

A polymorphism in the nuclear receptor coactivator 7 gene and breast cancer susceptibility

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Abstract. The nuclear receptor coactivator 7 (NCoA7) gene codes for an estrogen receptor-associated protein that plays a significant role in the cellular response to estrogens. Given that NCoA7 is expressed in the mammary gland, alterations in this gene may affect breast cancer risk. In this study, we compared the genotype and allele frequencies of the missense single nucleotide polymorphism (SNP) rs1567, located in the coding region of the NCoA7 gene and resulting in an amino acid exchange from asparagine to glutamine, in 305 women with sporadic breast cancer and 346 women without any malignancy. Statistical analysis of the observed frequencies did not reveal a significant difference between the cancer and control groups, nor did a comparison between histological breast cancer subgroups. In conclusion, the results of our phenotype-genotype association study indicate that NCoA7 SNP rs1567 does not affect breast cancer susceptibility.

Introduction

Multiple hormone-related mechanisms have been reported to be involved in breast cancer onset and progress (1). Markedly high levels of estrogens are known to increase the risk of developing this disease (1,2). The cellular response to estrogens is mediated by specific estrogen receptors (ERs), ER α and ER β . These receptors regulate transcription of the target genes by binding directly to estrogen response elements or by interaction with other transcription factors. Binding to coregulatory proteins, which comprise corepressors as well as coactivators, is essential for ER function. These proteins bind to specific domains of the ligand-bound estrogen receptors and are therefore capable of controlling the transcription of

target genes (3). In addition to communicating with the transcriptional machinery, certain coregulators are also capable of altering chromatin function (4). It has been suggested that coregulatory proteins perform virtually all of the reactions required to control enhancer-dependent gene expression. These proteins regulate subfunctions of numerous transcription factors in addition to cell processes including translation, energy generation and motility (5).

Nuclear receptor coactivator 7 (NCoA7) is an ER-coactivator protein also known as ERAP140 (ER-associated protein 140) (6). Ligand-bound ER α or ER β recruits NCoA7 to the promoter region of ER target genes (7). Since NCoA7 has little structural homology with other nuclear receptor cofactors, it may represent a distinct class of nuclear receptor coactivators. NCoA7 is expressed in a cell- and tissue- specific manner. It is most abundantly expressed in the brain, but is also found in organs including the mammary gland, bone marrow, testis, ovary, uterus, prostate, stomach, bladder, spinal chord, pancreas and thyroid (6). The specific physiological function of NCoA7 is not completely understood. Certain studies have indicated that NCoA7 may be a favourable prognostic indicator for neuroblastomas. On the other hand, other authors identified an isoform that appears to be ovulation-associated and reproduction system-specific; another study found NCoA7 to possess an activity that contributes to oxidation resistance (8,9,10). Given that NCoA7 is expressed in the mammary gland and thus plays a role in the response of this tissue to estrogens, molecular alterations in the NCoA7 protein may affect breast cancer risk.

The most frequent molecular variations of the DNA are single nucleotide polymorphisms (SNPs). SNPs are point mutations occurring in at least 1% of the population. SNPs located in exon regions may alter protein function, whereas SNPs in the gene promoter are capable of modifying gene expression levels. Various genome-wide association studies detected a number of gene polymorphisms postulated to influence development of various diseases, as well as breast cancer susceptibility (11-17).

In this study, we tested whether the missense SNP rs1567, located in the last exon of the coding region of the NCoA7 gene and thus leading to an amino acid exchange from asparagine to glutamine in the coactivator protein, affected breast cancer risk. For this purpose, we performed a genotype-phenotype association study and compared genotype and

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allele frequencies of this SNP in 305 women with sporadic breast cancer and 346 without any malignancy.

Patients and methods

Patients. This study included blood samples from 305 Caucasian women with sporadic breast cancer and 346 age-matched Caucasian women without any malignancy. The median ages of the patient collective and the control group were 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 I. Samples of breast cancer case participants were provided by the Institute of Pathology, University of Regensburg, in an anonymous and randomised manner or were collected at the Clinic of Obstetrics and Gynecology, University Medical Center Regensburg after informed consent was obtained from the patients. Included were Caucasian female sporadic breast cancer patients with available information on tumour size, grading and nodal status, as well as receptor status of ER α , PR and HER2, between 2002 and 2007. The control participants originated from the same geographic area as the tumour patients, the Oberpfalz area of Regensburg, Bavaria, Germany. The inclusion criterion for the control subjects was the absence of known malignancies. The study was approved by the Ethics Committee of the University of Regensburg.

