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MicroRNAs: Novel Breast Cancer Susceptibility Factors in Caucasian and African American Women

So far, we have completed the genotyping analysis in 800 Caucasian and 800 African American breast cancer patients, and 800 Caucasian and 800 African American healthy controls. A total of 275 SNPs were analyzed, including 112 SNPs in the microRNA genes, and 163 SNPs in microRNA processing genes. We have identified several SNPs in microRNA processing genes and microRNA genes are associated with breast cancer risk in either Caucasian Americans or African Americans.
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INTRODUCTION

In current proposal, we propose to conduct a molecular-based case-control study to evaluate the genetic polymorphisms in selected miRNA genes, responsive elements in target genes, and miRNA processing genes as predictors of breast cancer risk. The study population will consist of 1000 breast cancer patients and 1000 healthy controls. The proposed research will utilize biological specimens and epidemiological data from breast cancer patients and healthy controls systematically collected by an existing study. We will integrate epidemiologic and clinical data with the genetic data from the studies. In further exploratory analysis, we will evaluate whether SNPs in miRNA genes that are predicted to regulate key breast cancer genes, SNPs in responsive elements in these key genes, and haplotypes in miRNA processing genes (Drosha, Dicer, DGCR8, XPO5, TRBP and AGO2) are associated with early age at diagnosis and aggressive disease characteristics (high-grade tumors and ER-negative status) in AA women.

BODY

So far, we have completed the genotyping analysis in 800 Caucasian American breast cancer patients and 800 African American breast cancer patients, 800 Caucasian American healthy controls and 800 African American healthy controls. A total of 275 SNPs were analyzed, including 112 SNPs in microRNA genes, and 163 SNPs in microRNA processing genes. Because the advance of genotyping technology and the drop of the genotyping cost, we are able to genotype more SNPs and more study subjects compared to what were proposed in the original proposal. The data are briefly presented below:

Identification of significant miRNA processing gene SNPs
In our preliminary analysis of the 74 miRNA processing gene SNPs, we excluded 1 SNP that was out of Hardy-Weinberg equilibrium in the controls (PACT: rs9283487), resulting in a total of 73 SNPs for analysis. SNPs were characterized as significant in an analysis if they met at least one of the following criteria: a significant odds ratio (OR), a significant p-value (p) for within ethnicity comparisons, or a significant p value for interaction (p-int), representing the p-value for interaction between ethnicities.

In our analysis of significant miRNA processing gene SNPs associated with breast cancer within AA cases and controls, we identified three significant SNPs in two genes. In the AA population, two SNPs were significant in AGO4. First, the combined CA and AA variants in AGO4 rs7354931 was associated with a decreased risk of developing breast cancer (OR=0.64, 95%CI=0.42-0.96, p=0.03) while the GC/CC combined variant in AGO4 rs3820276 was associated with a modest increased risk of de novo breast cancer (OR=1.32; 95% CI=1.03-1.71, p=0.03) (Table S1). The GG gene variant in CCND2 rs3217926 was associated with a greater than 3.5 fold risk of developing breast cancer in the AA population (OR=3.74, 95%CI=1.22-11.49) and was found to be associated with a differential risk of developing breast cancer in multiple comparisons (p-int=0.074). ESR1 rs2234693 was also found to be associated with a differential risk of developing breast cancer in multiple comparisons (p-int=0.045). Within the EA cases and controls, one SNP in DGCR8 (rs9606241, GG variant) was associated with an increased risk of developing breast cancer (OR=1.77, 95%CI=1.02-3.09). Within the EA cases and controls, a second SNP in DGCR8 (rs443678, AA variant) was associated with a decreased risk of developing breast cancer (OR=.54, 95%CI=0.30-0.98).

In our analysis of significant SNPs associated with breast cancer within premenopausal AA cases and controls, we identified two significant SNPs in two genes: one in AGO4 rs7354931, CA/AA combined variants (OR=0.6; 95%CI=0.36-0.99, p=0.05) and one in PACT rs10930831, GC variant (OR=1.63;
95%CI=1.09-2.42). We also identified a significant SNP in premenopausal EA cases and controls: DGCR8 rs443678 AA variant (OR=0.38; 95%CI=0.18-0.83).

