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Current Genomics, 2018, *19*, 395-410

RESEARCH ARTICLE

Impact of p53 arg72pro SNP on Breast Cancer Risk in North Indian Population

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ARTICLE HISTORY

Received: October 30, 2016
Revised: October 25, 2017
Accepted: October 26, 2017

DOI:
10.2174/1389202919666171205104137

Abstract: ***Background*:** Genetic changes in p53 gene contribute to breast cancer susceptibility.

***Objective and Methods*:** A case-control study and a meta-analysis were performed to investigate the role of p53 codon72 SNP with breast cancer susceptibility in Indian women.

***Results*:** p53 heterozygous arginine variant was associated with decreased risk of breast cancer in total cohort. In meta-analysis, Allelic and GG *vs*. CC genetic comparison model were found to be associated with breast cancer risk. Moreover, recessive comparison model indicated a protective correlation with breast cancer occurrence.

***Conclusion*:** The findings of our case-control study and meta-analysis suggest a significant association between p53 Arg72Pro polymorphism and an increased risk of breast cancer in Indian population.

Keywords: p53 codon72 SNP, Breast cancer, Indian populations, Case-control, Meta-analysis, p53 Arg72Pro polymorphism.

1. INTRODUCTION

Breast cancer accounts for 23% of the total cancer incidence causing 14% cancer related deaths among women, worldwide [1]. In India, breast cancer has surpassed cervical cancer incidence and is now the most common cancer in women with an estimated 1:4 incidence ratio between urban and rural population, respectively [2]. Alarmingly, prognosis is poor with high mortality rate estimated to be nearly 50% [3]. Diagnosis at advanced stage along with incidence at young age (40-50 years average age of Indian breast cancer patients *versus* 60-70 in western countries) contributes to high mortality rate [4-7]. Quite intriguingly, majority of breast cancer cases in India are young mothers (<40 years of age) with long history of breast feeding their children, which should protect them from developing this enigmatic disease.

Certain genetic/epigenetic changes producing an aberrant gene product and altering a pathway or function eventually leads to the development of breast cancer [8]. Abnormalities in cancer genes can be of germline and/or somatic origin [9, 10]. The loss of function mutations in the p53 tumor suppressor gene commonly leads to tumor formation [11] discussed in a great number of studies [12, 13].

Early reports have shown the involvement of p53 gene mutations in more than half of all human cancers [14]. The p53 function as a tumor suppressor is neutralized upon its interaction with certain cellular and viral proteins like viral E6, T-antigen, and mdm2 [15].

rs1042522 polymorphism present at codon 72 in wt p53 gene affects a substitution of proline for arginine (Arg72Pro) [16] and disturbs a PXXP motif that resembles SRC homology 3 binding domain [17]. This proline-rich region plays an important role in apoptosis and regulates uncontrolled proliferation. Codon 72 polymorphism produces two variants with distinct biological and biochemical properties [18]. After an early study suggested an association of rs1042522 polymorphism with cervical cancer [19], a plethora of studies have replicated the results in various human cancers such as non-Hodgkins lymphoma [20], lung [21], colorectal [22], ovarian [23], colon [24], cervical [25, 26], urinary bladder [27], skin [28], esophageal [29] and breast [30-36] cancer.

The significant role of p53 gene in cancer occurrence has led to assumptions that the presence of Arg72Pro SNP renders an individual susceptible for breast cancer onset and

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progression. As mentioned above, few epidemiological studies have tried to elucidate the risk association of rs1042522 with breast cancer in Indian population but the reports lack consensus. To analyse the possible risk association of rs1042522 with breast cancer in our population, we carried out a case-control study and meta-analysis based on earlier published studies from India in order to generate a meaningful result by increasing statistical power.

The objectives of the study were:

1. To evaluate the p53 Arg72Pro SNP distribution in an Indian cohort; 2. To define the association between the p53 Arg72Pro SNP and breast cancer in India by performing a meta-analysis

2. MATERIALS AND METHODS

2.1. Case-Control Study

2.1.1. Ethical Statement

The study was approved and cleared by the Ethics Committee of Jamia Millia Islamia (A Central University), New Delhi and All India Institute of Medical Sciences, New Delhi. The consent was obtained from all participants (patients and controls) before collecting any sample. All the patients and participants were provided with Patient Information sheet and Participant Information sheet (English/Hindi), respectively.

2.1.2. Sample Size

One hundred and fifteen (115) blood samples from the Breast Cancer patients attending the OT of B.R. Ambedkar-Institute Rotary Cancer Hospital (BRA-IRCH), AIIMS, New Delhi, India and the even number of control samples from unrelated normal healthy women of same age group and without a family history of cancer were taken for polymorphic studies. Blood was collected directly into a BD Vacutainer tube. These controls were recruited from medical indoor patients who were undergoing treatment for conditions such as diabetes, hypertension, *etc*. The patients belonged to age group of 25-75 years. The blood collected was used for DNA analysis and this collection did not compromise the availability of sufficient material for routine pathology and other tests were performed as part of patient care.

2.1.3. DNA Isolation

DNA was isolated from the peripheral blood of the subjects using standard protocol. Briefly, contents of BD Vacutainer tube were added with RBC Lysis Buffer in a 50 ml centrifuge tube. After centrifugation, 6 ml of Nucleic Acid Lysis Buffer was added to pellet and dissolved by gentle vortexing. Subsequently, 300 ul of Proteinase-K was added before incubation for 48 hours at room temperature. NaCl solution was mixed with the incubated sample and centrifuged after a brief incubation at ice. The obtained supernatant was added to a tube containing 20 ml of chilled absolute ethanol. The sample was kept at room temperature to allow DNA precipitation. A sterile inoculation loop was used to transfer the precipitated DNA into an eppendorff tube. Chilled 70% ethanol was used to wash the precipitated DNA by centrifuging before adding TE buffer to the pellet.

2.1.4. Determination of Genomic DNA Concentration

DNA concentration was assessed using a dual beam UV spectrophotometer (Cecil, USA) using the formula: Absorbance 260 X Dilution Factor X 50 = DNA µg/ml µg/ml. Alternatively, DNA concentration was estimated using electrophoresis technique [37].

2.1.5. PCR Amplification of Codon72 Polymorphism

The PCR amplification for the codon72 analysis was performed using the specific primers (Table **1**) as described previously [19]. The 141 and 177 bp product for p53Arg and p53Pro, respectively, were visualized using Gel documentation system, Bio-Rad Laboratories, CA, USA.

2.1.6. Statistical Analysis

The data was tabulated and analyzed using SPSS software. The mean ±SD was calculated for different groups. Two-way analysis of variance was employed to test for the difference in mean values. Student t-test was employed to compare the mean difference wherever appropriate. Simple correlation coefficient was estimated to quantify the relationship between clinicopathological variable and status of p53 alteration.

