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Male breast cancer (MBC) is rare, with an incidence rate of 0.5 - 1/100,000 per year. The objective of this grant is to study a series of unselected population-based MBC cases and their relatives to characterize the role of *BRCA2* in MBC and to estimate the attributable risk of MBC due to *BRCA2* mutations. At the end of this second year of funding, we have collected DNA samples on 124 MBC cases and paraffin-embedded tissue on 21 of those. Of the 80 MBC cases with available family history data, 44 (55%) have a family history of breast cancer in first or second degree relatives. To detect germline mutations in *BRCA2*, single strand conformational analysis (SSCA) of the DNA samples is being performed for seventy-four amplicons spanning the entire coding region and intron/exon boundaries. Ninety percent of the gene has been screened for mutations in 10 samples, 60% for 48 samples, and 50% for 66 samples. We have identified five frameshift mutations and three missense mutations of which two appear to be polymorphisms and one is of unknown significance. During the next year, we will continue to screen for mutations and to accrue additional participants with MBC.

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Susan Neuhausen 7-13-98

PI - Signature Date

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Annual Progress Report
Grant DAMD17-96-1-6266

Period: June 15, 1997-June 15, 1998

Introduction.

Breast cancer in men was first described in the 14th century and the first familial case was reported in 1889 (Kozak et al., 1986). Breast cancer is a rare disease in men, affecting approximately 0.1% over their lifetime, as compared to 12% in women. However, male and female breast cancers are similar in presentation and response to treatment.

BRCA2 was isolated in 1995 (Wooster et al., 1995; Tavtigian et al., 1996). We estimate that 10-15% of male breast cancer (MBC) may be due to mutations in *BRCA2*. In a study of loss of heterozygosity (LOH) of markers spanning the *BRCA2* gene, 16 of 24 (67%) MBC cases showed LOH in at least one of the two markers, suggesting a role for *BRCA2* in the development and/or progression of MBC (Prechtel et al., 1998). In a Swedish study of 34 MBC patients, seven (21%) *BRCA2* germline mutations were found. Of those seven cases, only one had a family history of breast cancer (Haraldsson et al., 1998). In a British study of 28 MBC cases, 2 (7%) deleterious mutations were identified (Mavraki et al., 1997). In a US study of 54 MBC cases, no germline *BRCA1* mutations were found and two (4%) BRCA2 mutations were found. One of those two cases had a family history of breast cancer (Friedman et al., 1997). Interestingly, the remaining eight cases with a family history of breast and/or ovarian cancer did not have a mutation in either *BRCA1* or *BRCA2*. Combining all three of these studies, the proportion of MBC cases, unselected for a family history of breast cancer, with a germline mutation in *BRCA2* is 9.5%.

The objective of this proposal is to study MBC cases to characterize the role of *BRCA2* in MBC and to estimate the attributable risk of male breast cancer due to *BRCA2* germline mutations. Secondly, identification of *BRCA2* mutations in these MBC cases,will allow us to ascertain a set of high-risk families which can be used to further examine the genetic epidemiology of *BRCA2*-related cancers including what underlying risk factors are related to incidence of cancer in mutation carriers. This is a population-based study. The results to date are discussed below, as well as future work to be performed.

Body.

Our goals for the past year included continued ascertainment of male breast cancer cases and screening for mutations in the *BRCA2* gene.

Progress in ascertainment of male breast cancer cases. During the past year, we continued to enroll MBC cases in Utah through the Utah Cancer Registry (UCR), a population-based registry. The UCR is the agency designated to record all cancer diagnosed in the state. We also obtained a set of population-based MBC cases from the Imperial Cancer Research Fund (ICRF) Genetic Epidemiology Laboratory at St. James's University Hospital in England. We are not obtaining new MBC cases from Memorial Sloan Kettering Cancer Center (MSKCC), because of a New York State law requiring that the participants be offered testing at a clinical laboratory. There are no funds in the grant to pay for commercial testing.

