# Hereditary Ovarian Cancer and Germline Mutations: Review Article

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## ABSTRACT

*Ovarian cancer is the most lethal gynecologic malignancy, nearly 80% of patients are diagnosed at advanced stage disease (III/IV). Different risk factors are associated with ovarian cancer Obesity, High fat diet, Hormone replacement therapy, and most importantly a family history of breast/ovarian cancer and/or colon cancer. Close relative with ovarian cancer increases a woman's risk of developing ovarian cancer by ~3 times. Women with family history of ovarian cancer should consider regular clinical exams. Moreover, Women who carry BRCA1 or BRCA2 mutation are at a very high risk and surgical removal of the ovaries and fallopian tubes are recommended. Different types of mutations were found either BRCA1 or BRCA2ranging in size from 1bp (point mutation and/or frameshift mutations) up to hundreds or thousands of base pairs, such as Large genomic rearrangements (LRGs). Therefore, Identifying women with higher risk is essential for surveillance, surgical removal of the ovaries and for earlier diagnosis.*

**Keywords:** *Ovarian Cancer; BRCA1; BRCA2; LRGs*

## INTRODUCTION

Ovarian cancer is the most common fetal gynecologic malignancy worldwide. Yearly, over 140,000 cases are diagnosed worldwide, representing over 4% of all cancer cases in women. In U.S. alone, 24,500 cases each year are diagnosed with more than 14,300 deaths (Fig.1). The high rate of mortality is due to the late stage, at which ovarian cancer is diagnosed. Overall, the probability that a woman will be diagnosed with ovarian cancer in her lifetime is 1 in 70. Multiple risk factors are associated with ovarian cancer, and these include age, race, infertility and most importantly family history.
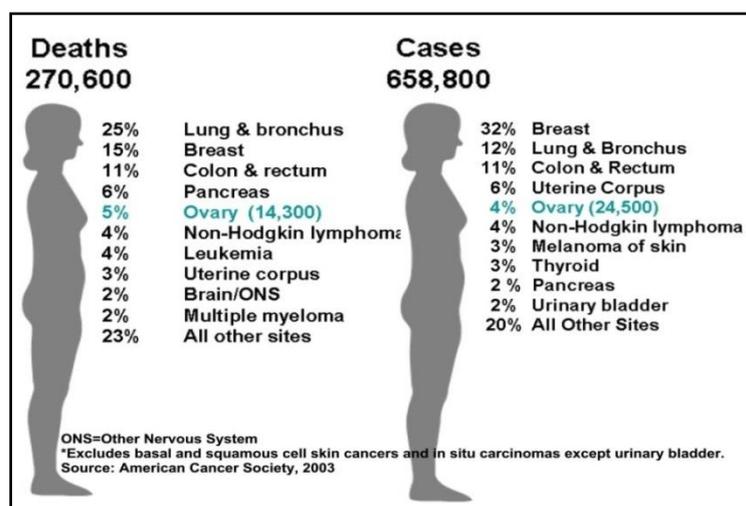


**Figure1.** *The epidemiology of ovarian cancer: Ovarian cancer is the fifth cancer type in terms of both incidence and mortality.*

In general, ovarian tumors are classified pathologically into three categories, i.e., epithelial carcinoma (derived from the cells on the surface of the ovary) which accounts for 90% of ovarian neoplasms, germ cell tumors (derived from the egg producing cells within the body of the ovary) which accounts for <10% of ovarian cancer and the sex cord stromal tumors, which are very rare.

Cancers of the breast and ovary seemingly share many etiological factors. For instance, women with breast cancer have doubled risk of ovarian cancer, and women with ovarian cancer have 3-4 fold increase in the risk of subsequent breast cancer (Bergfeldt et al., 2002). Close relative of ovarian cancer will increase the women's risk almost 3-4 fold times compared to general population (Fig. 2).
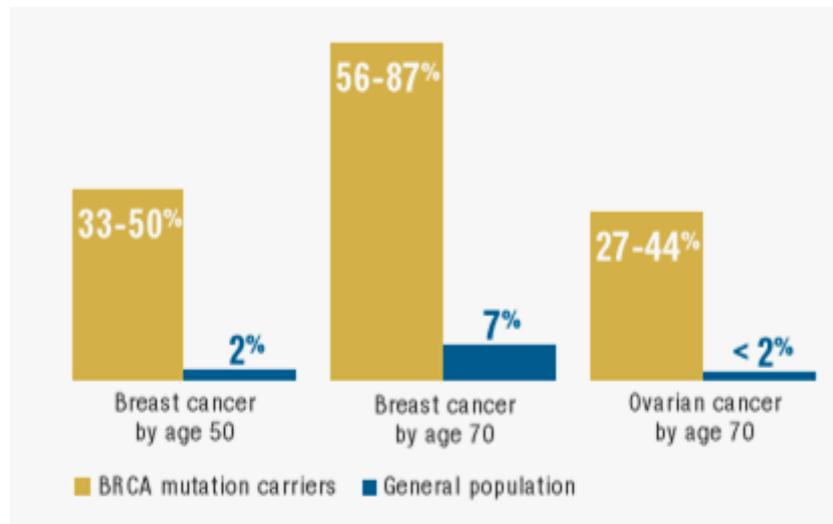


