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ORIGINAL PAPER

Frequency of pathogenic germline mutations in cancer susceptibility genes in breast cancer patients

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Abstract

In this study, we evaluated the incidence of pathogenic germline mutations in 30 breast cancer susceptibility genes in breast cancer patients. Our aim was to understand the involvement of the inherited mutations in these genes in a breast cancer cohort. Two hundred ninety-six female breast cancer patients including 4.5% of familial breast cancer cases were included in the study. 200 ng of genomic DNA was used to evaluate the pathogenic mutations, detected using Global Screening Array (GSA) microchip (Illumina Inc.) according to the manufacturer's instructions. The pathogenic frameshift and nonsense mutations were observed in BRCA2 (10.9%), MLH1 (58.6%), MTHFR (50%), MSH2 (14.2%), and CYTB (52%) genes. Familial breast cancer patients (4.5%) had variations in BRCA2, MLH1, MSH2, and CYTB genes. 28% of patients with metastasis, recurrence, and death harbored mono/biallelic alterations in MSH2, MLH1, and BRCA2 genes. The results of this study can guide to develop a panel to test the breast cancer patients for pathogenic mutations, from Malwa region of Punjab. The screening of MSH2, MLH1, and BRCA2 should be carried in individuals with or without family history of breast cancer as these genes have been reported to increase the cancer risk by tenfold.

Keywords Breast cancer · Alterations · Mutations · Frameshift · Nonsense

Introduction

Breast cancer is the most common tumor malignancy among women. Genetic and non-genetic factors have been reported to be involved in the etiology of the breast cancer [1]. Genetic factors play a crucial role in augmenting the risk in the individuals with a family history of breast cancer via some inherited genetic mutations [2]. Familial breast cancer has been reported in 10% of cases, whereas the rest are all sporadic [3]. The mutations in high penetrance genes like Breast cancer 1 (BRCA1), breast cancer 2 (BRCA2), phosphatase and tensin homolog (PTEN), tumor protein 53 (TP53) and serine/threonine kinase 11 (STK11), moderate penetrant genes including ataxia-telangiectasia mutated (ATM), checkpoint kinase 2 (CHEK2), partner and localizer of BRCA2 (PALB2), BRCA1 interacting protein C-terminal helicase1 (BRIP1) and 19 low penetrance genes including TOX high mobility group box family member 3 (TOX3), estrogen receptor 1 (ESR1), etc., are implicated in the pathogenesis of the disease [2]. All these genes play a vital role in various cell signaling pathways as the cross-talk between these proteins maintains a delicate balance for proper functioning of the cellular machinery. If one of these signaling molecules becomes non-functional, this balance gets disturbed and thereby assists in the progression of carcinogenesis [1].

The national average cancer prevalence in India has been reported to be 800/million/year [4]. The Malwa region of Punjab in India grabbed national attention a couple of years ago, when its steeply rising cancer graph came into picture [5]. Breast cancer has been reported to be the second most common cancer after lung cancer in this region [6]. The cancer prevalence (per million/year) in this region (reported by Department of Health & Family Welfare in 2013 is 1089/million/year) is higher in comparison with other two regions, i.e., Majha (647/million/year) and

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Doaba (881/million/year) of the state [7]. The mutation spectrum in breast cancer patients from this region has not been evaluated till date. In this study, we evaluated the incidence of pathogenic germline mutations in 30 breast cancer susceptibility genes in sporadic and familial breast cancer patients.

Materials and methods

Two hundred and ninety-six female breast cancer patients diagnosed at Guru Gobind Singh Medical College and Hospital, Faridkot (the main referral center for this region), Punjab, were included in the study. Ethical clearance was taken from study hospital as well as Central University of Punjab, Bathinda. The diagnosis was carried out by fine needle aspiration, cytology, mammography, and histopathology. Patients with major cardiac, renal, skeletal disorders, hepatic, neurological disorders, and other cancers were excluded from this study. Information on demographic features and risk factors was collected using a structured questionnaire.

DNA isolation and genotyping

Two milliliter of peripheral blood was collected in EDTA-coated vacutainers with the written informed consent of the patients. Isolation of genomic DNA was performed using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Qualitative and quantitative analysis of DNA was carried by gel electrophoresis and Nano-Drop ND-1000 UV–Vis Spectrophotometer (Thermo Scientific), respectively. Genotyping was performed on Illumina Infinium HD assay platform using Global Screening Array (GSA) microchip (Illumina Inc.) according to the manufacturer's instructions with 200 ng of genomic DNA. GSA microchip contains > 700,000 up-to-date markers, optimized for human genome-wide backbone for unparalleled genomic coverage, including clinically relevant content and all pharma GKB markers. Subsequent sample processing and array hybridization were performed according to the manufacturer's instructions (Illumina). Genome-Studio (Illumina, Inc.) was used for data pre-processing and analysis. Genotypes were called within Genome-Studio with the Gen Call algorithm of Genotyping Module v1.0. The final sample call rate was 99.99%. The data were subsequently exported to R/Bioconductor to calculate X^2 and odds ratio. Annotation was performed using ClinVar, 1000 Genomes, ExAC, Cosmic, and dbSNP databases. The p value $P \leq 5 \times 10^{-8}$ was considered statistically significant.

