Abstract
Cancer is a kind of disease which can be explained at the molecular level as triggered by accretion of damaged deoxyribonucleic acid. There are several genes involved in cancer, mainly belonging to two classes called tumour suppressors and oncogenes. Other than the well-known breast cancer susceptibility 1/2 genes, there are several other genes involved in the development of breast and ovarian cancers. However, since the past two decades the focus of research has been on breast cancer susceptibility 1/2 genes. The current review was planned to delve into the structure and function of breast cancer susceptibility 1/2 genes to augment research on the genetics of breast cancer. The understanding of tumour suppressor genes is also helpful in the analysis of mutational spectra and to determine the treatment strategies in clinical interventional studies.

Keywords: Oncogenes, Tumour suppressor genes, BRCA1, BRCA2.

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Introduction
At the molecular level, cancer is characterised by the accumulation of damaged and unrepaired deoxyribonucleic acid (DNA). In the human body, there are around 50,000 suspected endogenous mediators which are considered to be the cause of DNA damage per cell per day. Moreover, there are hundreds of exogenous agents, like cancer-causing chemicals and various harmful radiations which are responsible for damage to DNA. Due to the mechanism provided by nature, living things stay within sustainable level of DNA damage without any severe level of morbidity. In case of defect of DNA repair process due to hereditary, epigenetic or somatic alterations, the result is the accumulation of un-repaired DNA which is the actual cause of cancer. The transformation of normal cell to cancerous cell requires a series of several mutations in specific classes of genes. Genes that are involved in cell proliferation, differentiation and death must mutate to transform a normal cell into a malignant cell. Genetic alterations are likely to occur at any stage of life or at any level of development; variations could fluctuate from whole chromosome to only a single nucleotide. Similarly, epigenetic changes result in activating or silencing of microribonucleic acid (RNA) which in turn is responsible for the expression of hundreds of genes.

Major classes of genes contributing to carcinomas
In the study of cancer genetics over several decades, the concept of Mendelian recessive and dominant was integrated. Biologists working in the field of cancer genetics identified two major classes of genes involved in this disease known as proto-oncogenes (POGs) and tumour suppressor genes (TSGs).

Proto-Oncogenes
POGs are normal genes present in every human male, female and many other organisms. It is involved in normal cell growth and proliferation, but can mutate to oncogenes. Since these are dominant genes, therefore it means that the activation of only one allele is enough for normal cellular growth. Cancerous cells form due to mutation or over-expression of these genes. In most of the cases, morbidity related to oncogenes is caused by mutation in some other genes such as TSGs or environmental factors, like viral infection.

Tumour Suppressor Genes (TSGs)
TSGs are further divided into caretaker TSGs and gatekeeper TSGs. A third class of TSGs is called landscape genes, the mutated form of which has been found to promote angiogenesis.

These genes affect the functions of cell, such as genomic stability, DNA repair, senescence, inter-cellular communication, interactions of cell with matrix mortality and angiogenesis. In contrast to POGs, caretaker and gatekeeper TSGs act in a recessive manner. Theoretically speaking, at the cellular level, loss of function or inactivation of both alleles is required for pathogenicity, but actually for some TSGs, like breast cancer susceptibility genes (BRCA)1 and 2, there is evidence of haplo-insufficiency. Thus, only one mutated gene is enough for the onset and progression
of the disease. Normal functioning of gatekeeper TSGs is related to the direct prevention of growth of tumours by apoptosis, differentiation and suppression of proliferation, whereas caretaker TSGs indirectly suppress tumorigenesis and neoplastic development. However, this classification of TSGs somewhere has become arbitrary because some genes like tumour suppressor gene 53 (TP53) and BRCA2 have exhibited functions of caretaker as well as gatekeeper genes. Recent research is focussing on synergy of functional and regulatory role of oncogenes and TSGs mediated by microRNA. Meta-analyses and research about epigenetic methylation of promoters of TSGs also play a vital role in the development of cancer treatment techniques.