3. META ANALYSIS

3.1. Literature Identification and Data Extraction Strategy

PubMed, Web of Science, CGEMS and EBSCO database (prior to March 2016) were searched for the research articles using key words: "p53 codon 72 Arginine/ Proline", "polymorphism", "breast cancer" and "India". Relevant studies were also identified using reference lists of the selected articles. Two reviewers independently assessed the quality of the extracted data by following inclusion-exclusion criteria strictly. Another reviewer also par-

Table 1. Oligonucleotide primer sequences used for the analysis *p53*:72 Proline/Arginine alleles.

Name	Consensus Sequence	Annealing Temperature (^{o}C)	Amplicon Size (bp)
*p53*Pro+/*p53*- FP	5′-GCC AGA GGC TGC TCC CCC-3′	61	177
*p53*Pro+/*p53*- RP	5′-CGT GCA AGT CAC AGA CTT-3′		
p53+/*p53*Arg-FP	5′-TCC CCC TTG CCG TC CCA A-3′	61	141
p53+/*p53*Arg-RP	5′-CTG GTG CAG GGG CCA CGC-3′		

ticipated to reach a final concurrence in cases of difference between two primary reviewers on any piece of the data collected. Author's name, year of publication, the number of cases and controls, subject ethnicity, type of study, and allelic and genotypic distribution among subjects were extracted from the selected studies.

3.2. Inclusion and Exclusion Criteria

Case-control studies that analyzed the association between codon72 polymorphism and breast cancer risk by recruiting clinically confirmed breast cancer cases and cancer-free controls, and published in English language were included in the current meta-analysis. The studies having overlapping of the data or codon72 SNP analysis in breast cancer cell line, or case-only design were excluded.

3.3. Statistical Analysis

Assessment of risk association between codon72 SNP and breast cancer was done by analyzing ORs from all eligible studies along with their 95% CIs through allelic, dominant and recessive genetic models. Between-study heterogeneity of the studies was calculated by chi-square-based Q-statistic test [38]. Random- or fixed-effects model was employed for ORs analysis in cases of significant or not significant between-study heterogeneity, respectively [39, 40]. Larger values of I^2 statistics reflected larger heterogeneity [41]. The departure of frequencies of p53codon 72 Arginine/ Proline polymorphism from Hardy-Weinberg Equilibrium (HWE) was assessed by chi-square test. Presence or absence of publication bias was calculated using funnel plot asymmetry and egger's linear regression test. Significance of the intercept having p-value less than five in t-test showed significant publication bias [42]. Statistical analyses in the present meta-analysis were done using the Comprehensive Meta-Analysis 2.0 (Biostat, USA).

4. RESULTS

4.1. Case-control Study

4.1.1. Clinicopathologic Attributes

Various clinicopathologic variables like basic demographics and tumor characteristics recorded are represented in Table **2**.

4.1.2. Clinical Stage

Clinical staging of the tumor was done according to AJCC which showed that of all 31 were of stage II and 64 cases of stage III and 20 were of stage IV. The majority of the patients (55.65%) were in clinical stage III. The distribution of clinical staging of the breast cancer patients is presented in Table **3**.

4.1.3. Correlation of Codon72 SNP with Clinicopathological Variables

Homozygous arginine variant of p53 was found associated with 51 poorly differentiated histological grade breast cancer cases amounting to 83.61% of total cases, 61 clinical stage III & IV (100%), 45 lymph node positive cases (73.77%), 39 estrogen receptor negative cases (64%) and more than 50% of the total premenopausal stage and progesterone receptor negative cases. Heterozygous arginine variant of p53 was found associated with 17 well differentiated histological grade breast cancer cases amounting to 68% of total cases and 22 clinical stage II (88%) cases (Table **4**).

Table 2. Clinicopathologic attributes.

Clinicopathological Variables	No. of Patients	Percentage (%)
Age Distribution 25-77 years, average 35-50 years	115	-
Age		
< 50	77/ 115	66.95
> 50	38/ 115	33
Menstrual status		
Pre-Menopausal	69/ 115	60
Post-Menopausal	46/ 115	40
Nodal status		
Positive	77/ 115	67
Negative	38/ 115	33
Histological grading		
PD	62/ 115	61
MD	37/ 115	15
WD	16/ 115	24.35
Histological status		
Invasive Ductular Carcinoma (IDC)	107/ 115	93
Invasive Lobular Carcinoma (ILC)	8/ 115	7
Tumor Size		
pT1 (<2)	6/ 115	5. 2
pT2 (<5)	41/ 115	35.65
pT3 (<15)	68/ 115	59.13
Estrogen Receptor (ER) status		
+ve	43/ 115	37.39
-ve	72/ 115	62.61
Progesterone Receptor (PR) status		
+ve	53/ 115	46.09
-ve	62/ 115	53.91
Clinical Stage TNM		
I	0/ 115	0.00
II	31/ 115	27
III + IV	84/ 115	73

Table 3. Clinical stages of the breast carcinoma patients.

Clinical Stage	No. of Cases (n=115)	Percentage
I	0	0.00
II	31	27
III	64	55.65
IV	20	17.39

4.1.4. Codon72 SNP in p53 Gene

Arg/Pro variant was found significantly linked with decreased breast cancer risk in total cohort as well as in pre- and post-menopausal women stratification. ORs for Arg/Pro (G/C) genotype in total cohort, pre- and post-menopausal women were 0.17 (95% CI, 0.097-0.307, p-value 1.852e-09), 0.32 (95% CI, 0.162-0.665, p-value 3.208e-03) and 0.05 (95% CI, 0.018-0.154, p-value 1.367e-08), respectively. Arg/Arg (G/G) genotype was also found linked with increased breast cancer risk in total cohort and postmenopausal women with ORs 3.06 (95% CI, 1.768-5.3, p-value 9.49e-05) and 6.17 (95% CI, 2.395-15.864, p-value 2.408e-04), respectively (Table **5**).

5. META- ANALYSIS

5.1. Characteristics of Eligible Studies

Of 8 studies selected initially, 2 were excluded during data extraction, because one of them studied p53 codon 72 polymorphism in breast cancer cell lines [33], while the other study provided results in a confusing manner [34]. An attempt was made to get the clarification from corresponding author without yielding a result. A total of six research articles were used to estimate the role of codon72 SNP in breast cancer susceptibility in Indian population, involving 1249 cases and 1838 controls [35, 36, 43-46]. Distribution of genotypes showing concurrence with Hardy-Weinberg equilibrium (Table **6**), and Minor Allele Frequency (MAF) among subjects is shown in Table **7**.

5.2. Role of p53 Codon72 SNP in Breast Cancer Risk

Overall analyses show a significant association of p53 codon72 SNP in breast cancer susceptibility. An elevated risk was found in 2 genetic comparison models namely Allelic (G *vs*. C: OR=1.26, 95% CI=1.139 to 1.401, p-value 0.000*) and GG *vs*. CC (OR=1.39, 95% CI=1.148 to 1.687, p-value 0.001*). Recessive genetic comparison model showed a protective correlation with breast cancer (CC *vs*. GG+GC: OR=0.79, 95% CI=0.668 to 0.939, p-value 0.007*) (Table **8**) (Figs. **1** & **2**).