Results

Demographic profile

Two hundred ninety-six female breast cancer patients from Malwa region of Punjab were included in the study. The average age of patients at the time of disease diagnosis was found to be 53.62 ± 12.31 years. The youngest patient with breast cancer was 23 years old. More than half of the patients (55%) were residents of urban areas. None of the patients was pregnant at the time of diagnosis. Obesity was observed in 44.9% of patients. Since Punjab is a leading grain producer in India with maximum use of pesticides, 48% had an exposure to pesticides (Table 1). Left breast carcinoma was observed in 55% of cases, whereas in the remaining cases the right breast was affected. Lumpectomy and modified radical mastectomy (MRM) was performed in 5 and 63% of patients, respectively. All the patients underwent adjuvant chemotherapy (Cyclophosphamide and doxorubicin).

Prevalence of breast cancer subtypes

Infiltrating ductal carcinoma, histopathological subtype of breast cancer was most prevalent among patients followed by infiltrating lobular carcinoma, in situ lobular, and ductal carcinoma. ER/PR-positive subtype was observed in 37.6%. 17.5% of patients were suffering from triple-negative breast cancer which is considered as the most aggressive type of the breast cancer. Most of the breast cancer cases had Grade 2 (69%) followed by Grade 3 (27%). However, none of the patients was found to be in Grade 1.

Table 1 Demographic profile of breast cancer patients

Personal data	Item	Number	%
Gender	Female	296	100
Menopausal status	Premenopausal	101	34.12
	Postmenopausal	184	62.16
	Hysterectomy	11	3.71
Parity	Parent	286	96.62
	Nulliparous	10	3.37
Weight	Obese	133	44.9
	Normal	140	47.2
	Underweight	23	7.77
Family history	Breast cancer	13	4.5
	Other cancers	22	7.5
Pesticide exposure	Exposed	138	46.6

Global screening analysis

The breast cancer patients were screened for germline mutations using GSA. Pathogenic mutations were found in fifteen genes including ATM, BRCA2, STK11, PTEN, BRIP1, CHEK2, RAD51C, NBN, MLH1, MSH2, MSH6, MUTYH, CDK2NA, and MTHFR. The alterations were either synonymous or non-synonymous and were found to be pathogenic in the dbSNP repository. The most common variations among patients were nonsense and missense.

Frequency of genomic mutation burden

Among 283 patients, 10.9% (31) had mono/biallelic variations in BRCA2 gene. Out of these, 11 patients had a biallelic nonsense mutation (rs80358815) in this gene. In addition to this nonsense mutation, 9 patients were found to be monoallelic for one or other BRCA2 mutations. The remaining 20 non-carriers of rs80358815 were found to bear other BRCA2 alterations in monoallelic condition (Table 2). Further, 80.9% of patients were mono/biallelic for non-BRCA2 genes including MUTYH, CYTB, BRIP1, MTHFR, NBN, PTEN, ATM, CDK2NA, RAD51C, STK11, MLH1, MSH2, MSH6, and CHEK2. Majority (58.6%) of the patients had either monoallelic (44.4%) or biallelic (14.2%) variation in MLH1 gene (rs1800734), whereas 44.4% of patients exhibited at least one monoallelic variation in this gene. Monoallelic variations in both MSH2 and ATM genes were observed in 17.8% patients. BRIP1 gene carried monoallelic variation for rs587780875 in 10.7% of patients. In addition, 52% patients were mono/biallelic for MSH6, PTEN, CHEK2, NBN, MUTYH, STK11, RAD51C, MTHFR, and CYTB genes. The details have been summarized in Table 2. Out of 283 patients, 8.2% of patients harbored mono/biallelic alterations in both BRCA2 and non-BRCA2 genes (Table 2).

Death, recurrence, and metastasis were observed in 28% of patients included in the study. Most of these patients were found to bear at least one or the other monoallelic/biallelic variation in MLH1, MSH2, and BRCA2 genes. None of the patients was mutated for other known breast cancer susceptibility genes including BRCA1, PMS2, EPCAM, APC, BAP1, MITF, TP53, CDH1, BAPR1A, SMAD4, GREM1, POLD1, POLE, PALB2, and RAD51D.

Prevalence of variations by age and receptor subtypes

The patients ($n=31$; 10.9%) bearing BRCA2 mutations were found to fall in the age group of 50–56 years. Out of these, 26 patients were negative for ER/PR, whereas the remaining five were found to be positive for the receptor status. The patients exhibiting CHEK2 gene variations were in the age group of 49–71 years. Majority of these except for 20 (7.1%) were ER/PR positive. MLH1 gene variations were observed in ER/PR-positive as well as ER/PR-negative patients. Individuals with alterations in MLH1 were found to be in the age group of 50–73 years. Variations in MUTYH, PTEN, ATM, MTHFR, CYTB, RAD51C, and STK11 genes were observed in the age group of 36–84 years.

