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Polymorphisms in the promoter region of *ESR2* gene and breast cancer susceptibility

Oliver Treeck[a,*], Esma Elemenler[a,1], Christina Kriener[a], Felicitas Horn[a], Anette Springwald[a], Arndt Hartmann[b], Olaf Ortmann[a]

[a] *Department of Obstetrics and Gynecology, University of Regensburg, 93053 Regensburg, Germany*
[b] *Institute for Pathology, University of Regensburg, 93053 Regensburg, Germany*

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ABSTRACT

Genetic variations like single nucleotide polymorphisms (SNPs) in genes involved in estrogen biosynthesis, metabolism and signal transduction have been suggested to affect breast cancer susceptibility. In this study we tested the hypothesis that polymorphisms in the promoter of *ESR2* gene may be associated with increased risk for breast cancer. We analyzed three SNPs in the promoter region of human *ESR2* gene by means of allele-specific tetra-primer PCR. A total of 318 sporadic breast cancer cases and 318 age-matched controls were included in the study. With regard to homozygous genotypes, women with sporadic breast cancer more frequently carried the CC genotype of *ESR2* promoter SNP rs2987983 (OR 1.99, $p = 0.005$). Calculation of allele positivity demonstrated that presence of T allele of this SNP was more frequent in healthy women. Our data suggest that a SNP in the promoter region of *ESR2* gene might be able to affect breast cancer risk. These results further support the emerging hypothesis that ERβ is an important factor in breast cancer development.

1. Introduction

Breast cancer is the most frequently diagnosed cancer and the second-most common cause of cancer related death in women. Estrogens are pivotal in the growth and development of both normal and neoplastic mammary tissues, and mediate most of their action via ligand-dependent transcription factors, the estrogen receptors (ER). Despite the fact that ER expression is an important prognostic and predicitve factor in breast cancer [1], the situation seems to be far more complex. Identification of the second estrogen receptor gene *ESR2* and its gene product, ERβ [2] has led to a re-evaluation of estrogen action in target tissues such as breast tumors. ERβ is expressed both in normal and neoplastic human breast tissue [3–4], but its role in either is not fully understood. In animal studies, while ERα has been shown to be essential for normal mammary gland development, ERβ effects are more subtle, with roles in terminal differentiation [5] and modulation of ERα activity being described [6–8]. In contrast to ERα, published data suggest that ERβ expression declines during breast tumorigenesis [3,9]. This down-regulation of ERβ in breast cancer compared with normal breast tissue suggests a role for ERβ as a tumor suppressor [10]. Nevertheless, ERβ expression in breast tumors varies widely [11], [4] and attempts to correlate ERβ with various biomarkers have resulted in varied, often contradictory conclusions [12]. This might also be due to differential detection of variant non-ligand binding ERβ proteins which have been detected in breast tissues [13–14] and which code for proteins exerting functions distinct from that of the full-length ERβ 1 protein [8,15].

Single nucleotide polymorphisms (SNPs) are the most frequent sequence variations in the human genome. SNPs located in exon regions may alter protein function, whereas SNPs in the gene promoter can modify gene expression levels [16–22]. Polymorphisms in genes involved in estrogen biosynthesis, metabolism and signal transduction have been suggested to play a role in breast cancer risk [23–31]. In the last years, a multitude of SNPs both in *ESR1* and *ESR2* gene have been identified and different genotype–phenotype association studies have been published examining the significance of randomly chosen SNPs in different hormone-dependent diseases [18,32–35]. In this study, for the first time three SNPs were selected which are located in the promoter region of *ESR2* gene and which thus could be able to modify expression level of this receptor. We compared allele frequencies of these SNPs in 318 healthy women and 318 women with sporadic breast cancer.

* Corresponding author at: Klinik für Frauenheilkunde und Geburtshilfe, Universität Regensburg, Landshuter Str. 65, 93053 Regensburg, Germany.
Tel.: +49 941 7827520; fax: +49 941 7827515.
E-mail address: otreeck@caritasstjosef.de (O. Treeck).
[1] Both authors contributed equally.

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2. Patients and methods

2.1. Patients

Blood samples from 318 Caucasian women with sporadic breast cancer and such as many age-matched Caucasian women not having any malignancy were included in this study. The median age of the patients collective and the control group was 55.6 and 54.9 years, respectively (age at diagnosis for case patients and age at inclusion for control subjects). The histopathological characteristics of the patients are shown in Table 1. Samples of breast cancer case participants were provided by the Institute of Pathology, University of Regensburg, in an anonymous and randomized manner. Included were Caucasian female sporadic breast cancer patients with available information on grading, tumor size, nodal status, and receptor status of ERα, PR and HER2 from 2002 to 2007. Control subjects were selected from the same geographic origin as the cases, the Oberpfalz area of Regensburg, Bavaria, Germany. Inclusion criterion for the control subjects was the absence of known malignancies. The study was approved by the Ethical Committee of the University of Regensburg.