5.3. Sensitivity Analysis

Systematic deletion of one study at a time did not significantly modify the pooled ORs in any of six genetic models *i.e*., allelic, dominant and recessive (Fig. **3**), as well as GC *vs*. CC, GG *vs*. CC and GG *vs*. GC (Fig. **4**) suggesting the statistical significance of our findings.

5.4. Publication Bias Diagnosis

Egger's test and Begg's funnel plot were performed to assess the publication bias among the eligible studies. Begg's funnel plot did not show an evidence of publication bias in any of six genetic models *i.e*., allelic, dominant and recessive (Fig. **5**), as well as GC *vs*. CC, GG *vs*. CC and GG *vs*. GC (Fig. **6**). Additionally, the findings of funnel plot were numerically supported by Egger's test (Table **9**).

5.5. Heterogeneity Calculation

Random effects model was applied in four genetic models to compute the data having heterogeneity as reveled by Q-test and I^2 statistics. Fixed effects model was used to analyze the data in Allelic (G *vs*. C: $P_{heterogeneity}$ 0.090; I^2 45.12) and GG *vs*. CC ($P_{heterogeneity}$ 0.171; 33.70) genetic comparisons (Table **9**).

6. DISCUSSION

Breast cancer incidence has risen by approximately 2% per annum in India across all age groups except younger age groups (< 45 years) which is being affected in higher percentage [47]. The disease is affecting Indian patients a decade earlier when compared with the western patients. 50% of all breast cancer cases in India affects premenopausal women whereas postmenopausal women constitutes the majority of breast cancer in western countries [48]. More than 80% of breast cancer cases in India occurred at age less than 60 years with a significant proportion affected before 35 years of age [48]. Furthermore, large and poorly defined tumor, high hormone receptor negative condition, frequent relapses and poor prognosis is correlated with less age. [49, 50]. Family history of cancer, presence of *BRCA1* mutation, oral contraceptive use and hormonal exposure are major risk factors for premenopausal breast cancer occurrence in young

Table 4. Correlation of p53 (codon 72) polymorphism with clinicopathological variables (n= 115).

-	Histological Grading			Clinical Staging			Nodal Stage		Menopausal Status		Estrogen Receptor (ER) Status		Progesterone Receptor (PR) Status	
	PD	MD	WD	I	II	III & IV	+ve	-ve	Pre	Post	+ve	-ve	+ve	-ve
GG (61)	51	5	5	-	-	61	45	16	35	26	22	39	27	34
GC (25)	5	3	17	-	22	3	13	12	13	12	11	14	14	11
CC (29)	14	9	6	-	9	20	19	10	21	8	10	19	12	17

Table 5. Allelic and genotypic frequencies of p53 (codon 72) gene polymorphism in case control and breast cancer patients.

Total Women	**Patient Frequency (n= 115)**	**Control Frequency (n= 115)**	**Odds Ratio (Confidence interval 95%)**	**p-value**
Allele Frequency (Total number of alleles)	-	-	-	-
G	0.64 (147)	0.58 (133)	1.292 (0.888 - 1.879)	0.214
C	0.36 (83)	0.42 (97)	0.774 (0.532 - 1.126)	
Genotypic Frequency (Total number of geno-types)	-	-	-	-
GG	0.53 (61)	0.27 (31)	3.061 (1.768 - 5.300)	9.490e-05
GC	0.22 (25)	0.62 (71)	0.172 (0.097 - 0.307)	1.852e-09
CC	0.25 (29)	0.11 (13)	2.646 (1.306 - 5.351)	0.010
GC + CC	0.47 (54)	0.73 (84)	0.327 (0.189 - 0.566)	9.490e-05
Total Premenopausal Women	**Patient Frequency (n= 69)**	**Case Control Frequency (n= 69)**	**Odds Ratio (OR) (95% CI)**	**p-value**
Allele Frequency (Total number of alleles)	-	-	-	-
G	0.64 (89)	0.60 (83)	1.204 (0.740 - 1.957)	0.535
C	0.36 (49)	0.40 (55)	0.831 (0.511 - 1.351)	
Genotypic Frequency (Total number of geno-types)	-	-	-	-
GG	0.50 (35)	0.33 (23)	2.059 (1.039 - 4.081)	0.057
GC	0.28 (19)	0.54 (37)	0.329 (0.162 - 0.665)	3.208e-03
CC	0.22 (15)	0.13 (9)	1.852 (0.762 - 4.488)	0.261
GC + CC	0.50 (34)	0.67 (46)	0.486 (0.245 - 0.963)	0.057
Total Postmenopausal Women	**Patient Frequency (n= 46)**	**Case Control Frequency (n= 46)**	**Odds Ratio (OR) (95% CI)**	**p-value**
Allele Frequency (Total number of alleles)	-	-	-	-
G	0.63 (58)	0.54 (50)	1.433 (0.797 - 2.578)	0.295
C	0.37 (34)	0.46 (42)	0.698 (0.388 - 1.255)	
Genotypic Frequency (Total number of geno-types)	-	-	-	-
GG	0.57 (26)	0.17 (8)	6.175 (2.395 - 15.864)	2.408e-04
GC	0.13 (6)	0.74 (34)	0.053 (0.018 - 0.154)	1.367e-08
CC	0.30 (14)	0.09 (4)	4.594 (1.436 - 14.516)	0.0180
GC+ CC	0.43 (20)	0.83 (38)	0.162 (0.063 - 0.418)	2.408e-04

Table 6. Major characteristics of the studies included in the meta-analysis.

S. No.	Author(s)	Year	Reference Number	Ethnicity	Study Design	Cases	Controls
1	Samson *et al*.	2007	[43]	Indian	HB	250	500
2	Gochhait *et al*.	2007	[36]	Indian	HB	243	333
3	Singh *et al*.	2008	[44]	Indian	HB	104	105
4	Rajkumar *et al*.	2008	[45]	Indian	HB	250	500
5	Suresh *et al*.	2011	[76]	Indian	HB	37	35
6	Surekha *et al*.	2011	[35]	Indian	HB	250	250
7	Current study	2011	-	Indian	HB	115	115

Table 7. Distribution of p53 Arg72Pro polymorphism of seven studies included in the meta-analysis.

Author	Cases				Control			
	Genotype			**Minor Allele**	Genotype			**Minor Allele**
	GG	**GC**	**CC**	**MAF**	**GG**	**GC**	**CC**	**MAF**
Samson *et al*.	66	125	59	0.49	135	224	141	0.51
Gochhait *et al*.	86	109	48	0.42	76	160	97	0.53
Singh *et al*.	46	45	13	0.34	28	65	12	0.42
Rajkumar *et al*.	66	125	59	0.49	135	224	141	0.51
Suresh *et al*.	11	19	7	0.45	10	22	3	0.40
Surekha *et al*.	144	0	106	0.42	118	0	132	0.53
Current study	61	25	29	0.36	31	71	13	0.42

Table 8. Summary of the Odds Ratios (ORs) for the six genetic comparison models.

