RESEARCH NOTE

Oestrogen receptor beta (ERβ) polymorphism and its influence on breast cancer risk

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Introduction

Oestrogen, a steroid hormone plays an important role in the development and maintenance of female sexual characters in humans. It also plays a crucial role in the pathogenesis and progression of breast cancer. The biological effects of oestrogen, such as growth stimulation and differentiation of normal mammary tissue are mediated primarily through high-affinity binding of oestrogen receptor beta (ERβ) (Roodi *et al*. 1995). ERs are nuclear receptor proteins that have an oestrogen-binding domain (Rayter 1991). There are two types of ERs: ERα (ESR1) and ERβ (ESR2). The *ERβ* gene is located on chromosome 14q22-24. ERβ regulates genes that function as tumour suppressors. The *ESR2* gene comprises of eight exons spanning ~40 kb. *ESR2* has a high homology to *ESR1* in the DNA-binding and ligand-binding domains, but encodes a distinct transcriptional activating function-1 (AF-1) domain. ER signalling plays a major role in development and maturation of mammary gland. Low percentage of epithelial cells (7%–10%) in normal human breast express ERα, whereas relatively higher percentage of breast cells express ERβ which is inversely related to cell proliferation, indicating its protective role in tumorigenesis. While *ERα* expression fluctuates during menstrual cycle, the expression of *ERβ* remains stable. The protective role of ERβ was evident from the inverse relation observed between expression level and grade of tumour of ductal carcinoma *in situ* (DCIS). Data from protein analysis of ERβ indicated that the expression of ERβ is a favourable prognostic marker in breast cancer. While the role of ERβ in breast cancer is unclear, the recently emerging hypothesis is that the increased *ERβ* expression is associated with increased likelihood of good response to endocrine therapy (Herynk and Fuqua 2004).

Numerous studies have reported the expression of *ERβ* in various cancers including lung, breast and stomach. Immunohistochemical studies suggest that *ERβ* tends to be expressed in ERα-positive breast cancers and there are *ERα* and *ERβ* coexpressing cells in breast cancer. The existence of various variants forms of ERβ had been reported in breast cancer cells (Leygue *et al*. 1999). Various observations obtained from experiments using *ERβ*-expressing stable transformants in breast cancer cell lines suggested that ERβ and ERβcx (truncated at the C-terminal region but has extra 26 aminoacids) specifically suppress the function of ERα through different mechanisms. These ERβ isoforms could be an important functional modulators of oestrogen-signalling pathways in breast cancer cells and might affect the clinical outcome of patients with primary breast cancer (Ogawa *et al*. 1998).

The A1730G polymorphism is present in the 3′UTR of *ERβ* gene. It is believed that single nucleotide polymorphisms when situated in the untranslated region might even cause different-structural folds of mRNA and thus influence gene expression (Shen *et al*. 1999). It was reported that this polymorphism presumably had no functional implication, but its allele might be in linkage disequilibirium with relevant mutations in the gene. The specific control sequence of the *ERβ* mRNA degradation pathway was found to be located in the 3′UTR region (Kenealy *et al*. 2000). Analysis of 1730 A–G polymorphism at *ERβ* showed no significant association with anorexia, bulimia nervosa and obesity in children and adolescents (Rosenkranz *et al*. 1998) and other diseases. The *G* allele of the A1730G polymorphism was more frequent in patients with an early age of onset than in patients with a late age of onset of Parkinson disease ($P = 0.006$) (Westberg *et al*. 2004).

Keeping in view the protective role offered by ERβ, the present study has attempted to evaluate the potential functional significance of A1730G polymorphism in 3′UTR of *ERβ* gene.

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Keywords. oestrogen receptor; polymorphisms; breast cancer; PCR-RFLP; HER2/neu 3′UTR.

Materials and methods

In the present study, 250 breast cancer patients were selected. Two hundred and fifty healthy and age-matched women without family history of breast cancer or any other cancers were selected as controls. Cases were chosen from Nizam's Institute of Medical Sciences and controls included healthy volunteers after confirmed diagnosis. The diagnosis of breast cancer was established by pathological examination, mammography, fine needle aspiration (FNAC) and biopsy. Epidemiological history, such as age at onset of breast cancer, diet, socioeconomic status, occupation, reproductive history, family history and consanguinity were obtained through personal interview with breast cancer patients. The patients were screened for receptor status of oestrogen, progesterone and HER-2/neu (human epidermal growth factor receptor 2) by using immunohistochemical assay. The clinical history such as size of the tumour, presence of auxiliary nodes, extent of metastasis, stage and type of the breast cancer, chemotherapeutic drugs used and prognosis of the disease were collected with the help of oncologist. Informed consent was taken from all patients and controls. The approval of ethical committee was taken before initiation of the work.