In our analysis of significant miRNA processing gene SNPs associated with breast cancer within postmenopausal AA cases and controls, we identified two SNPs that were significant only between postmenopausal AA cases and controls: AGO4 rs7354931 (p=0.05) and PACT rs10930831 GC variant (OR=0.59; 95%CI=0.36-0.98). XPO5 rs11077 was significant in AA postmenopausal cases and controls and was associated with a greater than half a reduction in risk of developing breast cancer (p=0.01), and AA/EA interaction (p=0.03). XPO5 rs1106841 was also associated with a significant reduction in risk of developing breast cancer in AA cases and controls (p=0.01), and AA/EA interaction (p-int=0.04). CCND2 rs3217926 was significant and was associated with a 10-fold increase in risk between postmenopausal cases and controls in AA women (GG variant, OR=10.68; 95%CI=1.29-88.61, p=0.04) and it was also significant between postmenopausal cases and controls in EA women (p=0.05). Two SNPs were significant in two genes in only the EA postmenopausal population: DGCR8 GG variant (OR=2.73; 95%CI=1.08-6.87), and IL16 GG variant (OR=0.46; 95%CI=0.22-0.98, p=0.05).

Identification of significant microRNA SNPs

In our preliminary analysis of the 97 pre-miRNA SNPs, we excluded 2 SNPs that were out of Hardy-Weinberg equilibrium in the controls (hsa-miR-27a: rs895819; hsa-miR-146a: rs2910164), resulting in a total of 95 SNPs in 80 miRNAs. SNPs were characterized as significant if they met at least one of the following criteria: a significant odds ratio (OR), a significant p-value (p-trend) for within ethnicity comparisons, or a significant p-int.

In our analysis of significant miRNA SNPs associated with breast cancer within AA cases and controls, we identified 4 SNPs that were significant within the AA population: hsa-miR-641 rs11880261 AA variant (OR=8.61; 95%CI=1.06-69.84); hsa-miR-624 rs11156654 TA variant (OR=1.58; 95%CI=1.01-2.47; p=0.03); hsa-miR-199-3p rs219 AA variant (OR=1.58; 95%CI=1.01-2.47; p=0.03); hsa-miR-758 rs12586258 GA variant (OR=1.68; 95%CI=1.08-2.60; p=0.02), p-int=0.03. Two SNPs were significant in both the EA population and in the interaction between AA and EA populations: hsa-miR-487 rs19512032 GA/AA combined variants (OR=1.57; 95%CI=1.12-2.21), p-int=0.01 and hsa-miR-573 rs7696197 AG/GG combined variants (OR=3.48; 95%CI=1.19-2.30; p=0.04), p-int=0.01. In our analysis of significant miRNA SNPs within EA cases and controls, we identified 4 SNPs that were significant within the EA population: hsa-miR-548a-2 rs878175 (p=0.03).

In our analysis of significant SNPs in miRNAs associated with breast cancer within premenopausal AA cases and controls, we identified 4 significant SNPs within the AA population: hsa-miR-105 rs970292 GA variant (OR=2.04; CI=1.10-3.75); hsa-miR-548a-3 rs11997039 AG/GG combined variants (OR=0.54; 95%CI=0.33-0.89; p=0.02); hsa-miR-659 rs5750504 TA variant (OR=0.61; 95%CI=0.41-0.91); hsa-miR-758 rs12586258 GA variant (OR=0.53; 95%CI=0.33-0.85; p=0.001). We identified 2 SNPs that were only significant in the interaction between AA and EA populations: hsa-miR-573 rs7696197; p-int=0.05; hsa-miR-100 rs1834306; p-
In our analysis of significant SNPs in miRNAs associated with breast cancer within premenopausal EA cases and controls, we identified 9 SNPs that were significant within the EA population: hsa-miR-487 rs4906032 variant GA (OR=1.60; 95%CI=1.05-2.44; p=0.05); hsa-miR-487 rs1951032 GA/AA combined variants (OR=1.80; 95%CI=1.13-2.85; p=0.01); hsa-miR-518a rs4470257 variant AG (OR=1.76; 95%CI=1.03-3.01; p=0.04); hsa-miR-331 rs11107973 variant GG (OR=2.04; 95%CI=1.16-3.58; p=0.02); hsa-miR-106b rs1527423 (OR=1.69; 95%CI=1.07-2.68); hsa-miR-128a rs11888095 variant GA (OR=0.61; 95%CI=0.37-0.99); hsa-miR-544 rs10144193 variant AT (OR=2.00; 95%CI=1.29-3.11); hsa-miR-576 rs6856291 variant GA (OR=0.64; 95%CI=0.41-0.99); hsa-miR-206 (p=0.05).

