our projects

BREAST CANCER AND GENE ASSOCIATION STUDIES

- Gene Variants And Their Significant Increase Risk For Breast Cancer
- Genetic Polymorphisms In Proinflammatory Genes And Risk Of Breast Cancer
- Original Article: A Multigene Test For The Risk Of Sporadic Breast Carcinoma
- Identification Of Breast Cancer Risk Predicting Genes

GENE VARIANTS AND THEIR SIGNIFICANT INCREASE RISK FOR BREAST CANCER

Background:

Mutations in genes with large effects on breast cancer risk have been identified from our previous studies. In this study, few additional genes showing significant association with the disease and its progression are reported. We investigated the following seven genes based on the previous studies and the relevance of these gene mutations in predisposition to breast cancer. Androgen receptor (AR) gene polymorphisms which render the more transcriptionally active receptors have been related to a lower risk of breast cancer.

The CDKN2A tumor-suppressor locus on chromosome band 9p21, which encodes p16 (INK4A), a negative regulator of cyclin-dependent kinases, and p14(ARF1), an activator of TP53, is inactivated in many human cancers by point mutation. KLF family members are involved in cell proliferation and differentiation control in normal as well as in pathological situations. Cyclin-dependent kinase inhibition by the KLF6 tumor suppressor protein through interaction with cyclin D1 was demonstrated in an earlier study. Overexpression of the ERBB2 protein leads to constitutive activity of the HER2 receptor and breast tumor development through enhanced cell proliferation, survival, motility and adhesion. Our recent study showed significant association of KLF6 gene with prostate cancer. Since breast cancer is hormone related cancer we predicted the association of these genes with the disease.

Materials & Methods:
Peripheral blood was drawn and seven gene polymorphisms (AR, CCR5, CDKN2A, HER2, KLF6, MTHFR and TNFB) were screened in 195 breast cancer patients and 134 female normal healthy controls using PCR restriction enzyme assay. Gene expression studies were performed using FFPE tissue sections.

Results:
Significant association of five gene variants (AR- $p< 0.025$, CDKN2A- $p< 0.0005$, KLF6- $p<0.0032$, HER2 - $p<0.0039$, and TNFB - $p<0.010$) with breast cancer were found. A high frequency of the associated variant alleles were found in breast cancer patients with other types of cancer as compared to patients with only breast cancer.CCR5 and MTHFR gene polymorphisms did not show significant association even though a high frequency of variant gene was found in breast cancer patients as compared to normal healthy controls.

Conclusions:
The significant association of AR, CDKN2A, KLF6 gene variants with breast cancer may be correlated with other studies. Epidemiological and clinical evidences suggest that higher levels of circulating androgen increase the risk of developing breast cancer. Physical interaction between CDKN2A/p16 and CDK4 proteins regulates the cell cycle progression through the G1 phase. Previous studies demonstrated that the G1 cell cycle checkpoint in carcinomas of the breast is frequently abrogated by loss of p16, the product of the CDKN2/INK4A gene. KLF family members are involved in cell proliferation and differentiation control in normal as well as in pathological situations. Studies using FFPE tissue showed significantly low KLF6 gene expressions in malignant tissue as compared to normal tissue suggesting its possible role in tumorigenesis. The data showing correlations of these gene expressions and the associated gene variants with breast cancer will be discussed.

GENETIC POLYMORPHISMS IN PROINFLAMMATORY GENES AND RISK OF BREAST CANCER

Background:
The proliferation of malignant breast epithelial cells is regulated by various stimuli including cytokines and growth factors, and thus the variants of their respective genes may modify the breast cancer risk. Tumor necrosis factor (TNF), which regulates inflammatory responses and combats tumor growth, can induce cell death in tumor cells. High concentrations of RANTES are produced in human breast and breast cells produce this chemokine under specific physiological stimuli. It is also known that tumor cells such as ovarian tumors that are under hormonal regulation produce RANTES.

We hypothesize that the proinflammatory gene polymorphisms, in combination with proinflammatory conditions, may influence the development of breast cancer. To evaluate the potential influences of these gene polymorphisms in breast cancer risk as well as in disease progression, a case-control study using polymorphisms in the promoter of the TNF gene, in the promoter of RANTES and IL1β were investigated.

Materials & Methods:
Genomic DNA was isolated from whole blood of 163 breast cancer patients and 200 normal healthy age matched controls. Polymorphism analysis was done by PCR-RFLP method. The collected data was analyzed using statistical package software SPSS 10.1.

Results:
The relative association between patients and control subjects for genotype prevalence was assessed by Chi-saqure test. Genotype distributions were significantly different in both the groups. Significant association of TNF-β (P=0.002) and TNF-α (-308; p= 0.037), IL1-β (p=0.035) and RANTES (P=0.042) gene polymorphisms were found with breast cancer when compared to control subjects.

