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Original Article

'BRCA1' RESPONSIVENESS TOWARDS BREAST CANCER-A POPULATION-WISE PHARMACOGENOMIC ANALYSIS

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ABSTRACT

Objective: In the present pharmacogenomic work, the genetic, epigenetic and environmental factors associated with BRCA1 induced breast cancer, cancer proneness and its variants across different populations like Indian, Netherland, Belgium, Denmark, Austrian, New Zealand, Sweden, Malaysian and Norwegian and the 'mutation and methylation-prone' region of BRCA1 have been computed.

Methods: The global variations associated with the disease have been identified from the 'Leiden open variation database (LOVD 3.0)' and 'Indian genome variation database (IGVDB)'. The variants, 'single nucleotide polymorphisms (SNPs)' are then characterized. The epigenetic factors associated with breast cancer have been identified from the clinical reports and further scrutinized using EpiGRAPH tool. The various contributing environmental factors responsible for the variations have been considered.

Results: All the variants across different populations such as Indian, Netherland, Belgium, Denmark, Austrian, New Zealand, Sweden, Malaysian and Norwegian are found to be in a specific transcript of BRCA1 that ranges within 41,196,312-41,277,500 (81,189 base pairs) of the chromosome 17. Two 'single nucleotide variations (SNVs)' (5266dupC: rs397507246 and 68_69delAG: rs386833395) have been identified as risk factors in hereditary breast and ovarian cancer syndrome in the global population and 39 SNPs have been identified as pathogenic and deleterious. 'Evolutionary history' seems to be the most significant attribute in the predictability of methylation of BRCA1. Unhealthy dietary habits, obesity, use of unsafe cosmetics, estrogen exposure, 'hormone replacement therapy (HRT)', use of oral contraceptives and smoking are the major environmental risk factors associated with breast cancer incidence.

Conclusion: This chromosome location (41,196,312-41,277,500 (81,189 base pairs)) can be considered as the population-specific sensitive region corresponding to BRCA1 mutation. This supports the fact that stabilization within the region can be a promising technique to control the epigenetic variants associated with the global position. The global variation in the proneness of the disease may be due to a cumulative effect of genetic, epigenetic and environmental factors subject to further experimentations with identical variations and populations.

Keywords: BRCA1, Epigenetic factors, Environmental factors, Mutation, Breast cancer, Population analysis

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INTRODUCTION

Cancer is one of the leading causes of death worldwide, and the incidence rate is increasing day by day according to information gathered from the 'National Health Portal of India.' The most familiar cancer type seen among women in the world is breast cancer [1]. The distribution of annual death rate due to breast cancer across various countries is included in fig. 1 and is worth focusing.

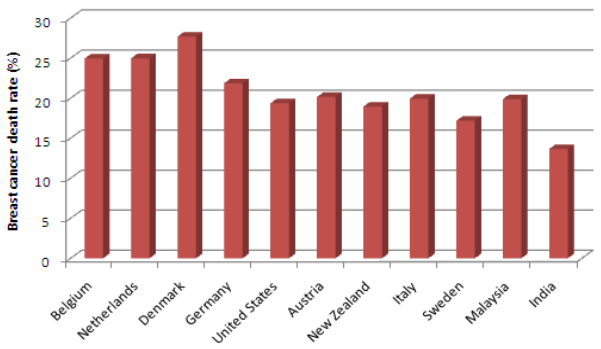

Fig. 1: Annual death rate of breast cancer across different countries [2]

Along with the global population, the Indian population is also prone but comparatively less to breast cancer with an annual death rate of 13.65% [2]. On the basis of a survey performed by 'Indian Council of Medical Research (ICMR)', it has been found that the occurrence of breast cancer in India has doubled during the period of 1982-2005.

BRCA1 is the prominent mutation leading to breast cancer [3]. In normal cells, this gene is involved in DNA repair, transcription regulation, and tumor suppression. The BRCA1 can be spotted at the 21st position of chromosome 17 at the q arm. Mutations in BRCA1 gene are involved in the formation of abnormal BRCA1 proteins that result in fallopian tube cancer, ovarian cancer, breast cancer, prostate cancer, pancreatic cancer, etc. The 'single nucleotide polymorphism (SNP)' has been considered as the major DNA marker, corresponding to the genetic signature of the disease [4-6]. Hence, identification and characterization of SNP corresponding to proneness of breast cancer are helpful in making a global analysis of the situation.

