UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

1. **Project report No.:** **Final**
2. **UGC Reference No.:** **F.No.40-293/2011 (SR)**
3. **Period of report:** **From July 1, 2011** to **December 31, 2014**
4. **Title of research project**: **Assessment of *HIF-1α* and *TP53* polymorphisms and their serum levels in Breast Cancer Patients**
5. **a. Name of the Principal Investigator: Dr. Kamlesh Guleria**

   b. Deptt. and University where work has progressed: Human Genetics

   Guru Nanak Dev University,

   Amritsar, Punjab (India) 143005
6. **Effective date of starting of the Project**: **July 1, 2011**
7. **Grant approved and expenditure incurred during the period of the report**:

a. Total amount approved: Rs. 14,74,738/

Grant Received: Rs.13,47,594/

b. Total expenditure: Rs. 13,47,594/ **(Annexure I)**

Balance Amount to be Released by UGC: **Rs. 1,27,144**

c. Report of the work done: (Please attach a separate sheet) (**Annexure II)**

i. Brief Objectives of the Project:

- To evaluate the polymorphisms in *HIF-1α* and *TP53* Breast cancer patients and unrelated healthy control individuals to assess whether these polymorphisms are associated with breast cancer.
- To study the serum p53 and HIF-1α levels using ELISA kit in normal healthy unrelated controls and breast cancer patients prior to any therapy / surgery.
- To find relationship between *HIF-1α* and *TP53* polymorphisms and their expression levels in serum.
- To find correlation if any, of HIF-1α and TP53 levels in serum, and genetic polymorphisms to assess their prognostic or diagnostic utility.

ii. Work done so far and results achieved and publications, if any, resulting from the work

- Sharma S, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, Singh NR, Bansal D, Gupta A (2014). TP53 Polymorphisms in Sporadic North Indian Breast Cancer Patients. Asian Pacific Journal of Cancer Prevention; 15 (16): 6871.
- Sharma S, Kapahi R, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, Singh NR (2014). No Association of Hypoxia Inducible Factor-1α Gene Polymorphisms with Breast Cancer in North-West Indians. Asian Pacific Journal of Cancer Prevention; 15 (22): 9973-9978.
- Guleria K, Sharma S, Manjari M, Uppal MS, Singh NR, Sambyal V (2012). p.R72P, PIN3 Ins16bp Polymorphisms of TP53 and *CCR5*Δ32 in North Indian Breast Cancer Patients**.** Asian Pac J Cancer Prev; 13(7):3305-11.

iii. Has the progress been according to original plan of work and towards achieving the objective? if not, state reasons

iv. Please indicate the difficulties, if any, experienced in implementing the project:

Delay in Release of Second Installment of grant

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet NA

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission

Salient Features of Results:

- Breast cancer incidence was higher among individuals more than 40 years of age (80%) compared to those less than 40 years (20%).
- For p.P47S polymorphism, we observed the PP genotype in 99.5% of the patients and PS genotype in only 1 patient. All the controls had the wild type PP genotype.
- Heterozygous (RP) genotype was increased in breast cancer patients as compared to controls (51.5 vs 45.5%) and showed 1.61 folds significantly increased risk for breast cancer (OR=1.61, 95% CI, 1.01-2.58, p=0.04). Carrier of P allele (RP+PP) also demonstrated 1.64 folds increased risk for breast cancer (OR=1.64, 95% CI, 1.06-2.54; p= 0.02).
- For PIN3 Ins16bp polymorphism, carriers of A2 allele (A1A2+A2A2) were higher in patients as compared to the controls but the results were not statistically significant (p=0.74).
- For p.R213R (c.639A>G), all individuals had homozygous wild type genotype.
- For *TP53* r.13494g>a polymorphism, no significant difference between genotype and allele frequency in the breast cancer patients and controls was observed.

- Interaction between p.R72P and PIN3 Ins16bp polymorphism (RP-A1A1) showed significant risk of breast cancer (OR=1.65, 95%CI: 0.98-2.78, p=0.05).
- The genotype combination RP-GG of p.R72P and r.13494g>a polymorphism showed 1.72 folds risk for breast cancer (OR=1.72, 95%CI: 1.01-2.92, p=0.04).
- Analysis of genotype combinations of p.R72P, PIN3 Ins16bp and r.13494g>a polymorphisms of *TP53* showed marginally significant risk for breast cancer in individuals with RP-A1A1-GG genotype combination (OR=1.67, 95%CI: 0.97-2.88, p=0.06).
- The CC and CA genotype frequency of *HIF-1α* g.C111A polymorphism was 100 vs 99% and 0 vs 1% in breast cancer patients and healthy controls. AA genotype of g.C111A polymorphism was observed neither in patients nor in control subjects.
- For g.C1772T polymorphism, no significant difference in genotype and allele frequencies of *HIF-1α* g.C1772T polymorphism between cases and control individuals was observed (p>0.05).
- For g.G1790A genotypes, all patients and controls had GG genotype; GA and AA genotype was not observed in patients and control individuals.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as

***Manpower Trained*:**

Project Fellow Ms. Sarika Sharma working on topic entitled **"*HIF-1α, TP53* Polymorphisms and Chromosomal Instability in Breast Cancer Patients"**

***Publication of Results*:**

- Sharma S, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, Singh NR, Bansal D, Gupta A (2014). TP53 Polymorphisms in Sporadic North Indian Breast Cancer Patients. Asian Pacific Journal of Cancer Prevention; 15 (16): 6871.
- Sharma S, Kapahi R, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, Singh NR (2014). No Association of Hypoxia Inducible Factor-1α Gene Polymorphisms with Breast Cancer in North-West Indians. Asian Pacific Journal of Cancer Prevention; 15 (22): 9973-9978.
- Guleria K, Sharma S, Manjari M, Uppal MS, Singh NR, Sambyal V (2012). p.R72P, PIN3 Ins16bp Polymorphisms of TP53 and *CCR5*Δ32 in North Indian Breast Cancer Patients**.** Asian Pac J Cancer Prev; 13(7):3305-11.