Involvement of different TSGs in breast cancer (BC)
The most widespread genetic cancers found globally are breast, ovarian and prostate cancers caused by TSGs. There are several somatic and germline mutations involved in the process of tumorigenesis. More widely explored high penetrance genes are TP53 and BRCA1/2. TP53 is known to express in 20–35% of breast tumours for which almost 1400 variants have been described to be involved in Li-Fraumeni cancer syndrome. The cadherin 1 (CDH1) gene was found to be involved in lobular type of breast tumours in BC. The carrier women of CDH1 exhibit an elevated rate of 50% of having BC in their lifetime. However, the prognosis in under-expression of CDH1 was found to be poorly associated and greater rate of metastasis was revealed in oestrogen receptor (ER)-positive BC patients. The carriers of phosphatases protein (PTEN) gene mutation have 4% more chance of breast carcinoma involving clathrin signalling. Mutant form of this gene is involved in triple negative and ductal carcinomas. The somatic mutation caused by liver kinase B1 (LKB1), a serine threonine kinase 11 (STK11)-interacting protein, which is directly responsible for Peutz-Jeghers syndrome (PJS) and mutation in this locus is accompanied by around 5 times more risk of BC in PJS patients. LKB1 and PTEN genes are responsible for the carcinoma of squamous cell in lungs. There is another TSG present on chromosome 22 at q21.1 called checkpoint kinase 2 (CHK2) that contains 14 exons where the gene product consisting of 543 amino acids with three important conserved functional domains which are: sickle cell disease (SCD)-regulatory domain on N-terminus rich in threonine, serine and glutamine, forhead-associated (FHA) domain which is protein-to-protein interaction, and kinase domain on C terminus. The CHK2 gene plays a significant role to respond the cell on double stranded breaks (DSBs). The mode of action of the gene is to act on all checkpoints of the cell cycle, and to accordingly regulate it by DNA repair or apoptosis.

There are evidences of involvement of some other genes like partner and localiser of BRCA2 (PALB2, RAD51 Recombinase and BRCA1 interacting protein (BRIP1) in hereditary BC, because of interconnection of these genes with BRCA1/2 functioning. The ataxia telangiectasia protein mutated (ATM) gene encodes ataxia telangiectasia protein, mutant form of which impairs the vitally important function of DSB repair of DNA and cell cycle; increased risk of BC is also reported in carriers of ATM gene though not much significantly. It was demonstrated that the polymorphism of 5557G>A at exon 39 of this gene is more frequent in BC patients with significant difference compared to the control group. In certain populations, astrocytoma development is also found in the 5557G>A in conjunction with some other genes.

Hereditary breast ovarian cancer by BRCA1/2
Most commonly influencing genes in breast/ovarian carcinomas are BRCA1 and BRCA2. BRCA1 and BRCA2 genes are found to be associated with more aggressive and higher-grade breast tumours. Cancer progression elevates up to 80% by involvement of BRCA1/2 genes. Loss of function of BRCA1/2 is also involved in elevated risk of ovarian, prostate, pancreatic and male BC. Moreover, there is specific pattern of genetic aberrations associated with BRCA1/2 that demonstrate specific behaviour during tumorigenesis. Differential expression levels of Proto-oncogene (P53), transcription factor (MYB) human epidermal growth factor receptor-2 (HER2) and cyclin D1 (CCND1) was found, while compared BC tumour cells with BRCA1 defective variant with sporadic BC tumour cells.

Historical Account of BRCA1/2 genes
BRCA1/2 genes were discovered nearly 20 years ago after which lots of progress has been witnessed. A major landmark regarding its treatment was coined after about 2 decades of discovery of BRCA when the United States Food and Drug Administration (FDA) approved olaparib as the chemo-therapeutic agent for ovarian carcinoma patients with BRCA-positive mutation. Major milestones are the pre-clinical studies for testing olaparib drug therapy in 2005, ruling out patents right of Myriad Lab for genetic testing in 2013 and approval of olaparib by FDA for BRCA mutant-positive patients. In recent years, new treatment option specified olaparib tablets for BRCA mutants with HER2-negative BC.