Mutations among familial breast cancer patients

Family history of cancer including breast and another type of cancers was observed in 13 (4.5%) and 22 (7.5%) patients, respectively. Thirteen patients with family history of breast cancer belonged to nine families (Fig. 1). Out of these, we were able to collect blood samples from affected individuals belonging to only two families designated as F1 and F2.

In F1 family, both the affected sisters were found to bear mutations in MLH1 (biallelic) and MSH2 (monoallelic) genes. The sister carrying with MLH1 gene alteration was also suffering from ovarian cancer earlier (A in Fig. 1). In F2 family, both the affected sisters were found to bear common biallelic mutations in BARD1 (rs587782681) and CYTB (rs527236043). The rest of the families designated as C-I (Fig. 1) had only one affected individual alive, whereas the other affected individual in the family has already expired. Therefore, we were able to collect samples only from alive affected individuals belonging to these families. All these were found to be monoallelic for one or the other variant of MLH1, MSH2, or BRCA2 genes (Fig. 1).

Discussion

In the current study, we carried out a detailed analysis of variation in 30 genes, implicated in the pathogenesis of breast cancer among 296 patients, including 4.5% familial breast cancer patients from Malwa region of Punjab. Breast cancer is widely feared in this region of Punjab. The genes were screened using GSA (Illumina Inc.) which contains around 7 lakh biomarkers including breast cancer susceptibility genes. Although few studies have been carried in India on the genetic susceptibility of breast cancer, the present study is the first one from India exploring most of the breast cancer susceptibility genes in the patients affected with the disease.

Among 30 breast cancer susceptibility genes evaluated, the highest number of pathogenic, monoallelic frameshift, and nonsense variants were observed in BRCA2 gene. Mono/biallelic variants were observed in BRCA2 gene in 10.9% patients. BRCA2 gene variants were also common in patients (4 families) with the family history of the disease (Fig. 1C, D, and H). BRCA2 has been implicated in the pathogenesis of familial breast cancer in a large

Table 2 Frequency of pathogenic mutations in breast cancer patients

S. no	Gene	AA change	Heterozygous mutated %	Homozygous mutated %	Nature	Type of mutation
1	ATM	Intronic	14.8		Pathogenic	Intronic
		K1646N	11.5		Pathogenic	Frameshift
		F1209L	3.7		Pathogenic	Frameshift
		L1542F	7.4		Pathogenic	Frameshift
2	BRCA2	Y839Ter	2.7		Pathogenic	Nonsense
		S1955Ter		3.7	Pathogenic	Nonsense
		R2520Ter	3.7		Pathogenic	Nonsense
		L2972Ter	3.7		Pathogenic	Nonsense
		I591M	3.7		Pathogenic	Frameshift
		G602E	3.7		Pathogenic	Frameshift
		S552P	3.7		Pathogenic	Frameshift
		K585R	3.7		Pathogenic	Frameshift
		E1397K	3.7		Pathogenic	Frameshift
		N1626S	7.6		Pathogenic	Frameshift
		W1692N	3.7		Pathogenic	Frameshift
		I1874R	3.7		Pathogenic	Frameshift
		S1943L	3.7		Pathogenic	Frameshift
		Q2009A	3.7		Pathogenic	Frameshift
		Q2157I	3.7		Pathogenic	Frameshift
		Y2215Ter	3.7		Pathogenic	Frameshift
		C311F	3.7		Pathogenic	Frameshift
		E2918E	3.7		Pathogenic	Synonymous
		T1647S	3.7		Pathogenic	Frameshift
		C2212L	3.7		Pathogenic	Frameshift
		K1860Ter	7.6		Pathogenic	Nonsense
		L1732P	3.7		Pathogenic	Frameshift
		Q2530K	3.7		Pathogenic	Frameshift
		A1922C	7.4		Pathogenic	Frameshift
3	BRIP1	Y461Y	10.7		Pathogenic	Synonymous
4	CDKN2A	G101R	3.7		Pathogenic	Missense
		Intronic	3.7		Pathogenic	Intronic
5	CHEK2	R224H	3.7		Pathogenic	Missense
		Intronic	3.7		Pathogenic	Splice variant
		Intronic	22.2	3.7	Pathogenic	Intronic
6	MLH1	Intronic	44.4	14.2	Pathogenic	5′utr variant
		S131Ter	3.7		Pathogenic	Nonsense
		F80V	3.7		Pathogenic	Missense
		L658F	3.7		Pathogenic	Frameshift
		P495R	3.7		Pathogenic	Frameshift
		Y625Ter	3.7		Pathogenic	Nonsense
		K70I	4.0		Pathogenic	cds indel
		I229D	3.7		Pathogenic	Frameshift
		D484M	3.7		Pathogenic	Frameshift
		E78K	3.7		Pathogenic	Frameshift
		E71I	11.1		Pathogenic	Frameshift