Papers presented in Conferences

- Sharma S, Guleria K, Sambyal V (2012). Association of *TP53* Polymorphisms with Breast Cancer. International Conference on Genes, Genetics& Genomics: Today & Tomorrow-Human Concerns 37th Annual Conference of The Society of Human Genetics. Panjab University Chandigarh. March 3-5, 2012.
- Sambyal V, Guleria K, Sharma S, Manjari M, Uppal MS, Singh NR, (2013). p.R72P, PIN3 Ins16bp Polymorphisms of *TP53* and *CCR5*Δ32 in North Indian Breast Cancer Patients. 32nd Annual Convention of Indian Association for Cancer Research "Emerging Trends in Cancer Research: Road to Prevention & Cure" International Symposium on: Infection & Cancer. Dr. B.R. Ambedkar Center for Biomedical Research (ACBR) University of Delhi. February 13-16, 2013.
- Sharma S, Singh NR, Sudan M, Uppal MS, Manjari M, Guleria K, Sambyal V, (2014). HIF-1α, TP53 Polymorphisms and Chromosomal Instability in North-Indian Breast Cancer Patients. 5th International Conference on Translational Cancer Research, Vigyan Bhawan, New Delhi (India). February 6-9, 2014.
- Sharma S, Singh NR, Sudan M, Uppal MS, Manjari M, Guleria K, Sambyal V, (2015). TP53 Polymorphisms and Chromosomal Instability in Breast Cancer Patients: A Follow up Study. International Symposium on "Genomics in Health and Disease" 40th Annual Conference of Indian Society of Human Genetics, National Institute of Immunohaematology, Mumbai. January 28-30, 2015.

Principal Investigator
Signatures with Seal

Dr. Kamlesh Guleria
Assistant Professor
Department of Human Genetics
Guru Nanak Dev University
Amritsar-143005 (India)

Registrar
Signatures with Seal

Registrar,
Guru Nanak Dev University,
Amritsar.

Annexure I

GURU NANAK DEV UNIVERSITY, AMRITSAR

(Established by the State Legislature Act No. 21 of 1969)

Accredited at "A" grade level by NAAC and awarded "University with Potential for Excellence" status by UGC

UTILISATION CERTIFICATE

Certified that the grant of Rs. 13,47,594/- (Rupees Thirteen Lac Forty Seven Thousand Five Hundred Ninety Four Only) sanctioned to Guru Nanak Dev University, Amritsar by the University Grants Commission, New Delhi vide letter Nos.:-

S. No.	Sanction Letter No.	Amount (Rs.)
1.	F.40-293/2011 (SR), dated 29-06-2011	5,84,800.00
2.	F.40-293/2011 (SR), dated 18-10-2014	7,62,794.00
	Total	**13,47,594.00**

pertaining to Research Project entitled, " **Assessment of HIF-1x and TP53 polymorphism and their serum levels in Breast Cancer Patients"** undertaken by **Dr. Kamlesh Guleria, Department of Human Genetics"** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the Commission.

Deputy Controller
(Local Audit Deptt., Punjab)
Guru Nanak Dev University,
Amritsar.

Registrar
Guru Nanak Dev University
Amritsar

ਗੁਰੂ ਨਾਨਕ ਦੇਵ ਯੂਨੀਵਰਸਿਟੀ, ਅੰਮ੍ਰਿਤਸਰ

Guru Nanak Dev University, Amritsar

(Established by the State Legislature Act No. 21 of 1969)

Expenditure Statement

Statement showing expenditure incurred out of the grant sanctioned by **University Grants Commission, New Delhi** for major research project entitled, "**Assessment of HIF-1x and TP53 polymorphism and their serum levels in Breast Cancer Patients**" undertaken by **Dr. Kamlesh Guleria, Department of Human Genetics**, Guru Nanak Dev University, Amritsar **for the period 01-07-2011 to 31-12-2014**.

Grant received	Amount (Rs.)
2011-12	5,84,800/-
2014-15	7,62,794/-
Total	**13,47,594/-**

S. No.	Particulars	Amount Allocated (Rs.)	Expenditure (Rs.)
A.	**Non- Recurring**		
1.	Books & Journals	10,000.00	10,000.00
	Total	**10,000.00**	**10,000.00**
B.	**Recurring**		
1.	Project Fellow	4, 97,032.00 +99,406.00 5,96,438.00	5,95,819.00
2.	Contingency	45,000.00	38,239.00
3.	Chemicals	6,00,000.00 +1,00,000.00 7,00,000.00	5,99,677.00
4.	Travel/Field work	30,000.00	10,559.00
5.	Overhead Charges	93,300.00	93,300.00
	Total	**14,64,738.00**	**13,37,594.00**
	Grand Total of (A) and (B)	**14,74,738.00**	**13,47,594.00**

(Rupees Thirteen Lac Forty Seven Thousand Five Hundred Ninety Four Only)

Deputy Controller (Local Audit Deptt., Punjab)
Guru Nanak Dev University
Amritsar

Registrar
Guru Nanak Dev University
Amritsar

PABX: 2258802 to 2258809; Extension O: 3011, R : 3012; FAX (0183) 2258819, 2258820; GRAMS: University, Amritsar

Annexure II

Work Done

A questionnaire was prepared after the comprehensive survey of literature. The detailed information about the patients and unrelated control individuals along with the family history was recorded in the questionnaire. The patient samples were collected from Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar and healthy control samples were collected from various localities after obtaining written informed consent from these individuals. 10 ml blood sample was collected in pre-labelled vials containing 0.5M EDTA as an anticoagulant and also in plain vials to separate the serum for ELISA analysis.

General characteristics of subjects

In this case-control study 200 sporadic breast cancer patients (197 females and 3 males) and 200 gender and age matched unrelated healthy control individuals (197 females and 3 males) were analyzed. The mean age was 49.4±11.9 years (Range 25-85 years) for the cases and 47.1±12.5 years (Range 24-80 years) for the controls. Breast cancer incidence was higher among individuals more than 40 years of age (80%) compared to those less than 40 years (20%). Out of 200 patients, 22 had stage I, 110 had stage II, 53 had stage III and 15 had stage IV tumors.