Comparison Models	**ORs**	**CI (95%)**		**Z-value**	**p-value**
		Lower Limit	**Upper Limit**		
Allelic (G *vs*. C)	1.26	1.139	1.401	4.417	0.000*
Dominant (GG *vs*. CC+GC)	1.50	1.095	2.073	2.519	0.012
Recessive (CC *vs*. GG+GC)	0.79	0.668	0.939	-2.688	0.007*
GC *vs*. CC	0.77	0.439	1.359	-0.896	0.870
GG *vs*. CC	1.39	1.148	1.687	3.371	0.001*
GG *vs*. GC	1.63	0.956	2.786	1.794	0.073

OR, Odd's Ratio; CI, Confidence Interval; *statistically significant.

women [51, 52]. Contrary to popular belief in India, early childbearing and multiparity are breast cancer risk factors in young women aged less than 35 years [53]. A recent study showed that almost 50% of early age breast cancer cases carrying *BRCA1, BRCA2*, and *TP53* mutations had strong family histories of breast cancer. On the other hand the same mutations were found in less than 10% of cases without a family history of breast cancer [54].

A large population-based study showed a significant correlation of fatty diet, obesity and little activity with breast cancer risk at an early age [55]. An early age at menarche, prior mantle irradiation for Hodgkin lymphoma, high intake of red meat and alcohol also contribute significantly to breast cancer risk in young women [51, 56]. Genetic mutations or SNPs in p53 gene often contributes to cancer risk in cervical, lung, colorectal and breast cancer among many others [57].

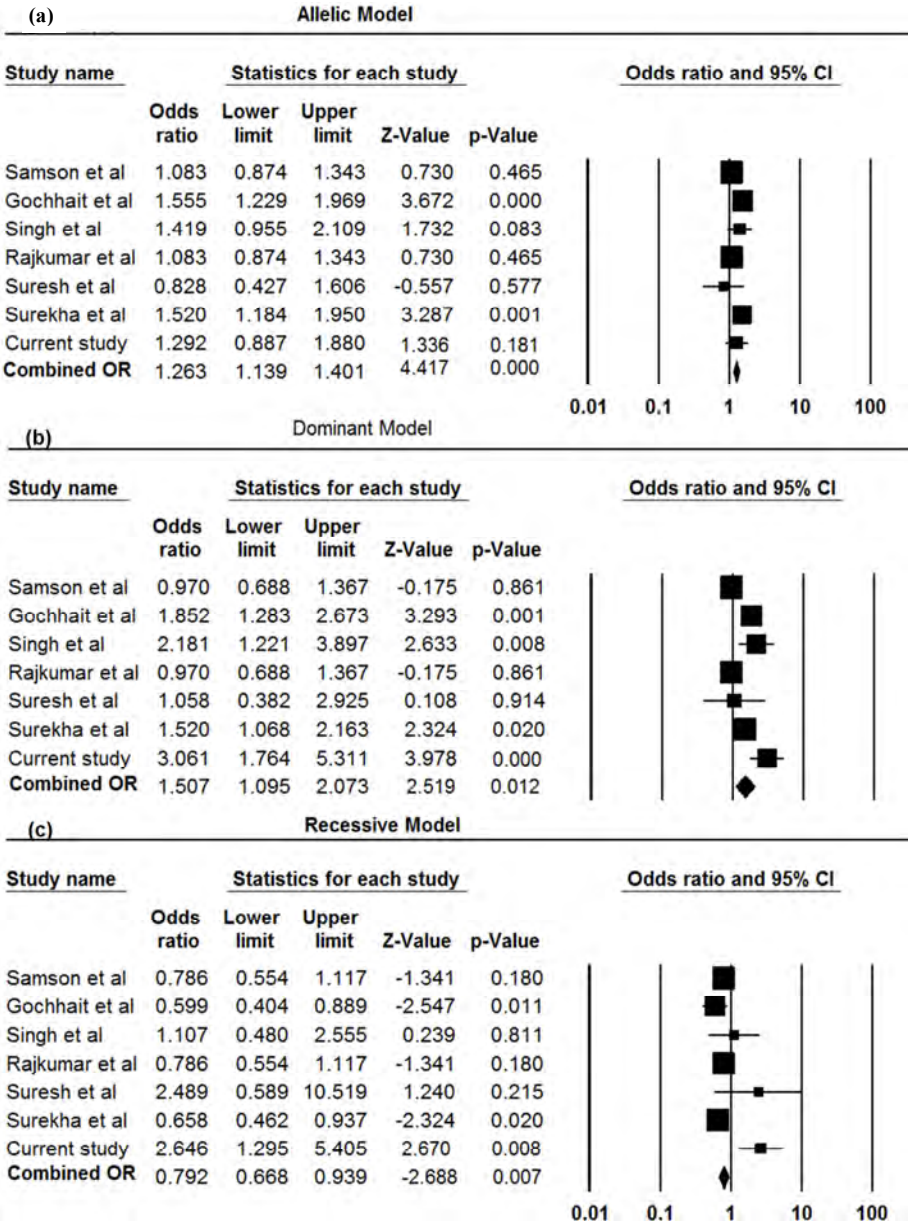

Fig. (1). Forest plot of OR with 95% CI of breast cancer associated with the p53 Arg72Pro polymorphism in Indian population by fixed and random effect models. Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. (**a**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (G *vs*. C; allelic model). (**b**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (Dominant (GG *vs*. CC+GC) model). (**c**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (Recessive (CC *vs*. GG+GC) model).

Codon 72 polymorphism of exon 4 (Arg72Pro; rs1042522) introduces proline in place of arginine [58] and produces two variants of p53 protein differing in biochemical and functional properties. The p53 variants have variable ability to modulate gene transcription, DNA repair or apoptosis, suppression of the transformation of primary cells, reduction of genomic instability and eventually increases susceptibility to cancer occurrence [18, 30, 59-65].

Recent studies have found both Arg or Pro allele presence in breast cancer tumor tissue. However, several studies have presented contradictory data regarding the relation between polymorphism and selective allele retention indicating that the Arg/Arg, Arg/Pro and Pro/Pro prevalence essentially hinges on the racial composition of the target population [66-70]. The two variants namely p53Arg72 and p53Pro72 proteins variably modulate transcription process leading to variable cancer risk [67, 71].

The Pro allele induces an enhanced transcription of *p53* downstream effector genes and influences tight control at G1 phase of cell cycle when compared with Arg allele [60]. On the other hand, Arg allele triggers faster apoptosis and checks transformation in a better way [57, 59, 60, 72] by interacting with iASPP [73].

