

Null Results in Brief

Cyclooxygenase 2 Polymorphism (Val⁵¹¹Ala), Nonsteroidal Anti-inflammatory Drug Use and Breast Cancer in African American Women

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAID) have been associated with reduced risks of colon cancer, breast cancer, and other cancer sites (1, 2). We previously reported a strong inverse relationship between NSAID use and breast cancer in a North Carolina study, with a suggestion of stronger associations among African Americans (3). One hypothesized mechanism for the reduction in cancer risk by NSAIDs is the inhibition of cyclooxygenase 2 (COX2), which is overexpressed in various cancer types and is thought to stimulate angiogenesis and inhibit apoptosis (1).

A polymorphism in the *COX2* gene [valine to alanine at residue 511 (Val⁵¹¹Ala)] has been identified in African Americans that results in a conformation change in the enzyme near its active site, and it has been hypothesized this polymorphism could modify biochemical function or change the response to NSAIDs (4, 5). In a recent paper, carriers of this polymorphism seemed to be at reduced risk for colon adenomas [odds ratio (OR), 0.56; 95% confidence interval (95% CI), 0.25-1.27] and colon cancer (OR, 0.67; 95% CI, 0.28-1.56; ref. 5). In this report, we describe the relationship between the *COX2* Val⁵¹¹Ala polymorphism, NSAIDs, and breast cancer in a case-control study in North Carolina.

Materials and Methods

These analyses were based on 1,441 African American participants in the Carolina Breast Cancer Study, a population-based, case-control study conducted between 1993 to 2001 (3, 6). Cases were 20 to 74 years old and had either invasive breast cancer or carcinoma *in situ*. Control women were selected from Division of Motor Vehicle and Health Care Financing Administration lists and matched by race and age to cases.

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Genotyping was done according to previously described procedures using the Taqman system (6). The Ala⁵¹¹ (C) allele-specific probe was labeled on the 5' end with the VIC reporter dye and contained the nucleotide sequence 5'-TGCTCCAgCTTCTAC-3' with a melting temperature of 68.5°C and a G-C content of 53.3%. The Val⁵¹¹ (T) allele-specific probe was labeled on the 5' end with the 6-FAM reporter dye and contained the nucleotide sequence 5'-TGCTCCAaCTTCTAC-3' with a melting temperature of 67.5°C and a G-C content of 46.7%. Both probes were minor groove binding and used a nonfluorescing quencher on the 3' end. Forward and reverse primers were used to amplify the region surrounding the Val⁵¹¹Ala polymorphism. The nucleotide sequences for the forward and reverse primers were 5'-AGAAAAGCCTCGGC-CAGATG-3' and 5'-GGCAGGAGAACATATAACATTACC-CATAA-3', respectively.

Logistic regression analyses were done using the GENMOD procedure in SAS (Cary, NC). We calculated ORs and 95% CIs to evaluate the association between breast cancer and the Val⁵¹¹Ala polymorphism and the joint effects of NSAIDs and genotype.

Results

Genotyping results were available for 763 cases (673 invasive and 90 *in situ*) and 678 controls. The frequency of the Ala allele was 4.3% in cases and 4.0% in controls (Table 1), which is very similar to the allele frequency of 4.5% reported by Lin et al. (5). There were no significant departures from Hardy-Weinberg equilibrium among either cases ($P = 0.16$) or controls ($P = 0.36$). Few women were homozygous for the Ala allele; therefore ORs for breast cancer were calculated comparing carriers of any Ala allele (Ala/Ala or Ala/Val) to individuals who were homozygous for the Val allele. There were no significant genotype differences between cases and controls with an overall OR associated with carrying an Ala allele of 1.2 (95% CI, 0.8-1.7). The ORs for invasive cases and *in situ* cases were 1.1 (95% CI, 0.7-1.6) and 1.6 (95% CI, 0.4-7.0), respectively. We also did not find any statistically significant associations when stratifying by menopausal status or estrogen receptor status (data not shown).