Techniques:

Following techniques were standardized with few modifications:

- **DNA Extraction:** The genomic DNA has been extracted from all these samples using standard phenol-chloroform method (Adeli and Ogbonna 1990) with few modifications. The quality and quantity of DNA has been checked using agarose gel electrophoresis.
- **PCR Analysis:** The PCR has been optimized for three [p.P582S (rs11549465), p.A588T (rs11549467) and p.S28Y] polymorphisms of *HIF-1α* and five (p.Pro47Ser, p.R72P (rs1042522), p.R213R, *PIN3 ins16bp* (rs17878362) and r.13494g>a) polymorphisms of *TP53*.

Table 1. Details of *TP53* Polymorphisms and Screening Conditions

Variant (RefSNP)	Genotyping Method	Primers References	PCR product size (bp)	Annealing Temperature, $MgCl_2$	Restriction enzyme	Allele Size (bp)	Expected Fragment
p.P47S (rs1800371)	PCR-RFLP	Pinto et al., 2008	201/185*	59°C, 1.5 mM	MspI	S	201/185
						P	156/140 and 45
p.R72P (rs1800371)	PCR-RFLP	Kazemi et al., 2009	279	59°C, 1.5 mM	BstUI	P	279
						R	160 and 119
PIN3 Ins 16bp (rs17878362)	PCR	Costa et al., 2008	119 or 135	61°C, 1.5 mM	-	A1	119
						A2	135
p.R213R (rs1800372)	PCR-RFLP	Pilger et al., 2008	1621	59°C, 1.0 mM	TaqI	A	926, 383, 312
						G	926, 695
r.13494g>a (rs1625895)	PCR-RFLP	Pilger et al., 2008	1621	59°C, 1.0 mM	MspI	G	356, 299, 277, 277, 168, 124, 120
						A	633, 299, 277, 168, 124, 120

*Size divergence is due to 16bp ins/del polymorphism in intron 3

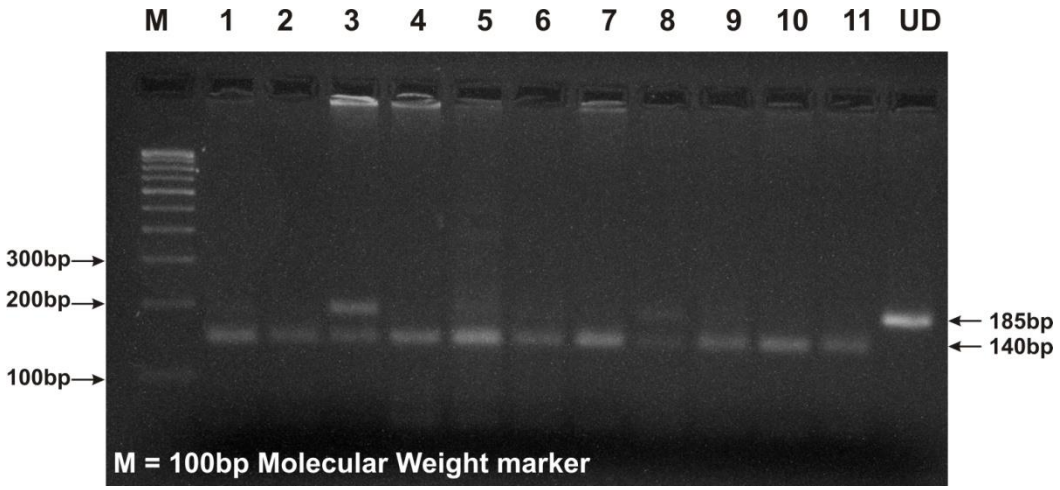

Figure 1. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of p.P47S Polymorphism of TP53. Lane 1,2, 4-7, 9-11 represents wild type homozygous PP individuals and Lane 3 and 8 represents heterozygous (PS) individuals. UD = Undigested PCR product.

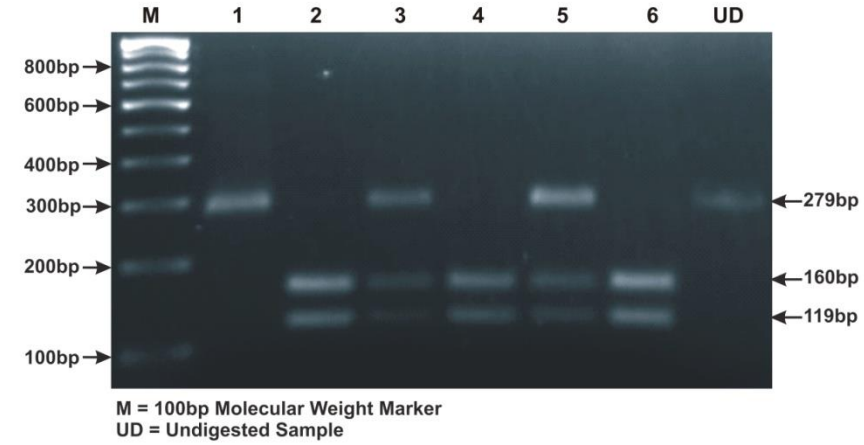

Figure 2. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of TP53. p.R72P polymorphism of *TP53*. Lane 1 shows homozygous proline, lanes 2, 4 and 6 show homozygous arginine and lanes 3 and 5 show heterozygous form.

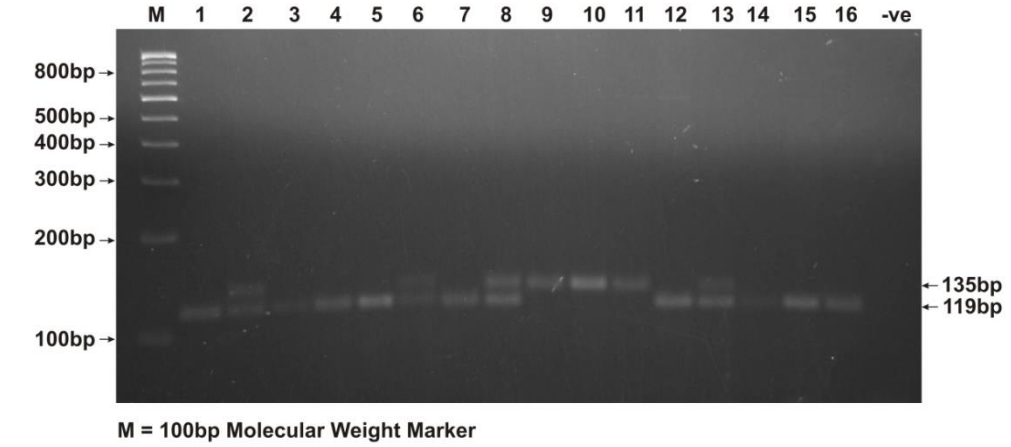

Figure 3. A photograph of Gel Demonstrating the Different Genotypes of PIN3 Polymorphism of *TP53*. Lanes 1, 3, 4, 5, 7, 12, 14, 15 and 16 show A1A1 genotype, lanes 2, 6, 8 and 13 show A1A2 and lane 9-11 show A2A2 genotype of PIN3 Ins16bp polymorphism.

