Cancer Susceptibility for Male Breast Cancer Assessed by SNP-A Analysis and Risk Alleles of TP53, MDM2, VEGF, VEGFR1, HIF1A and BRCA1

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Abstract

Male Breast Cancer (MBC) has a familial component thus identification of polymorphic risk alleles of candidate genes and/or cytogenetic anomalies may help to predict the risk for the offspring of MBC patients. The conventional metaphase cytogenetics can indicate loci that are hotspots while analysis by single nucleotide polymorphism arrays (SNP-A) can identify chromosomal defects which may play a role in the etiology of cancer. A cumulative genotype risk due to each allele of candidate genes of the signaling pathways regulating c-MYC, HIF1A, TP53 and BRCA1 may be a factor facilitating cancer development. Cancer risk was assessed in a 35-year-old healthy son of a 60-year-old MBC patient with a family history of cancer by metaphase cytogenetics, SNP-A and analysis of 25 polymorphisms in six genes TP53, MDM2, VEGF, VEGFR1, HIF1A and BRCA1. The risk genotype GG-TT of MDM2 309T > G and VEGF-417C/T polymorphisms along with chromosomal instability shown by cytogenetic analysis and SNP-A, rare de novo duplication Yp, deletion in 7q pericentric region indicate an increased risk of cancer in the healthy son of an MBC patient.

Keywords

Breast Cancer, Aneuploidy, Polymorphism, CN-LOH, SNP-A

1. Introduction

A family history of breast cancer confers a relative risk of 2.5 with 20% of men with breast cancer having a positive family history [1]. A first-degree relative of a
cancer patient may have a 2 - 3 fold higher risk of developing cancer at the same site as family members tend to share genetic background along with environment, i.e., food, lifestyle, infections, pollutants [2]. Polymorphisms in both high and low penetrance genes contribute to breast tumorigenesis in combination with exogeneous (diet, pollution) and endogeneous (hormones) factors [3]. A cumulative genotype risk due to each allele may be a factor facilitating cancer development. The oncogenic changes involving non-random gene deletion, amplification or mutation in any oncogene and/or tumor suppressor genes cluster along the signalling pathways that regulate c-MYC, HIF1A and TP53. HIF1A can bind and stabilize p53, also stimulate angiogenesis and induce adaptation to hypoxia whereas p53 mediates hypoxia-induced apoptosis [4]. MDM2 is a negative regulator of p53. HIF1A up-regulates expression of VEGF during hypoxia in the majority of solid tumors [5]. VEGF is a potent mitogen with a vital role in normal physiological and tumor angiogenesis [6]. Angiogenesis also plays a crucial role in BRCA1/BRCA2 breast cancers [7]. VEGF binds to its receptor VEGFR1 (FLT1). Latter regulates VEGF expression as well as mediates migration of monocytes, macrophages and proliferation of endothelial cells [8]. Individuals carrying mutations in BRCA1/2 have a 47% - 55% probability of developing breast cancer [9] [10]. Apart from inherited mutations in BRCA2, in about 4% of patients BRCA1 tends to increase the risk of male breast cancer [11]. HIF1A expression has also been associated with male breast cancer [12].

Single Nucleotide Polymorphisms (SNPs) in the regulatory or coding regions can alter gene expression or affect protein functions and also influence different characteristics among individuals. The functional polymorphisms which alter the gene expression have been reported to be associated with the development of complex diseases like cancer [13]. Single Nucleotide Polymorphism Array (SNP-A) can identify even those chromosomal defects that are not apparent by metaphase cytogenetics, thus complementing the latter [14]. Considering the polygenic component of breast cancer, assessment of known risk-associated variants along with cytogenetic analysis can also be a useful predictor of breast cancer susceptibility.