**Figure2.** *lifetime risk estimate of ovarian cancer for women in general population is 1.7% compared to 25-60% of women with altered BRCA1 or BRCA2 genes.*

According to the International Federation of Gynecology and Obstetrics (Moeder et al., 2007) guideline for effectiveness screening, women with high risk of familial ovarian cancer is defined as a women with first degree relative(s), such as mother, father, sister, brother, daughter, or son affected by cancer and meets one of the following criteria:

- One individual with ovarian cancer at any age and one with breast cancer diagnosed at age younger than 50 years, who are the first-degree relative of each other.

- One relative with ovarian cancer at any age and two with breast cancer diagnosed younger than age 60 years, who are connected by first-degree relationships.

- Known *MLH1* or *MSH2* mutation carrier.

- Two or more individuals with ovarian cancer, who are first-degree relatives of each other.

- An individual with both breast and ovarian cancer.

- Three or more individuals with breast or ovarian cancer over three generations (one must have ovarian cancer).

- Known *BRCA1* or *BRCA2* mutation carrier.

Genetic factor is considered the main basis of cancer risk in hereditary ovarian cancer. Specifically, germline mutations in one of the *BRCA1*, *BRCA2* tumor suppressor genes are known to significantly increase the susceptibility to familial ovarian cancer (Godwin et al., 1993). We describe below the mutation analyses for these genes in context of their relationship to breast/ovarian cancer.

### BRCA1 (Breast Cancer Associated Gene 1)

*BRCA1* was mapped in 1990 (Hall et al., 1990) and subsequently cloned by Miki (Miki, et al.1994). *BRCA1* gene is located on chromosome 17q12, and it has 24 coding exons encoding a 220 KD large nuclear protein with 1863 amino acids. Two major motifs are identified at the N-terminal of the protein (1-112 AA) containing a RING finger domain (Fig. 3) (Yarden& Papa, 2006) and the C-terminus containing a BRCT (breast cancer C-terminal) domain. The RING finger domain specifically interacts with another RING finger protein known as *BARD1*. Both *BRCA1* and *BARD1* have a BRCT domain in their C-terminal domain. *BRCA1* functions as a multifunctional tumor suppressor through its interaction with various cellular regulatory

proteins, which play an important role in cell cycle, DNA repair, transcriptional regulation, replication and recombination. For instance, *BRCA1* was found to play role in cell cycle regulation through binding with *BACH1* gene,

which functions in DNA damage-induced cell cycle checkpoint control that is necessary for efficient double-strand break repair (Cantor et al., 2004).
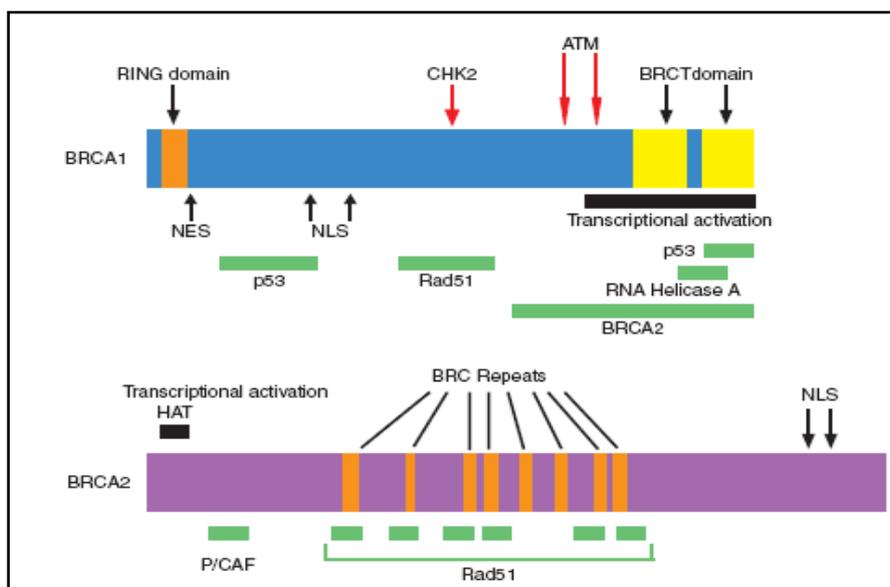


