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13. ABSTRACT *(Maximum 200)* In pursuing our aims to characterize *BRCA2*, we continue to screen for *BRCA2* mutations in 40 high-risk breast cancer families. With 85% of the coding sequence examined, we have identified one missense mutation and several polymorphisms. A search for large deletions and/or rearrangements in *BRCA2* in 10 families detected no variants. We are now performing long-range PCR across the gene to confirm those results. In characterizing *BRCA2*, we found no difference in prognosis between mutation carriers and breast cancer patients with no known mutations, even though the carriers have worse prognostic indicators than the controls. A second study found that oral contraceptive use reduced ovarian cancer risk by 50% in *BRCA1* and *BRCA2* mutation carriers. A study of recurring *BRCA2* mutations, determined that they likely were founder mutations as there was no evidence for multiple origins for identical mutations. Our results were also consistent with previous reports of an ovarian cancer cluster region in *BRCA2*, and in this region, breast cancer cases had a significantly older mean age at diagnosis than seen outside the region. An examination of the role of the founder I1307K *APC* mutation on occurrence of breast cancer in Ashkenazi Jewish women found the association largely limited to those with either a *BRCA1* or *BRCA2* mutation.

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FOREWORD

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Susan Neuhansen 9-18-98
PI - Signature Date

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Annual Progress Report
Grant DAMD17-94-J-4260
Period: August 22, 1997-August 21, 1998

Introduction

Hereditary breast cancer is believed to account for up to 10% of breast cancer. We isolated *BRCA1* in 1994. We and our past collaborator on this grant localized *BRCA2* in 1994, and our collaborator isolated *BRCA2* in 1995, thus accomplishing the initial goal of this grant. The current aims are to continue to identify mutations and to characterize *BRCA2*. Since the time when *BRCA1* and *BRCA2* were isolated, we and others have devoted much effort and time to identify mutation carriers and collect epidemiological and family history data. Currently, there are a large number of mutation carriers available for study, so that we and other researchers can investigate the roles of specific mutations in age-specific penetrance and occurrence of other cancers, evaluate other low penetrant genes which may modulate penetrance and expressivity, and assess the effects of preventative measures on the risk of developing breast and ovarian cancers.

Body

Our goals for the previous year were to 1) identify mutations in *BRCA2* which are due to rearrangements or large deletions; 2) examine tumors from familial breast cancer cases and obligate carriers to assess frequency of loss at *BRCA2* versus *BRCA1*; 3) continue to identify additional mutation carriers within our *BRCA2* families for use in epidemiological studies of risk factors for *BRCA2* mutation carriers; 4) continue to examine shared haplotypes; and 5) screen our families with no *BRCA1* or *BRCA2* mutations for putative candidate loci for *BRCA3*.

Aim 1: To identify mutations in *BRCA2* due to large deletions or rearrangements.

Last year at this time, we had just constructed a restriction map of the entire *BRCA2* gene by using published *BRCA2* sequences in Genbank and identifying restriction sites. We had selected five restriction endonuclease enzymes and designed 14 probes for examining Southern blots of DNAs from families linked to *BRCA2* for which no mutations had been identified after sequencing of the entire coding region. In addition to the 5 initial families, we included samples from 5 Utah families and from 4 families from our previous collaborator on this grant, Mike Stratton at ICRF. Unfortunately, Dr. Stratton was only able to send 10 ug of DNA per sample, so that his samples were only examined with one restriction enzyme. We had many technical difficulties trying to make the probes with a sufficiently high radioactivity count in order to detect the signal on the Southern blots. We identified what appeared to be aberrant bands for

several individuals on different restriction enzymes using different probes. However, we did not identify any consistent variants that were present either with two different restriction enzymes or with two different probes as would be expected if the variants were due to a deletion or rearrangement. The result was that we did not detect any large deletions (> 1 kb) or rearrangements.

We have just begun a project to reexamine these individuals using long-range PCR of 15 kb overlapping fragments spanning *BRCA2*. We are also trying to obtain additional blood samples in order to extract RNA, make cDNA, and then examine the cDNA for deleted exons or unspliced introns. To detect loss of transcript for an exon, we will compare cDNA to genomic DNA at 7 polymorphisms within *BRCA2*. A positive result will be that the genomic DNA is heterozygous at the polymorphism and the cDNA product is either homozygous or one allele is of reduced intensity. By examining a series of polymorphisms at different positions in the gene, we may be able to determine that there is a transcript that it is truncated or contains a deletion, based on cDNA heterozygous for a polymorphism in one exon and homozygous for an informative polymorphism in another exon. We will also amplify the cDNA using primers which span exons to look for smaller fragment sizes than expected which would indicate deletion of an entire exon.