For all patients and controls, 5 mL of blood was collected in an EDTA vaccutainer. DNA was isolated (Nuremberg and Lahari 1991) and used for amplification of A1730G single nucleotide polymorphism in the 3′UTR of exon eight of *ERβ* gene.

ERβ polymorphism

PCR-RFLP was done for the identification of *ERβ* polymorphism using specific primers (Wang *et al*. 2004). The amplified product was digested with 1 unit of *Alu*I enzyme (New England Biolabs, Ipswich, USA) at 37°C for overnight and electrophoresed on 2% agorose gel.

A–G substitution at nucleotide 1730 in exon eight introduces a recognition site for *Alu*I which is present at 3′UTR. Digestion by *Alu*I produces one band of 307 bp in the normal *ERβ* sequence (*AA*). Three separate bands of 307, 240 and 67 bp in the heterozygous individual (*AG*), and two separate bands of 240 and 67 bp in mutant homozygotes (*GG*) (figure 1).

Statistical analysis

The results were analysed using appropriate statistical tests by SPSS version 14. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. Differences in genotype frequency distribution between disease and control groups were done using 2×2 χ^2 and χ^2 test for heterogeneity. Multivariate analysis was done to find out the combined risk of two to three factors.

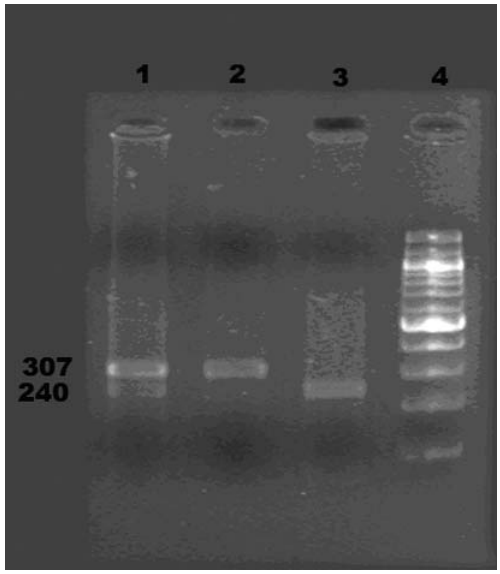

Figure 1. Gel picture of ERβ polymorphism. Lane 1, *AG*; lane 2, *AA*; lane 3, *GG*; lane 4, 100-bp ladder.

Results and discussion

ERβ is one of the most important factors in the mechanism of oestrogen action and it is found in many tissues, including the central nervous system, cardiovascular system, immune system, urogenital tract, gastrointestinal tract, kidneys and lungs (Kupier *et al*. 1997). Table 1 shows the frequency distribution of *ERβ* genotype in both cases and controls. The frequency of *GG* genotype (8.47%) was increased significantly in breast cancer as compared to controls (3.6%) with corresponding increase in an allele frequency ($P < 0.05$; table 2), suggesting that *GG* genotype confers risk for development of breast cancer. Both cases ($\chi^2 = 0.44$; $P = 0.8025$) as well as controls ($\chi^2 = 0.11$; $P = 0.9465$) did not show a significant deviation from Hardy–Weinberg equilibrium.

The frequency of *GG* genotype was found to be elevated in premenopausal breast cancer patients (12.2%) as compared to postmenopausal breast cancer patients (4.3%), which indicates that the presence of *GG* genotype might predispose to an early onset of breast cancer. An increased frequency of *GG* genotype was also observed in patients with family history of cancer (12.3%) when compared with nonfamilial cases (6.9%). The lowered *ERβ* expression of *GG* genotype might be resulting in loss or reduced tumour suppressor function leading to inherited predisposition to develop cancer (table 1).