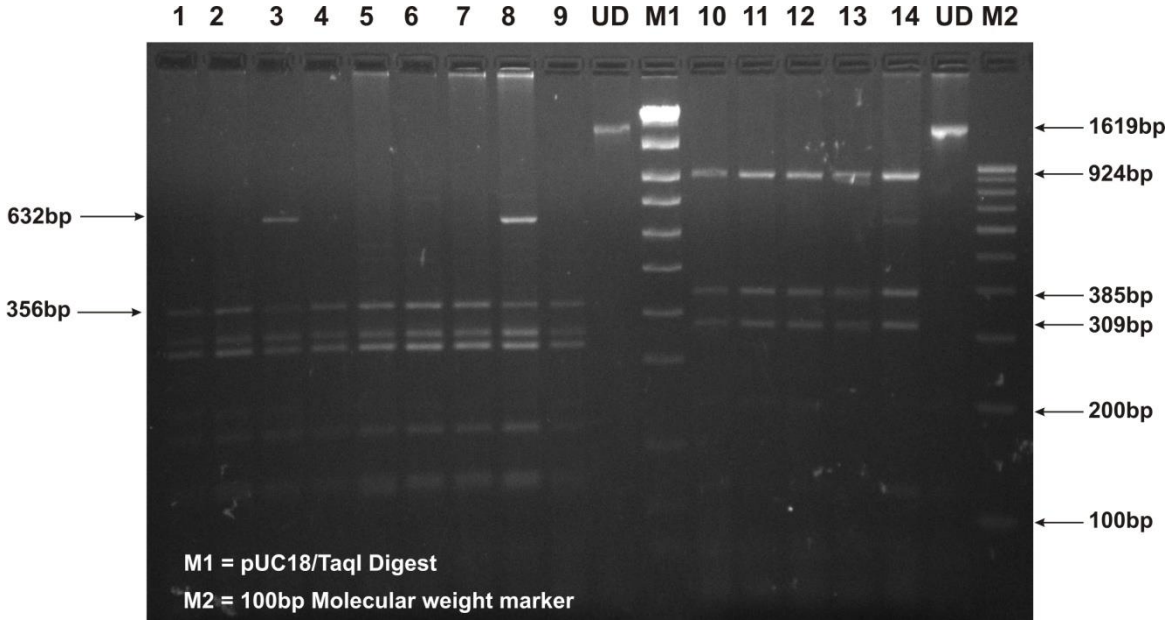

Figure 4. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of p.R213R and r.13494g>a Polymorphisms of *TP53*. Lane 1,2, 4-7 and 9 represents wild type homozygous GG individuals and Lane 3 and 8 represents heterozygous (GA) individuals for r.13494g>a polymorphism. Lane 10-14 represents wild type homozygous AA individuals for p.R213R polymorphism. UD = Undigested PCR product.

Table 2. Details of *HIF-1α* Polymorphisms and Screening Conditions

Variant (RefSNP)	Genotyping Method	Primers Reference	PCR Product Size (bp)	Annealing Temperature	Restriction Enzyme	Allele	Expected Fragment Size (bp)
g.C111A	PCR-RFLP	Apaydin *et al*. 2008	187	59°C	*Bgl*II	C A	143, 44
g.C1772T (rs11549465)	PCR-RFLP	Apaydin *et al*. 2008	346	55°C	*Hph*I	C T	228, 118 346
g.G1790A (rs11549467)	PCR-RFLP	Apaydin *et al*. 2008	346	55°C	*Aci*I	G A	201, 145 346

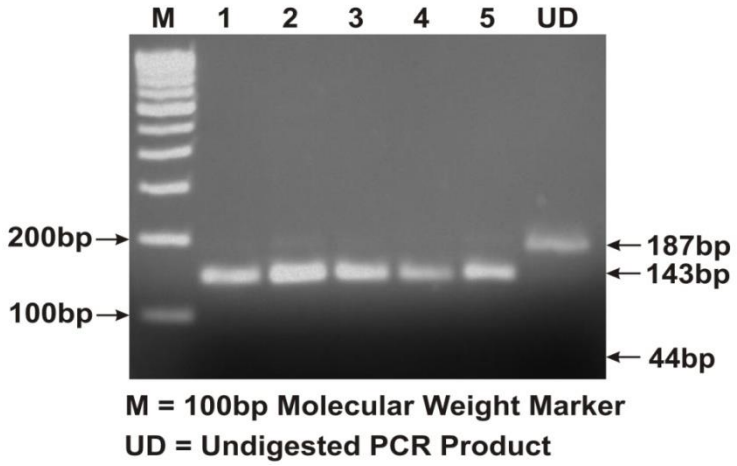

Figure 5. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of g.C111A **polymorphism of *HIF-1α*. Lane M = 100bp molecular weight marker, Lane 1, 2, 3, 4, 5 = CC genotype and Lane UD = Undigested PCR product.**

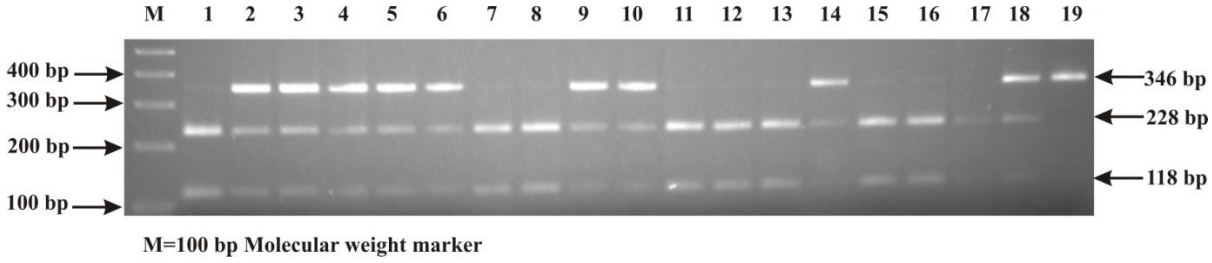

Figure 6. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of g.C1772T polymorphism of *HIF-1α*. Lanes 1, 7, 8, 11-13, 15-17 show CC genotype, Lanes 2-6, 9, 10, 14, 18 show CT genotype and Lane 19 = Undigested PCR product.