Male breast cancer frequency is approximately 2% among breast cancer patients in India. The risk of breast cancer in a healthy male offspring of an MBC patient was estimated to be approximately 22% with a 10% chance of identifying a mutation in BRCA1 and BRCA2. In the absence of any specific tool available for assessing breast cancer risk in the Indian population, the pedigree assessment tool, the Ontario family assessment tool, Manchester scoring system available for the USA population were used to estimate the risk (https://www.uspreventiveservicestaskforce.org/apps/). Hence a multidimensional approach was used to estimate genetic risk for cancer in the healthy male offspring of Indian Male Breast Cancer (MBC) patients using classical cytogenetic analysis by G-banding, SNP-A and study of twenty-five variants in six genes (TP53, MDM2, HIF1A, VEGF, VEGFR1 and BRCA1) involved in proliferation, apoptosis, angiogenesis and tumor progression. The SNPs selected in the present study (Table 1)
### Table 1. Detail of BRCA1, TP53, VEGF, HIF1A, MDM2, VEGFR1 variants and reaction conditions used for screening.

| Gene     | Variant                  | Location | Genotyping Method | PCR Product (bp) | Annealing Temperature, MgCl₂ concentration | Restriction enzyme | Restriction digestion pattern | Primers Reference |
|----------|--------------------------|----------|-------------------|------------------|--------------------------------------------|-------------------|-------------------------------|-------------------|
| **BRCA1** | p.Pro871Leu (c.2612C>T) | Exon 10  | PCR-RFLP          | 125              | 59°C, 1.5 mM                               | *Hpa* II          | C allele-99 and 26 T allele-125 | [15]              |
|          | p.Cys64Arg (c.190T>C)   | Exon 5   | PCR-RFLP          | 193              | 59°C, 1.5 mM                               | *Stu* I           | T allele-193 C allele-124 and 69 | [16]              |
|          | 130delT                  | Exon 11  | PCR-RFLP          | 151              | 59°C, 1.5 mM                               | *Dde* I           | Wt-151 Mut-96 and 55           | [17]              |
|          | p.G1738R (g.5331G>A)    | Exon 20  | PCR-RFLP          | 233              | 59°C, 1.5 mM                               | *Bsa* XI          | G allele-112, 91, 30 A allele-233 | [18]              |
| **TP53** | p.P47S                   | Exon 4   | PCR-RFLP          | 201/185          | 59°C, 1 mM                                 | *Msp* I           | S allele-201/185 P allele-156/140, 45 | [19]              |
|          | p.R72P                   | Exon 4   | PCR-RFLP          | 279              | 59°C, 1 mM                                 | *Bst* U           | P allele-279 R allele-160 and 119 | [20]              |
| **PINS Ins 16 bp** | Exon 3 | PCR      | -                 | 61°C, 1 mM      |                                            |                   | A1 allele-119 A2 allele-135    | [21]              |
|          | p.R213R                  | Exon 6   | PCR-RFLP          | 1621             | 59°C, 1.5 mM                               | *Taq* I           | A allele-312, 383 and 926 G allele-695 and 926 | [22]              |
|          | r.13494g>a               | Intron 6 | PCR-RFLP          | 1621             | 59°C, 1.5 mM                               | *Msp* I           | G allele-356, 277, 277, 299, 168, 124 and 120 A allele-633, 299, 277, 168, 124, 120 | [22]              |
| **VEGF** | –2549I/D                 | Promoter | PCR               | 229/211          | 59°C, 1.5 mM                               | -                 | I allele 229 D allele 211      | [23]              |
|          | –2578C/A                 | Promoter | PCR-RFLP          | 459              | 59°C, 1.5 mM                               | *Bgl* II          | C allele-459 A allele-247, 212 | [24]              |
|          | +936C/T                  | Intron 3 | PCR-RFLP          | 207              | 59°C, 1.5 mM                               | *Nla*I II         | C allele-207 T allele-122,85  | [25]              |
|          | –417C/T                  | Promoter | Sequencing        | 486bp            | 62°C, 1.5 mM                               | -                 | -                            | [26]              |
|          | –172C/A                  | Promoter | Sequencing        | -                |                                            | -                 | -                            | [26]              |
|          | –165C/T                  | Promoter | Sequencing        | -                |                                            | -                 | -                            | [26]              |
|          | –160C/T                  | Promoter | Sequencing        | -                |                                            | -                 | -                            | [26]              |
|          | –152G/A                  | Promoter | Sequencing        | -                |                                            | -                 | -                            | [26]              |
|          | –141A/C                  | Promoter | Sequencing        | -                |                                            | -                 | -                            | [26]              |
|          | –116G/A                  | Promoter | Sequencing        | -                |                                            | -                 | -                            | [26]              |
|          | –7C/T                    | Intron 5 | ARMS-PCR           | 59°C, 1.5 mM    |                                            |                  | Control-425 C and T allele-183 | [27]              |
| **HIF1A**| g.C111A                  | Exon 2   | PCR-RFLP          | 187              | 59°C, 1.5 mM                               | *Bgl* II          | C allele-44, 143 A allele-187  | [28]              |
|          | g.C1772T                 | Exon 12  | PCR-RFLP          | 346              | 55°C, 1.5 mM                               | *Hpa* I           | C allele-118, 228 T allele 346 | [28]              |
|          | g.G1790A                 | Exon 12  | PCR-RFLP          | 346              | 55°C, 1.5 mM                               | *Ac* I           | G allele 145, 20, 21 A allele 346 | [28]              |
| **MDM2** | SNP309T>G                | Promoter | PCR-RFLP          | 351              | 59°C, 1.5 mM                               | *Msp* All         | T allele-232, 88, 31 G allele-186, 88, 46, 31 | [29]              |
| **VEGFR1**| –710C/T                  | Promoter | PCR-RFLP          | 665              | 65°C, 1.5 mM                               | *Nla* III         | C allele-665 T allele-520, 145 | [30]              |