**Figure3.** *Schematic representation of BRCA1/2 binding proteins: BRCA1 (upper panel) and BRCA2 (lower panel) interact with each other and with various DNA repair and cell cycle regulatory proteins such as CHK2, P53 and ATM. RAD51 as a DNA repair protein interacts with both BRCA1/2 in case of DSB repair (Yoshida & Miki, 2004).*

*BRCA1* directly interacts with *BRCA2*, *RAD51* and many other proteins that are involved in cell

cycle and in maintaining genetic stability in response to DNA damage (Fig. 4).
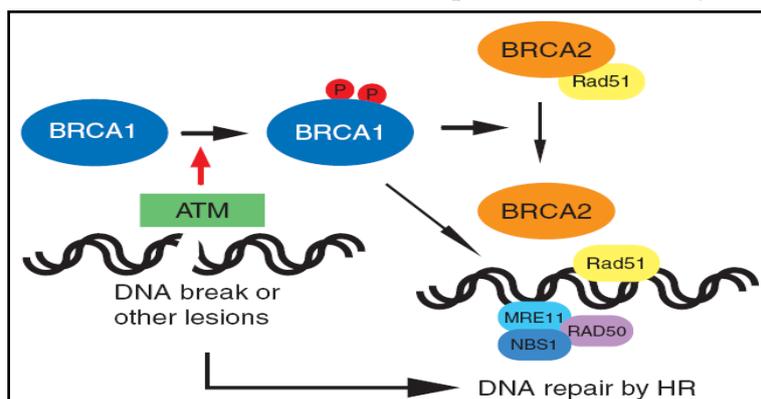


**Figure4.***A model for the role of BRCA protein in DNA damage repair: ATM phosphorylates BRCA1 in response to DSBs and activates DNA repair via HR, in cooperation with BRCA2 and RAD51. BRCA1 also recruits RAD50-MRE11-NBS complex to the sites of DNA damage (Yarden& Papa, 2006).*

In summary, *BRCA1* gene acts as a gatekeeper through its role in DNA damage response, cell cycle regulatory, DNA repair, transcription and chromatic remodeling.

Germline mutations in *BRCA1* have been described in breast and/or ovarian cancer families. These mutations involve different types and sizes including point mutations, frameshift mutations and LGRs. Human Gene Mutation Database (HGMD) has documented the presence of over more than 1000 mutations

in *BRCA1* in association with hereditary breast and ovarian cancer (Table1). These mutations include 362 point mutations, 456 frameshift mutations (338 deletions, 118 insertions) mostly in exon 11 which constitutes >60% of the coding region, 15 small indels, 133 LGRs (110 deletions, 23 duplications/triplications) and 14 complex mutations. As mentioned above, some of these mutations have a founder effect in certain populations (Petrij-Bosch A et al., 1997; Neuhausen, 2000; Ferla R et al., 2007).

## BRCA2 (Breast Cancer Associated Gene 2)

In 1994, Wooster and his team identified another breast cancer susceptibility gene named *BRCA2* (Wooster et al., 1994), which was mapped to chromosome 13q12.3 (Wooster et al., 1995). *BRCA2* gene encodes a 384 KD nuclear protein. It has similar gene structure as *BRCA1* with 27 exons (with exon 11 being the largest) spreading over 70 kb of genomic DNA (Fig. 3). Various studies have demonstrated the localization of *BRCA1*, *BRCA2* and *RAD51* as a complex to the site of recombination and DNA damage-induced HR for DSBs. This strongly suggests that *BRCA2* has a role in DSB detection and repair.

So far, the majority of mutations reported are frameshift small insertions/deletions and non-sense mutations leading to truncated non-functional proteins. Germline mutations in *BRCA2* genes have been found in a large number of families with multiple cases of early-onset breast and ovarian cancer (Shattuck-Eidens et al., 1995; Wooster et al., 1995; Tavtigian et al., 1996; Gayther et al., 1997). The number of mutations reported in *BRCA2* is comparable to those in *BRCA1* in terms of nonsense, splice site alterations, frameshift mutations spreading along the entire coding region. The discovery of LGRs has broadened the mutation spectrum in *BRCA2* (Casilli et al., 2002), but its frequency is much lower in compared to *BRCA1*. HGMD® database has reported over 772 mutations in *BRCA2* that are associated with breast and/or ovarian cancer (Table 1), and these include 225 point mutation, 446 frameshift (325 deletions, 121 insertions, and 15 indels), 29 LGRs (21 deletions, 8 duplications), 116 splicing site and 6 complex mutations. A small number of founder mutations in all types in specific populations have also been described (Ferla R et al., 2007).