Table 2 (continued)

S. no	Gene	AA change	Heterozygous mutated %	Homozygous mutated %	Nature	Type of mutation
7	MSH2	L595Y	4.1		Pathogenic	Frameshift
		Intronic	14.1		Pathogenic	Splice variant
		Intronic	3		Pathogenic	Splice variant
		E177V	4.15		Pathogenic	Frameshift
		E48Ter	3.7		Pathogenic	Nonsense
		Q215Ter	4.3		Pathogenic	Nonsense
		Y43Ter	3.7		Pathogenic	Nonsense
8	MSH6	K545R	5.8		Pathogenic	Frameshift
		V907R	5.7		Pathogenic	Frameshift
		G237D	6.0		Pathogenic	Frameshift
		L1330V	6.2		Pathogenic	Frameshift
9	MUTYH	L406M	5.9		Pathogenic	Missense
		A371P	6.4		Pathogenic	Frameshift
10	NBN	F20L	6.0		Pathogenic	Frameshift
11	PTEN	Y68N	7.6		Pathogenic	Frameshift
		Y65D	3.7		Pathogenic	Missense
12	RAD51C	F32S	6.2		Pathogenic	Frameshift
13	STK11	Intronic	3.7		Pathogenic	splice variant
14	MTHFR	A263V	37.5	12.5	Pathogenic	Missense
15	CYTB	G99G		52	Pathogenic	Synonymous

number of studies [8]. Germline BRCA1 or BRCA2 mutations account for 20–40% of breast cancer that clusters in families and < 5% of overall breast cancer. These are also associated with a high lifetime risk of up to 60–85% for breast cancer as well as an increased risk for ovarian cancer [9]. Lahad et al. reported that BRCA2 carriers are at breast cancer risk of 84% and an ovarian cancer risk of 27% [10]. BRCA2 maintains the genome stability by homologous recombination or by interacting with various other proteins including RAD51, DSS1, RPA, MRE11, PALB2, and p53. BRCA2 is involved in centrosome duplication specifically in the metaphase to anaphase transition. Inactivation or depletion of BRCA2 leads to centrosome amplification that results in unequal separation of chromosomes. BRCA2 localizes with central spindle and mid-body during telophase and cytokinesis [11]. It is a caretaker gene and mutation in this gene leads to the disruption of other downstream genes. In the present study, five nonsense mutations were observed in BRCA2 gene in 7.1% patients. These mutations were nonsense and therefore likely to result in non-functional truncated protein. Pathogenic frameshift alterations in BRCA2 gene were found at a frequency of 10.2% in the diseased patients. BRCA2 has emerged as a significant susceptibility gene in sporadic as well as familial breast cancer patients from Malwa region of Punjab, and therefore, the patients and their first-degree relatives with or without family history of breast cancer must be screened for BRCA2 variants.

However, surprisingly we did not find any individuals with BRCA1 mutations which is in contradiction with a study carried out in a South-Indian population [12]. This might be on account of the fact that the genetic architecture of north and south Indian is different. Our study is in consensus with a previous study carried out in Asian patients in which BRCA2 mutations have been reported to be more frequent than BRCA1 [13].

Among 28% of the patients with metastasis and death, 7.9% were found to carry either a mono- or biallelic pathogenic alterations in BRCA2 gene. Therefore, patients should be screened for these alterations for early intervention. Further, we also found BRCA2 variants more common prevalent in ER/PR-negative breast cancer. Our study is in consistency with a previous study that reported higher prevalence of BRCA2 mutations in receptor-negative breast cancer [14]. The individuals with BRCA2 variations and receptor-negative breast cancer respond to cisplatin-targeted therapy and PARP-1 inhibitors better in comparison with adjuvant therapy (combination of doxorubicin and cyclophosphamide) [15]. However, majority of the patients from Malwa region of Punjab are treated with AC therapy. Therefore, patients with receptor-negative status should be screened for appropriate treatment strategies.

