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The original goal of this project is to determine the frequency of genetic polymorphisms for carcinogen metabolism and the p53 mutational spectra in a previously conducted breast cancer study designed to assess nutritional risk factors, seeking to identify risk factors related to inheritable susceptibilities and chemical etiologies. We have determined that the NAT2 slow acetylator genotype and cigarette smoking is a risk factor for postmenopausal, but not premenopausal, women. Rapid acetylators are at risk if they consume processed meats. There was no association for CYP2E1 and breast cancer. Other genetic polymorphisms are either in progress or are completed (CYP2D6, MEH, ADH, APOE). Other parts of this study, including the p53 mutational spectra analysis and the ancillary studies corroborate these findings and develop smoking prevention strategies are currently in progress. Thus far, the findings of these studies are important because they are identifying new etiologies for breast cancer where behavior modification would lead to a decreased risk.

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INTRODUCTION

The original goal of this project is to determine the frequency of genetic polymorphisms for carcinogen metabolism and the p53 mutational spectra in a previously conducted breast cancer study designed to assess nutritional risk factors, seeking to identify risk factors related to inheritable susceptibilities and chemical etiologies. The workscope was subsequently expanded to include the same goals, but for other epidemiological studies of breast cancer, and to perform studies of breast metabolism, p53 and smoking (including smoking cessation). We were awarded to examine a variety of risk factors (hormonal and non-hormonal; environment and diet; carcinogens and anticarcinogens) in relationship to p53 mutations and breast cancer with genetic polymorphisms as effect modifiers. The frequency of genetic polymorphisms themselves in relation to breast cancer and to p53 mutations are being determined.

A population-based case-control study of breast cancer was conducted between 1986 to 1991; blood and tissue have been stored. There were 371 postmenopausal and 301 premenopausal women with breast cancer and 438 and 316 age-matched controls, respectively. Genotyping for GSTM1, CYP1A1, CYP2D6, CYP2E1, APOE, aldehyde dehydrogenase, glutathione-*S*- transferase theta (GSTT) and N-acetyltransferase 1 and 2 is being determined for all subjects. The p53 mutational spectra is being determined for informative cases, who will be identified by single stranded conformational polymorphism analysis and immunohistochemical staining. Persons with mutations will be categorized by mutation and hypothesized chemical etiology will be compared to persons with other types of p53 mutations (four for each case) and also to controls without cancer (ten for each case). Odds ratios and logistic regression will address the association of genetic polymorphisms and exposures as a risk for p53 mutation and breast cancer, adjusting for other risk factors. We also will examine effect modification for other risk factors by genetic polymorphisms.

The current workscope has been expanded to perform additional studies relating to findings in the first year of the award, specifically as they relate to smoking, smoking-related

carcinogens and breast cancer. Thus, we are culturing human breast epithelial cells and examining the rate of adduct formation from cigarette-smoke carcinogens, as well as the p53 and apoptosis response. Interindividual variation will specifically be addressed. The purpose of these studies is to corroborate our epidemiological findings. We will also reproduce our findings in additional epidemiological studies. Finally, we will examine nicotine addiction and genetic risk factors for addictive behaviors, in the context of a smoking cessation project, in order to identify smoking cessation strategies that will reduce the incidence of breast cancer in susceptible populations.

BODY

1. Collection of Tissue Samples and Tissue Preparation

- DNA has been extracted from blood clots of 300 premenopausal cases and additionally, we identified 80 additional postmenopausal cases for which we now have extracted the DNA.

- Tumor blocks for 93 cases have been obtained and sectioned. An additional 200 have been identified.

- A mechanism for receiving fresh breast tissues from autopsy cases and reduction mammoplasties is in place. We have received four to date, 2 of which are suitable for cell culture. Additionally, we have collected 120 frozen breast tissues (100 female and 20 male).

- Collection of blood samples from 500 persons enrolled in a smoking cessation project and non-smokers have been collected. 100 samples have been extracted to date.

2. Genetic Polymorphism analysis

- NAT2 genotyping was completed for all premenopausal and additional postmenopausal cases. We found that there was a significant association of breast cancer and smoking for postmenopausal women who are slow acetylators. The risk was greatest for smoking one

pack per day, and intensity of smoking was a greater risk factor than duration. We performed several types of analyses including a case-series analysis and a smoking-matched nested case control study. All results were consistent, with odds ratios of approximately 8.0 in the highest quartiles of smoking. However, we did not find a smoking effect for premenopausal women. Whether this is due to different risk factors for what are essentially different diseases (pre- versus postmenopausal), latency or different capacities for metabolism remain to be determined. The biological mechanism for this finding is likely and impaired capacity to detoxify aromatic amines. Laboratory studies confirming this finding, the analysis in other study groups and a study of smoking addiction are currently being conducted (see below). A manuscript summarizing these findings have been submitted to the Journal of the American Medical Association.

We also have completed the analysis of NAT2 and diet. We found that there was risk of breast cancer in premenopausal women who were rapid acetylators and consumed processed meats. There was no increased risk for consumption of red meat, poultry or fish. No association was found for postmenopausal women. The biological basis for this finding is likely related to the consumption of heterocyclic amines, and laboratory studies are now in progress to further investigate this. A manuscript has been submitted to Cancer Research.