**Table1.** *Summary of mutations for BRCA1/2 in HGMD® database*

| Gene | Missense/Nonsense | Splicing | indels | Small deletion | Small insertion | LGRs/ deletions | LGRs/ amplifications | Complex |
|---|---|---|---|---|---|---|---|---|
| **BRCA1** | 362 | 94 | 15 | 338 | 118 | 110 | 23 | 14 |
| **BRCA2** | 225 | 52 | 15 | 325 | 121 | 21 | 8 | 5 |

Different other genes have been reported to be associated with breast cancer such as, a study by Zhang reported an effective algorithm to identify key pathways associated with breast cancer utilizing Logistic regression with graph Laplacian regularization. Also found a key biomarkers used to categorize breast cancer subtypes, These sub networks potentially reflect relationships with clinical or biologic significance. For example, one of sub networks identified multiple genes includes, (*AR, ESR1, MED1, MED24, RARA, PRAME, and HMGA1*) involved in steroid hormone signaling (Zhang W et al., 2013), confirming other report published an analyses shown that *GATA3* mediates genomic *ESR1*-binding upstream of *FOXA1* (Theodorou V et al., 2013).

## TYPES OF GERMLINE GENETIC MUTATIONS AND THEIR CONTRIBUTION TO FAMILIAL OVARIAN CANCER

Different types of genetic mutations either hereditary or somatic have been identified in cancer genes; below we discuss various types and resolutions of mutation and the methods for their detection.

### Point Mutations

It was estimated that over 15 million loci in our genome have a nucleotide that is different from one person or population to another. These variations are called Single Nucleotide Polymorphisms (SNPs). If a SNP is associated with a disease, it is considered a point mutation. Base pair substitutions can generate either nonsense stop codons resulting in truncated non-functional protein or missense point mutations where a single nucleotide has changed to cause substitution of the encoded amino acid and lead to a protein product with modified sequences. The consequences of a point mutation may range from no effect on the protein level to lethal when change in the activity and expression of the protein, as in the case of hemoglobin gene mutation in sickle-cell disease (Taylor et al., 2008), depending on the location and the effect of the sequence change in relation to gene function. This type of mutations has been the focus of most mutation detection studies performed in the past.

Point mutations may arise spontaneously during DNA replication. Mutations may be introduced each time the cell replicates. During DNA replication, polymerases copy three billion base pairs of the entire human genome with impressive, but limited fidelity. Although the majority of polymerase errors are corrected by mismatch repair process, the repair system does not function with 100% efficiency. Mutations

can also be introduced as a result of DNA damage through environmental agents (mutagens), including sunlight (UV-rays), X-rays, radiation and chemical agents (Fig. 3). Such agents represent risk factors for cancerous transformation. For example, germline mutations in genes involved in maintaining genomic integrity can be responsible for different phenotypes including Xeroderma Pigmentosum (XP) (De Vrieset al., 1995), Ataxia Telangiectasia (AT), Nijmegen Breakage Syndrome (NBS), Hereditary non-polyposis colorectal cancer (HNPCC) and Bloom Syndrome (BS) (Prollaet al., 1994 & 1998).

In 2007, the international Hap Map project characterized over 3 million SNPs that are useful to identify genes involved in complex diseases and estimate their prevalence in different genetic studies via Genome Wide Association Studies (GWAS). For instance, a GWAS by a French group analyzed DNA from over 3,200 participants revealed an association between a version of a gene for a protein that transports zinc in the pancreas and the increased the risk of type 2 diabetes diseases (Cauchi et al., 2006).

Different methodologies have been developed for the detection of all forms of point mutations. Examples of these include Single Strand Conformation Polymorphism (SSCP), direct sequencing by Polymerase Chain Reaction (PCR) and Protein Truncation Test (PPT).

## Small Deletion/Insertion Mutations

Another common type of mutations is named "indel", which involves insertion and/or deletion of one or more base pairs. Detection/insertion mutations often cause a shift in the open-reading frame with generation of a premature stop codon resulting in truncated protein product, and this case, we call them frameshift mutations. Another possible mechanism is the activation of Non-sense mediated Decay (NMD) by mutations; NMD activation eventually will lead to mRNA degradation.

Frameshift mutations usually occur by strand slippage and mispairing during DNA replication. Following strand slippage, a strand can reanneal with one or more base pairs bulged out with the downstream nucleotides correctly base paired. This mispaired strand is elongated by DNA polymerase and the mutation is "fixed" in the new sequence. When this double-stranded DNA is subsequently replicated during a cell division, it will produce one daughter cell with

the frameshift mutation and one with the wild type sequence (Farabaugh, 1996; Dangel J et al., 1999; Raue at al., 2007). Typically, a frameshift mutation in a coding region can have one of the three outcomes: l) mutations cause shift in the reading frame and therefore, alter or suppress the function of the encoded product and eventually lead to disease phenotype;2) nonsense frameshift mutation generates a premature stop codon, leading most often to loss of function; 3) it might be restored to a wild type by a nearby compensatory frameshift mutation.