It is prevailing that breast cancer is caused by a blend of genetic, epigenetic and environmental factors [7, 8]. The epigenetic regulation normally comprises of the components, DNA methylation, histone modifications and the existence of miRNA. These mechanisms seem to play a major role in altering the gene expression levels within a living cell. Epigenetic factors can be modified by external as well as internal environmental factors such as obesity, excess dietary fats, heavy metals, estrogen content, alcohol, etc. [8, 9].

In the present manuscript, the genetic, epigenetic and environmental factors associated with BRCA1 induced breast cancer, cancer proneness and its variants across different populations like Indian, Netherland's, Belgium, Denmark, Austrian, New Zealand, Sweden, Malaysian and Norwegian has been provided.

MATERIALS AND METHODS

The frequency of mutation in the BRCA1 gene is identified using 'human genome mutation database (HGMD)' [10]. The variations within the gene observed in different geographical origins have been collected from 'Leiden open variation database (LOVD 3.0)' [11] and the corresponding SNPs from the 'national center for biotechnology information (NCBI)' database [12]. The SNPs have been further characterized using 'sorting intolerant from tolerant (SIFT)' [13] and 'polymorphism phenotyping (POLYPHEN-2)' [14] tools. The CpG island regions in the gene have been identified using 'database of CpG islands and analytical tool (DBCAT)' [15]. The meSNPs, which are the sites for a high probability for methylation, have been located in the CpG islands.

The epigenetic factors associated with breast cancer have been identified from the clinical reports and further scrutinized using 'EpiGRAPH' tool [16]. The proneness of methylation has been predicted using 847 attributes coming in 10 groups, genes, transcriptome, regulatory regions, epigenome-chromatin structure, DNA structure, repetitive DNA, chromosome organization, evolutionary history and population variation. The 'support vector machine (SVM)' based machine learning approach has been used for the predictions. Promising results are obtained while making predictions using linear kernel SVM towards mutability of BRCA1. The global variations associated with the disease have been identified from the LOVD and 'Indian genome variation database (IGVDB)' [17].

RESULTS AND DISCUSSION

According to HGMD, a total of 1464 mutations has been reported in the gene comprising of missense, nonsense, insertion, deletion, duplication and complex rearrangement (fig. 2).

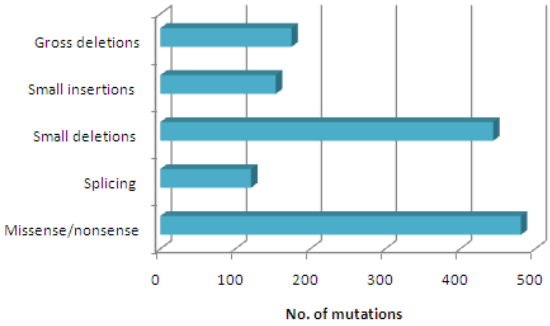

Fig. 2: Significant mutations and corresponding frequencies of BRCA1

The 'single nucleotide variations (SNVs)' and SNPs resulted from the mutations in the BRCA1 gene across the populations have been collected and included in table 1.

Table 1: Some of the variations observed in individuals of different countries

Country	SNVs/SNPs
Netherlands	54G>A, 6G>A, 66dupA (rs80357783), 2393C>T (rs876660005), 2405_2406del, 2477C>A (rs28897683), 2523G>C, 2475delC (rs80357970)
Belgium	2359dupG (rs80357739), 2380dupG, 2393C>T (rs876660005), 2475delC (rs80357970), 2603C>G (rs80356925), 2634A>G (rs730881451), 2649_2650insGGCA
Austria	676del, 1687C>T (rs80356898), 1874_1877dup (rs80357516)
Norway	697_698del, 5407-25T>A, 5096G>A (rs41293459), 3228_3229del, 1556del, 1016dup
Sweden	3048_3052dup
New Zealand	1074C>G, 19-115T>C (rs3765640), 19-134T>C, 1067A>G (rs1799950), 1106_1108del, c.131G>T (rs80357446)
Denmark	5213G>A (rs80357450), 5559C>A (rs80357336), 4862A>G, 3008_3009del, 1486C>T (rs28897676), 5297T>A (rs752476527)
Malaysia	181T>C (rs28897672), 115T>C (rs80357164), 2521C>T (rs1800709), 190T>C (rs80357064), 5057A>G (rs730882166), 5072C>A (rs80357034), 5108A>G (rs876660071)
India	5193+1354G>A (rs8176265), 075-237C>A (rs8176257), 4185+112C>A (rs2070833), 4308T>C (rs1060915)

All the variants are found to be in a specific transcript of BRCA1 that ranges within 41,196,312-41,277,500 (81,189 base pairs) of the chromosome 17. This chromosome location can be considered as the population-specific sensitive region corresponding to BRCA1 mutation. This supports the fact that stabilization within the region can be a promising technique to control the epigenetic variants associated with the global position.