In addition to BRCA1 and BRCA2 which are the main repair pathway genes under focus, some new candidate genes including NBN, RAD51C, and BRIP1 have been added to the list recently [16]. In the present study also

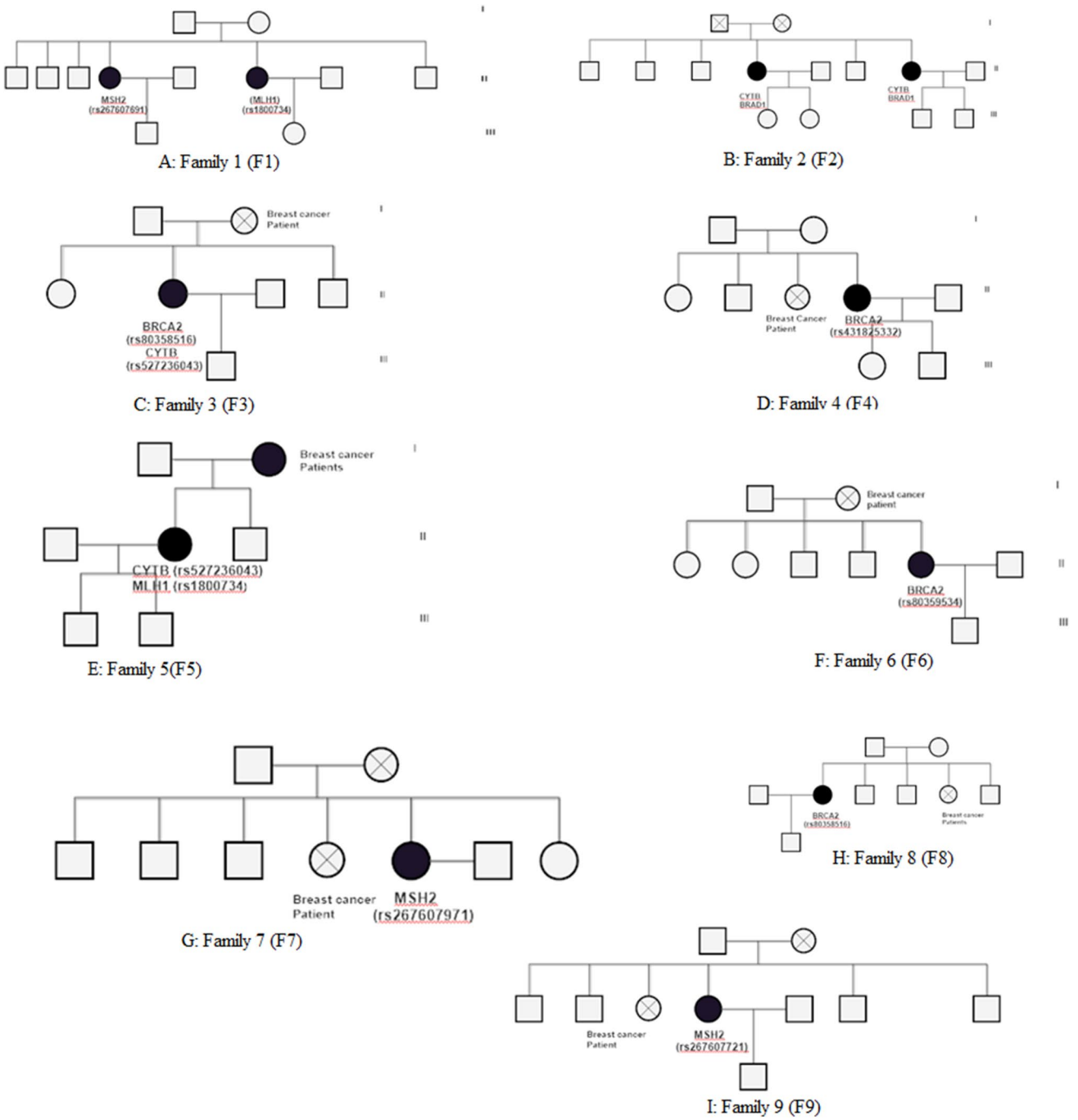

Fig. 1 Pedigrees of familial breast cancer patients

monoallelic alterations in these genes were observed in the patients. However, the penetrance and clinical spectrum of these genes need to be explored more. Some previous studies have also reported the implication of these genes in inter-individual drug response (platinum and, poly [ADP-ribose] polymerase [PRAP]-1 inhibitors) among BRCA1/BRCA2-deficient cancers [17].

Monoallelic variants in MUTYH gene (rs144079536 and rs587778536) were observed in 6% of the patients, whereas 3.7% of patients carried alterations in CDK2NA gene. Both these genes are involved in DNA repair pathway. MUTYH gene encodes MYH glycosylase, which is involved in DNA repair mechanism. Rennert et al. reported high prevalence of founder mutations in MUTYH gene in Sephardi Jews. They

demonstrated a significantly elevated risk of breast cancer associated with a heterozygous G396D variant in MUTYH gene [18]. In addition, few other studies also demonstrated an association of MUTYH alterations with breast cancer in populations with familial breast cancer or polyposis [19, 20]. The current study suggests that bearers of MUTYH gene mutations must be counseled for their possible increased risk of breast cancer and measures to prevent cancer. Further, the researchers have reported that although mutations in these two genes are incidental in breast cancer but their screening can prevent metastasis causing colon, lung, prostate, and extracolonic cancer [16].