SNP analysis. The analysed SNP in the coding region of the last exon in the NCoA7 gene was identified using the websites www.genecards.org and <http://www.ncbi.nlm.nih.gov/projects/SNP>. SNP rs1567 (G/T) is located at position 126291607 (plus strand) of chromosome 6. The basis for the selection of this SNP was its location in the coding region of the NCoA7 gene. At this position, the nucleotide polymorphism causes a missense mutation and therefore a change of amino acids in the resulting NCoA7 protein. Genomic DNA was isolated from 100 μ l EDTA-blood after the addition of 300 μ l lysis buffer [1% v/v TritonX (Sigma, St. Louis, MO, USA), 0.32 M sucrose, 0.01 M Tris (pH 7.5) and 5 mM MgCl₂ (Roth, Karlsruhe, Germany)] and 2-fold centrifugation (13000 rpm) for 30 sec. The pellet was resuspended in 50 μ l PCR buffer (GoTaq colorless buffer, Promega, Madison, WI, USA) containing 0.5% Tween-20 (Sigma) and 10 mAnson units proteinase K (Merck, Darmstadt, Germany), followed by incubation at 50°C overnight and 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 (18) allowing allele-specific amplification using the PCR primers listed in Table II (synthesised at Metabion, Martinsried, Germany). For this purpose, 2 μ l of 5X GoTaq buffer, 0.2 μ l of dNTP mix (10 mM) (Fermentas, St. Leon-Rot, Germany), 0.2 μ l of primer 567-1, 567-2 and 567-T (10 μ M), 0.4 μ l of primer 567-G (10 μ M) and 0.1 μ l GoTaq polymerase (Promega, Madison, WI, USA) was added to 10 ng of genomic DNA and the PCR reaction was carried out using a T1 thermocycler (Biometra, Goettingen, Germany). The PCR program comprised denaturation of 10 min at 94°C followed by 38 PCR cycles of 94°C (30 sec), 63°C (30 sec) and annealing at 72°C (60 sec), followed by a final extension for 5 min at 72°C. PCR products were analysed

Table I. Histopathological characteristics and receptor status of breast cancer cases included in this study (n=305).

Characteristics	Patient numbers
Tumour size	
pT1	164
pT2	107
pT3	11
pT4	12
pTx	11
Histological grade	
G1	10
G2	99
G3	161
Gx	35
Nodal status	
N0	178
N1-3	103
Nx	24
ER α status	
Negative (≤ 4)	52
Positive (> 4)	243
ERx	10
PR status	
Negative (≤ 4)	118
Positive (> 4)	178
PRx	9
Her2/neu status	
Negative (0-2)	238
Positive (3)	43
Her2/neuX	24

using 1.5% agarose gel electrophoresis. Allele-specific PCR product sizes for SNP rs1567 were 154/196 bp (G/T). As a quality control for genotyping, a negative water control and a previously characterised DNA sample representing the heterozygous and the two homozygous genotypes were analysed in addition to the unknown samples in each PCR run.

Statistical analysis. Deviation from the Hardy-Weinberg equilibrium was estimated using the Fisher's exact and χ^2 tests, and all values were subjected to one-way ANOVA to achieve homogeneity of variance. Statistical tests for association [confidence interval (CI) 95%] and significance were carried out using Microsoft Office Excel 2003 and the Internet websites <http://www.quantitativeskills.com/sisa/statistics/twoby2.htm> and <http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>. $P < 0.05$ was considered to be statistically significant. After tests for deviation using the Hardy-Weinberg equilibrium were conducted, allele frequency, allele positivity and genotype frequencies were determined. The odds ratio (OR) was calculated using the more frequent homozygous genotypes as a reference group.

Table II. Oligonucleotides used for SNP genotyping with tetra primer PCR.

SNP	Primer	Sequence (5'-3')
rs 1567	567-T	AGGATCTGGAGGTGTGGGCATTTAAT
	567-G	CAGTCTTTTAAATGTTATATTTTAAGGCAGTCTGAATTTTAC
	567-1	TAGATGCTGATTTATACCACGGACGAAG
	567-2	ACCTTCCAGAATCTTTCTGCAATGTGAT
PCR product sizes (bp)	rs1567:	T allele, 196; G allele, 154; outer primers, 283.

Table III. No significant differences were observed between the breast cancer and the control groups with regard to genotype frequency, allele frequency or allele positivity of NCoA7 SNP rs1567^a.

rs1567	Genotype frequency						Allele frequency				Allele positivity for T-allele				Allele positivity for G-allele			
	TT		TG		GG		T		G		TT+TG		GG		TT		TG+GG	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Case	229	75.1	69	22.6	7	2.3	527	86.4	83	13.6	298	97.7	7	2.3	229	75.1	76	24.9
Control	255	73.7	81	23.4	10	2.9	591	85.4	101	14.6	336	97.1	10	2.9	255	73.7	91	26.3
P-value				0.7781		0.6182				0.6092				0.6347				0.6868
Odds ratio				1.0542		1.2829				1.0851				1.267				1.0753
CI 95%				0.73-1.52		0.48-3.42				0.79-1.48				0.75-1.53				0.47-3.37

^aAfter tests for deviation using the Hardy-Weinberg equilibrium were conducted, genotype and allele frequency were determined, as well as allele positivity. The odds ratio and p-values were determined using the more frequent allele as a reference group. CI, confidence interval.