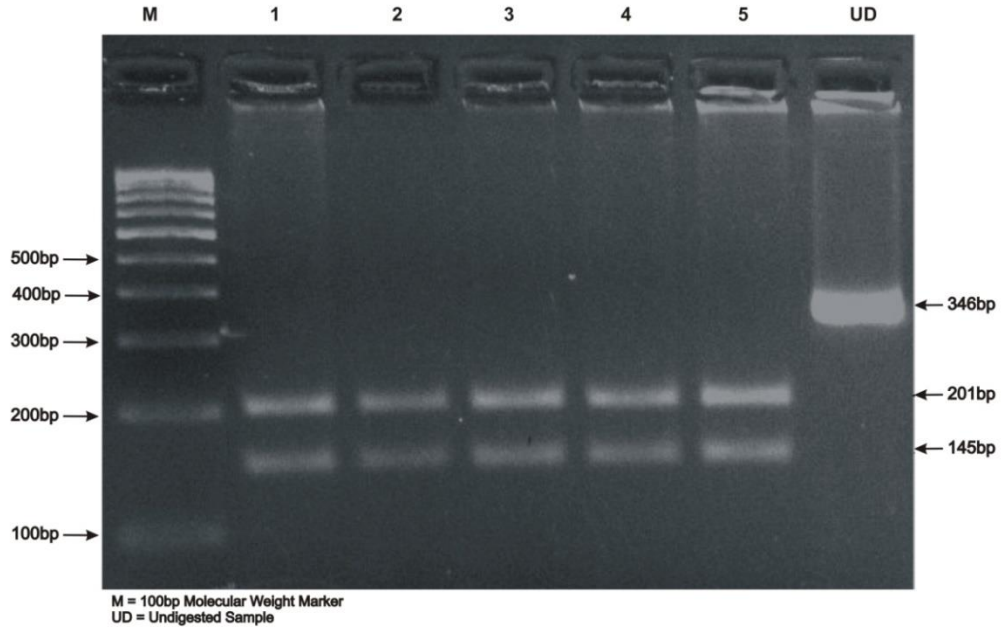

Figure 7. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of g.G1790A polymorphism of *HIF-1α*. Lanes 1-5 represent wild type homozygous GG genotype.

Association between *TP53* polymorphisms and breast cancer risk

The genotyping results of the five polymorphisms of *TP53* [p.P47S, p.R72P, PIN3 Ins16bp, p.R213R and r.13494g>a] are presented in **Table 3**. The observed genotypes frequencies of two polymorphisms (p.R72P and r.13494g>a) were in HWE ($p>0.05$). In PIN3 Ins16bp polymorphism, we observed deviation from HWE in patients ($p<0.05$) which could be attributed to selection bias. For p.P47S polymorphism, we observed the PP genotype in 99.5% of the patients and PS genotype in only 1 patient. All the controls had the wild type PP genotype.

The frequencies of RR, RP and PP genotype of p.R72P was found to be 23.5% vs 33.5%, 51.5% vs 45.5% and 25% vs 21% in patients and controls respectively. Heterozygous (RP) genotype was increased in breast cancer patients as compared to controls (51.5 vs 45.5%) and showed 1.61 folds significantly increased risk for breast cancer (OR=1.61, 95% CI, 1.01-2.58, p=0.04). Carrier of P allele (RP+PP) also demonstrated 1.64 folds increased risk for breast cancer (OR=1.64, 95% CI, 1.06-2.54; p= 0.02). The frequencies of R and P allele were 49.3 vs 56.3% and 50.7 vs 43.7% in patients and controls respectively.

Table 3. Genotype and allele distribution of *TP53* polymorphisms in breast cancer patients and control individuals

Polymorphism (RefSNP)	Genotype	Allele	Patients n (%)	Controls n (%)	OR (95% CI)	p-value
p.P47S	PP		199 (99.5)	200 (100.0)	-	-
(rs 1800371)	PS		1 (0.5)	0 (0.00)	-	-
	SS					
		P	399 (99.75)	400 (100.0)	-	-
		S	1 (0.25)	0 (0.00)	-	-
p.R72P	RR		47 (23.5)	67 (33.5)	Reference	
(rs1042522)	RP		103 (51.5)	91 (45.5)	1.61 (1.01-2.58)	**0.04**
	PP		50 (25.0)	42 (21.0)	1.70 (0.97-2.95)	0.84
	RP+PP		153 (76.5)	133 (66.5)	1.64 (1.06-2.54)	**0.02**
		R	197 (49.3)	225 (56.3)	Reference	
		P	203 (50.7)	175 (43.7)	1.32 (1.00-1.75)	0.04
PIN3 Ins16bp	A1A1		134 (67.0)	137 (68.5)	Reference	
(rs17878362)	A1A2		52 (26.0)	55 (27.5)	0.97 (0.62-1.51)	0.88
	A2A2		14 (07.0)	8 (04.0)	1.79 (0.73-4.40)	0.19
	A1A2+A2A2		66 (33.0)	63 (31.5)	1.07 (0.70-1.63)	0.74
		A1	320 (80.0)	329 (82.3)	Reference	
		A2	80 (20.0)	71 (17.7)	1.15 (0.81-1.65)	0.41
p.R213R	AA		200 (100.0)	200 (100.0)	-	-
(rs1800372)	AG		0 (0.00)	0 (0.00)	-	-
	GG		0 (0.00)	(0.00)	-	-
		A	400 (100.0)	400 (100.0)	-	-
		G	(0.00)	(0.00)	-	-
r.13494g>a	GG		124 (62.0)	135 (67.5)	Reference	
(rs1625895)	GA		66 (33.0)	56 (28.0)	1.28 (0.83-1.98)	0.25
	AA		10 (05.0)	9 (4.5)	1.21 (0.48-3.08)	0.90
	GA+AA		76 (38.0)	65 (32.5)	1.27 (0.84-1.92)	0.24
		G	314 (78.5)	326 (81.5)	Reference	
		A	86 (21.5)	74 (18.5)	1.20 (0.85-1.70)	0.28