Out of the 1202 pathogenic SNVs of BRCA1, two of them (5266dupC: rs397507246 and 68_69delAG: rs386833395) have been identified as risk factors in hereditary breast and ovarian cancer syndrome in the global population.

The genetic variants (SNPs) of the BRCA1 gene present within this specific 'mutation-prone' region of the global population have been searched out. There are 292 pathogenic SNPs identified within the region from the global population out of which 39 are found to be deleterious (rs28897686, rs80356988, rs80357477, rs80356937, rs80356950, rs80357005, rs80357202, rs80357251, rs55770810, rs28897672, rs28897696, rs41293459, rs41293463, rs45553935, rs55851803, rs80356880, rs80356890, rs80356907, rs80356913, rs80356914, rs80356915, rs80356945, rs80357003, rs80357043, rs80357061, rs80357065, rs80357069, rs80357107, rs80357111, rs80357222, rs80357239, rs80357276, rs80357327, rs80357382, rs80357389, rs80357438, rs80357446, rs80357463, rs80357498). For the Indian population, the SNPs, rs8176265, rs8176257, rs2070833 and rs1060915 have been identified as the genetic signature. These variants are also reported in the same variation-prone region of BRCA1. But none of them has been found to be pathogenic or deleterious in nature.

Nineteen CpG islands have been located in the BRCA1 supporting a high probability for methylation. The CpG islands, its position and the meSNPs of each CpG Island is being provided in table 2.

During the analysis of epigenetic factors using EpiGRAPH, a linear kernel SVM has been recognized as the most efficient platform for the prediction of the proneness of methylation. The mean correlation, sensitivity, and specificity with respect to the above-described attributes have been provided in fig. 3.

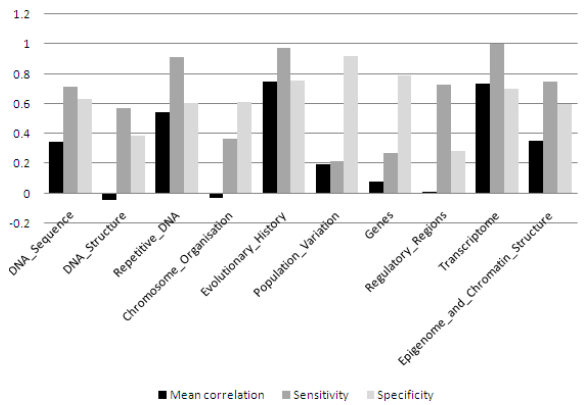

Fig. 3: Factors affecting methylation in BRCA1

Table 2: CpG islands, its position and the meSNPs of each CpG island

S. No.	CpG island starts position	CpG island ends position	meSNPs
1	41196400	41197231	--
2	41200388	41201401	rs80356969, rs80357055, rs80357065, rs80357069, rs80357219, rs80357284, rs80358028, rs80358126, rs397509268
3	41204153	41204416	--
4	41210054	41210306	--
5	41211675	41211895	--
6	41214505	41214837	--
7	41219324	41219829	rs80187739, rs80356879, rs80356890, rs80357043, rs80357061, rs80357204, rs397507239, rs730882165, rs730882166, rs786203754
8	41220109	41220502	--
9	41243717	41243937	rs28897686, rs80356903, rs80357162, rs80357208, rs80357269, rs80357310, rs80357356, rs80357455, rs786203884
10	41249868	41249761	--
11	41249868	41250166	--
12	41253599	41253857	--
13	41254320	41254615	--
14	41256749	41256969	--
15	41263958	41264382	--
16	41268041	41268387	--
17	41269940	41270220	--
18	41271200	41271488	--
19	41273376	41273785	--

The major attributes evolutionary history, repetitive DNA and DNA sequence are associated with DNA methylation and histone modification. The transcriptome includes all types of RNAs including the non-coding/miRNA suggesting the involvement of miRNA in the mutation. The evolutionary history seems to be the most significant attribute in the predictability of methylation of BRCA1. The meSNPs act as genetic signatures in the conserved region of the gene which can be inherited to the next generation.

The epigenetic variations can be evolved by some environmental factors also [18, 19]. So, these factors play a significant role in causing the disease [20, 21].