Frameshift mutations frequently result in severe phenotype as cancer, since they have frequently been found to be present in major DNA repair genes in cancer including genes that are responsible for hereditary cancer syndromes. For example, frameshift mutations in *BRCA1* and/or *BRCA2* are considered the leading mechanism for familial breast and ovarian cancer development. Some of the recurrent mutations are considered founder mutations due to their presence in high frequency in individuals from distinct population or ethnics. So far, over 21 *BRCA1* and *BRCA2 f*ounder frameshift mutations have been described in breast and ovarian cancer-prone kindred in numerous populations. For example, 185delAG and 5382insC in *BRCA1* or 6174delT in *BRCA2* are founded in 232 of individuals with Ashkenazi Jewish heritage reported to be associated with approximately 20% with breast cancer in that population (Neuhausen, 1996 & 2000). Likewise, small deletions or insertions are also common in DNA repair genes such as *MLH1* and *MSH2*, resulting in hereditary colon cancer, for example, HNPCC (Colella et al., 1998). The methodologies that are used for the detection of small deletion insertion are similar to those used for the detection of point mutations or SNPs.

## Large Genomic Rearrangements (Lgrs)

Existing mutation detection studies have been mostly focusing on small size mutations such as missense and small deletions and/or insertions. Recently, studies have demonstrated that other types of genetic changes are also responsible for cancer etiology. Among these are the Large Genomic Rearrangements (LGRs), mainly involving large loss or gain (hundreds or thousands) of DNA bases that may encompass one or more exons of genes and sometimes the entire gene. LGRs could lead to complete loss of gene expression by generating a shift in the

reading frame causing a premature stop codon and eventually a truncated protein. LGRs are in most part overlooked by most mutation detection methodologies due to their large size and being heterozygous in nature. Mutation analysis using regular screening techniques may fail to detect the mutant allele in the case of large intragenic deletions avoiding primer binding site in the deleted copy. In the case of duplication/insertion, the amplification of the mutated allele can either be failed or strongly disfavored due to it large size. Genomic rearrangements are currently of interest due to their important role in the etiology of different genetic disease including sporadic cancer (Lupski, 2007) or hereditary cancer such as familial breast/ovarian cancer (Engert et al., 2008) and HNPCC (Van der Klift et al., 2005). Studies have reported that genomic rearrangements in *BRCA1/2* are more frequent among European populations compared to other populations and it is likely due to some major founder effect, such as *BRCA1*exon 13 duplication in British breast and ovarian cancer families.

Apart from cancer, LGRs are also responsible for a variety of other type of genetic diseases such as DiGeorge syndrome, Williams-Beuren syndrome and Prader-Willi syndrome (Shaikh et al., 2007; Bayés et al., 2003). The direct cause of this group of disorders is not from single nucleotide substitutions, but from recurrent chromosomal aberrations, which give rise to DNA copy number changes or disruption of the structural integrity of a dosage sensitive gene(s).

Repetitive DNA sequences in different forms are believed to be the major mediator in generating genetic in stability that results in genomic deletions or duplications. LGRs are no exceptions; they are believed to be mediated by stretches of DNA segments that are highly similar in sequences, such as Short and Long Interspersed Elements (SINEs and LINEs, respectively) by segmental duplication or any other repeat or by homologous forms with more than 99.5% sequence identity. Various studies suggested that the major mechanisms led to LGRs are DNA double strand breaks (DSBs) followed by DNA repair by either Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ) (Fig. 4).
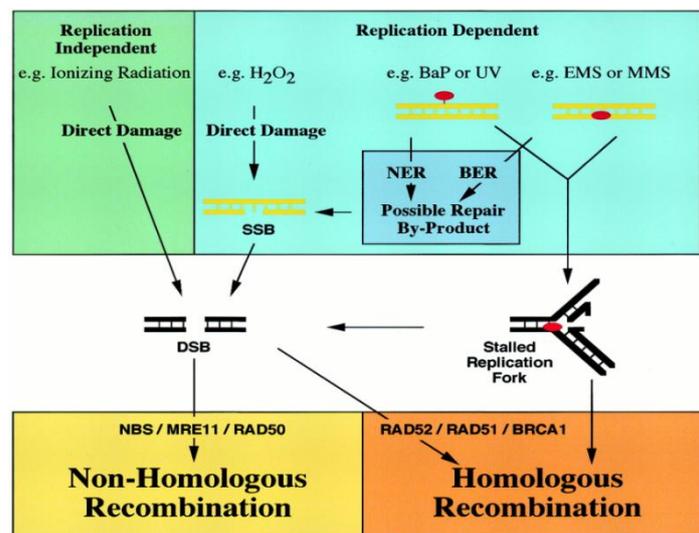