We currently have DNA samples from 124 MBC cases (Table 1). The age at diagnosis ranges from 31-82 years. Of the 80 MBC cases with family history data, 55% have a family history of breast cancer in at least one first degree relative. We have also collected blood samples from 103 first degree relatives of 42 MBC cases. We currently have 21 tissue blocks from Utah MBC cases with another 21 requests pending at area hospitals. Sets of slides are being sent from six MSKCC MBC cases. Questionnaires have been sent to 50 Utah MBC cases and 20 have been returned. Unfortunately, seven men have died since we obtained their blood samples.

Table 1. Number of male breast cancer cases sampled and number with a family history.

		Family history		
Source	# cases	Positive	Negative	Unknown
Univ. of Utah	57	23	26	8
MSKCC	27	17	10	0
ICRF	40	4	--	36
Total	124	44	36	44

As described in our report submitted July 1997, the MBC cases have been more difficult to ascertain than we had anticipated, as approximately 55% of the cases in the UCR prior to December 1997 had addresses which were unknown, refused to participate in this study, or were deceased. We have included rapid-reporting of recently diagnosed cases in order to obtain a larger response rate. Two MBC cases diagnosed in 1997 have just agreed to participate. In addition, we are ascertaining cases through the Idaho Cancer Registry using the same protocol. Letters were sent to 14 living MBC cases asking if they are interested in participating in our study. We have also contacted and sent information to the New Mexico Cancer Registry, the Arizona Cancer Registry, and the Colorado Cancer Registry to ask them to contact the MBC cases diagnosed in those states. Once the protocol for each of the surrounding states is completed, we expect to ascertain approximately 25-35 male breast cases from those registries. We received IRB permission and have posted our study on the website of a Male Breast Cancer Support Group, inviting all males who have had breast cancer to participate in our study.

Progress in screening for *BRCA2* mutations. During the past year, we have optimized our set of primers for amplifying the entire *BRCA2* coding regions and intron/exon boundaries. Single strand conformational analysis (SSCA) is now being performed on 74 amplicons, with an average size of 250 basepairs and a maximum size of 300 basepairs. For ten samples, 67 amplicons have been completed. For 66 samples, one-half of the amplicons have been screened, and for 48 samples, 45 amplicons have been completed.

In our 1997 Progress Report, we had identified 3 frameshift mutations (4075delT, 4360ins5, 6174delT in 3 samples), one missense mutation (D1420Y), and one missense mutation (K1132K) known to be a polymorphism. In the last year, we have identified two additional frameshift mutations (9481insA and 9325insA) in two MBC cases from England. Five of the seven mutation carriers with a frameshift mutation have a family history of breast cancer and one is unknown. The D1420Y missense mutation identified last year appears to be a polymorphism and has been identified in an additional 3 MBC cases. A missense mutation, T2005A, was identified in one individual. We will examine the frequency of this missense mutation in a set of 100 (200 chromosomes) unrelated DNA samples to see if this appears to be a polymorphism based on frequency in an unaffected population.

Plans for the third year of funding. During the next year, our primary focus will continue to be to ascertain MBC cases and to screen for mutations in all our samples. We will also begin to examine variants in putative low penetrance genes which may affect the risk of breast cancer, including those genes involved in hormone metabolism, such as the estrogen and progesterone receptors.

Conclusions.

Male breast cancer is a relatively rare disease, as shown by our difficulty in rapidly accruing a large number of living cases for this study. Based on preliminary results, in which on average only 57% of *BRCA2* has been screened for mutations in these 124 MBC cases, 6% of the MBC have mutations in *BRCA2*. The actual proportion of mutation carriers will be higher, as this percentage is clearly an underestimate as only slightly more than half the gene has been screened in the majority of the cases. Five of the seven mutation carriers have a family history, and one is unknown. As more data are available, we will examine family history as a predictor of carrying a *BRCA2* mutation.

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