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Research Article

Genomic Analysis of BRCA2, and a Comparative Phylogenetic Analysis of BRCA2 in Humans along Different Species: A Candidate for Breast Cancer in Humans

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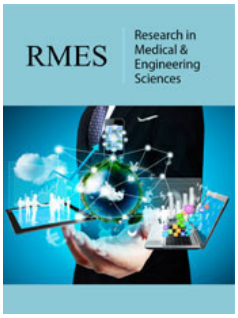

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Abstract

In DNA homologous recombination repair (HRR) several proteins are encoded by BRCA1 and BRCA2. Any mutation in HRR pathway increases risk of tumor formation as a result of DNA destroying factors like salts of platinum, anthracyclines and novel agents that can damage the DNA homologous repair pathway in the form of inhibitors. Hereditary mutation in BRCA2 gene is linked with germline mutation that contribute to develop breast cancer in women. In current study, phylogenetic analysis of BRCA2 gene in 3 different species is compared to obtain similarities in that gene. Further analysis of gene structure to find exons and introns in these species is done. This proposed study supports the divergence of these 3 species from their common ancestors due to diversity in genes on chromosomes as a result of gene distribution. This current research on BRCA2 gene in humans as compared to other species help us to observe evolutionary changes in that gene through phylogenetic.

Keywords: BRCA2 gene; Germ-line mutations; Gene structures; Phylogenetic analysis

Introduction

BRCA2 gene is considered in human as a tumor suppressor gene (specifically, a responsible caretaker form of a gene), present in all humans and its protein form, is also termed as breast cancer type 2 susceptibility protein, is observed for repairing damages in DNA. BRCA2 are genes that produce such proteins to repair damaged DNA. Every organism gets two copies of these genes that are inherited from parents [1]. Sometimes BRCA2 genes are also referred as tumor suppressor genes because when they undergo mutation, they develop into cancer. When people inherit such harmful variants, they are at greater risk of several forms of cancer like ovarian and breast cancer [2].

Harmful variant can be inherited from either parent in BRCA1 and BRCA2 genes. Any mutation in any of these genes carried by child has 50% chances to develop into cancer. Such inherited mutations are termed as germline mutations that pass from parents to offspring. If a woman inherits any mutation in BRCA1 and BRCA2 genes, then the chance of ovarian and breast cancer increases in that woman's lifetime [3].

On chromosome number 13, BRCA2 gene is present. Mutation in this gene is also passed by autosomal pattern of inheritance. As BRCA1 and BRCA2 are responsible to suppressor tumors so they have ability to control cell death and cell division [4]. Any mutation in these genes, ultimately enhance the chances of breast cancer in both men and women. Everyone inherits two copies of BRCA2 genes, one from mother and one from father. It has been observed that if a mutation occurs in one copy of gene in a person that is inherited from one parent, then still a normal copy of that gene is also present in that individual from another parent [5].

Mutation in both of these genes BRCA1 and BRCA2 causes ovarian, breast and other types of cancers. If a family undergo mutation in BRCA2 gene, then all family members show same type of mutation [6]. DNA binding part of BRCA2 gene contain 4 domains and 27 exons that consist of 82.4 kb part of genomic DNA. 11386 bp of mRNA transcript is encoded by BRCA2 gene. Site of transcription is present on 227 bp upstream region of the BRCA2 ORF on first ATG [7]. Recent research shows that only 5 to 10% cases of breast cancer develop through inherited mutation in BRCA2, while risk of breast cancer or other cancer in BRCA2 mutation depend on several other factors. Menand women carrying a mutation in BRCA2 gene are greater risk of breast, ovarian, prostate, fallopian and pancreatic cancer [8].

Material and Methods

Sequence retrieval of BRCA2 gene

Sequence of BRCA2 gene for 3 different species such as Homo sapiens, Pan troglodytes and Gorilla is retrieved from NCBI (https://www.ncbi.nlm.nih.gov/).Protein sequence of BRCA1 was used as a query from databases. Then BLASTP was used to check the predicted protein in mammalian species including Homo sapiens, Pan troglodytes and Gorilla. Then all the conserved domains were observed for these candidate sequences by using SMART tool (http:// smart.embl-heidelberg.de/) and pfam domain (PF00170) (http://pfam.xfam.org/). Then sequences were checked to find out the BRCA2 gene in all these mammalian species. Then to predict the conserved domains of candidate gene, multiple sequence alignment was performed [9]. The full-length BRCA2 proteins from humans, chimpanzee and pan troglodytes were aligned in MEGA 7.0.21 and a phylogenetic tree was constructed that showed the sequence alignments.