It is being reported that Asian population has 10% lower incidence of breast cancer while comparing with Western countries probably due to the variation in lifestyle and food habits. Interestingly, the occurrence rate of the disease increases while the Asians migrate to Western countries eliciting the importance of environmental factors in causing breast cancer [1].

Dietary habits, especially foods rich in methionine or folic acid like red meat is found to be having a major role in causing breast cancer [22]. Methionine (or folic acid which is catabolized to methionine) serves to contribute towards the hypermethylation of CpG island of BRCA1 leading to lowering of gene expression [20]. Also, the release of 'heterocyclic amines (HCAs)' during the cooking of red meat also contributes towards the tumor development [23]. An additional cause of breast cancer is the consumption of tinned food or the food kept in plastic containers. Most of these containers may release the endocrine disruptor, bisphenol A (BPA), which mimics the natural cell responses of estradiol [24] leading to the disruption of the normal cell growth.

Consumption of high-fat diet and obesity are two other reasons for breast cancer [25], particularly in post-menopausal women. The estrogen biosynthesis in post-menopausal women predominantly occurs in different sites including adipose tissue of the breast, thighs, etc. leading to the presence of higher amount of estrogen in the mammary tissues than in the circulation [26]. Based on the report of 'World Health Organization (WHO)', the prevalence of obesity in 2014 among females of different countries has been depicted in fig. 4. Vegetarian diet, especially fruits and vegetables rich in antioxidants are found to reduce the risk of breast cancer [27].

Exposure to estrogen by 'hormone replacement therapy (HRT)' is another contributing factor towards breast cancer development [28]. HRT is a treatment given to the post-menopausal women to restore the functions of the female sex hormones. It increases the levels of estrogen within the body. Similarly, oral contraceptives (birth control pills) are also reported to increase the chance of breast cancer [29].

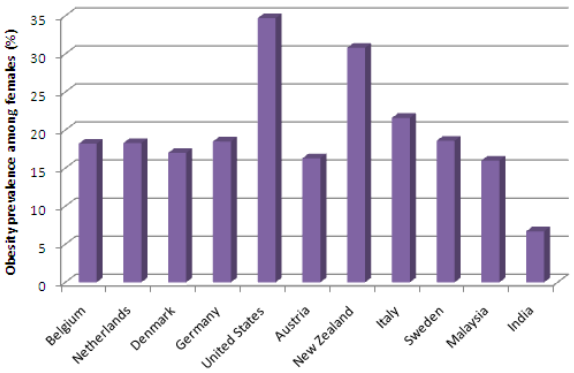

Fig. 4: Prevalence of obesity among females in 2014

The use of cosmetics seems to be another major cause of breast cancer. Most of the cosmetics such as anti-aging creams, shampoos, moisturizers, nail coloring materials, lipstick, etc. contain endocrine disrupting chemicals and hormones. Estrogen is the significant hormone that is being added to anti-aging creams to reduce the wrinkles [30]. A preservative chemical of the class 'parabens' is added to most of the cosmetic products, including moisturizers, shaving creams, gels, hair-care products, etc. Parabens act as weak estrogens leading to cellular proliferation [31]. The phthalates are included in lingering of scents and nail color products. These are endocrine disruptors interacting with the normal balance of hormonal system within the body [32].

Tobacco smoke is found to be associated with increased risk of breast cancer. It is primarily due to the presence of endocrine disruptors, 'polycyclic aromatic hydrocarbons (PAH)' such as dioxins in the tobacco smoke [33].

CONCLUSION

BRCA1 has been identified as the frequently mutated genes in breast cancer. The variations associated with this gene have been identified in different populations. All the population wise variants of the gene

have been found in the 41,196,312-41,277,500 (81,189 base pairs) of the chromosome 17. Thirty-nine pathogenic, deleterious SNPs have been reported in this region. The unique region is also found to be highly susceptible to methylation. Indian population has also reported four variants in the same region. But none of them were either pathogenic or deleterious. From the study, it is found that the SNP variants associated with the gene are frequently modified by epigenetic as well as environmental factors. These epigenetic variations can alter the expression levels of the genes that finally code for the diseases. Unhealthy dietary habits, obesity, use of unsafe cosmetics, estrogen exposure, HRT, use of oral contraceptives and smoking are the major environmental risk factors associated with breast cancer incidence. The reduced genetic and epigenetic susceptibility, healthy food habits, less prevalence of obesity and avoiding smoking reduces the incidence of breast cancer. The global variation in the proneness of the disease may be due to a cumulative effect of all the above factors subject to further experimentations with identical variations and populations.

CONFLICT OF INTERESTS

Declared none

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