In the present study, 11 alterations were observed in MLH1 gene. However, rs1800734 was the most frequently observed alteration in patients (monoallelic in 44.8% and biallelic in 14.2%). rs1800734 has been reported to be associated with colorectal cancer and lung cancer [21]. In addition, seven variants were found in MSH2 gene and rs267607691 (14.1%) was the most frequent among patients. Both these genes are involved in mismatch repair and alterations in this gene lead to impaired mismatch repair. Out of 28% patients with metastasis and death, 21% of the patients harbored pathogenic mono/biallelic mutations in either MSH2 or MLH1 gene. Harkness et al. reported that female carriers of MLH1 variant are at a moderate risk of breast cancer and this risk increases by 18.6% at the age of 70 years [22]. They further added that the risk of breast cancer in case of MSH2 carriers for pathogenic variants to be 11.2% at the age of 70 years [22]. In the current study, the patients bearing rs1800734 (MLH1) and rs267607691 (MSH2) were found in the age group of 50–73 years.

CHEK2 and ATM genes are moderate penetrance genes and heterozygous pathogenic variants in these genes have been reported to increase the risk of breast cancer by two- to threefold, respectively [23–25]. The main monoallelic variant found in the patients of Malwa region was an intronic mutation in ATM gene (rs587779866) observed at a frequency of 14.8%. The mutation results in aberrant splicing, i.e., skipping of exon 54 and thereby alters the normal functioning of the ATM protein [26]. Mutations in CHEK2 and ATM genes are implicated in defective DNA repair pathway also [27]. Currently in India, the patients are mainly screened for BRCA1 and BRCA2 gene mutations. However, CHEK2 mutations are not screened in breast cancer patients although it is one of the important genes involved in the pathogenesis of the breast cancer. Previous studies have reported that CHEK2 mutation carriers are at an increased risk of developing receptor-positive breast cancer [28]. In our study, CHEK2 mutations were more prominent in patients with receptor-positive status that is in consistency with this study. Therefore, physicians must add this gene to the screening list for individuals belonging to Malwa region of Punjab.

Although MTHFR gene is not directly involved in the pathogenesis of breast cancer, it is a key enzyme involved in folate metabolism pathway. MTHFR irreversible catalyzes 5–10 methylenetetrahydrofolate to 5 methyltetrahydrofolate, which is a co-substrate for homocysteine remethylation to methionine. The genetic alterations in MTHFR have been reported to impact folate metabolism, DNA methylation, synthesis, and repair. Low intake of folate is associated with increased risk of several type of cancers including breast cancer [29, 30]. The common functional polymorphism in the MTHFR gene, C677T (rs1801133) is associated with low levels of plasma folate and reduced activity of the MTHFR enzyme [31]. The folate deficiency might lead to misincorporation of uracil in place of thymidine during DNA replication, resulting in DNA strand breaks and chromosomal translocations and deletions [32]. It has been reported that TT genotype of MTHFR (C677T; rs1801133) polymorphism increases the risk of breast cancer by 2.5-fold [33]. In the present study, 12.5 and 37.5% patients were homozygous and heterozygous for this alteration, respectively.

CYTB is a protein found in mitochondria of eukaryotic cells and is a part of the complex that is involved in the electron transport chain of oxidative phosphorylation. It is the only component of the complex that is encoded by a mitochondrial gene. Previous studies suggest that the frequency of mtDNA mutations is higher than nDNA in a variety of human cancers including bladder, head and neck, lung, and breast [34, 35]. In the present study, 52% of the patients including both familial and non-familial cases were found to bear a homozygous mutation in CYTB gene (15034 G/A).

BARD1 mutations have been found in families suspected to be affected with a form of Hereditary Breast and Ovarian Cancer syndrome (HBOC). Female carriers of mutated BARD1 have a 10.2% increased risk of breast cancer at the age of 80 years [36]. In the present study, a single-nucleotide polymorphism (rs587782681) in this gene was observed in two affected members of one family (F2). The alteration is a nonsense mutation and must have resulted in a truncated protein. BARD1 forms a complex with BRCA1, which is involved in tumor suppression [37]. Therefore, mutations in BARD1 gene, might in turn lead to inefficient tumor suppressor complex of BRCA1/BARD1 and induce tumorigenesis.

A recent study involving European population identified 65 novel variants among breast cancer patients [38]. However, none of the variants reported in this study were found in the patients from Malwa region of Punjab. Therefore, each ethnic group needs to be explored for mutations implicated in the pathogenesis of breast cancer.

Nonsense, missense, and frameshift alterations were detected in the tumor suppressor (BRCA2, STK11, ATK, PTEN, and CHEK2) and DNA repair (MTHFR, CYTB, BARD1, BRIP1, MSH1, MSH2, MSH6, NBN, and

RAD51C) genes. All the alterations detected in the patients are reported to be pathogenic in the dbSNP repository. These alterations in tumor suppressor and DNA repair genes might result in protein malfunctioning and, therefore, lead to excessive cell proliferation and defective DNA repair, respectively.