BRCA1 Nomenclature and Resources
Homo sapiens breast cancer susceptibility gene 1 is also known as familial breast/ovarian cancer gene 1, located
on chromosome number 17 q-21 possessing gene identification (ID) 672. This gene has various other synonyms such as BRCC1, PSCP, IRIS and RNF53 (Alias symbols of BRCA1). Ensemble ID is ENSG00000012048. This gene is present on the negative strand. Genomic coordinates are: 17: 43045678 to 43124096. There are 27 different three-dimensional (3D) structures which can be viewed on Catalogue of Somatic Mutations In Cancer-3D (COSMIC-3D). So far, 47341 unique samples have been recorded. Alternate assembly of transcripts is annotated by BRCA1_ENST0000047118. Various sequences for this gene can be viewed on complementary DNA (cDNA-ENST00000357654). Transcription and protein alignment can be observed at ENST00000357654+BRCA1. Mutation data and drug sensitivity can be seen on PF-4708671. This Information about gene can be viewed on official website of National Centre for Biotechnology Information.35

Besides NCBI, external links and resources include genome browsers Ensemble and UCSC. Link related to bioinformatics resources of BRCA1 is Online Mendelian Inheritance in Man (OMIM). Transcript ID is ENST00000357654. Copy Number Analysis (CONAN) command would be used for observation of copy number. Gene name in Atlas of Genetic Oncology is BRCA1, while human genome nomenclature (HGNC) ID is 1100, detail of such information can be viewed by browsing at home page of Human Genome Nomenclature Committee.36

**Structure of BRCA1**

BRCA1 was mapped by Hall et al.37 in 1990 and was found to be linked to Chromosome17 q-21 by linkage method. The structure was further revealed by Miki et al.17 in 1994 through cloning method since they observed the strong predisposition in five out of eight kindred who were probable candidates to segregate BRCA1 allele. In the mentioned initial investigative study for cloning this gene, 1 base pair insertion, 11 base pairs deletion, missense substitution and formation of stop codon was observed. Discovery of the gene has greatly revolutionised the understanding of BC biology. The gene comprises of 24 exons (Figure-1). The protein products encoded by BRCA1 consist of 1863 amino acids.17 More than 40% of BRCA1 gene is composed of Alu repeats sequences and some other repeated sequences with low frequency.18 The gene is spread over 117kbp of genome with exon 11 covering 3426 base pairs, making it the biggest human exon.39

BRCA1 gene produces three isoforms by alternative splicing; one including all exons, other formed by skipping of exon 11, and the third includes only 117 bases of exon 11 along with rest of all exons respectively called full isoform, Δ11 isoform and Δ11q isoforms; the last one is also called in-frame of BRCA1 intron 11 splice(IRIS) form consisting of 1399 amino acids.40,41 BRCA1 full isoform consists of many conserved crucially functional domains, including ring domain on N terminus, two nuclear localisation regions and BRCA1 C Terminus (BRCT) domain on the C terminus.42 BRCA1 is one of the important genes belonging to tumour suppressor class categorised as "guardians of genome", therefore protein products have some important DNA-binding domains for the regulation of DNA repair pathways and apoptosis.43 There are around 15 other target genes, including BRCA2, ATM, interacting protein (CtIP) and Tumor Protein P53which are harbour by BRCA1 for such regulation and, thus, it is said to be the key regulator for the maintenance of genomic integrity by control on DNA repair and apoptosis.44

**Summary of BRCA1 Functions**

The wild type BRCA1 gene products play crucial role in the control of checkpoints of the cell cycle.45 Most of the products of mutations consist of truncated protein. Functional features of BRCA1 have been investigated by gene knockout experiments using different organism
models.\cite{46} Crucially vital role of BRCA1 could be explained by its association with various important DNA repair proteins like RAD1, P53, RNA helicase, holoenzyme of RNA polymerase II, terminal Binding Protein (CtBP) interacting protein, BRCA1 associated RING Domain 1 (BARD1),MYC Proto-Oncogene, Basic helix-loop-helix (BHLH) Transcription Factor-myc and BRCA2.\cite{47} All these associated proteins strongly predict the important role of this gene in transcriptional transactivation, DNA repair and control on cell cycle.\cite{29} The BRCA1 plays its role in DNA DSB repairs, mediated by homologous-recombination.\cite{48} Thus, summarising the functions of BRCA1, it can be said to act as a vehicle for converging cell regulatory proteins. Mutations in BRCA1, therefore, affect the composition of formation of complexes which results in deregulation of cellular repair processes and, eventually, the development of malignant tumours.

