. Rate of BRCA mutation detection was varying according to the screening method and much higher rate of gBRCA mutation was found in recent SOLO-1 study.

Summary

- With modern technology, rapid BRCA mutation screens are moving from highly specialized cancer clinics to the mainstream setting.

- A family history of cancer is an influential factor for performing a genetic test, but BRCA gene test should also be considered **in the presence of high-risk feature such as high-grade serous ovarian cancer phenotype.**

- Knowledge of BRCA status may influence the management of the patient, including decisions about specific targeted therapy like PARP inhibitor.

Thank you for your attention!

bgkim@skku.edu