Another approach for detecting deletions is to examine parent-offspring to look for unequal transmission of alleles suggestive of hemizygous individuals. At the time, we had only identified two common polymorphisms in the gene. As we have screened for *BRCA2* mutations (see Aim 3 below), we have identified several other polymorphisms. We will examine five families with 5 additional polymorphisms in order to detect hemizygotes.

Aim 2. To examine tumors from familial breast cancer cases and obligate carriers to assess frequency of loss at *BRCA2* versus *BRCA1*

We have just recently had slides cut from paraffin-embedded tissue blocks from 10 mutation carriers and had the pathologist identify non-admixed areas of tumor and normal tissue. As part of this aim, we became interested in examining tumors from Hodgkins lymphoma cases who later developed breast cancer. As *BRCA2* interacts with RAD51, it may be that *BRCA2* mutation carriers are more sensitive to radiation and therefore develop breast cancer. We are currently examining 10 Hodgkins cases with breast cancer and 10 controls for loss of heterozygosity (LOH) at *BRCA2* using a set of 5 polymorphic markers which flank the gene. LOH would give us an initial indication that a mutation in *BRCA2* might be involved, prior to expending the time and cost to screen for mutations. This aim will be accomplished during this last year of funding.

Aim 3. To continue to identify additional mutation carriers within our *BRCA2* families for use in epidemiological studies of risk factors for *BRCA2* mutation carriers

a. Identifying *BRCA2* mutations in additional high risk breast cancer families.

As part of expanding our set of *BRCA2* female mutation carriers, we have continued to look for

mutations in *BRCA2* in high risk breast cancer families for which no *BRCA1* mutations have been identified. We have been examining one individual with breast or ovarian cancer from 40 families by single strand conformational analysis (SSCA). In order to screen all the coding regions and intron/exon boundaries with overlapping amplicons, 75 primer pairs were made. We have screened for mutations in 85% of the amplicons and have identified only one missense mutation a Ser2533Cys. We are currently examining this mutation in a set of 100 unrelated controls to see if it is a rare polymorphism. We will finish screening the remaining amplicons during this last year of funding.

b. Further expansion of families.

We have focused on obtaining up-to-date questionnaires from female *BRCA2* mutation carriers and now have a set of 39 entered into the database. We are still trying to extend these families, and enrolled an additional 11 females in the previous year.

c. Collaborative studies with *BRCA1* and *BRCA2* mutation carriers.

In two studies, using only a subset of our *BRCA1* and *BRCA2* mutation carriers, we examined the response to radiation therapy and prognosis (Gaffney et al., 1998) and examined pathobiologic characteristics of hereditary breast cancer (Lynch et al., 1998). We found that there was no difference in prognosis between mutation carriers and breast cancer patients with no known germline mutations (Gaffney et al., 1998), even though mutation carriers presented with higher nuclear grade, had increased expression of DNA topoisomerase II-alpha, lacked hormone receptors, and were more likely to have mutations in p53 (Lynch et al., 1998).

In a collaborative case-control study of 207 women with ovarian cancer due to *BRCA1* or *BRCA2* mutations and 161 of their sisters, we examined the effects of oral contraceptive use on risk of ovarian cancer (Narod et al., 1998). The adjusted odds ratio for ovarian cancer associated with any use of oral contraceptives was 0.5 (95% confidence interval, 0.3-0.8). The risk decreased with increasing duration of use; when used for six or more years there was a 60% reduction in risk. Other collaborative projects are still ongoing, including to evaluate hormone metabolizing genes which may modify age-specific penetrance, to determine if there is a reduction in risk of a contralateral breast cancer following tamoxifen treatment for the first breast cancer, to assess if prophylactic oophorectomies protect against breast cancer, and to examine risks of other cancers in only *BRCA2* mutation carriers.