ARMS-PCR: Amplification refractory mutation system—Polymerase chain reaction; PCR-RFLP: Polymerase chain reaction—Restriction fragment length polymorphism.
had been previously reported to be related to cancer risk due to their effect on protein expressions or functions.

2. Methods

Case Presentation

The proband was healthy son aged 35 years (IV:2) of a 60 year old male breast cancer (MBC) patient (III:1) (Figure 1(I)). The proband sought information about his own susceptibility to cancer as he also had a positive family history; his grandfather (II:4) and paternal grand uncle (II:3) had died of throat and blood cancer respectively. His father, the MBC patient (III.1, Figure 1(I)) had presented with a growth (2 × 1 cm) in outer upper quadrant of left breast with a history of inflammation on left breast for past two years at the time of diagnosis. The clinical examination and histopathology confirmed infiltrating ductal carcinoma of breast, stage II (ER+, PR+, Her-2neu –ve). After written informed consent, blood samples of both subjects were collected in EDTA and heparin vacutainers. Heparinised blood was used to set up standard 72 hour peripheral blood culture [31]. Cultured cells were G-banded, karyotypes were made and described as per ISCN, 2016 [32]. Genomic DNA was extracted from EDTA anticoagulated blood using standard phenol-chloroform method [33]. Twenty five variants of six genes (TP53, MDM2, HIF1A, VEGF, VEGFR1 and BRCA1) were screened by PCR-RFLP or direct PCR method using previously published primer sequences (Table 1). The samples were also analyzed using Illumina Human Cyto SNP array and data was analyzed using KaryoStudio (v 1.2). This study was approved by the Ethics Committee of Guru Nanak Dev University, Amritsar, Punjab, India.

3. Results

Karyotypic analysis by G-banding showed increased frequency of chromosomal aberrations in MBC patient (79.9%) especially numerical aberrations than proband, his healthy son (73.9%). The proband had low level mosaicism and increased chromosomal instability; he had a monosomy of chromosome 11, numerical and structural anomalies in chromosome 6 along with structural aberrations in chromosome 13, 14, 21, 22, marker chromosomes and ring chromosomes (Table 2). The proband also had copy neutral LOH (CN-LOH) in adrenal hyperplasia associated region on 6p22.3 - 6p21.2, duplication in Yp11.2 and Yp11.3 and deletion in 7q11-21 pericentromeric region (Figures 1(II)-(A)-(D)). In MBC patient, monosomy of chromosome 12, 17, 19, loss of Y and trisomy of 21 was observed. Chromosome 21 was also involved in translocations with chromosomes 1, 14 and 15 (Table 2). The SNP-A of MBC patient showed a loss of Yq11.22.2 and gain in Yq11.22.1 in the azoospermia (AZF) region (Figure 1(III)-(a) and Figure 1(III)-(b)).