*n- Number of subjects, Figures in parentheses represents frequency of each genotype and allele; OR- Odds ratio; CI- confidence interval. Statistically significant p-values ($p<0.05$) are indicated in bold

In breast cancer patients the frequencies of A1A1, A1A2 and A2A2 genotypes of PIN3 Ins16bp polymorphism was 67%, 26% and 7% respectively whereas in controls the genotype frequencies were 68.5%, 27.5% and 4% respectively. No significant difference was observed in the genotype and allele frequency between the breast cancer patients and controls. Carriers of A2 allele (A1A2+A2A2) were higher in patients as compared to the controls but the results were not statistically significant (p=0.74). For p.R213R (c.639A>G), none of the breast cancer patients and controls analyzed exhibited A to G nucleotide substitution at position 639, and all individuals had homozygous wild type genotype. The frequencies of GG, GA and AA genotypes of *TP53* r.13494g>a polymorphism were 62 vs 67.5%, 33 vs 28% and 5 vs 4.5% in patients and controls respectively. There was no significant difference between genotype and allele frequency in the breast cancer patients and controls.

To study the association between breast cancer and possible combinations of the *TP53* polymorphisms, we performed genotype - genotype combination analysis of three (p.R72P, PIN3 Ins16bp and r.13494g>a) polymorphisms (**Table 4**). We observed that interaction between p.R72P and PIN3 Ins16bp polymorphism (RP-A1A1) showed significant risk of breast cancer (OR=1.65, 95%CI: 0.98-2.78, p=0.05). The genotype combination RP-GG of p.R72P and r.13494g>a polymorphism showed 1.72 folds risk for breast cancer (OR=1.72, 95%CI: 1.01-2.92, p=0.04). Analysis of genotype combinations of p.R72P, PIN3 Ins16bp and r.13494g>a polymorphisms of

TP53 showed marginally significant risk for breast cancer in individuals with RP-A1A1-GG genotype combination (OR=1.67, 95%CI: 0.97-2.88, p=0.06) (**Table 4**). The associations between the p.R72P, PIN3 Ins16bp, r.13494g>a polymorphisms and the risk of breast cancer were further examined with stratification on age at onset, menopausal status and clinical stage. No significant association was observed.

Table 4. Interaction between p.R72P, PIN3 Ins16bp and r.13494g>a polymorphisms in breast cancer patients and healthy controls

Combination	No. of patients n (%)	No. of controls n (%)	OR (95% CI)	p-value
p.R72P-PIN3 Ins16bp				
RR-A1A1	46 (23.0)	60 (30.0)	Reference	
RR-A1A2	1 (0.5)	7 (3.5)	0.19 (0.02-1.57)	0.14
RP-A1A1	71 (35.5)	56 (28.0)	1.65 (0.98-2.78)	**0.05**
RP-A1A2	30 (15.0)	33 (16.5)	1.18 (0.63-2.22)	0.60
RP-A2A2	2 (1.0)	1 (0.5)	NC	NC
PP-A1A1	17 (8.5)	21 (10.5)	1.06 (0.50-2.23)	0.89
PP-A1A2	21 (10.5)	15 (7.5)	1.82 (0.85-3.93)	0.13
PP-A2A2	12 (6.0)	7 (3.5)	2.24 (0.81-6.13)	0.11
PIN3 Ins16bp-r.13494g>a				
A1A1-GG	118 (59.0)	124 (62.0)	Reference	
A1A2-GG	6 (3.0)	11 (5.5)	0.57 (0.20-0.60)	0.28
A1A1-AG	16 (8.0)	13 (6.5)	1.29 (0.60-2.80)	0.51
A1A2-AG	41 (20.5)	41 (20.5)	1.05 (0.64-1.73)	0.84
A2A2-AG	9 (4.5)	2 (1.0)	NC	NC
A1A2-AA	5 (2.5)	3 (1.5)	NC	NC
A2A2-AA	5 (2.5)	6 (3.0)	0.87 (0.26-2.95)	0.82
p.R72P-r.13494g>a				
RR-GG	44 (22.0)	62 (31.0)	Reference	
RR-AG	1 (0.5)	4 (2.0)	NC	NC
RR-AA	0 (0.0)	1 (0.5)	NC	NC
RP-GG	66 (33.0)	54 (27.0)	1.72 (1.01-2.92)	**0.04**
RP-AG	37 (18.5)	37 (18.5)	1.41 (0.77-2.56)	0.26
RP-AA	2 (1.0)	0 (0.0)	NC	NC
PP-GG	14 (7.0)	19 (9.5)	1.04 (0.47-2.29)	0.92
p.R72P- PIN3 Ins16bp - r.13494g>a				
RR-A1A1-GG	45 (22.5)	59 (29.5)	Reference	
RP-A1A2-AG	24 (12.0)	26 (13.0)	1.21 (0.61-2.38)	0.58
RP-A1A1-GG	60 (30.0)	47 (23.5)	1.67 (0.97-2.88)	0.06
PP-A1A2-AG	17 (8.5)	12 (6.0)	1.85 (0.80-4.27)	0.14
PP-A1A1-GG	13 (6.5)	18 (9.0)	0.95 (0.42-2.13)	0.89
PP-A1A2-AA	3 (1.5)	2 (1.0)	-	-
RP-A1A2-AA	2 (1.0)	-	-	-
PP-A2A2-AG	7 (3.5)	1 (0.5)	-	-
PP-A1A1-AG	4 (2.0)	2 (1.0)	-	-
RP-A1A1-AG	11 (5.5)	10 (5.0)	1.44 (0.56-3.69)	0.44*
PP-A2A2-AA	5 (2.5)	6 (3.0)	-	-
RR-A1A1-AG	1 (0.5)	1 (0.5)	-	-
RP-A1A2-GG	4 (2.0)	-	-	-
PP-A1A2-GG	1 (0.5)	1 (0.5)	-	-
RP-A2A2-AG	2 (1.0)	1 (0.5)	-	-
RP-A1A2-GG	1 (0.5)	7 (3.5)	-	-
RR-A1A2-AG	-	3 (1.5)	-	-
RR-A1A2-AA	-	1 (0.5)	-	-
RR-A1A2-GG	-	3 (1.5)	-	-