**Figure4.**Repair of DNA damage induced double strand break (BSB) by HR and NHEJ mechanisms: Two typess of DSBs can be formed, either directly by IR independent of replication recombination, in which SSB may form as a result of DNA damaging agent (e.g.H2O2) or indirectly as in incomplete product of DNA repair (e.g. Benzo a pyrene, UV irradiation) forming Nucleotide excision repair (NER) or Ethylmethanesulfonate (EMS) and Methylmethanesulfonate (MMS) forming base excision repair (BER). Replications of this lesion may cause formation of DSB. DNA damage may be result directly in replication fork stalling, which would be processed into DSB (lupski et al., 2005).

### *Homologous Recombination (HR) and Genomic Rearrangements*

For many years, scientists studied DSB as a mechanism for maintaining genetic integrity. The two main mechanisms for DSB repair are either homolog or non- homolog based

recombination events. HR is a known mechanism for promoting diversity and DNA repair through mitotic recombination in diploid cells (Fig. 5a). In yeast, DSB is thought to initiate meiotic HR via a mechanism that is conserved through mammals. The mechanism of HR relies on the use of template DNA sequence

such as homologous sequences, preferably in the sister chromatid to re-synthesize the damaged or missing copy at the break site. Several homology-directed pathways have been identified, yet all of them are initiated by 5'→3' direction at the DSB end.
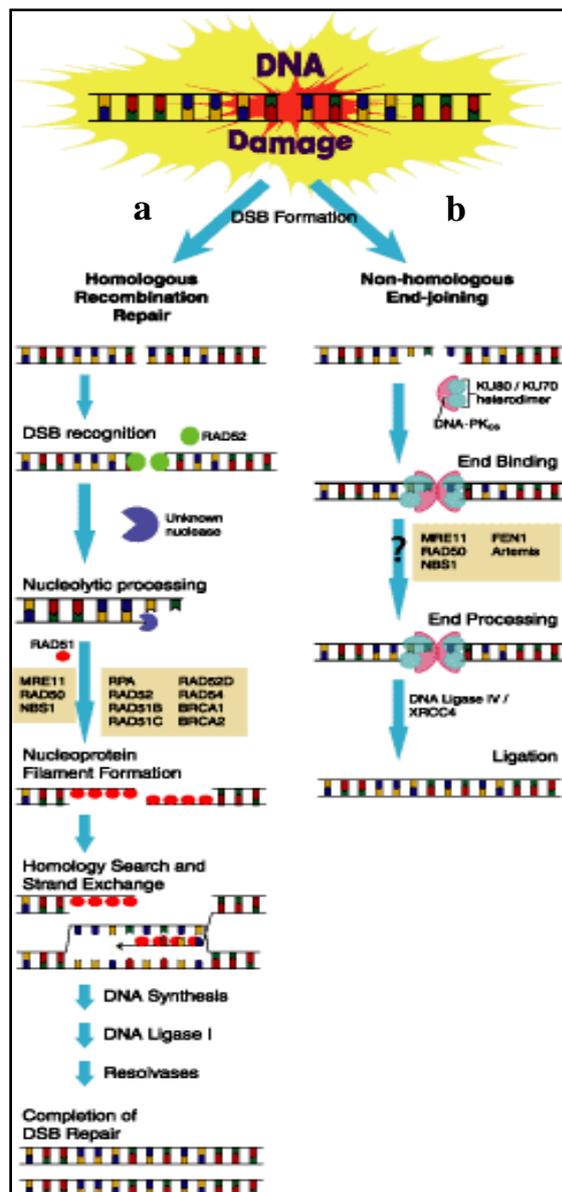


**Figure5.** *Double Strand Break (DSB) Repair mechanisms: An overview of steps required for two main DNA DSB repair pathways, either by (a) homologous recombination, or by (b) non-homologous end-joining (Jackson, 2002).*

The process is facilitated by Mre11/Rad50/Nbs1 complex (Fig. 4) (Paull & Gellert, 1998). Synthesis-dependent strand annealing pathway is the classical double-Holliday Junction model for DSB repair (Szostak et al., 1983) and single-strand annealing, all of which contribute to the repair of two-ended DSBs.