Among polymorphic variants analyzed, the MBC patient was homozygous for variant allele of 5 polymorphisms; MDM2 SNP 309T > G and four polymorphisms of VEGF (−2578C/A, −417C/T, −152G/A, −116G/A). The proband was
Figure 1. (I) Family Pedigree; (II) SNP-A analysis showing CN-LOH (a), loss (b) and gains (c) and (d) in proband; (III) SNP-A analysis showing gain (a) and loss (b) in male breast cancer patient.
Table 2. Cytogenetics profile of MBC patient and his healthy son.

| Classical Cytogenetics with GTG banding |  |
|----------------------------------------|------------------|
| **Representative Karyotypes** | **MBC Patient (Father)** |
| 43,Y,-X,-11,-12,-13,-16,+2mar/44,XY,-11,t(13;?)q?,+13 | 45,X,-Y[2]/45,XY,-19/46,XY,-17,+21/45,XY,tas(1;21)(q44q1 |
| -14/45,XY, dic(2;6)(qter-p24;q23-qter), +6, chtb(9)(q2?), -11/45,XY, dic(2;22)(qter | dd(4)(q?), add(7)(q?), +9, +12, +16, -22/84,XXYY, +X, +1,-1, -5, |
| -p13?:p13?:qter)/45,XY, rob(13;14)(q10;q10)/45,XY,-4,+ | +7,-9,-10,-11,-12,-14,-17,+19,-20,+21,-22/Polyploidy[10], |
| Triradials, Polyploidy/46,XY[11] |  |

| SNP-A Profile |  |
|---------------|------------------|
| **Gain** | **Loss** |
| Yp11.2, Yp11.3 | 7q11-21 |
| Yq11.22.1 | Yq11.22.2 |
| CN-LOH |  |
| 6p22.3-6p21.2 | - |

homozygous for variant allele of VEGF -417C/T and MDM2 309T > G polymorphisms. The proband was heterozygous for 8 polymorphisms, three in TP53 (p.R72P, PIN3 Ins 16bp and r.13494g > a) and four in VEGF (−2549I/D, −2578C/A, −152G/A, −116G/A) and one in BRCA1 (p.Pro871Leu). For VEGF +936C/T and HIF1A g.C1772T polymorphism, the MBC patient was heterozygous while his son (proband) had wild type genotype (Table 3).

4. Discussion

Polymorphisms have an important role in promoting susceptibility to diseases as well as the response of the individuals to various drugs [34]. As individual polymorphism may confer a minor increase of disease risk, collectively the common cancer associated SNPs can cause a substantial elevated risk. In current study both MBC patient and his healthy son had RP-GG genotype for TP53 p.R72P and MDM2 309T > G polymorphism. The MDM2 GG genotype has been associated with deficiency in p53 response, being a negative regulator of p53. It is also associated with a significantly reduced age of onset for p53 dependent cancer. The R72 allele has 15-fold greater capacity for inducing apoptosis than P72 allele. Though both PP genotype of TP53 p.R72P and GG genotype of MDM2 309T > G polymorphisms have not been independently associated with overall breast cancer risk [35], they are believed to act as effect modifier instead of being causal [36].