Nevertheless, the p53 codon72 SNP can be used as a biomarker for genetic screening of susceptible subjects

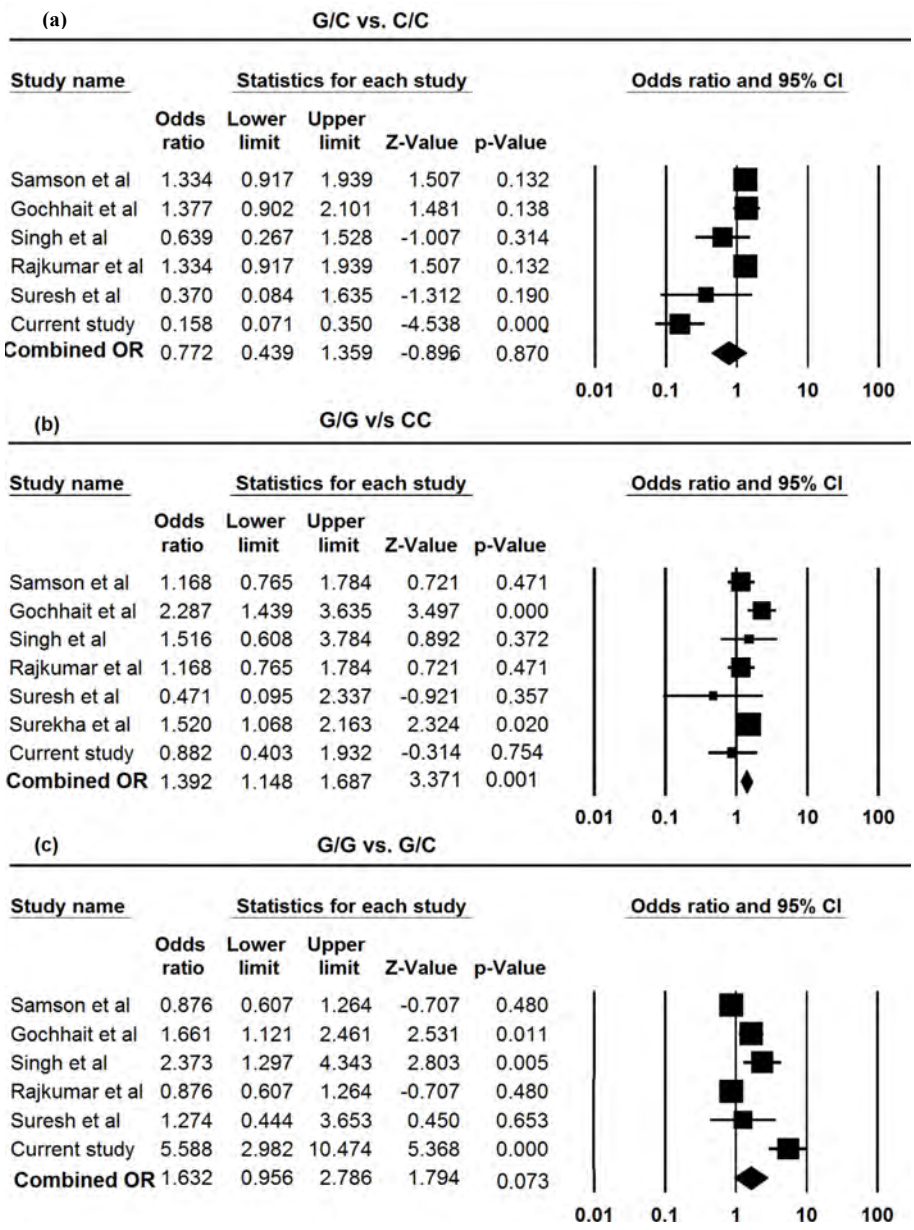

Fig. (2). Forest plot of OR with 95% CI of breast cancer associated with the p53 Arg72Pro polymorphism in Indian population by fixed and random effect models. Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. (**a**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (GC *vs*. CC model). (**b**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (GG *vs*. CC; homozygous model). (**c**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (GG *vs*. GC model).

[74, 75]. In our case-control study, G/C genotype was found significantly correlated with decreased breast cancer risk in total cohort, premenopausal and postmenopausal women. Our results are in agreement with an early study from India showing correlation of G/C variant with decreased risk of breast cancer in postmenopausal women [44]. In contrast, high p53 Arg72Pro heterozygous variant frequency was found in breast cancer cases, though the association failed to reach statistical significance [43, 45, 76]. Lately, Suresh *et al*. also reported high prevalence of *arg/pro* genotype in breast cancer cases from south India. But the prevalence again failed to reach statistical significance [76]. However, few other Indian studies report an elevated breast cancer risk associated with *p53* codon 72 Arg homozygous genotype [35, 36].

Further, we observed significant correlation of Arg/Arg (G/G) genotype with increased breast cancer risk in total cohort and postmenopausal women. Our results are in agreement with an early Indian study showing high p53 Arg72Arg homozygous variant frequency in breast cancer patients, though the association failed to reach statistical significance [43, 76].

Many early reports showing high frequency of allele G in breast cancer cases from Indian, Turkish and Caucasian population suggest that G allele predisposes a person to high