Gene structure and annotations

The gene structure display server (GSDS) program (http://gsds.cbi.pku.edu.cn/) was used to find the intron/exon regions in the gene structure of Homo sapiens, Pan troglodytes and Gorilla. Exact locations on chromosomes for the BRCA2 genes that code for BRCA2 proteins were observed from the GENE database of NCBI [10]. At the short arm telomere position to the long arm ends of telomere, the genes were organized separately on chromosome number 13 that is linked in ascending order of physical positioning. Data observed on NCBI (www.ncbi.nlm.nih.gov) was used to find all the initial GOS analysis of orthologs in three different mammalian species. The gene annotations of BRCA2 gene in humans, chimpanzee and pan troglodytes were retrieved from BLAST [11]. Each sequence of candidate specie was used as a query, to separate the BLAST searches and to form outcomes more authentic and accurate. These sequences help us to find the closer sequences and more distant sequences. After aligning all sequences, repetitive sequences were excluded from the data {Azeem, 2020 #315}.

Phylogenetic analysis of BRCA2 gene

Phylogenetic analysis of BRCA2 geneis was done to find the orthologs and homologs between human BRCA2 and two other mammalian BRCA2 genes. Using the BLAST sequence of BRCA2 genes of mammals in MEGA7, a green tree is constructed. Phylogenetic trees show evolutionary relationship between human and other mammalian species [12]. Characterization of BRCA2 gene in three different mammalian species has done by using Clustal Omega 7. By using Maximum likelihood method, we inferred the evolutionary history of BRCA2 gene in three different species. This method is based on JTT (Jones Taylor Thornton) model that is alternative model for protein alignment. The evolutionary tree is constructed according to scale with branch lengths showing evolutionary history [13].

Result

Identification of BRCA2 gene in Homo sapiens, Pan Troglodytes and Gorilla

The protein sequence of BRCA2 in candidate species was retrieved from NCBI-BLAST search. In BLAST searches, every sequence was considered as a query to obtain outcomes more precise and accurate. Through these retrieved sequences more possible matches are identified. After aligning all these sequences, repetitive sequences were removed. The signature domain of BRCA2 in these BLAST sequences was observed by using an online tool (SMART http://smart.embl-heidelberg.de) and MEME motif that showed the existence of the conserved BRCA2 domains in all candidate species (Figure 1&2).

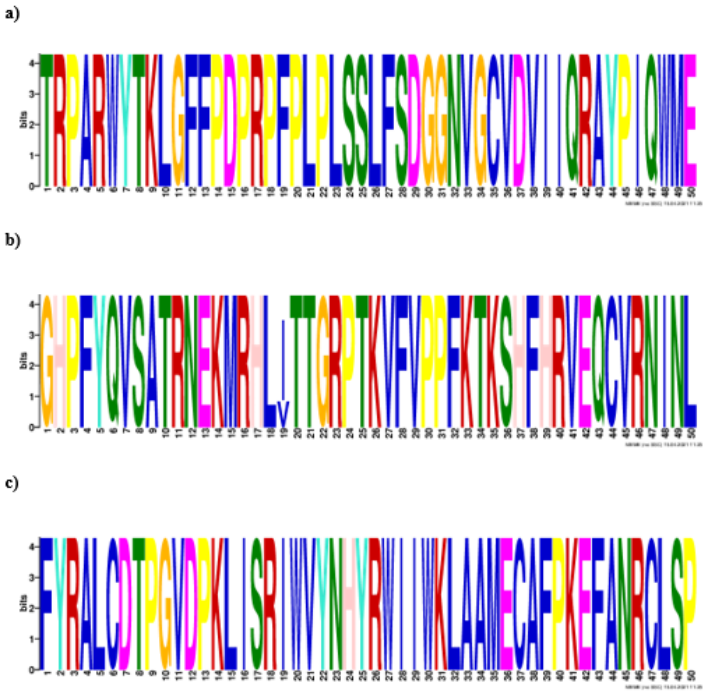

Figure 1: Conserved BRCA2 domains
a) Homo sapiens.
b) Pan troglodytes.
c) Gorilla gorilla.