In our analysis of significant SNPs in miRNAs associated with breast cancer within postmenopausal AA cases and controls, we identified 6 significant SNPs within the AA population: hsa-miR-202 rs22185743 variant GA (OR=0.63; 95%CI=0.41-0.98); hsa-miR-302d rs13136737 variant AA (OR=0.11; 95%CI=0.01-0.99); hsa-miR-500 rs17174054 variant AG (OR=0.62; 95%CI=0.40-1.0; p=0.02); hsa-miR-578 rs17624836 AG/GG combined variants (OR=0.56; 95%CI=0.35-0.91; p=0.02); hsa-miR-598 rs4840516 variant GC (OR=0.62; 95%CI=0.39-0.95; p=0.03); hsa-miR-576 rs6856291 (p=0.04). We identified 2 SNPs that were significant both within the AA postmenopausal population and between the AA and EA postmenopausal populations: hsa-miR-766 rs5909648 CA/AA combined variants (OR=0.62; 95%CI=0.40-0.96; p=0.03) p-int=0.05; hsa-miR-606 rs1367290 GA and GG variants (OR=0.39; 95%CI=0.19-0.78 and OR=0.43; 95%CI=0.22-0.83, respectively), p-int=0.02. One SNP was only significant in the interaction between the AA and EA postmenopausal populations: hsa-miR-487 rs1951032, p-int=0.03. Two SNPs were significant in the EA postmenopausal population as well as in the interaction between the AA and EA postmenopausal populations: hsa-miR-598 rs289254 AA variant (OR=0.23; 95%CI=0.07-0.76; p=0.01), p-int=0.01, and hsa-miR-659 rs5750504 AA variant (OR=2.51; 95%CI=1.2 3-5.12; p=0.01), p-int=0.04. We also identified 4 SNPs that were significant in the EA postmenopausal population only: hsa-miR-608 rs4919510 CG variant (OR=0.53; 95%CI=0.30-0.93); hsa-miR-641 rs1180261 (OR=1.69; 95% CI=1.02-2.81); hsa-miR-139 rs754042 (p=0.05); hsa-miR-196a-1 rs718079 (p=0.04).

**KEY RESEARCH ACCOMPLISHMENTS**

- Significant associations have been observed between SNPs in microRNA genes and breast cancer risk in either African American or Caucasian American women. These SNPs include rs7696197, rs6856291, rs878175, rs4919510, rs12586258, rs10144193, rs1951032, rs5750504, rs2018562, rs5970292.
- Stratified by menopausal status, significant associations have been observed between SNPs in microRNA genes and breast cancer risk in either pre-menopausal African American or pre-menopausal Caucasian American women. These SNPs include rs6856291, rs1527423, rs11997039, rs11107973, rs12586258, rs10144193, rs1951032, rs4906032, rs4470257, rs5750504, rs5970292, rs42039.
- Stratified by menopausal status, significant associations have been observed between SNPs in microRNA genes and breast cancer risk in either post-menopausal African American or post-menopausal Caucasian American women. These SNPs include rs17624836, rs4840516, rs2898254, rs1367290, rs4919510, rs754042, rs3217926, rs11156654, rs718079, rs5750504, rs17174054, and rs5909648.
- Stratified by ER status, significant associations have been observed between SNPs in microRNA genes and ER positive breast cancer risk in either African American or Caucasian American women.
These SNPs includes rs6771018, rs7696197, rs6856291, rs11134527, rs9396886, rs2898254, rs2060133, rs12266981, rs4919510, rs754042, rs11613504, rs11156654, rs5750504, and rs718079.

- Stratified by ER status, significant associations have been observed between SNPs in miRNA genes and ER negative breast cancer risk in either African American or Caucasian American women. These SNPs include rs1970801, rs107822, rs3025039, rs16882131, rs4919510, rs7141987, rs9324030, rs2281611, and rs9875.

REPORT OUTCOMES
We have presented a poster at DOD Era of Hope meeting in this August. The tile of the poster is “Novel Breast Cancer Susceptibility Factors in Caucasian and African American Women”.

A manuscript is under working.

CONCLUSION
So far, the study moves smoothly. We don’t expect any problem to complete the study. In addition, we expect to genotype additional 200 Caucasian American breast cancer patients and 200 African American breast cancer patients, 200 Caucasian American healthy controls and 200 African American healthy controls. In addition, we will include another 20 SNPs to genotype in the whole study population.