Aim 4: To examine shared haplotypes

During the last year, we have continued to examine founder mutations. The I1307K *APC* variant was found to occur in approximately 6% of Ashkenazi Jews. In a collaborative study, we examined the role of this mutation on occurrence of breast cancer in Ashkenazi Jewish women by determining the frequency of this variant in a set of 632 women with primary invasive breast cancer and a set of 146 familial breast cancer cases (Redston et al., 1998). We concluded that the effect of the I1307K allele on breast cancer risk was largely limited to those with either *BRCA1* or *BRCA2* mutations.

As reported last year, we were in the process of examining 9 recurrent *BRCA2* mutations in 111 families in order to determine whether there was a common founder for each mutation, to estimate the age of the mutation, and to compare mutations to examine mutation specific phenotypes (Neuhausen et al., 1998). Ten polymorphic markers spanning a distance of 6 cM around the *BRCA2* gene were used to establish genotypes and subsequent haplotypes for each set of samples. Only those mutations (six) for which there were five or more families could be analyzed. We estimated that six mutations arose from 400-2,000 years ago. There was no evidence for multiple origins of identical *BRCA2* mutations. Our study data were consistent with the previous report of a higher incidence of ovarian cancer in families with mutations in a 3.3 kb region of exon 11 called the ovarian cancer cluster region (OCCR). When the age at diagnosis of the breast cancer cases was examined by OCCR, cases associated with mutations in the OCCR had a significantly older mean age at diagnosis than was seen in those outside this region (48 years versus 42 years).

Aim 5: Progress in examining other putative genes predisposing to breast cancer:

We genotyped 122 DNA samples from 11 families to analyze linkage for putative candidate regions and genes. The candidate chromosomal regions to be analyzed for linkage included 8p, 11p15, 11q13 and 11q22, 15q14, 16q22-24, 18q11-13, 20q13, 22, and the candidate genes include the progesterone receptor, BCSC1, DAB2, e-cadherin, and PTEN/MMAC. There was no evidence for linkage to 8p. The other data have not yet been analyzed but the genotypes have been generated and checked. The analysis will be completed during this final year of funding. Unlike *BRCA1* and *BRCA2* where there are specific phenotypes associated with each gene, e.g. ovarian cancer and breast cancer for *BRCA1* and male and female breast cancers for *BRCA2*, no phenotype with which to stratify families has been identified. It may be that localization of additional breast cancer genes will need to await more knowledge of genes which interact with *BRCA1* and *BRCA2* or a larger set of families.

Conclusions:

As demonstrated by our five publications last year, we have continued to make progress in characterizing and examining *BRCA2*. However, there are many more interesting facets to explore, with projects still on-going in this last year of funding. Results from studies of phenotype-genotype correlations; of breast cancer risk associated with hormonal factors including variants in hormone-metabolizing genes, oral contraceptives, and prophylactic surgeries; and of prognostic indicators can be used to target specific areas of *BRCA2* for basic biology research. Better understanding of *BRCA2* should allow for development of better therapeutics and preventative measures, as well as for more accurate individual cancer risk assessment for women at high risk to develop cancer.

New publications in the last year resulting from funding from this grant.

Gaffney DK, Brohet RM, Lewis CM, Holden JA, Buy SS, Neuhausen SL, et al. Response to radiation therapy and prognosis in breast cancer patients with *BRCA1* and *BRCA2* mutations. 1998. *Radiotherapy and Oncology* 47:129-136.

Lynch BJ, Holden JA, Buys SS, Neuhausen SL, Gaffney DK. Pathobiologic characteristics of hereditary breast cancer. 1998. *Human Pathology*: In Press.

Narod SA, Risch H, Moslehi R, Darum A, Neuhausen S, Olsson H et al. Oral contraceptive use and the risk of hereditary ovarian cancer. 1998. *New Eng J Med* 339: 424-428.

Neuhausen SL, Godwin AK, Gershoni-Baruch R, Schubert E, Garber J, Stoppa-Lyonnet et al. Haplotype and phenotype analysis of nine recurrent *BRCA2* mutations in 111 families: results of an international study. 1998. *Am J Hum Genet* 62: 1381-1388.

Redston, M, Nathanson KC, Qiang Z, Neuhausen SL, Satagopan J, Wong N, et al. The APC I1307K allele and breast cancer risk. 1998. *Nature Genetics* 20: 13-14.