Null Results in Brief

Polyglutamine Repeat Length in the *NCOA3* Does Not Affect Risk in Familial Breast Cancer

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Introduction

The nuclear receptor coactivator 3 (NCOA3) is a protein that binds to nuclear hormone receptors and thereby facilitates the expression of downstream genes. The *NCOA3* gene has been found to be overexpressed in breast tumors (1). On exon 21, the *NCOA3* gene contains a polymorphic region with a trinucleotide repeat encoding a polyglutamine repeat in the COOH terminus of the NCOA3 protein. Longer repeat lengths have been shown to correlate with higher breast cancer risk in *BRCA1* and *BRCA2* (*BRCA1/2*) mutation carriers, whereas shorter alleles seem to have a protective effect (2, 3). However, the NCOA3 polyglutamine repeat length alone has not been found to alter the breast cancer risk among unselected postmenopausal women (4). Unlike these studies, we used for the first time a large set of DNA samples from women with familial breast cancer, where the likelihood to detect possible risk factors is much higher than in unselected cases. To prove if the *NCOA3* polymorphism has an impact on the breast cancer risk, we analyzed 591 breast cancer samples from German and Polish women together with 536 matched controls.

Materials and Methods

The region of a trinucleotide repeat (CAG, CAA) in the *NCOA3* gene was genotyped by fragment analysis using a case-control group of 1,127 women. This group composed of 591 women with familial breast cancer that were negative for *BRCA1/2* mutations and 536 ethnically and geographically matched controls from Poland and Germany (5). Seventy-five percent of the women with breast cancer were diagnosed *under age 50 years*. The cases were consecutive cases collected during the years 1997 to 2003 according to the criteria described in Jin et al. (5) through the Chemotherapy Clinics and the Genetic Counseling (Gliwice, Poland) and the Institute of Human Genetics, University Heidelberg (Germany). Ninety percent of the patients approved the participation to the study. The controls were recruited to earlier studies with comparable participation rates. Additionally, 62 Polish samples from *BRCA1/2* mutation carriers with breast cancer were available for this study. This study was approved by the ethical committee of Karolinska Institute Syd. For PCR, genomic DNA (1 ng) isolated from blood was used. The primers (forward 5′-CGACAACAGAGGGTGGCTATG-3′, reverse 5′-GAGGAGCTTGTGGCATTGTGG-3′) were purchased from MWG (Ebersberg, Germany). For detection, the forward primer was 5′ labeled with a FAM fluorophore. PCR was done with a 5-minute incubation at 94°C followed by 32 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds and a final extension at 72°C for 5 minutes. *NCOA3* repeat alleles were determined with an automated ABI PRISM 3100 genetic analyzer with performance-optimized polymer 6 and sized by Genescan 3.0 software (Applied Biosystems, Weiterstadt, Germany). Ten percent of the samples were repeated and sequenced to confirm the predicted repeat length. To estimate the breast cancer risks associated with *NCOA3* genotypes, we used χ^2 test and determined odds ratios (OR) with 95% confidence intervals. In the joint analysis, each series was treated as a separate stratum applying the Mantel-Haenszel adjustment. All statistical tests were carried out with the Epi Info 3.2 software (http://www.cdc.gov/epiinfo/).

Results and Discussion

The most common alleles in the polymorphic region of *NCOA3* contained 26 (12%), 28 (35%), and 29 (52%) CAG/CAA repeats, with 28/29 and 29/29 as most frequent genotypes (35% and 28%, respectively). No significant differences were found between populations or cases and controls. Allele distribution was similar to the ones reported previously for Caucasian women (3, 4). The genotype distribution among our familial breast cancer cases without *BRCA1/2* mutations followed the same pattern as shown earlier among unselected postmenopausal women with breast cancer (4). No correlation was found between breast cancer risk and genotypes with longer or shorter *NCOA3* alleles, respectively (Table 1). Women diagnosed for breast cancer at age ≥50 years tended to carry at least one allele shorter than 28 repeats. In German women, this effect was significant with an OR (95% confidence interval) of 2.17 (1.05-4.51; $P = 0.023$). However, in the joint population, this effect was not significant (OR, 1.45; 95% confidence interval, 0.9-2.31; $P = 0.133$).

In *BRCA1/2* mutation carriers, an elevated breast cancer risk has been reported for women carrying longer *NCOA3* alleles (2, 3). We found a comparable trend for 62 Polish samples from *BRCA1/2* mutation carriers that were compared with the group of Polish controls. However, due to small sample size, woman carrying at least 29 repeats in both alleles had no statistically increased risk for breast cancer

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Table 1. NCOA3 associated to the polyglutamine repeat lengths in NCOA3 in familial breast cancer

Group	Gln repeats	Cases (%)	Controls (%)	OR (95% confidence interval)	*P*
Polish 646 samples	1 allele <28	66 (18.7)	59 (20.1)	0.87 (0.56-1.34)	0.507
	28/28, 28/29, 28/30	184 (52.1)	143 (48.8)	1.00	
	2 alleles ≥29	103 (29.2)	91 (31.1)	0.88 (0.61-1.28)	0.481
German 481 samples	1 allele <28	68 (28.6)	56 (23.0)	1.25 (0.79-1.98)	0.324
	28/28, 28/29, 28/30	113 (47.5)	116 (47.7)	1.00	
	2 alleles ≥29	57 (23.9)	71 (29.2)	0.82 (0.52-1.30)	0.383
Combined 1,127 samples	1 allele <28	134 (22.7)	115 (21.5)	1.03 (0.75-1.41)*	0.905
	28/28, 28/29, 28/30	297 (49.8)	259 (48.8)	1.00	
	2 alleles ≥29	160 (27.1)	162 (30.2)	0.86 (0.64-1.14)*	0.304

*Mantel-Haenszel adjustment.

(OR, 1.31; 95% confidence interval, 0.71-2.40; $P = 0.355$) compared with women carrying shorter alleles.

The strength of our study was the use of DNA samples from women selected for familial breast cancer. With our sample size and a frequency of the rare genotype of ~0.25, we had a 90% power to detect an OR of ≥1.6. Yet, according to Antoniou and Easton (6), the power of a study with familial breast cancer cases is at least twice higher than in a study using unselected cases. Therefore, we would even have a 90% power to detect an OR of ≥1.3. Due to the lack of information about other factors than familial history, we cannot exclude the possibility that the NCOA3 polymorphism would have an effect on breast cancer risk in combination with hormonal or other risk factors. In conclusion, our moderately large, case-control study strongly suggests that the polyglutamine repeat length in NCOA3 alone is not influencing the breast cancer risk in women.

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