In conclusion, the results of this study can guide to develop a panel to test the breast cancer patients for pathogenic mutations, from Malwa region of Punjab. Especially, the present study suggests that the screening of BRCA2, MSH2, and MLH1 should be carried out in general population since previous studies have suggested that these mutations increase the risk of breast cancer by tenfold and accordingly evidence-based management recommendations can be given to the individuals bearing these mutations. Further, the patients need to be screened for BRCA2, MSH2, and MLH1 since these have been implicated in metastasis by previous studies. Familial breast cancer patients need to be screened for BARD1, MSH2, MLH1, BRCA2, and CYTB genes as these alterations were observed in familial breast cancer patients.

However, the drawback of the current study is that we could not detect novel mutations since the GSA detects only the known markers. The GSA identified many genes that might provide clinical guidance to ensure appropriate interventions. The cancer spectrum and phenotype may vary for different mutations in each gene, and therefore, a large number of mutation carriers need to be assessed before elucidating specific predictor for each gene.

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Compliance with ethical standards

Conflict of interest The authors do not hold any conflict of interest.

Ethical approval The study was carried out only after the ethical clearance by Institutional Ethics Committee (IES) of CUPB and the study hospital.

Informed consent The samples and information were collected from the participants only after their written informed consent.

References

1. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol. 2010;4(3):174–91.
2. Lalloo F, Evans DG. Familial breast cancer. Clin Genet. 2012;82(2):105–14.
3. Saxena S, Chakraborty A, Kaushal M, Kotwal S, Bhatanager D, Mohil RS. Contribution of germline BRCA1 and BRCA2 sequence alterations to breast cancer in Northern India. BMC Med Genet. 2006;4:75.
4. Blaurock-Busch E, Busch YM, Friedle A, Buerner H, Parkash C, Kaur A. Comparing the metal concentration in the hair of cancer patients and healthy people living in the Malwa region of Punjab, India. Clin Med Insights Oncol. 2014;8:1.
5. Thakur J, Rao B, Rajwanshi A, Parwana H, Kumar R. Epidemiological study of high cancer among rural agricultural community of Punjab in Northern India. Int J Environ Res Public Health. 2008;5(5):399–407.
6. Ali I, Wani WA, Saleem K. Cancer scenario in India with future perspectives. Cancer Ther. 2011;8(1):56–70.
7. Bedi J, Gill J, Aulakh R, Kaur P, Sharma A, Pooni P. Pesticide residues in human breast milk: risk assessment for infants from Punjab, India. Sci Total Environ. 2013;463:720–26.
8. Berzina D, Nakazawa-Miklasevica M, Zestkova J, Aksenoka K, Irmejs A, Gardovskis A, Kalniete D, Gardovskis J, Miklasevics E. BRCA1/2 mutation screening in high-risk breast/ovarian cancer families and sporadic cancer patient surveilling for hidden high-risk families. BMC Med Genet. 2013;14(1):61.
9. Ripperger T, Gadzicki D, Meindl A, Schlegelberger B. Breast cancer susceptibility: current knowledge and implications for genetic counselling. Eur J Hum Genet. 2009;17(6):722–31.
10. Levy-Lahad E, Friedman E. Cancer risks among BRCA1 and BRCA2 mutation carriers. Br J Cancer. 2007;96(1):11–5.
11. Shailani A, Kaur RP, Munshi A. A comprehensive analysis of BRCA2 gene: focus on mechanistic aspects of its functions, spectrum of deleterious mutations, and therapeutic strategies targeting BRCA2-deficient tumors. Med Oncol. 2018;35(3):18.
12. Vaidyanathan K, Lakhotia S, Ravishankar HM, Tabassum U, Mukherjee G, Somasundaram K. BRCA1 and BRCA2 germline mutation analysis among Indian women from south India: identification of four novel mutations and high-frequency occurrence of 185delAG mutation. J Biosci. 2009;34(3):415–22.
13. Kim H, Choi DH. Distribution of BRCA1 and BRCA2 mutations in Asian patients with breast cancer. J Breast Cancer. 2013;16(4):357–65.
14. Peshkin BN, Alabek ML, Isaacs C. BRCA1/2 mutations and triple negative breast cancers. Breast Dis. 2011;32(1–2):25–33.
15. Meyer P, Landgraf K, Högel B, Eiermann W, Ataseven B. BRCA2 mutations and triple-negative breast cancer. PLoS ONE. 2012;7(5):e38361.
16. Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K, Timms K, Garber JE, Herold C, Ellisen L, Krejdovsky J. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 2015;121(1):25–33.
17. Dedes KJ, Wilkerson PM, Wetterskog D, Weigelt B, Ashworth A, Reis-Filho JS. Synthetic lethality of PARP inhibition in cancers lacking BRCA1 and BRCA2 mutations. Cell Cycle. 2011;10:1192–9.
18. Rennert G, Lejbkowicz F, Cohen I, Pinchev M, Rennert HS, Barnett-Griness O. MutYH mutation carriers have increased breast cancer risk. Cancer. 2012;118(8):1989–93.
19. Vogt S, Jones N, Christian D, Engel C, Nielsen M, Kaufmann A, Steinke V, Vasen HF, Propping P, Sampson JR, Hes FJ. Expanded extracolonic tumor spectrum in MUTYH-associated polyposis. Gastroenterology. 2009;137(6):1976–85.
20. Wasielewski M, Out AA, Vermeulen J, Nielsen M, van den Ouweland A, Tops CM, Wijnen JT, Vasen HF, Weiss MM, Klijn JG, Devilee P. Increased MUTYH mutation frequency among Dutch families with breast cancer and colorectal cancer. Breast Cancer Res Treat. 2010;124(3):635–41.
21. Lo YL, Hsiao CF, Jou YS, Chang GC, Tsai YH, Su WC, Chen KY, Chen YM, Huang MS, Hsieh WS, Chen CJ. Polymorphisms of MLH1 and MSH2 genes and the risk of lung cancer among never smokers. Lung Cancer. 2011;72(3):280–6.