*n- Number of subjects, Figures in parentheses represents frequency of each genotype and allele; OR- Odds ratio; CI- confidence interval. Statistically significant p-values (p<0.05) are indicated in bold

Association between HIF-1α polymorphisms and breast cancer risk

The genotype and allele frequencies of g.C111A, g.C1772T and g.G1790A polymorphisms of *HIF-1α* in the patients and controls are shown in the **Table 5**.

Table 5. Genotype and Allele Frequencies of *HIF-1α* polymorphisms in Breast Cancer Patients and Controls

Variant			Patients n(%)	Controls n(%)	OR(95% CI)	χ^2 value	p value
g.C111A	Genotype	CC	200 (100)	198 (99.0)	-		
		CA	-	2 (1.0)	-	NC	NC
		AA	-	-	-		
		Allele					
		C	400 (100)	398 (99.5)	-		
		A	-	2 (0.5)	-		
g.C1772T	Genotype	CC	152 (76.0)	149 (74.5)	1(Reference)		
(rs11549465)		CT	38 (19.0)	42 (21.0)	0.89(0.54-1.45)	0.23	0.63
		TT	10 (5.0)	9 (4.5)	1.09(0.43-2.76)	0.03	0.86
		Allele					
		C	342 (85.5)	340 (85.0)	1(Reference)	0.04	0.84
		T	58 (14.5)	60 (15.0)	0.96(0.65-1.42)		
g.G1790A	Genotype	GG	200 (100)	200 (100)	-	NC	NC
(rs11549467)		GA	-	-	-		
		AA	-	-	-		
		Allele					
		G	400 (100)	400 (100)	-		
		A	-	-			

*NC: Not calculated; OR: odds ratio; CI: Confidence intervals

The CC and CA genotype frequency of *HIF-1α* g.C111A polymorphism was 100 vs 99% and 0 vs 1% in breast cancer patients and healthy controls. AA genotype of g.C111A polymorphism was observed neither in patients nor in control subjects. For g.C1772T polymorphism, the frequency of CC, CT and TT genotype was 76 vs 74.5%, 19 vs 21% and 5 vs 4.5% in breast cancer patients and control individuals respectively. There was no significant difference in genotype and allele frequencies of *HIF-1α* g.C1772T polymorphism between cases and control individuals ($p>0.05$). For g.G1790A genotypes, all patients and controls had GG genotype; GA and AA genotype was not observed in patients and control individuals. Analyses of various genetic models (**Table 6**) showed no association of *HIF-1α* g.C1772T polymorphism with breast cancer risk in the studied subjects ($p>0.05$).

Table 6. Association Analyses of *HIF-1α* g.C1772T Polymorphism with Breast Cancer Risk

Genetic Model		OR(95% CI)	p value
Dominant model	CT+TT *vs* CC	0.92(0.59-1.45)	0.73
Over dominant model	CT *vs* CC+TT	0.88(0.54-1.44)	0.62
Recessive model	TT *vs* CC+CT	1.12(0.44-2.81)	0.81
Homozygous codominant	TT *vs* CC	1.09(0.43-2.76)	0.86
Heterozygous codominant	CT *vs* CC	0.89(0.54-1.45)	0.63
Allele contrast	T *vs* C	0.96(0.65-1.42)	0.84

*OR: odds ratio; CI: Confidence intervals

We stratified the study subjects to investigate the relationship of *HIF-1α* g.C1772T polymorphisms with age, menopausal status, habitat, habit and tumor stage of breast cancer patients and observed significant difference in genotype distribution of CC and combined CT+TT genotypes of *HIF-1α* g.C1772T in vegetarian and non vegetarian breast cancer patients (p=0.02).

Evaluation of serum HIF-1α levels using ELISA

Serum HIF-1α levels were measured with a commercially available enzyme linked immunosorbent assay kit (Cusabio Biotech Co., LTD), according to the manufacturer protocol. The lower limit of detection for HIF-1α was <7.8 pg/ml. Serum HIF-1α levels have been evaluated in 42 breast cancer patients. Mean HIF-1α levels was 21.66pg/ml.

Evaluation of serum TP53 levels using ELISA

Serum p53 levels were measured with a commercially available enzyme linked immunosorbent assay kit (Cusabio Biotech Co., LTD), according to the manufacturer protocol. The lower limit of detection for p53 was <3.0 pg/ml. Serum p53 levels have been evaluated in 42 breast cancer patients. Mean p53 levels was 82.5pg/ml.

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PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Name and address of the Principal Investigator: **Dr. Kamlesh Guleria**
   Department of Human Genetics,
   Guru Nanak Dev University,
   Amritsar, Punjab (India) 143005
2. Name and Address of the institution: Guru Nanak Dev University, Amritsar, Punjab (India) 143005
3. UGC Approval No. and Date: **F.No.40-293/2011 (SR), June 29, 2011**
4. Date of Implementation: **July 1, 2011**
5. Tenure of the Project: **Three Years + 6 month Extension**
6. Total Grant Allocated: **Rs. 14,74,738/**
7. Total Grant Received: **Rs. 13,47,594/**
8. Final Expenditure: **Rs. 13,47,594/**
9. Title of the Project: **Assessment of *HIF-1α* and *TP53* polymorphisms and their serum levels in Breast Cancer Patients**
10. Objectives of the Project:

- To evaluate the polymorphisms in *HIF-1α* and *TP53* Breast cancer patients and unrelated healthy control individuals to assess whether these polymorphisms are associated with breast cancer.
- To study the serum p53 and HIF-1α levels using ELISA kit in normal healthy unrelated controls and breast cancer patients prior to any therapy / surgery.
- To find relationship between *HIF-1α* and *TP53* polymorphisms and their expression levels in serum.
- To find correlation if any, of HIF-1α and TP53 levels in serum, and genetic polymorphisms to assess their prognostic or diagnostic utility.