- Apoliprotein E is involved in the production of VLDL and other parts of cholesterol metabolism. Several studies have related low cholesterol levels to breast cancer risk. The apoE gene is polymorphic, where some variants raise cholesterol levels and others lower them. We therefore measured apoE genotypes in both the pre- and postmenopausal women. The statistical analysis is currently being conducted.

- Alcohol consumption has been associated with breast cancer risk. Alcohol is metabolized in humans via aldehyde dehydrogenase to a reactive acetaldehyde. It is unknown, what if any, component of the alcoholic

beverage, or a metabolite, is related to breast cancer. To further refine the risk and assess metabolism, we are currently measuring a polymorphic site within ADH that lowers activity by 40%. The assays for the postmenopausal women are completed and we are currently assaying the premenopausal women.

- Our previous results indicated that a polymorphism in cytochrome P450IAI is related to breast cancer in persons with low tobacco use. There also was a non-significant trend for younger postmenopausal women. Both of the enzymes are involved in the activation and detoxification, respectively, of polycyclic aromatic hydrocarbons. Another enzyme involved in this pathway is microsomal epoxide hydrolase. There are two polymorphic sites that result in a decrease of activity by 40%. We are currently measuring this polymorphism and have approximately 100 postmenopausal cases completed.

- Cytochrome P450IID6 has been associated with lung cancer and breast cancer. Its metabolic substrate is unknown, but it may be a tobacco-specific nitrosamine. We are measuring the activity of this gene by PCR. Thus far, assays (4 different polymorphic sites) are completed for the postmenopausal women and 3 of 4 sites are completed for the premenopausal women.

- Cytochrome P450IIE1 is involved in the metabolic activation of carcinogenic *N*-nitrosamines. There is a polymorphism located in the non-coding region of the gene that has been associated with lung cancer. This site was measured in all pre- and postmenopausal women. We have thus far found that there was no relationship to family history or smoking. A manuscript summarizing this data has been submitted to Molecular Carcinogenesis. The data in relation to diet is now being studied.

3. P53 Mutational Spectra Analysis

- Blocks have been obtained and are being sectioned. We have identified appropriate controls to ensure quality.

control and no contamination of wild-type DNA. We have identified these controls from lung cancer samples. There are 20 controls that contain mutations in each of the 4 exons of interest. We have also prepared blocks of cell lines with known p53 mutations, which also will be used as controls. The PCR of samples is currently beginning.

4. Ancillary Studies

- We have developed the technique in our laboratory, based upon previously published methods, to isolate breast epithelial cells and culture them in a sterile environment. Thus far we have obtained four tissues and were successful in two. In these cells, we have determined that 4-aminobiphenyl is metabolically activated through cytotoxicity experiments. We plan to obtain viable cultures from 50 women, and examine the interindividual variation in relation to NAT2 acetylation. DNA adducts will be measured using the postlabeling ADAM procedure and radiolabeled compounds will also allow us to measure adducts using accelerator mass spectroscopy. We also will examine the p53 response and apoptosis. The studies also will utilize heterocyclic amines and benzo[a]pyrene.

- A collaboration has been initiated with Melissa Bondy, Ph.D. at the MD Anderson Cancer Center to perform a case-series study of NAT2 and smoking in women with breast cancer. We will analyze Caucasians, to follow-up our earlier results and also include Hispanics and African Americans. We are targeting a total of 400 samples. Genotyping will be performed from paraffin-embedded tissues. Samples are currently being collected.

- We have entered into a collaboration with Caryn Lehrman at Georgetown University and Neil Caporaso of the Genetic Epidemiology Branch at NCI to study neurobehavioral and metabolic risk factors for smoking addiction and ability to quit. Over 500 participants in a smoking cessation study and non-smoking controls have been enrolled and blood was collected. Over 100

samples have undergone DNA extraction. We have developed assays to measure polymorphisms in the D2 dopamine receptor, D4 dopamine receptor and tyrosine hydroxylase. These candidate polymorphisms have been linked to altered neurotransmitter levels, addictive behavior or psychiatric abnormalities. Thus, we will ask the question if women with specific psychological profiles (i.e., depression) and genetic polymorphisms have a greater risk for addiction and a decreased chance of quitting.

CONCLUSIONS

The findings of an association of smoking and breast cancer in Caucasian women with the slow NAT2 acetylation genotype is very important because approximately 50% of women are slow acetylators. This results in a large attributable risk. The findings need to be reproduced and examined in other races. We are currently doing that. Laboratory studies also need to corroborate this finding by examining the metabolic potential in rapid and slow acetylators. We also are currently doing this. The finding that cultured breast cells metabolize 4-aminobiphenyl indicates that we will be able to address the question directly about the role of this carcinogen in breast cancer.

Although less striking, the findings of increased risk in NAT2 rapid acetylators who eat processed meats also is important. The results suggest that heterocyclic amines play a role in breast cancer, as suggested by laboratory studies. Studies that can specifically develop an index of heterocyclic amine exposure are needed.

The lack of associations for the CYP2E1 is important as other investigators are planning to study this gene. The negative findings might lead some investigators to devote their resources elsewhere or utilize different study designs that might still elucidate a role for this polymorphism.

It is too early to make conclusions for the other studies as the assays and analyses are not completed.

Thus far, the findings of these studies are important because they are identifying new etiologies for breast cancer where behavior modification would lead to a decreased risk.

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