Some vitally important functions of BRCA1 gene need to be fully kept in mind.

**Role in control of cell division: G2-M checkpoint activation**

It has been found that DNA mismatch repair (MMR) is involved in the chemotherapeutic response to some drugs like 6-thioguanine (6-TG) during cancer treatments in human beings.\cite{49} Consistent resistance to 6-TG was observed in some MMR-deficient cells which reveals the G2-M checkpoint arrest, decreased rate of apoptosis and more resilience to thioguanine treatment and genotoxicity.\cite{50} Yamane et al.\cite{51} in 2007 investigated isogenic human BC cell line models, including a mutated BRCA1 cell line thyroid carcinoma, Hurthle cell (HCC-1937), and concluded that BRCA1 mutated cells showed more resistance to 6-TG than to BRCA1-positive cells and almost a complete loss of G2-M cell cycle checkpoint response induced by 6-TG.

**Role in differentiation and development**

BRCA1 mutated cells impair the process of differentiation and enhance proliferation in mammary epithelial cells. Furuta et al.\cite{52} in 2006 demonstrated the direct functioning of BRCA1 in the chemotherapeutic response of differentiation and development of acinus formation in mammary epithelial cells by using 3D in-vitro culture system. They concluded that BRCA1-deficient cells impair acinus formation and enhance proliferation by RNA interference. Recently, a similar kind of observation about BRCA1-mediated DNA repair has been made in humans regarding its involvement in the stabilisation of differentiation state inmucosae-associated epithelial chemokine (MEC) whose previous name was spliceosome factor (SMU1).\cite{53} In another investigation, BRCA1 wild type cell lines were compared with haplo-insufficient BRCA1 pathogenic variant which resulted in high-impact deficiency in the process of differentiation rendering cells more prone to malignancy.\cite{54}

**Genome instability**

BRCA1 gene is strongly associated with HR-mediated DNA repair, thus mutant gene products develop genomic instability as Tirkkonen et al.\cite{55} in 1997 observed high degree of aneuploidy in BRCA1 mutant cells compared to non-mutant cells. BC susceptibility gene 1 requires RAD1 during the assembly of subnuclear components, thus impaired functioning lead to genomic instability.\cite{56} BRCA1 was also explored to play a role in other DNA repair mechanisms like non-homologous end joining, nucleotide-excision repair, and base excision repair as it has an association with a large number of important proteins.\cite{49}

**Angiogenesis**

It was found during one of the investigations that the removal of BRCA1/interacting protein (CtIP) complex from Angiopoietin 1 (ANG1) promotor, accelerates the tumour growth in mammary tissues with noticeable vascularisation.\cite{52}

**Induction of apoptosis-escaping from programmed cell death**

Inactivation of extracellular signal-regulated kinase 2 (ERK1/2) functioning during BRCA1-driven apoptosis was explored by Yan et al.\cite{57} thus, indicating its role in escaping programmed cellular death. BRCA1-induced apoptosis was also found to be involved in activation of Jun N terminal kinase (JNK), Cell Surface Death Receptor (Fas-l/Fas) and caspases 8/9.\cite{57}

**Mis-localisation of cytosol to promote metastasis and cellular invasion**

In-vitro studies on genetically-induced BRCA1 mutant human cells revealed the cytosolic mis-localisation and increased cellular invasion activity.\cite{58} The feature of cytosol mis-localisation related to specific BRCA1 mutation could be utilised as a biomarker to predict the disease status, especially metastasis. BRCA1-deficient condition also relates to increased metastasis in brain tissue and shows DNA damage induction and sensitivity to Poly ADP-ribose polymerase (PARP), PARP is a family of proteins involved in a number of cellular processes involving mainly DNA repair and programmed cell death polymerase inhibitor.\cite{59}
Changes in the Bioenergetics
Wild type BRCA1 gene is also strongly associated with bioenergetics of the cell. In the year 2014, Jackson et al. investigated the role of BRCA1 gene product in different tissues, including breast tissue, by using translational approach. They found many isoforms of BRCA1 in human and mice muscle cells. In response to exercise, increased interaction between phosphorylated acetyl CoA carboxylase and BRCA1 was observed. The same study found that the decrease in the amount of BRCA1 resulted in decreased consumption of mitochondrial oxygen and increased production of reactive oxygen. Thus, BRCA1 plays a vital role in regulation of metabolic activities. Moreover, BRCA1 acts like a potentially important biomarker for insulin like growth factor 1 (IGF1) like growth-factor targeted therapy for BC.