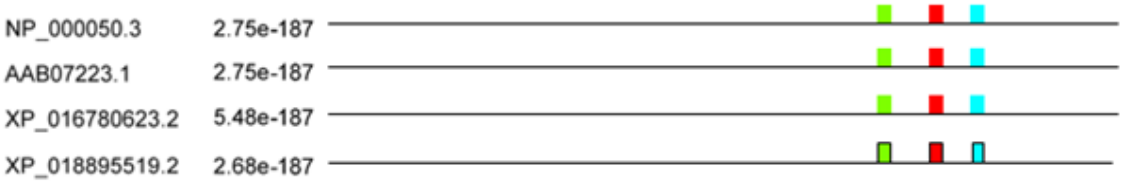

Figure 2: Motif locations of BRCA2 gene with their p-values.

Phylogenetic analysis of BRCA2 gene

The characteristics of the evolutionary relationship between BRCA2 from Homo sapiens, Pan troglodytes and Gorilla gorilla was confirmed by the alignment of gene BRCA2 from all these species by using MEGA 7.0.21 to create rooted tree via the maximum likelihood (ML) method. Gene was found in all three candidate species. The following analyses of conserved motifs and structure of genes reinforced the characterization of BRCA2 in all species [14]. The SMART tool analysis manifested that most of the proteins of BRCA2 genes is conserved in all three candidate species while some additional domains are also present (Figure 3).

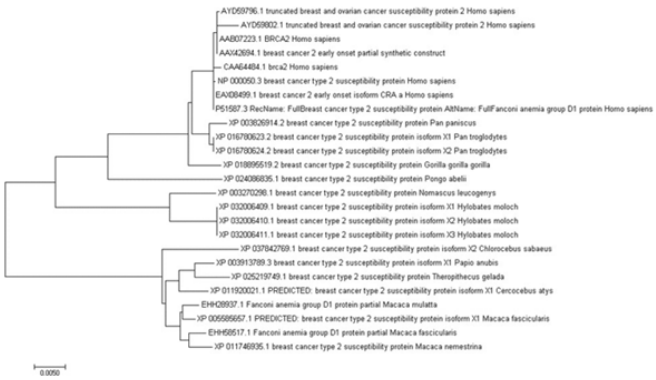

Figure 3: Phylogenetic analysis of 25 species by using MEGA-7.

Gene structure analysis of BRCA2 gene

In evolutionary analysis, the locations of introns/exons in orthologous genes are particularly well-conserved, while in paralogous genes the location of introns and exons is found approximately less conserved. In order to confirm the structural variation of BRCA2 genes, we determined the positions of introns/exons by making comparison of CDS and genomic sequences. Although, there was substantial diversity found in the number and length of exons in all these candidate species including Homo sapiens, Pan troglodytes and Gorilla (Figures 4-6). Although, sequences retrieved at the subfamily level of these three species showed resemblance in gene structures in respect to intron number, chromosome number and exon length. Conserved motifs of BRCA2 proteins were identified and proved useful to determine the protein functions, and the additional motifs of BRCA2 gene were found functional in activating the functions of BRCA2 proteins in humans [15]. On 13 chromosome number BRCA2 gene is present in Homo sapiens. The distribution of BRCA2 gene was found dispersed on 13 chromosomes. The evolution of BRCA2 gene was further observed in all three mammalian species, while gene duplication events were examined for tandem and segmental duplications [16] (Table 1) (Figure 7).