The MBC patient was heterozygous for BRCA1 p.Pro871Leu, TP53 p.R72P, VEGF +936C/T and HIF1A g.C1772T polymorphisms. BRCA1 acts as a transcriptional co-activator and increases p53 dependent transcription from p21 and BAX promoters [37]. BRCA1 has been reported to block VEGF promoter activity by estrogen receptor alpha [38]. An upregulated local concentration of estrogen selectively supports survival and proliferation of breast cancer cells with BRCA1 mutations [39]. The MBC patient was ER + ve, PR + ve and HER-2-neu negative. MBC patients usually have high ER (90%) and PR (81%) positivity and lower Her-2-neu positivity (2% - 15%) [40]. Hormonal, environmental factors
Table 3. Molecular genetic profile of male breast cancer patient and his healthy son.

| Gene       | Variant                        | RefSNP       | Proband | MBC Patient | Functional Relevance                                                                 |
|------------|--------------------------------|--------------|---------|-------------|--------------------------------------------------------------------------------------|
| **BRCA1**  | p.Pro871Leu (c.2612C > T)      | -            | CT      | CT          | Associated with increased BRCA1 expression                                           |
|            | p.Cys64Arg (c.190T > C)        | -            | TT      | TT          | Prevents BRCA1-BARD1 binding                                                         |
|            | 130delT                        | -            | WT      | WT          | Premature stop codon at AA residue 409                                               |
|            | p.G1738R (g.5331G > A)         | -            | GG      | GG          | Destabilizes protein folding                                                         |
| **TP53**   | p.R72P                         | rs1042522    | RP      | RP          | Plays role in apoptosis                                                               |
|            | PIN3 Ins 16bp                  | rs17878362   | A1A2    | A1A1        | Affects function and expression of p53                                               |
|            | p.P47S                         | rs1800371    | PP      | PP          | Plays role in apoptosis                                                               |
|            | p.R213R                        | rs1800372    | AA      | AA          | Role in activity of protein                                                          |
|            | r.13494g > a                   | rs1625895    | GA      | GG          | Affects function and expression of p53                                               |
| **VEGF**   | −2549I/D                       | rs35569394   | ID      | II          | D allele associated with increased transcriptional activity                          |
|            | −2578C/A                       | rs699947     | CA      | AA          | Alters the binding of GATA-2 transcription factor                                     |
|            | +936C/T                        | rs3025039    | CC      | CT          | Alters binding of transcription factor activating enhancer binding protein           |
|            | −417C/T                        | rs833062     | TT      | TT          | -                                                                                   |
|            | −172C/A                        | rs59260042   | CC      | CC          | Associated with ↑ VEGF m-RNA level                                                  |
|            | −165C/T                        | rs79469752   | CC      | CC          | -                                                                                   |
|            | −160C/T                        | -            | CC      | CC          | -                                                                                   |
|            | −152G/A                        | rs13207351   | GA      | AA          | -                                                                                   |
|            | −141A/C                        | rs28357093   | AA      | AA          | -                                                                                   |
|            | −116G/A                        | rs1570360    | GA      | AA          | A allele associated with reduced plasma VEGF level                                   |
|            | −7C/T                          | rs25648      | CC      | CC          | -                                                                                   |
| **HIF1A**  | g.C1772T                       | rs11549465   | CC      | CT          | Associated with higher transcriptional activities and enhanced angiogenesis          |
|            | g.G1790A                       | rs11549467   | GG      | GG          | -                                                                                   |
|            | g.C111A                        | -            | CC      | CC          | -                                                                                   |
| **MDM2**   | SNP309T > G                    | rs2279744    | GG      | GG          | Associated with enhanced MDM2 expression and attenuates function of TP53 protein     |
| **VEGFR1 (FLT1)** | −710C/T | - | CC | CC | - |

can be a cause of breast cancer as Polycyclic Aromatic Hydrocarbons (PAHs), benzo(a)pyrene have been found to reduce BRCA1 mRNA levels in MCF-7 human breast cells [41]. The MBC patient and his son were agriculturists. Former had a self reported long term exposure to agricultural chemicals especially chemical fertilizers and pesticides like organochlorines which are known carcinogens and tumor promoters.