2.2. SNP analysis

Three SNPs in the 0N promoter region of *ESR2* gene were identified using the Internet sites www.genecards.org and http://www.ncbi.nlm.nih.gov/SNP. The basis for SNP selection was their location in the 5′ region directly adjacent to the transcription start site of *ESR2* gene. Single nucleotide polymorphism rs2987983 (C/T) is located at position 63833406 of chromosome 14, rs3020450 (A/G) is located at position 63838055 and SNP rs3020449 (A/G) (formerly rs8004842) is located at position 63843145 of chromosome 14 (Fig. 1).

Genomic DNA was isolated from 100 μl EDTA-blood after addition of 300 μl lysis buffer (1%, v/v TritonX, 0.32 M Sucrose, 0.01 M Tris (pH 7.5) and 5 mM $MgCl_2$) and two-fold centrifugation (13,000 × *g*) for 30 s. Pellet was resuspended in 50 μl PCR buffer (GoTaq buffer, Promega, Madison, USA) containing 0.5% Tween 20 and 10 mAnson units proteinase K (Merck, Darmstadt, Germany) followed by incubation at 50 °C over night and finally heat inactivation of the enzyme for 10 min at 95 °C. The genomic DNA-containing lysate was subjected to a tetra-primer ARMS PCR approach [36] allowing allele-specific amplification using the PCR primers listed in Table 2 (synthesized at Metabion, Martinsried, Germany). For this purpose, to 100 ng of genomic DNA, 2 μl of 5 × GoTaq buffer, 0.2 μl of dNTP Mix (10 mM) (Fermentas, St. Leon-Rot, Germany), 0.2 μl of each PCR primer (10 μM) and 0.5 units GoTaq polymerase (Promega, Madison, USA) was added and PCR reaction was carried out using a T1 thermocycler (Biometra, Germany). PCR program was 10 min 94 °C followed by 38 PCR cycles of 94 °C (30 s), 56 °C (30 s) and 72 °C (60 s), followed by a final extension for 5 min step at 72 °C. PCR products were analyzed by 1.5% agarose gelelectrophoresis. Allele-

Table 1
Histopathological characteristics and receptor status of breast cancer cases included in this study (*n* = 318).

Characteristic	Patients numbers				
Tumor size	pT1 174	pT2 113	pT3 9	pT4 19	pTx 3
Histological grade	G1 36	G2 169	G3 113	Gx -	
Nodal status	N0 189	N1–3 118	Nx 11		
ERα status	neg. 67	pos. 238	ERx 13		
PR status	neg. 125	pos. 178	PRx 15		
HER2 status	neg. 203	pos. 45	HER2x 70		

The median age of patients was 55.6 years (range 24–82 years).

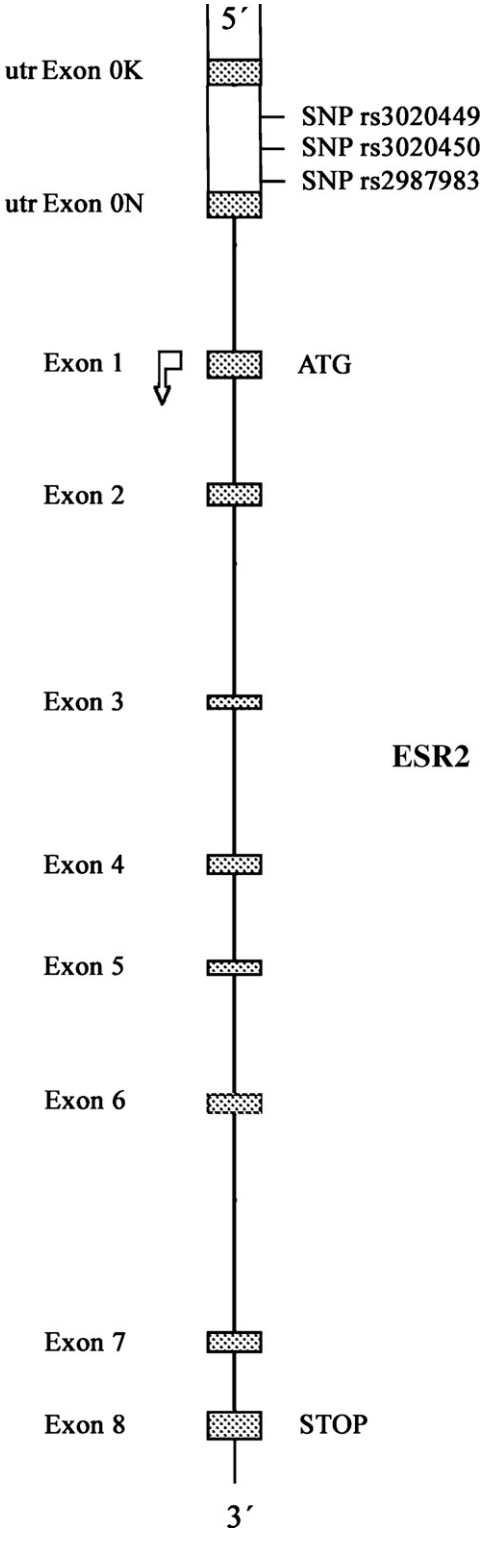

Fig. 1. The single nucleotide polymorphisms analyzed in this study are located in the 5′ region of estrogen receptor β gene (*ESR2*), namely in the promoter region of untranslated exon 0N.