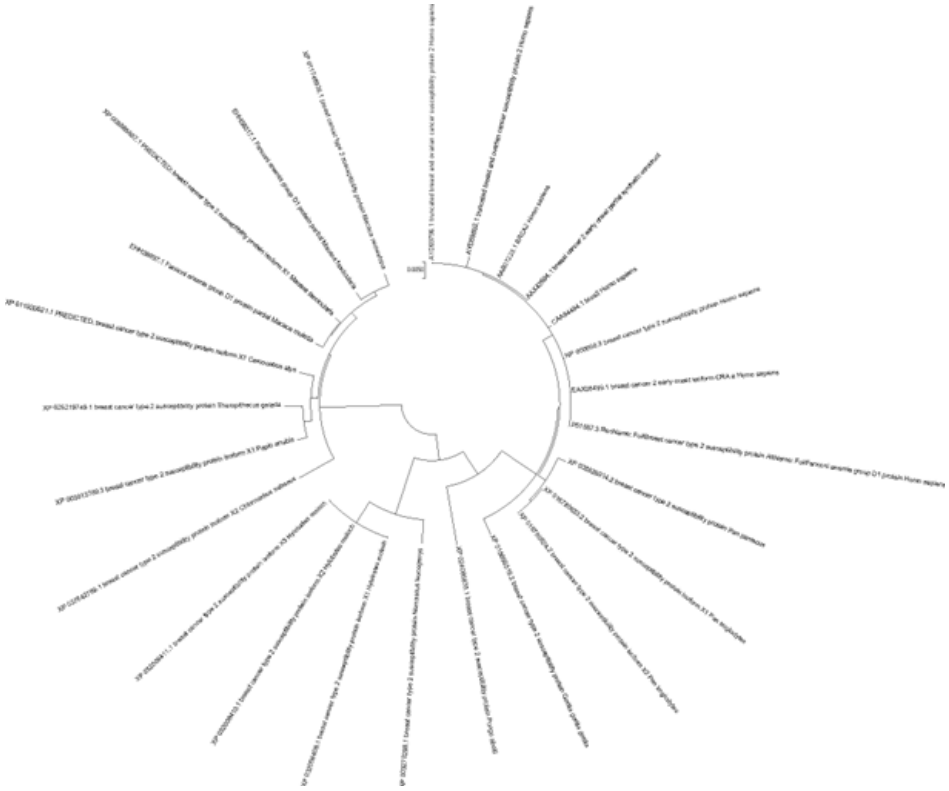

Figure 4: Phylogenetic analysis of 25 species in circular form by using ClustalW 7.

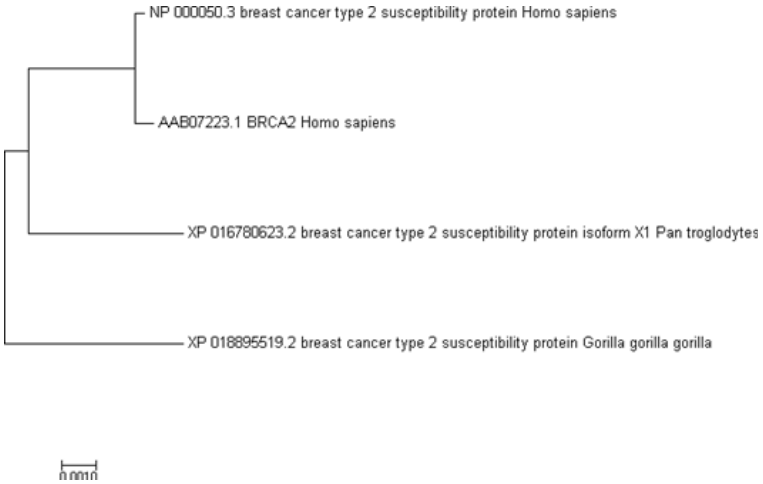

Figure 5: Phylogenetic analysis of candidate species by using MEGA-7.

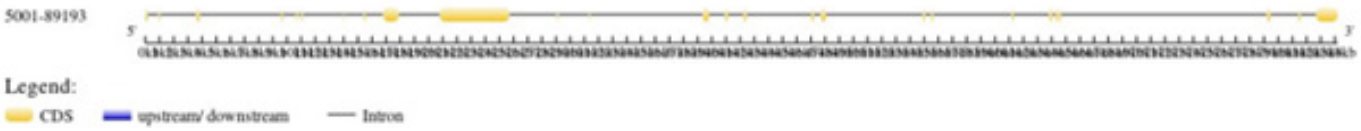

Figure 6: Positions of introns and exons are determined through gene structure display server, introns are marked by black colour while exons are marked by yellow colour.