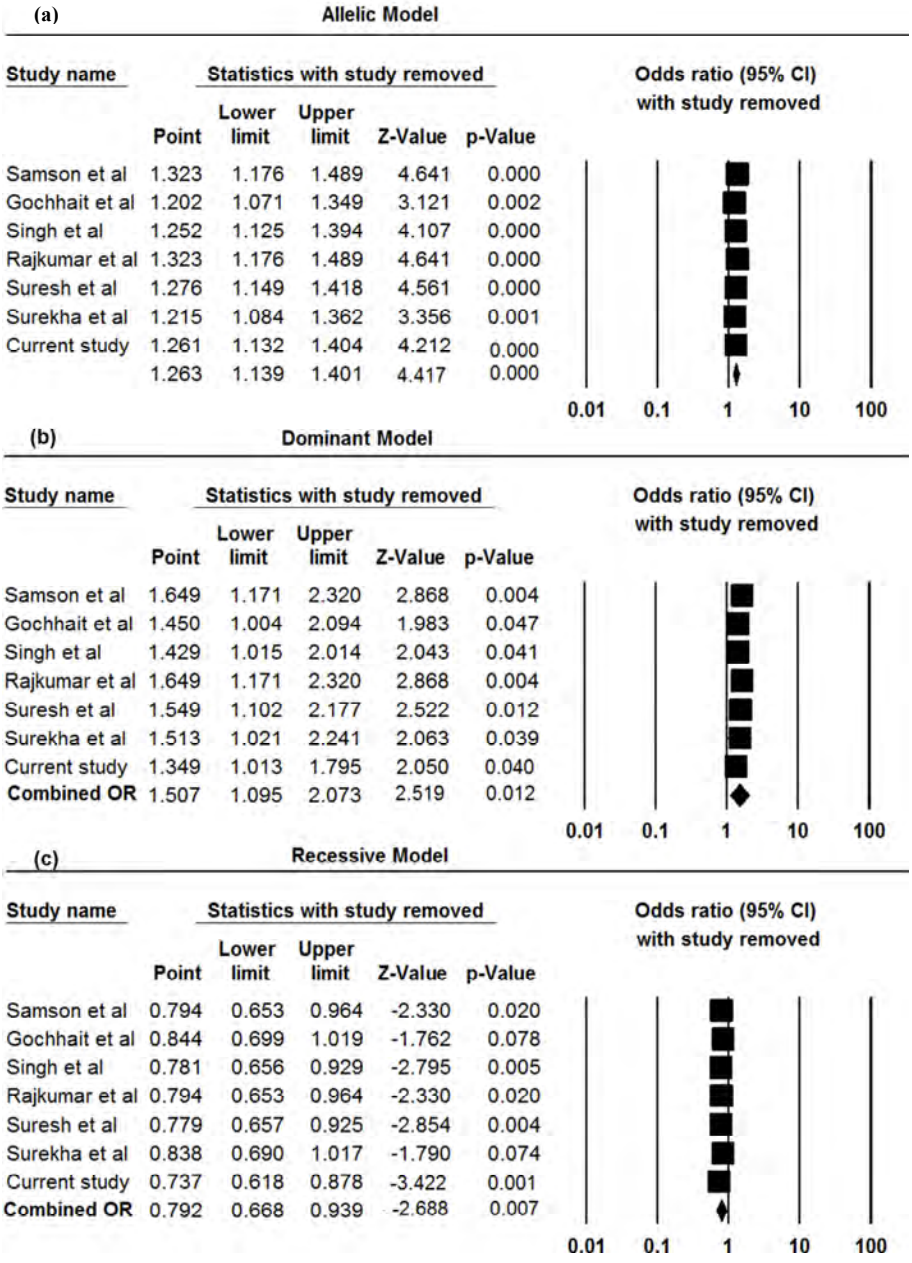

Fig. (3). Sensitivity analysis by showing forest plot of OR with 95% CI of breast cancer associated with the p53 Arg72Pro polymorphism in Indian population. Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. (**a**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (G *vs*. C; allelic model). (**b**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (Dominant (GG *vs*. CC+GC) model). (**c**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (Recessive (CC *vs*. GG+GC) model).

breast cancer risk [67, 76, 77]. However, contradictory reports suggest the association of homozygous C allele with an increased breast cancer risk [78, 79].

Other than breast cancer, many reports from India suggest an association of Arg72 variant with oral cancer [64] and an association of Pro72 variant with urinary bladder cancer risk [80]. The reason for the discrepant reports in the Indian studies mentioned above might be because of ethnic difference between the populations studied. Mitra *et al.* [64] drew the patients from Kolkata. Pandith *et al.* [80] studied ethnically diverse Kashmiri population, while many others studied Dravidian populations in south of India. Differential correlation of Arg72 or Pro72 polymorphic variant with cancer risk may also be dependent upon variable environmental exposures having modifier effect on the polymorphism.

Early reports show discrepant results about the association of p53 protein variants with the risk of a variety of human cancers including breast cancer globally [30, 59, 77, 81]. An association of Arg72 polymorphic variant with elevated risk for lung [21], colorectal [22], ovarian [23], colon [82], cervical [27] and breast [30, 31] cancers has been observed. However, many others report Pro72 variant association

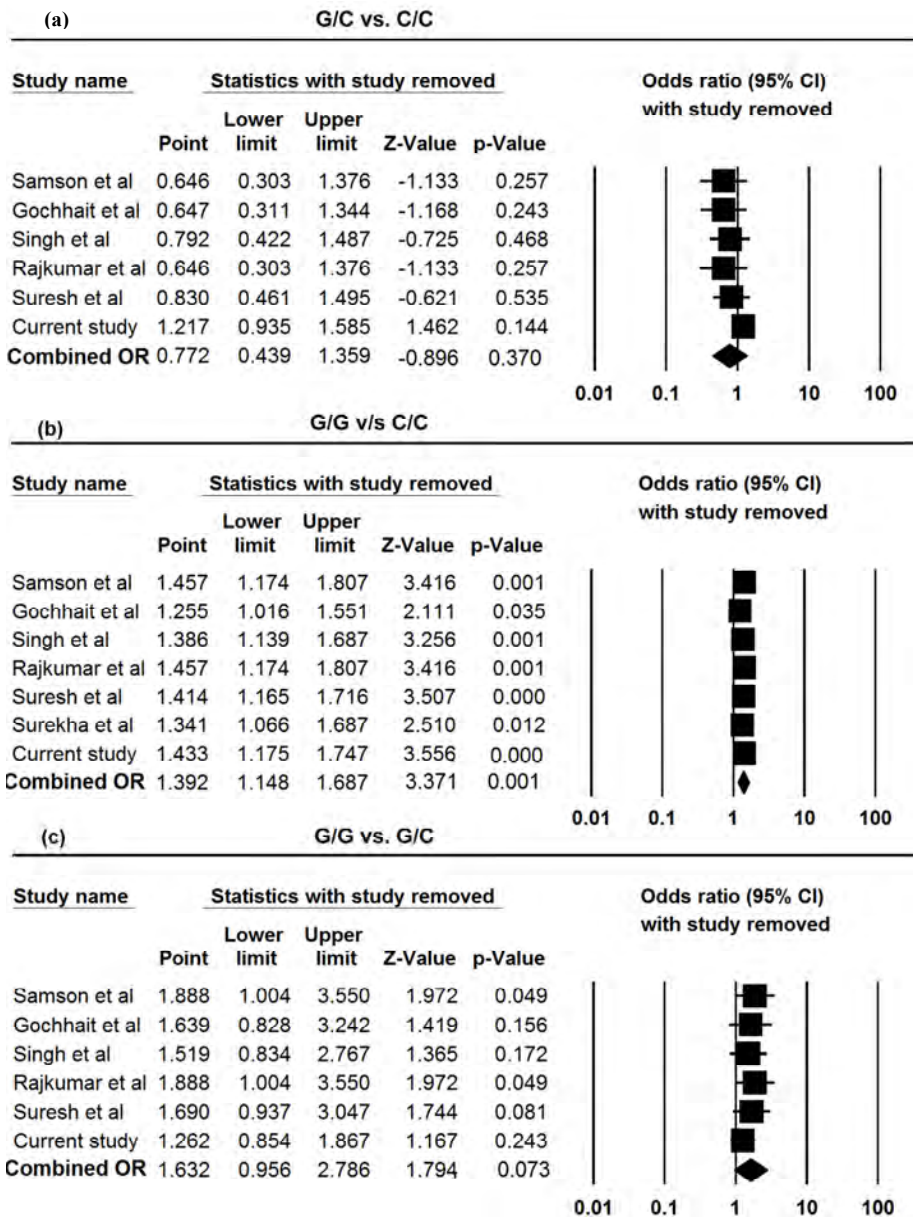

Fig. (4). Sensitivity analysis by showing forest plot of OR with 95% CI of breast cancer associated with the p53 Arg72Pro polymorphism in Indian population. Black square represent the value of OR and the size of the square indicates the inverse proportion relative to its variance. Horizontal line is the 95% CI of OR. The studies are listed by year of publication. (**a**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (GC *vs*. CC model). (**b**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (GG *vs*. CC; homozygous model). (**c**) Forest plot with ORs on breast cancer risk associated with p53 Arg72Pro polymorphism (GG *vs*. GC model).

with increased risk for cervical [25], urinary bladder [27], skin [28], esophageal [29], non-Hodgkin lymphoma [20] and breast [32] cancer.

Prospects of codon72 SNP use as a biomarker for breast cancer risk assessment [74, 75], has led to a large number of studies evaluating its association with breast cancer risk. A significant correlation of codon72 SNP with breast cancer risk has been shown [83-88], but contradictory reports are also present [89-92], and essentially, the reports are conflicting in nature [81, 88, 93, 94].