Using data from 462 cases and 367 controls for whom information on NSAID use was available (phase II participants), we evaluated the joint effect of genotype and NSAIDs using a composite variable. Women with the Val/Val genotype who reported no use of NSAIDs were the reference group and ORs were calculated for each of the other genotype-NSAID categories. As compared with the reference category, we observed ORs <1 for women who reported any NSAID use,

Table 1. Allele and genotype frequencies for COX2 among African American breast cancer cases (invasive and carcinoma *in situ*) and controls, the Carolina Breast Cancer Study

| | Cases, <i>n</i> (%) | Controls, <i>n</i> (%) | OR* (95% CI) |
|----------------------------------|------------------------|---------------------------|-----------------|
| COX2 allele frequencies | | | |
| Ala | 65 (4.3) | 54 (4.0) | |
| Val | 1,461 (95.7) | 1,302 (96.0) | |
| Missing | 10 | 6 | |
| COX2 genotype frequencies | | | |
| Ala/Ala | 0 (0) | 2 (0.3) | |
| Ala/Val | 65 (8.5) | 50 (7.4) | |
| Val/Val | 698 (91.5) | 626 (92.3) | |
| Missing | 5 | 3 | |
| Ala/Ala or Val/Ala | 65 (8.5) | 52 (7.7) | 1.2 (0.8-1.7) |
| Val/Val | 698 (91.5) | 626 (92.3) | 1.0 (reference) |
| Missing | 5 | 3 | |

*Adjusted for age and offset term used to oversample younger women and African American women.

regardless of genotype (Table 2). ORs ranged from 0.3 to 0.7, with 95% CIs that overlapped considerably. The OR for Ala carriers who were nonusers of NSAIDs was 0.2; however, there were only two controls and one case in this category, leading to an unstable estimate of the effect of the Ala allele alone.

Discussion

Although a previous report from a study of colon cancer suggested that the Val⁵¹¹Ala polymorphism had an effect on colon cancer risk comparable in magnitude (OR ≈ 0.6) to the protective effect that has been reported consistently for aspirin and other NSAIDs, our results showed no association between this polymorphism and breast cancer. We also found no evidence suggesting that the Val⁵¹¹Ala polymorphism modified the relationship between NSAID use and breast cancer; ORs <1 were observed for NSAID users regardless of genotype. This suggests that the role of COX2 may be different in breast cancer than colon cancer, a notion that is supported by data on NSAID use and breast cancer. Although most epidemiologic studies (3, 7-10) have reported inverse associations between NSAID use and breast cancer, the body of literature is less consistent than it is for colon cancer and the magnitude of the associations with aspirin/NSAID use have tended to be weaker than those reported for colon cancer.

The major strength of this investigation is the large number of African American women in our study, affording us >80% power to detect an OR of 0.7. A limitation is that we examined only one polymorphism in COX2. Based on the known conformational change associated with this polymorphism and the previous work suggesting an association with colon cancer, it was reasonable to examine its association with breast

Table 2. ORs and 95% CIs for the joint effects of COX2 genotype and NSAID use on breast cancer (invasive and carcinoma *in situ*) among African American women, the Carolina Breast Cancer Study

| NSAID use category | COX2 genotype | | | |
|--------------------|--|-----------------|--|-----------------|
| | Ala/Ala or Val/Ala | | Val/Val | |
| | Cases (<i>n</i>)/ controls (<i>n</i>) | OR* (95% CI) | Cases (<i>n</i>)/ controls (<i>n</i>) | OR* (95% CI) |
| Never | 1/2 | 0.2 (0.0-2.3) | 54/15 | 1.0 (reference) |
| Occasional | 27/11 | 0.7 (0.3-1.9) | 189/184 | 0.3 (0.1-0.5) |
| Regular | 18/17 | 0.3 (0.1-0.9) | 173/138 | 0.3 (0.2-0.6) |

*Adjusted for age and offset term used to oversample younger women and African American women.

cancer. Despite the lack of association between the Val⁵¹¹Ala variant and breast cancer, the multiple lines of evidence linking COX2 to breast cancer risk suggest that further research is warranted to gain insight into possible mechanisms for the chemopreventive effects of NSAIDs.

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