Chromosomal translocations are considered non-random and may lead to cancer by formation of oncogenic fusion proteins or activation of oncogenes by a promoter or enhancer [42]. The MBC patient had translocations involving chromosome 21 as well as copy number changes in 21. Chromosome 21 harbors
genes \textit{RUNX1} and \textit{DYRK1A} which have been implicated in tumorigenesis [43] [44]. Translocations have been reported to be influenced by spatial position of broken loci, recombinations or DNA repair elements [42] [45] [46]. In proband, chromosome 13 was involved in translocations and copy number changes. Chromosome 13 harbors two well known genes \textit{BRCA2} and \textit{RB} involved in tumorigenesis. Chromosome 6 showed copy number changes, was involved in translocations, formed a dicentric and also had copy neutral loss of heterozygosity (CN-LOH). The LOH region harbours various genes like \textit{ALDH5A1}, \textit{HFE}, \textit{CDSN}, \textit{NEU1}, \textit{C2}, \textit{SKIV2L}, \textit{CYP21A2}, \textit{TNXB}, \textit{TAP2}, \textit{TAP1}, \textit{TAPBP}, \textit{PSMB8}, \textit{COL11A2}, \textit{TULP1} and \textit{PNPLA1}, some of which have role in proliferation.

Many chromosomal regions showing uniparental disomy (UPD) are consistent and specific for tumor types and appear more frequently in solid tumors than leukemia [47]. UPD has been previously reported in breast cancer [48] [49] and other tumors. Mutated genes in UPD have been considered indicative of patient outcome with implications in response to chemotherapy. Constitutional UPD is associated with meiotic errors, resulting in developmental diseases, however, it can also be observed in healthy controls, probably because of early mitotic errors and autozygosity [50]. The CN-LOH at 6p22.3-p21.2 observed by SNP-A analysis in proband has been previously reported in patients of developmental delay as well as in healthy controls [14]. Loss of 6p22.3 has been associated with developmental delays and autism spectrum disorders with a possible haploinsufficiency of \textit{ATXN1} [51] [52]. Acquired UPD are common in both hematologic and solid tumors constituting 20% - 80% of LOH seen in human cancers [53] [54]. In myeloid malignancies CN-LOH has been associated with loss of normal allele of \textit{JAK2}, \textit{MPL}, \textit{cKIT} and \textit{FLI3} along with duplications of oncogenic mutations [14]. CN-LOH 6p22.1 and loss of chromosome 9, 15, 18 have been observed in tumor tissue of tubulocystic renal cell carcinoma [55]. The adrenal hyperplasia region on 6p21.3 also has gene for 21-hydroxylase enzyme which controls cortisol synthesis. The cortisol synthesis pathway shares steps with aldosterone, androgens and estradiol synthesis pathways. The 6p22.3-6p21.2 region harbours many genes like \textit{HFE}, \textit{EHMT2}, \textit{CLIC1}, \textit{DAXX}, \textit{DDR1}, \textit{E2F3}, \textit{ID4}, \textit{BAK1}, \textit{HLA-G}, \textit{IER3}, \textit{LTA}, \textit{MAPK14} with role as channel proteins or in apoptotic pathways [56]. Genetic variants in the region 6p21.1 - p22.3 along with \textit{VEGFA} and \textit{CDKAL1} have also been associated with type 2 diabetes [57]. Hypoandrogenism, liver disease causing hyperestrogenism, gynaecomastia, obesity and alcohol intake are among possible risk factors for breast cancer apart from family history [58]. The MBC patient did have a past history of alcohol consumption but the proband had no past history of alcohol intake or smoking.

The copy number variations, deletions and duplications in Y chromosome observed were different in both father and son. In MBC patient, the azoospermia factor region on Yq showed both gain and loss. The \textit{AZF} region has genes involved in germ cell differentiation and spermatogenesis. Though they are not directly linked to breast cancer yet, mosaic deletions in these genes have been pro-
posed as risk marker for non-Hodgkins lymphoma and testicular cancers. Postzygotic AZF deletions occur during early embryogenesis and may precede appearance of a testicular tumor by many years [59]. In the MBC patient, a complete loss of Y chromosome was also observed in karyotypic analysis of some metaphases. A study on colorectal and prostate cancer patients had found a strong association between the mosaic loss of Y chromosome in peripheral blood and carcinogenesis in males [60].