The frequency of *GG* genotype was found to be increased in patients with higher BMI (11/14 : 78.6%), indicating that both *GG* genotype and obesity might increase the risk of developing breast cancer (table 1). The breast cancer patients who were positive for oestrogen and progesterone receptor status had increased frequency of *GG* genotype as compared to those with receptor negative status. The frequency of *AG* genotype was found to be increased

Table 1. Oestrogen receptor β polymorphism with respect to breast cancer and epidemiological and clinical parameters.

Parameters	*AA*		*AG*		*GG*		Allele Frequency	
	n	%	*n*	%	*n*	%	*A*	*G*
Disease (248)	132	53.2	95	38.5	21	8.47	0.72	0.28
Controls (249)	159	63.9	81	32.5	9	3.6	0.80	0.20
	$\chi^2 = 8.142$ $P = 0.015^*$							
	OR (95%CI) (*GG* vs *AA*) = 2.8106 (1.245–6.345)							
	OR (95%CI) (*GG* vs *AG*) = 1.9895 (0.863–4.5866)							
Menopausal status								
Premenopausal (131)	60	45.8	55	41.9	16	12.2	0.67	0.33
Postmenopausal (117)	72	61.5	40	34.2	5	4.3	0.79	0.21
	$\chi^2 = 8.458$ $P = 0.015^*$							
	OR (95%CI) (*GG* vs *AA*) = 3.84 (1.329–11.0952)							
	OR (95%CI) (*GG* vs *AG*) = 2.3273 (0.787–6.8782)							
Familial history								
Familial (73)	36	49.3	28	38.4	9	12.3	0.68	0.32
Non-Familial (175)	96	54.9	67	38.3	12	6.9	0.74	0.26
	$\chi^2 = 2.119$ $P = 0.347$							
	OR (95%CI) (*GG* vs *AA*) = 2 (0.7771–5.1475)							
	OR (95%CI) (*GG* vs *AG*) = 1.7946 (0.6801–4.7356)							
BMI								
< 20(14)	5	35.7	8	57.1	1	7.1	0.64	0.26
20-26.4(27)	14	51.9	11	40.7	2	7.4	0.72	0.28
26.4-30(104)	58	55.8	37	35.6	9	8.7	0.74	0.26
> 30(44)	27	61.4	15	34.1	2	4.5	0.78	0.22
	$\chi^2 = 3.788$ $P = 0.705$							
Oestrogen receptor								
Positive (89)	48	53.9	32	36.9	9	10.1	0.72	0.28
Negative (97)	53	54.6	35	36.1	9	9.3	0.73	0.27
	$\chi^2 = 0.038$ $P = 0.981$							
	OR (95%CI) (*GG* vs *AA*) = 1.1042 (0.405–3.0108)							
	OR (95%CI) (*GG* vs *AG*) = 1.0938 (0.3863–3.0974)							
Progesterone receptor								
Positive (86)	42	48.8	33	38.4	11	12.8	0.68	0.32
Negative (100)	59	59	34	34	7	7	0.76	0.24
	$\chi^2 = 2.727$ $P = 0.256$							
	OR (95%CI) (*GG* vs *AA*) = 2.2075 (0.7905–6.1645)							
	OR (95%CI) (*GG* vs *AG*) = 1.619 (0.5599–4.6815)							
HER2/neu								
Positive (26)	13	50	10	38.5	3	11.5	0.69	0.31
Negative (27)	19	70.4	5	18.5	3	11.1	0.80	0.20
	$\chi^2 = 2.774$ $P = 0.249$							
	OR (95%CI) (*GG* vs *AA*) = 1.4615 (0.2542–8.4014)							
	OR (95%CI) (*GG* vs *AG*) = 0.5 (0.0728–3.4346)							
Node status								
Positive (121)	68	56.2	42	34.7	11	9.1	0.74	0.26
Negative (74)	40	54.1	30	40.5	4	5.4	0.74	0.26
	$\chi^2 = 1.272$ $P = 0.529$							

Table 1 (*contd.*)

Parameters	*AA*		*AG*		*GG*		Allele Frequency	
	n	%	*n*	%	*n*	%	*A*	*G*
	OR (95%CI) (*GG* vs *AA*) = 1.6176 (0.4827–5.4203)							
	OR (95%CI) (*GG* vs *AG*) = 1.9643 (0.5704–6.7648)							
Stage								
I (10)	1	10	8	80	1	10	0.50	0.50
II (96)	52	54.2	40	41.7	4	4.2	0.75	0.25
III (72)	44	61.1	21	29.2	7	9.7	0.76	0.24
IV (48)	26	54.2	16	33.3	6	12.5	0.71	0.29
	χ^2 = 14.325		P = 0.026*					

χ^2, chi-square; OR, odds ratio; CI, confidence interval.