Results

Genotype analysis. Analysis of 305 breast cancer patients and 346 women without any malignancy revealed that the homozygous genotype TT of ER-coactivator SNP rs1567 was most frequent (73.7% in the control group), whereas the GG genotype was relatively rare (2.3% in the control group). A heterozygous GT genotype was exhibited by 22.6% of the control population. Statistical analysis did not show significant differences in homozygous or heterozygous genotype frequencies between the case and the control group (Table III). Thus, no association of a specific rs1567 genotype with breast cancer risk was observed.

Allele frequency. Comparing allele frequencies of the NCoA7 SNP rs1567 in breast cancer patients and controls also showed no significant difference between the two groups (OR 1.0851, Table III). The clearly predominant allele in the two groups was the T allele (0.86 vs. 0.85), whereas only 13.6% of the tested chromosomes carried the G allele.

Allele positivity. Phenotype-genotype association analysis of the allele positivity of the rs1567 SNP revealed no significant difference between women with breast cancer and the control population (Table III).

Association between histological breast cancer characteristics and receptor status. We compared SNP genotype frequencies between breast cancer subgroups with various specific tumour characteristics. The respective subgroup size allowed statistical analysis of tumour size (T), nodal status (N) and histological grading (G). No significant difference was observed between the compared subgroups in any of these tests (data not shown). Since interactions between ER α , PR, and HER2 occur in breast cancer cells, we also compared the SNP genotypes between the subgroups of different ER α , PR and HER2 status. However, statistical analysis did not reveal a significant difference between the receptor status subgroups regarding genotype frequency, allele frequency or allele positivity.

Discussion

SNPs in genes of steroid hormone metabolism or signalling are potential factors that may affect cancer risk in hormone-dependent tissues. Various genome-wide association studies detected a number of gene polymorphisms, which are thought to affect breast cancer susceptibility (14-17). Since estrogens are known to affect breast cancer development, it is essential to analyse polymorphisms in the two estrogen receptor genes, ESR1 and ESR2, as well as their co-regulatory proteins, which may affect breast cancer risk. Co-activators and

co-suppressors determine the function of ERs as a transcriptional regulator. NCoA7 is an ER coactivator protein that has been shown to be expressed in the mammary gland, but has yet to be analysed in the context of breast cancer.

We did not observe any association of the missense SNP rs1567 with breast cancer risk or with histopathological characteristics of the cancer tissue. Even taking into account the fact that the tested sample numbers were limited and the frequency of the rare genotype was relatively small, the observed allele and genotype frequencies were so similar that our data clearly indicate that NCoA7 SNP rs1567 does not affect breast cancer susceptibility. One explanation for this lack of association is that the missense SNP tested only led to an amino acid exchange from asparagine to glutamine. The two amino acids have markedly similar properties, as they are neutral, polar and hydrophilic amino acids; thus, it appears unlikely that SNP rs1567 was able to strongly alter the structure or function of NCoA7 protein.

According to previous studies, the NCoA7 protein may be a new group of ER-coactivating proteins (7). Shao *et al* reported that NCoA7 is a tissue-specific coactivator to agonist-bound nuclear receptors, including the estrogen receptors. These authors also found that NCoA7 is expressed in breast tissue (6). In addition to its role as a coactivator the protein appears to be crucial for the reduction of oxidative damage of the DNA inside the nucleus. The NCoA7 protein contains similar functional domains to the mitochondrial OXR1-protein, which is known to reduce oxidative damage in DNA structure. In contrast to OXR-1, NCoA7 is not stress-induced but reacts to estrogens and interacts with the estrogen receptor (10). Given that NCoA7 is not estrogen receptor-specific but binds to other nuclear receptors as well, this coactivator is involved in a variety of other signalling pathways (7,19).

With regard to the function of NCoA7 in carcinogenesis, only limited data exist. Due to the high expression of NCoA7 in the human brain, previous studies focused on the role of NCoA7 in the context of brain diseases (8,20). NCoA7 expression is reportedly a favorable predictive factor in neuroblastoma (8). To the best of our knowledge, the only NCoA7 SNP study to be published reported a lack of association between the appearance of schizophrenia and five SNPs in this gene (20).

In conclusion, we did not observe any association between NCoA7 polymorphism rs1567 and breast cancer susceptibility. Our results do not preclude a significant function of NCoA7 in breast cancer development.

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