Many HR key regulatory proteins are considered cancer-associated genes, such as

*BRCA1*, *BRCA2*, *FANC*, *TP53*, *ATM* and *MMR* genes. Therefore, it is not surprising that any defect in these proteins will increase the risk of cancer (Thompson & Schild, 2002).For example, it was shown that homology-directed repair deficiency in *BRCA2*-nullcells results in the accumulation of chromosomal aberrations (Patel et al., 1998) (Fig. 6).
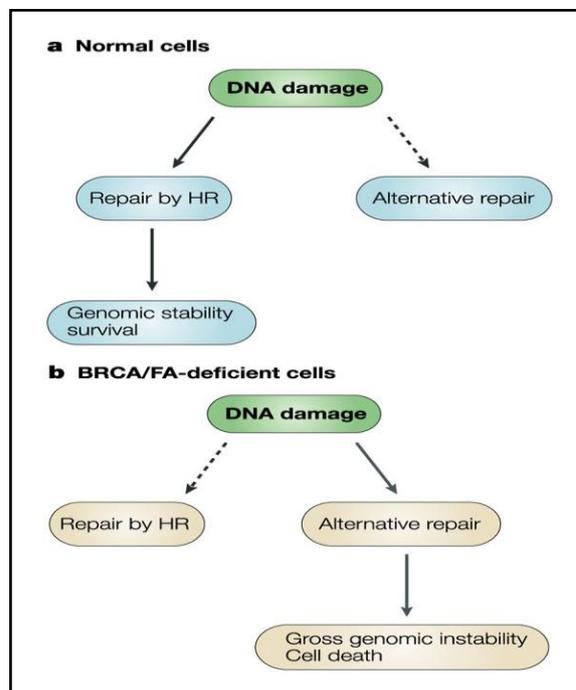


**Figure6.** *BRCA repair pathways and its implication of defect on HR: Defect in BRCA1 and/or BRCA2 pathways leads to a higher sensitivity to any type of DNA damage. a-DNA lesions that block replication or generate DNA double strand breaks during S/G2 phase are highly dependent on BRCA pathways for repair by HR. b- Although the details of the alternative mechanisms are unclear, yet the use of these alternative mechanisms lead to gross chromosomal instability and subsequent cell death (Turner et al., 2004).*

Another example is the Bloom syndrome gene (BLM). Unlike *BRCA2*, BLM mutant cells are proficient in initiating HR, but the outcome of these repair events is apparently shifted toward exchange-associated events (Chaganti et al., 1974). This increases the exchanges between homologous chromosomes and leads to a higher rate of loss of heterozygosity (LOH), which has been proposed to be the driving force behind the increased risk of cancer in Bloom's syndrome patients (Luo et al., 2000). Also it was suggested that suppression of HR capacity may be a late event in tumori genesis, as it has been observed in the case of pancreatic cancer (Bardeesy et al., 2001), and it is preceded by mutations that confer resistance to apoptosis, thus allowing highly unstable pancreatic cells to survive.

These examples demonstrate that deficiencies in HR initiation can lead to disease phenotype. It is important to emphasize that loss of HR in a normal cell is generally more toxic than mutagenic. Cells might tolerate a partial deficiency in HR, but not a complete loss of the function (Saleh-Gohari & Helleday, 2004).

Moreover, other studies showed that HR plays a role in promoting genetic instability due to the presence of highly repetitive elements in the human genome (Bishop & Schiestl, 2000). Nearly 50% of human genome contains repeat sequences including SINEs, LINEs, simple tandem and microsatellite repeats in addition to a chromosomal structural elements repeats, such as the centromeres and telomeres. Thus, the expected result of HR between different repetitive sequences types is the loss or gain of genetic material including gross rearrangements and genomic instability and eventually phenotype (van der Klift et al., 2005). The report by Toda Y, et al (1997) was first to suggest the involvement of SINE in form of Alu repeats in genomic rearrangements. The study suggested that large deletions or duplications are usually the result of either intra-chromosomal or unequal inter-chromosomal events. Both events canbe mediated by a stretch of homolog DNA sequences and may result in the removal or addition of large segments of DNA.

Since 41.5% of the *BRCA1* gene sequence is comprised of repetitive Alu elements (Petrij-Bosch et al., 1997; Perkowska et al., 2003), it suggests that *BRCA1* region is prone to more recombination events and subsequently to the occurrence of LGRs and genomic instability (Vasickova et al., 2007). Recent studies demonstrated the observation of Alu-mediated genomic rearrangements in both deletions and insertions (Hsieh et al., 2005). For example, a study by Montagna et al. (1999) using southern blot identified a 3 kb germline deletion of spanning exon 17 in *BRCA1* in a patient with a family history of breast and ovarian cancer. Such rearrangements have been implicated in the cause of several genetic diseases besides cancer, including X-linked ichthyosis, in which a 1.9 Mb of DNA deletion is mediated by the flanking S232elements (Yen et al., 1990). Hereditary neuropathy in which a 1.5 Mb deletion is mediated by *CMT1A-REP* (Chance et al., 1993). Similarly, HR-based LGRs are responsible for Prader–Willi syndrome (Ledbetter et al., 1981), DiGeorge syndrome (de la Chapelle et al., 1981) and hypercholesterolemia (Lehrman et al., 1985).