22. Harkness EF, Barrow E, Newton K, Green K, Clancy T, Lalloo F, Hill J, Evans DG. Lynch syndrome caused by MLH1 mutations is associated with an increased risk of breast cancer: a cohort study. J Med Genet. 2015;52(8):553–6.
23. Cybulski C, Wokołorczyk D, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Masojć B, Dębniak T, Górski B, Blecharz P, Narod SA. Risk of breast cancer in women with a CHEK2 mutation with and without a family history of breast cancer. J Clin Oncol. 2011;29(28):3747–52.
24. Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A, Byrd P, Taylor M, Easton DF. Cancer risks and mortality in heterozygous ATM mutation carriers. J Natl Cancer Inst. 2005;97(11):813–22.
25. Weischer M, Bojesen SE, Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. CHEK2*1100delC genotyping for clinical assessment of breast cancer risk: meta-analyses of 26,000 patient cases and 27,000 controls. J Clin Oncol. 2008;26:542–8.
26. Podralska MJ, Stembalska A, Ślęzak R, Lewandowicz-Uszyńska A, Pietrucha B, Kołtan S, Wigowska-Sowińska J, Pilch J, Mosor M, Ziółkowska-Suchanek I, Dzikiewicz-Krawczyk A. Ten new ATM alterations in Polish patients with ataxia-telangiectasia. Mol Genet Genomic Med. 2014;2(6):504–11.
27. Jalilvand M, Oloomi M, Najafipour R, Alizadeh SA, Saki N, Rad FS, Shekari M. An association study between CHEK2 gene mutations and susceptibility to breast cancer. Comp Clin Pathol. 2017;26:1–9.
28. Apostolou P, Papasotiriou I. Current perspectives on CHEK2 mutations in breast cancer. Breast Cancer: Targets Ther. 2017;9:331.
29. Kim YI. Folate and carcinogenesis: evidence, mechanisms, and implications. J Nutr Biochem. 1999;10(2):66–88.
30. Mason JB, Choi SW. Folate and carcinogenesis: developing a unifying hypothesis. Adv Enzyme Regul. 2000;40(1):127–41.
31. Ma LM, Ruan LH, Yang HP. Meta-analysis of the association of MTHFR polymorphisms with multiple myeloma risk. Sci Rep 2015; 5.
32. Beetstra S, Thomas P, Salisbury C, Turner J, Fenech M. Folic acid deficiency increases chromosomal instability, chromosome 21 aneuploidy and sensitivity to radiation-induced micronuclei. Mutat Res. 2005;578(1):317 – 26.
33. Ergul E, Sazci A, Utkan Z, Canturk NZ. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumor Biol. 2003;24(6):286 – 90.
34. Tan DJ, Bai RK, Wong LJ. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer Res. 2002;62(4):972–6.
35. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL, Sauter ER. Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. Carcinogenesis. 2005;26(1):145–52.
36. Ratajska M, Antoszewska E, Piskorz A, Brozek I, Borg Å, Kusmierek H, Biernat W, Limon J. Cancer predisposing BARD1 mutations in breast–ovarian cancer families. Breast Cancer Res Treat. 2012;131(1):89–97.
37. Klonowska K, Ratajska M, Czubak K, Kuzniacka A, Brozek I, Koczkowska M, Sniadecki M, Debniak J, Wydra D, Balut M, Stukan M. Analysis of large mutations in BARD1 in patients with breast and/or ovarian cancer: the Polish population as an example. Sci Rep. 2015; 5.
38. Michailidou K, Lindström S, Dennis J, Beesley J, Hui S, Kar S, Lemaçon A, Soucy P, Glubb D, Rostamianfar A, Bolla MK. Association analysis identifies 65 new breast cancer risk loci. Nature. 2017;551(7678):92–4.