Several inconsistent reports analyzing the association of codon72 SNP with the risk of breast cancer are due to low sample size and low statistical power. Meta-analysis, a quantitative technique, derives the information from early reports and provides a meaningful conclusion with increased statistical power [95]. The current meta-analysis includes the data from 6 early reports and from our present case-control study to analyses the association of p53 Arg72Pro polymorphism and breast cancer risk. The results of present analysis will provide a reliable assessment about the role of p53 Arg72Pro SNP in breast cancer susceptibility in Indian population by reducing random errors [96].

Our overall pooled analyses suggest a significant correlation between p53 Arg72Pro SNP and an elevated risk of breast cancer in Indian population. Significant breast cancer

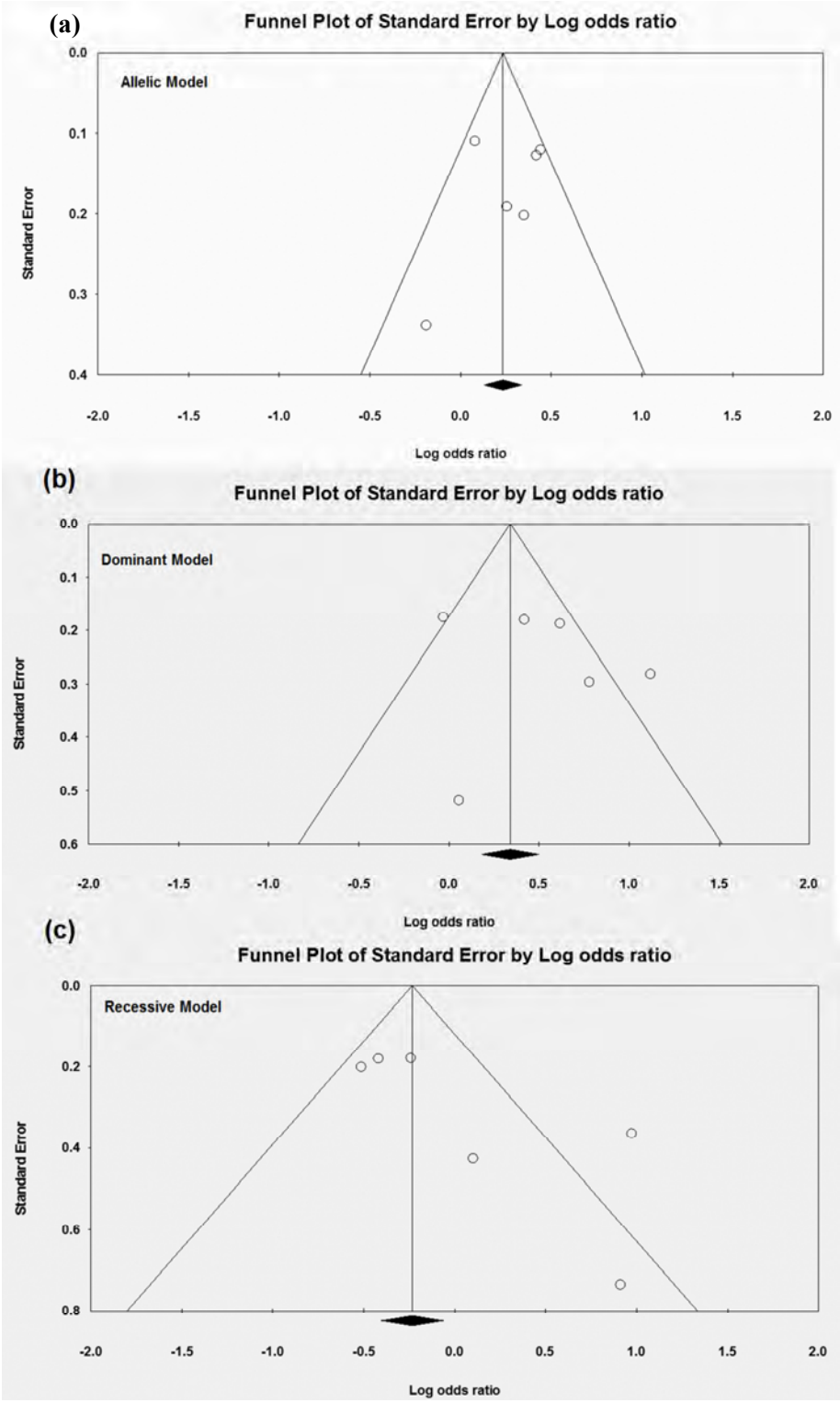

Fig. (5). Assessment of publication bias shown with Funnel plot in studies assaying odds of breast cancer associated with the p53 Arg72Pro polymorphism in Indian population. (**a**) Effect size against precision, the inverse of standard error (Allelic (G *vs*. C) model). (**b**) Effect size against precision, the inverse of standard error (Dominant (GG *vs*. CC+GC) model). (**c**) Effect size against precision, the inverse of standard error (Recessive (CC *vs*. GG+GC) model).

risk was found in 2 comparison models namely Allelic (G *vs*. C: OR=1.26, 95% CI=1.139 to 1.401, p-value 0.000*) and GG *vs*. CC genetic comparison model (OR=1.39, 95% CI=1.148 to 1.687, p-value 0.001*). A significantly decreased breast cancer risk was found in Recessive genetic model (CC *vs*. GG+GC: OR=0.79, 95% CI=0.668 to 0.939, p-value 0.007*). Our results corroborates the findings of an early meta-analysis [97] showing an increased breast cancer risk with the prevalence of GC and CC genotypes [45, 78]. Furthermore, the CC genotype has also been shown linked with poor prognosis [92].