Table 2. Chi-square and odds ratios (OR) for oestrogen receptor β polymorphism with respect to breast cancer, epidemiological and clinical parameters.

Parameters	Allele *A*	Allele *G*	Chi-square	*P*	OR	CI intervals (*G* vs *A*)
Disease	359	137				
Controls	399	99	7.8	0.0052*	1.538	1.1448–2.0662
Menopausal status						
Premenopusal	175	87				
Postmenopausal	184	50	8.08	0.0045*	1.8295	1.2205–2.7423
Familial history						
Familial	100	46				
Non-Familial	259	91	1.3	0.2542	1.3092	0.8576–1.9987
BMI						
< 20	18	10				
20 – 26.4	39	15				
26.4 – 30	153	55				
> 30	91	19	5.7	0.1272		
Oestrogen receptor						
Positive	128	50				
Negative	140	53	0	1	1.0318	0.6548–1.6258
Progesterone receptor						
Positive	117	53				
Negative	152	48	2.04	0.1532	1.4345	0.9065–2.2699
HER2/neu						
Positive	36	19				
Negative	73	14	0.91	0.3401	1.621	0.7138–3.681
Stage						
I	10	10				
II	144	56				
III	109	35				
IV	68	28	5.84	0.1197		

*Significant chi-square value ($P < 0.05$).

Table 3. Multivariate analysis of ERβ polymorphism for menopausal status, familial status and BMI.

	Low BMI								High BMI							
	Familial				Non-familial				Familial				Non-familial			
	AA	*AG*	*GG*	*T*	*AA*	*AG*	*GG*	*T*	*AA*	*AG*	*GG*	*T*	*AA*	*AG*	*GG*	*T*
Premenopausal	5 (55.6)	2 (22.2)	2 (22.2)	9	4 (26.7)	9 (60)	2 (13.3)	15	10 (41.7)	11 (45.8)	3 (12.5)	24	25 (52.1)	19 (39.6)	4 (8.3)	48
Postmenopausal	3 (60)	2 (40)	0	5	7 (53.8)	6 (46.2)	0	13	12 (52.2)	9 (39.1)	2 (8.7)	23	38 (71.7)	13 (24.5)	2 (3.8)	53
	$\chi^2 = 1.48$ $P = 0.4771$				$\chi^2 = 3.29$ $P = 0.193$				$\chi^2 = 0.56$ $P = 0.7558$				$\chi^2 = 1.4.2448$ $P = 0.12$			

T, total; the number in parenthesis are in percentage.

in HER2/neu positive patients (38.5%). Murphy *et al*. (2003) reported that greater than 50% of all tumours previously classified as ER negative express *ERβ*. Patients with *GG* genotype may not show good response to endocrine therapy due to lower expression of ERI (table 1).

When node status and stage of the patients were considered, the *GG* genotype frequency was associated with node positive tumour (9.1%) as well as advanced stage of the disease (13/18 : 72.2%), which suggest that the presence of *GG* genotype might result in bad progression of the disease (table 1).

Multivariate analysis for menopausal status, family history and BMI revealed that the elevated frequency of *GG* genotype in premenopausal breast cancer patients was independent of other two factors (table 3).

Hence, this mutation (A1730G) with reduced *ERβ* gene expression leads to an increased risk for breast cancer development. It might be possible that this polymorphism might be in linkage disequilibrium with other mutations in *ERβ* gene influencing the rate of gene expression.

In conclusion, our results suggest that the ERβ polymorphism, which leads to lower expression might predispose to the development as well as bad prognosis of the breast cancer.

Acknowledgement

This work was supported by University Grants Commission, New Delhi, India, and Nizam's Institute of Medical Sciences, Hyderabad, India.

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Received 29 October 2008; in final revised form 28 March 2009; accepted 15 April 2009.
Published on the Web: 3 August 2009