BRCA2 Nomenclature and Resources
Homo sapiens BRCA2 is located on chromosome 13 q-13.1, also known as familial breasts and/or ovarian cancer gene 2. Its gene ID is 675. This gene has various other synonyms, which are BRCC2, FAD, FAD1, FACD, FANCD1, FANCD and RPOVCA2 (Fanconi anemia, complementation group D1 alias names of BRCA1/BRCA2-containing complex, subunit 2).

Ensemble ID is ENSG00000139618. The gene is present on the positive strand. Genomic coordinates are 13:32316461 to13: 32398770(+). There are only two different 3D structures which can be viewed on Catalogue of Somatic Mutations In Cancer-3D (COSMIC-3D). BRCA2 possess numerous copies of motifs consisting of 70 amino acids named as activator of Rho GEF and GTPase (BRC). These motifs facilitate bonding of RAD51 to become functionally active in DNA repair. Loss of heterozygosity in BRCA2 tumours indicates its inclusion in class of TSGs.

Structure of BRCA2
The BRCA2 gene was first identified by Wooster in 1995 by using positional cloning method. This large gene comprises 27 exonic regions coding 3418 amino acids (Figure-2). This gene is categorised as TSG by further investigations on familial BC data. Various isoforms have been identified by exploring splice sites. BRCA2 mainly involves maintaining genomic integrity but also has important regulatory significance.

Summary of BRCA2 functions
Multiple range of functions has been explored for BRCA2 during the functional assays, a TSG with vital role in chromosomal stability. It controls the RAD51 during DNA repair by homologous recombination (HR) and mitotic advancement at G2/M checkpoint, spindle assembly and is also involved in cytokinesis. Loss of functions account for chromosomal aberrations in carcinoma cells driving neoplastic alterations and mutagenesis. BRCA2 also acts as modulator of process of proliferation, migration and cellular invasion by controlling Matrix Metallopeptidase 9 (MMP-9) expression. Duplication of centromere, gametogenesis, replication of telomeres, control on cytokinesis and regulation of transcriptional products are some other important processes where involvement of BRCA2 is important.

The following are some important functions carries out by BRCA2 gene.

Response to Replication Fork
Defects in BRCA2 functioning lead to both collapsed and stalled replication fork. The BRCA2 and RAD51 genes are
involved in the stabilisation of replication-stalled fork in single-stranded DNA. This function is not dependent on the activity of nuclease multiple response expansion 1 (MRE1). Moreover, BRCA2 shelters the nascent single-stranded DNA from degradation at the position of the stalled fork.

**Role in Programmed cell death**

Guaragnella et al. in 2014 used sensitised acid-induced apoptotic yeast cells and explored that the BRCA2 silencing causes decreased expression of anoikis. Cellular adhesion to matrix protein in extracellular environment is a necessary component of cell survival, and failure of such adhesion results in a specific type of apoptosis called anoikis. The process of anoikis is an important mechanism to prevent the dead cell from being misplaced, and plays a vital role in averting metastasis. Thus, the function of BRCA2 is modular of anoikis with the involvement of Receptor Tyrosine Kinase (ROS).

**Conclusion**

There are dozens of genes involved in breast and ovarian cancers among which BRCA1/2 genes are the most dominant contributors. The knowledge of structural and functional features of these genes plays a pivotal role in research plans to explore new horizons in cancer genetics and BRCA1/2 spectra of any specific population. Moreover, enhanced knowledge of cancer genetics and the involvement of BRCA1/2 genes in breast carcinoma would also augment the direction of research to improve treatment strategies.

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**References**