11. Whether Objectives were achieved: Yes

12. Achievements from the Project:

Publications:

- Sharma S, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, Singh NR, Bansal D, Gupta A (2014). TP53 Polymorphisms in Sporadic North Indian Breast Cancer Patients. Asian Pacific Journal of Cancer Prevention; 15 (16): 6871.
- Sharma S, Kapahi R, Sambyal V, Guleria K, Manjari M, Sudan M, Uppal MS, Singh NR (2014). No Association of Hypoxia Inducible Factor-1α Gene Polymorphisms with Breast Cancer in North-West Indians. Asian Pacific Journal of Cancer Prevention; 15 (22): 9973-9978.
- Guleria K, Sharma S, Manjari M, Uppal MS, Singh NR, Sambyal V (2012). p.R72P, PIN3 Ins16bp Polymorphisms of TP53 and *CCR5*Δ32 in North Indian Breast Cancer Patients**.** Asian Pac J Cancer Prev; 13(7):3305-11.

Papers presented in Conferences

- Sharma S, Guleria K, Sambyal V (2012). Association of *TP53* Polymorphisms with Breast Cancer. International Conference on Genes, Genetics& Genomics: Today & Tomorrow-Human Concerns 37th Annual Conference of The Society of Human Genetics. Panjab University Chandigarh. March 3-5, 2012.
- Sambyal V, Guleria K, Sharma S, Manjari M, Uppal MS, Singh NR, (2013). p.R72P, PIN3 Ins16bp Polymorphisms of *TP53* and *CCR5*Δ32 in North Indian Breast Cancer Patients. 32nd Annual Convention of Indian Association for Cancer Research "Emerging Trends in Cancer Research: Road to Prevention & Cure" International Symposium on: Infection & Cancer. Dr. B.R. Ambedkar Center for Biomedical Research (ACBR) University of Delhi. February 13-16, 2013.
- Sharma S, Singh NR, Sudan M, Uppal MS, Manjari M, Guleria K, Sambyal V, (2014). HIF-1α, TP53 Polymorphisms and Chromosomal Instability in North-Indian Breast Cancer Patients. 5th International Conference on Translational Cancer Research, Vigyan Bhawan, New Delhi (India). February 6-9, 2014.
- Sharma S, Singh NR, Sudan M, Uppal MS, Manjari M, Guleria K, Sambyal V, (2015). TP53 Polymorphisms and Chromosomal Instability in Breast Cancer Patients: A Follow up Study. International Symposium on "Genomics in Health and Disease" 40th Annual Conference of Indian Society of Human Genetics, National Institute of Immunohaematology, Mumbai. January 28-30, 2015.

13. Summary of the Findings:

- Breast cancer incidence was higher among individuals more than 40 years of age (80%) compared to those less than 40 years (20%).
- For p.P47S polymorphism, we observed the PP genotype in 99.5% of the patients and PS genotype in only 1 patient. All the controls had the wild type PP genotype.
- Heterozygous (RP) genotype was increased in breast cancer patients as compared to controls (51.5 vs 45.5%) and showed 1.61 folds significantly increased risk for breast cancer (OR=1.61, 95% CI, 1.01-2.58, p=0.04). Carrier of P allele (RP+PP) also demonstrated 1.64 folds increased risk for breast cancer (OR=1.64, 95% CI, 1.06-2.54; p= 0.02).
- For PIN3 Ins16bp polymorphism, carriers of A2 allele (A1A2+A2A2) were higher in patients as compared to the controls but the results were not statistically significant (p=0.74).
- For p.R213R (c.639A>G), all individuals had homozygous wild type genotype.
- For *TP53* r.13494g>a polymorphism, no significant difference between genotype and allele frequency in the breast cancer patients and controls was observed.
- Interaction between p.R72P and PIN3 Ins16bp polymorphism (RP-A1A1) showed significant risk of breast cancer (OR=1.65, 95%CI: 0.98-2.78, p=0.05).
- The genotype combination RP-GG of p.R72P and r.13494g>a polymorphism showed 1.72 folds risk for breast cancer (OR=1.72, 95%CI: 1.01-2.92, p=0.04).
- Analysis of genotype combinations of p.R72P, PIN3 Ins16bp and r.13494g>a polymorphisms of *TP53* showed marginally significant risk for breast cancer in individuals with RP-A1A1-GG genotype combination (OR=1.67, 95%CI: 0.97-2.88, p=0.06).
- The CC and CA genotype frequency of *HIF-1α* g.C111A polymorphism was 100 vs 99% and 0 vs 1% in breast cancer patients and healthy controls. AA genotype of g.C111A polymorphism was observed neither in patients nor in control subjects.
- For g.C1772T polymorphism, no significant difference in genotype and allele frequencies of *HIF-1α* g.C1772T polymorphism between cases and control individuals was observed (p>0.05).
- For g.G1790A genotypes, all patients and controls had GG genotype; GA and AA genotype was not observed in patients and control individuals.

14. Contribution to the Society:

There is no information about the *HIF-1α* or *TP53* polymorphisms in the population of Punjab, North-West India. These findings would help to draw a correlation with progression rate/aggressiveness of breast cancer. Genomic profiling can help in defining molecular targets for chemoprevention. This study would provide more precise molecular markers specific for early*TP53* alterations and enable mechanism-based early detection and personalized prevention strategies for breast cancer. Understanding of the biological mechanisms underlying breast cancer predisposition genes would offer exciting opportunities for new therapies.

15. Whether any Ph.D. Enrolled/Produced:

Project Fellow Ms. Sarika Sharma working on topic entitled "***HIF-1α, TP53* Polymorphisms and Chromosomal Instability in Breast Cancer Patients"**

16. No. of Publications out of the project: **Three**

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