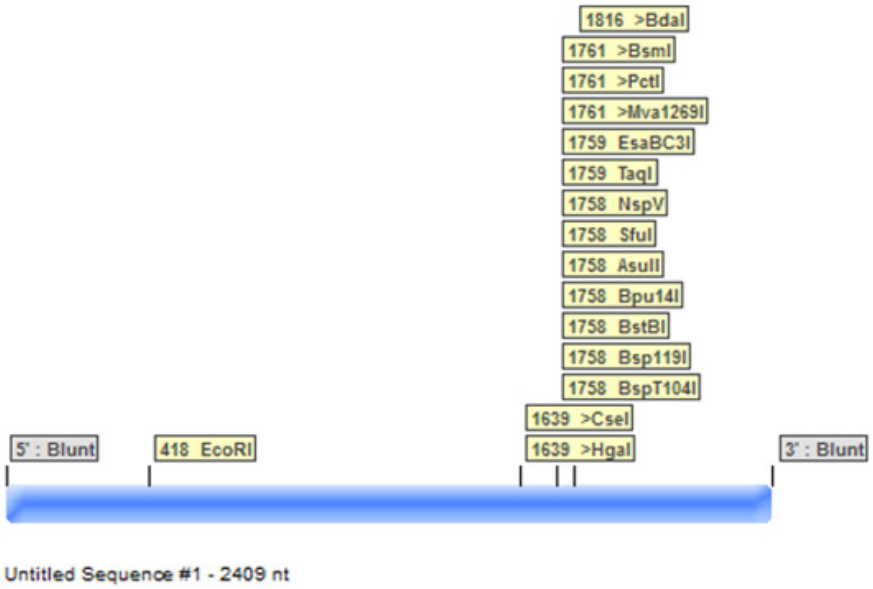

Figure 7: Graphical map of BRCA2 by using serial cloner.

Table 1: Proposed nomenclature and important features of BRCA2gene.

Proposed Names	Gene Locus	Protein Accession #	RNA Accession #	Exon	Chr #	ORF Length	Amino Acid Length	Start of Genomic Location	Conserved Domains in Protein Sequence
BRCA2 in *Homo sapiens*	50	P51587.3	59.4	27	13	10257	3418	Chr13:32	BRCA-2_ helical
BRCA2 *Pan stroglodytes*	16780623	16780623	16925134	29	13	10257	3418	Chr13	BRCA-2_ helical
BRCA2 in *Gorilla*	18895519	18895519	19039974	27	13	10167	3388	Chr13	BRCA-2_ helical

Mapping of BRCA2 gene

By using serial cloner, a graphical map of BRCA2 gene is obtained. By constructing a graphical map sequence length, nucleotides and base pairs are determined. Blunt ends are generated at 5 and 3 end. In serial cloner method protein sequence of BRCA2 in FASTA format is selected and mapping of gene has done. All genes are mapped with their respective restriction sites.

Discussion

This present study shows molecular analysis of BRCA2 gene in humans and animals. It has been observed that the patients who have mutations in BRCA2 gene are at greater risk of developing cancer than the noncarriers [17]. Through current advancement in research scientists are able to edit genes by DNA repair mechanism via knock out and can directly determine the effects of mutation. Mutation carriers present in genome provide valuable information about mechanism of mutation [18]. Different characteristics of mutant genes, sequence retrieved, conserved domains, positions of introns and exons are collectively meaningful to investigate relationship of brca2 gene in mammalian species. Further, by using gene structure analysis exons count from 0 to 27 are examined precisely. In Pan troglodytes and Gorilla (western gorilla) closely related genes are observed because of their similarity in structure of introns and exons that shows their close phylogenetic relationship [19]. Results of phylogenetic tree also depict that humans, chimpanzee and western gorilla share a common ancestor. The reason behind this hypothesis is similarity in their

intron exon structure. On chromosome number 13, large number of BRCA2 genes are found in all three species. That shows there is same chromosomal map for human, chimpanzee and gorilla. Subsequently, random distribution of genes on same chromosome is evidence of variation in genes. That results in an evolutionary process of three species which diverged from one another in their genome [20]. Through phylogenetic analysis, by comparing data in all three candidate species most closely related genes are observed. Hence, it is concluded that BRCA2 genes maintained their function even after divergence in humans, chimpanzee and western gorilla [21,22].

Conclusion

This research showed a high similarity of BRCA2 in structure of intron and exons present in mammalian species. Although, duplication of genes in candidate species is clearly examined. This research study depict the close evolutionary relationship between humans and chimpanzee genes as well as with western gorilla. Through this study the identified relatable genes can be used as informational source to manipulate the mutations in BRCA2 gene that are fatal.

Conflicts of Interest

The author has no conflicts of interest.

Acknowledgment

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