Alternatively, deletions may be due to an interchromatid recombination, such as unequal crossing over between misaligned homologous regions on sister chromatids or homologous chromosomes. Interestingly, Charcot Marie Tooth syndrome type 1A (CMTA1) resulted from a duplication of the same region that is deleted in hereditary neuropathy (Hoogendijk et al., 1992; Wise et al., 1993). Similar mechanism has been observed in cancer. Tandem duplication within the ALL-1 gene was found to be mediated by Alure combination and results in acute myeloid leukemia (AML) (Schichman et al., 1994). These duplications suggest an inter chromatid crossing-over mechanism in these events. It is also worth mentioning that HR between two homologous regions on different chromosomes could result in a translocation. For example, fine mapping of Philadelphia chromosome t (9/22) breakpoints, which is often found in chronic myelogenous leukemia (CML) patients, revealed the presence of two homolog Alu sequences at the breakpoints (Jeffs et al., 1998; Martinelli et al., 2000).

### Non-Homologous End Joining (NHEJ) and Genomic Rearrangements

Although HR is the major pathway for DSB repair, NHEJ represents a second form of DNA repair. NHEJ is a simple mechanism, in which broken sequences are directly join at their broken ends with little or no homology. A number of proteins are involved in NHEJ, including Ku heterodimer consisting of (*Ku70* and *Ku80*), forming a complex of DNA serine/threonine protein kinase catalytic subunit (*DNA-PKCS*), which is activated by the generation of DSB (Fig. 5b). After juxtaposition of two DNA ends, DNA-PKCS is auto phosphorylated (Ding et al., 2003) and the ends become ready for ligation and repair by the ligase IV complex (DNA ligase IV and the protein cofactor *XRCC4*) (Nick McElhinny et al., 2000). Importantly, NHEJ of DSB could lead to large-scale sequence rearrangements, since the ends can be joined from different loci.

Based on careful and comprehensive analysis of LGR breakpoint features in multiple cancer genes, NHEJ appears to be involved in this type of mutations. Even though many repetitive Alu elements have been identified at/or near genetic rearrangement breakpoints in patients with different genetic diseases and hereditary cancers, their role in NHEJ is still unclear. However, it was suggested that the nature and the size of the repeats on 5' and 3' breakpoints,

degree of homology, distance between repeats and their orientation with respect to each other, play important roles in affecting the frequency of HR, NHEJ, and their involvement in genomic rearrangements (Burma et al., 2006).

Deficiencies in NHEJ might contribute to carcinogenesis and most often lead to an increased risk of cancer with enhanced genomic and chromosomal instability. For example, heterozygosity at the liagse IV locus resulted in cancer-prone phenotype in an Ink4a deficient background (Sharpless et al., 2001).

HR and NHEJ function like a double-edge sword by being able to repair DNA damage, such as DSBs, but also responsible of creating genomic rearrangements leading to genetic abnormalities. So far, more than 62 genomic rearrangements with their exact breakpoints were reported to be found in *BRCA1* alone with different sizes (100 bp-37 kb) involved deletions and duplications of one or more exons. Interestingly, significantly smaller number of such rearrangements has been reported in *BRCA2*, despite the fact that these two paralogs have nearly similar gene structure and size. This might be due to either 1) less screening studies have been conducted in *BRCA2* in contrast to *BRCA1*; or 2) a lower number of repeats sequences are present in the intronic region of *BRCA2* gene. It is worth noting that the majority of discovered genomic rearrangements were from breast and ovarian families that were previously tested negative for point mutations and small deletions and insertions. LGRs can be detected by classical southern blot and more efficiently by quantitative mutation analysis using Multiplex Ligation dependent Probe Amplification (MLPA) and Quantitative Multiplex PCR Short fluorescent Fragments (QMPSF) and Quantitative Multiplex PCR Short fluorescent Fragments (QMPSF) and our modified assay universal-primer Quantitative Multiplex PCR Short fluorescent Fragments upQMPSF. (Azrak S, 2015).

## CONCLUSION

Different types of genetic abnormalities varying in size are present in cancer *BRCA1/BRCA2* genes susceptible to Hereditary ovarian cancer. These abnormalities involve changes in mutations sizes from 1 bp (e.g. point mutation/SNP, small deletion or insertions) up to hundreds of thousands of bases (e.g. chromosomal abnormalities). The need of their detection has motivated the development of various technologies with different resolutions

(e.g. SSCP, aCGH and FISH). However, LGRs involving one or more exon deletions and/or duplications that are responsible for many genetic diseases including hereditary cancers mostly went undetected. Until recently, with the development of MLPA and QMPSF and upQMPSF, more of LGRs are being uncovered.

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