Discussion:
Genetic polymorphisms of cytokine-encoding genes are known to predispose to malignant disease. In breast cancer patients, RANTES may be produced by protumorigenic activities and of proinflammatory cytokines, that may facilitate metastases rather than just by monocyte migration to the tumor site and contributes to disease progression. Our present results suggest that inflammatory cytokines and tumor derived RANTES may play a role in determining the risk of cancer in the breast. To better understand the role of these genes in the progression of breast carcinoma, the regulation of these gene expressions and the other mediators in the pathway need to be investigated in the malignant tumor tissue.

ORIGINAL ARTICLE: A MULTIGENE TEST FOR THE RISK OF SPORADIC BREAST CARCINOMA

Background:
Although the identification of the BRCA1 and BRCA2 genes have been of great interest, these genes account for less than 5% of all breast carcinoma cases. The remaining cases are sporadic. Reanalysis of a large twin study suggested that genetic factors may play a significant role in sporadic breast and other carcinomas. Sporadic breast carcinoma is polygenically inherited. Multiple genes are likely to have an additive effect, each gene accounting for a fraction of the variance.

One factor that may have an impact on the development of hormonally responsive breast tumors is the duration of exposure of the breast to estrogen. Therefore, one of the demographic risk factors for breast carcinoma is an early age of onset of menarche. The current study was based on the hypothesis that genes that play a role in demographic risk factors may be breast carcinoma risk genes in their own right.

The authors hypothesized that six genes relevant to the timing of the onset of menarche and related risk factors might be candidate genes for breast carcinoma. These were the leptin gene (LEP), the leptin receptor gene (LEPR), the catechol-0-methyltransferase gene (COMT), the dopamine D2 receptor gene (DRD2), the estrogen 1 receptor gene (ESR1), and the androgen receptor gene (AR).

Methods:

The authors examined 67 women with postmenopausal sporadic breast carcinoma and 145 gender and race-matched controls.

Results:
Five of these genes accounted for a significant percent of the variance (r2) of breast carcinoma. The following r2 and P values were calculated: LEP: 0.073, P 0.0001; LEPR: 0.064, P 0.0002; COMT: 0.073, P 0.0001; AR: 0.040, P 0.0035; and DRD2: 0.018, P 0.05. When evaluated in a multivariate regression analysis, they accounted collectively for 24% of the variance of breast carcinoma (P 0.0001).

These genes accounted for 40% of the variance (P 0.00001) in a subset of age-matched cases. Individual gene scores were added to form a breast carcinoma risk score (BCRS) that ranged from 0 to 17. When the BCRS was evaluated in a receiver operator characteristic plot, the area under the curve was 0.80 for the full set and 0.869 for the age-matched set. The relative breast carcinoma risk for the different BCRS scores ranged from 0.10 to 11.9.

Conclusions:
These results demonstrate a potentially powerful method of evaluating the additive effect of multiple breast carcinoma risk genes to form a potentially clinically useful assessment of women's risk for sporadic breast carcinoma. Cancer 2003;97:2160-70.

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IDENTIFICATION OF BREAST CANCER RISK PREDICTING GENES

Background:
Breast carcinoma is the most common malignant disease amongst women and the second most lethal one. Breast cancer progression may be affected by various cellular components expressed by the tumor cells and/or by micro environmental factors. Aging remains one of the single greatest risk factors for the development of breast carcinoma. Multiple genes are likely to have an additive effect, each gene accounting for a fraction of the variance.

We reported a multigene test for risk in sporadic breast carcinomas (Cancer. 2003 May 1; 97(9): 2160-70). Taking into account the data from the previous study, a multigenic case-control study with additional genes were performed, to define their role involved in breast cancer risk.

Methods:
Genomic DNA was isolated from whole blood of both breast cancer patients and controls.

By polymerase chain reaction analysis of genomic DNA, the genotypes were determined and statistical analysis done to evaluate the individual and additive effect of these 12 genes in breast carcinoma patients (HER2, LEPR, TNF alpha, CCND1, APC, RANTES403, TGF alpha, Interleukin 1Beta, CCR5, CDKN2A, MTHFR, PPARG 12.

Results:

Previous reports have shown the importance of certain protein expressions in tumor cell or gene associations predicting their role in cancer development. In this preliminary study, out of the 12 relevant genes screened individually for analysis only seven genes showed significance (HER2-p<0.002; LEPR-p<0.005; TNF alpha-p<0.001; CCND1-p<0.076; TGF alpha- p<0.000; APC-p<0.000; RANTES403-p<0.007). The present results indicate the above significant gene associations with breast cancer.

Conclusions:

Our preliminary results reported are promising. We propose to screen all these genes in a larger sample population with breast cancer and compare them with age-matched normal healthy controls. Use of ROC plots can validate the individual's risk level in developing cancer.