The son did not inherit the Yq deletions observed in his father but had a de novo duplication of Yp11.2-11.3. This duplication, a rare event, has been reported as a constitutional duplication of a portion of Yp in two brothers of Italian descent, one with B cell lymphoma and his healthy sibling, presumed to be inherited from their father [61]. Phylogenetic sequence comparisons show that duplications of the human Yp11.2/Yp11.1 region were already present in the macaque-human ancestor as multiple paralogs located predominantly in subtelomeric regions [62]. The short arm of the human Y chromosome harbor various genes viz. TSPY1, TSPY2, TSPY3, TSPY4, TSPY8, TBL1Y, TGIF2LY, AMELY, SRY, ZFY, etc. Among them variant expression of TSPY, a protooncogene, has been observed in gonadoblastoma [63], germ cell neoplasia, liver cancer, melanoma and prostate cancer along with another male specific gene TGIF2LY, found to be upregulated in liver cancer [64]. Thus, in the proband the duplication of the Yp region may lead to ectopic expression of genes influencing the development and progression of cancer.

Y chromosome loss and rearrangements have been associated with different types of cancer, such as bladder cancer [65], male sex cord stroma tumors [66], lung cancer [67] and esophageal carcinoma [68]. In a previous report, in an infertile man with severe oligoasthenoteratozoospermia, an approximate 2.4 Mb inherited duplication region in Yp11.2 and a de novo partial AZFb deletion (which spanned 5.25 Mb including eight protein coding genes and four non-coding transcripts) was observed which did not remove the RBMY gene family [69]. The proband in our study with Yp duplication was fertile and had three offsprings (Figure 1(I)).

The proband also had deletion in 7q11-21 region. The pericentromeric region of chromosome 7q contains intrachromosomal segmental duplications that give rise to recurrent constitutional genomic rearrangements. A majority of patients with Williams-Beuren syndrome (WBS) have a micro-deletion of about 1.5 Mb from chromosome 7q11. Also, deletions flanking the typical breakpoints of the WBS microdeletion in two genes, NCF1 and GTF2IRD2 have shown reduced expression levels, presumably because their transcription control elements are affected by the deletion [70].

Monosomy of chromosome 11 observed in proband is among the chromosome anomalies frequently observed in bladder cancer. The numerical aberrations of chromosome 11 in which Cyclin-D is located, deletion of 11p and also mutations of the H-RAS located on 11p might reflect alterations implicated in the genesis and pro-
gression of bladder cancer. Translocations of genetic material between chromosome 11 and other chromosomes have been associated with leukemias and lymphomas [71]. The cytogenetic analysis of malignant primitive neuroectodermal SK-PN-DW tumor cell line have revealed several chromosomal rearrangements like translocations involving chromosomes 1, 7, 11, 17, 22 and loss of chromosomes Y, 11, 13 and 18 [72]. Aneusomy of chromosome 1, 11, or 17 has been correlated significantly with some clinicopathologic features of breast tumors, such as lymph node status, histologic grade, or ER and PR status, indicating that chromosomal aneusomy can be a new biologic marker of breast carcinoma [73] [74].

5. Conclusion

In the proband, the risk genotype GG-TT of MDM2 309T > G and VEGF-417C/T polymorphisms along with chromosomal instability shown by cytogenetic analysis and SNP-A, rare de novo duplication Yp, deletion in 7q pericentromeric region indicate an increased risk in the proband (the son of MBC patient). Some of the additional anomalies observed in SNP-A analysis have not been reported in male breast cancer earlier. The results add to the database of anomalies associated with MBC and can have utility in counselling the relatives of MBC patients.

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Ethical Approval

All the study subjects gave their written consent to participate in the study. This study was approved by the Institutional Ethics Committee of Guru Nanak Dev University, Amritsar, Punjab, India.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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