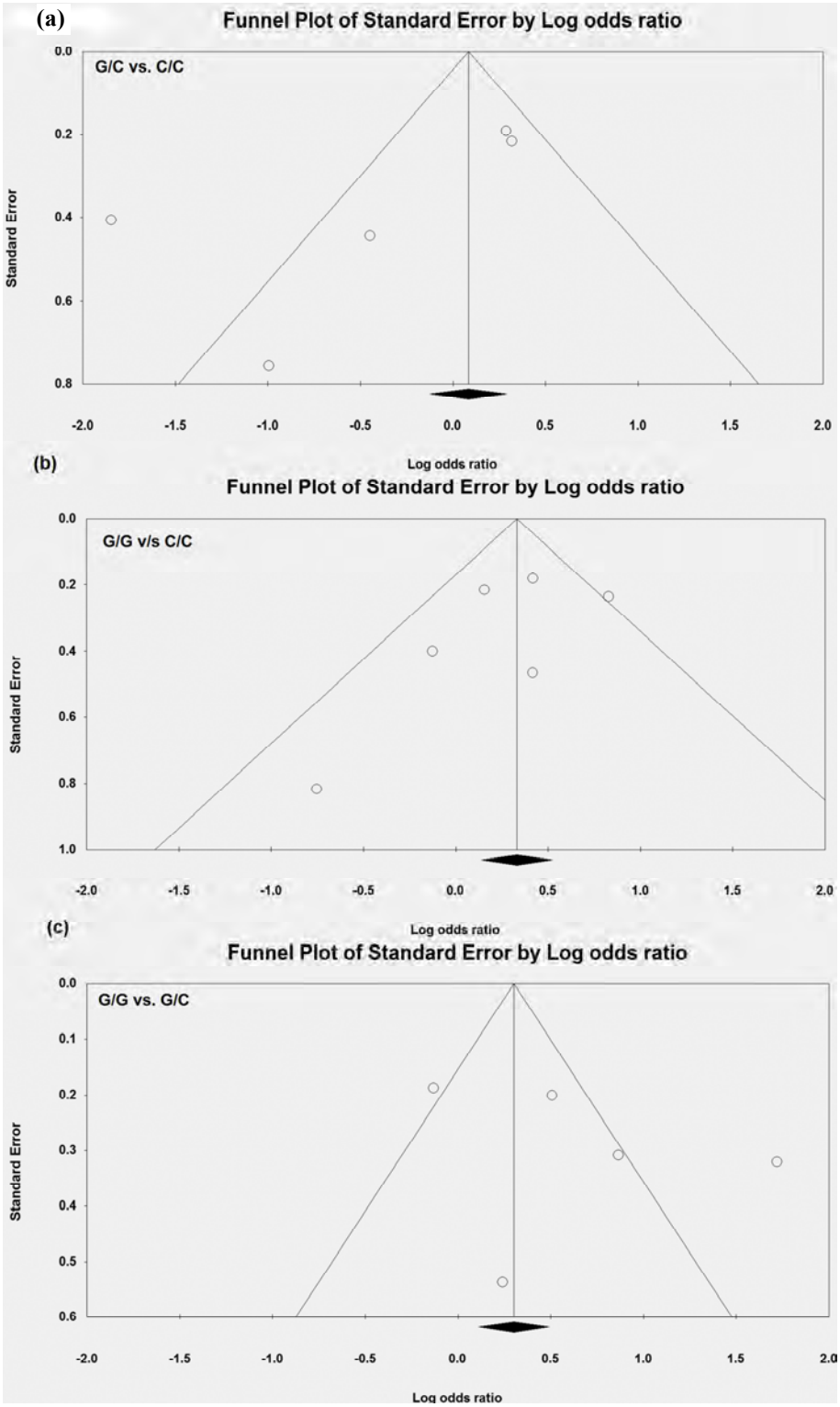

Fig. (6). Assessment of publication bias shown with Funnel plot in studies assaying odds of breast cancer associated with the p53 Arg72Pro polymorphism in Indian population. (**a**) Effect size against precision, the inverse of standard error (GC *vs*. CC model). (**b**) Effect size against precision, the inverse of standard error (GG *vs*. CC homozygous model). (**c**) Effect size against precision, the inverse of standard error (GG *vs*. GC model).

Our meta-analysis also corroborates an early study showing increasingly frequent Arg allele among Asian breast cancer cases. In contrast, significantly reduced risk of breast cancer related with GC *vs*. GG: OR = 0.91 and CC/GC *vs*. GG: OR = 0.90 has also been shown [98].

However the results largely pertained to European populations. Another meta-analysis showed no correlation of p53 Arg72Pro polymorphism with breast cancer susceptibility in overall pooled analysis, or in subgroups based on the race or controls [99]. Although this analysis included but only two

Table 9. Statistics to test publication bias and heterogeneity in the cumulative meta-analysis.

Comparison Models	Egger's Regression Analysis			Heterogeneity Analysis				Model Used
	Intercept	95% Confidence Interval	p-value (2-tailed)	Q-value	df (Q)	$P_{heterogeneity}$	I^2	
Allelic (G *vs*. C)	-0.25	-4.84 to 4.33	0.89	10.93	6	0.090	45.12	Fixed
Dominant (GG *vs*. CC+GC)	2.24	-4.01 to 8.50	0.39	21.48	6	0.002	72.07	Random
Recessive (CC *vs*. GG+GC)	3.04	-0.01 to 6.11	0.05	16.98	6	0.009	64.67	Random
GC *vs*. CC	-4.17	-9.04 to 0.68	0.07	29.43	5	0.000	83.01	Random
GG *vs*. CC	-1.27	-4.35 to 1.79	0.33	9.051	6	0.171	33.70	Fixed
GG *vs*. GC	4.29	-4.46 to 13.05	0.24	34.74	5	0.000	85.61	Random

Indian studies [26, 36] and the weights of both the studies in final analysis might not have amounted to much.

Few shortcomings of current study are as follows. First, we might have excluded some important data published in languages other than English. Second, owing to limited studies conducted on the subject matter, fewer studies included in the final analysis may render results sensitive to study selection. Breast cancer occurrence and progression involves intricate molecular mechanisms and multiple genes harboring many changes effects breast cancer susceptibility. Therefore many exhaustive studies are required to analyze the influence of p53 codon72 SNP on breast cancer risk. Third, the controls in different studies were not homogenously defined though they are exposed to variable risks of breast cancer development. The final conclusion would be more meaningful in the presence of certain details such as tobacco and/or alcohol consumption, menopausal status, obesity/overweight and exposure to varying environmental stress.

However, some strengths of our study are worthwhile to mention. Large number of breast cancer cases and healthy controls drawn from Indian population included in the present study substantially increased the statistical power of the analysis. More importantly, an absence of publication bias suggests the statistical reliability of the conclusion, which may elucidate the role of p53 72G/C polymorphism in breast cancer susceptibility.

CONCLUSION

In our case-control study, we found a significantly reduced breast cancer risk associated with p53 heterozygous arginine variant, (G/C) genotype in total cohort, pre- and post-menopausal women. Arg/Arg (G/G) genotype was found linked with the risk of breast cancer in total cohort and postmenopausal women. Our meta-analysis demonstrate significant breast cancer risk in 2 comparison models namely Allelic (G *vs*. C: OR=1.26) and GG *vs*. CC genetic comparison model (OR=1.39). Further, significantly reduced breast cancer risk related with Recessive genetic model (CC *vs*. GG+GC: OR=0.79). The findings of our meta-analysis suggest a significant association of p53 codon72 SNP with breast cancer susceptibility in Indian population. However, large multicentric studies considering the impact of multiple genes and environmental stresses on breast cancer risk are needed.

LIST OF ABBREVIATIONS

AJCC = American Joint Committee on Cancer
ASPP = Apoptosis-Stimulating of p53 Protein
CGEMS = Cancer Genetic Markers of Susceptibility
iASPP = Inhibitory member of the ASPP family
Mdm2 = Mouse Double Minute 2 Homolog
OR = Odds Ratio
SD = Standard Deviation
SNP = Single Nucleotide Polymorphism
SNP = Single Nucleotide Polymorphism
SV 40 = Simian Virus 40

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of Jamia Millia Islamia (A Central University), New Delhi; and All India Institute of Medical Sciences, New Delhi.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. All human research procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

A written informed consent was obtained from all participants (patients and controls).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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