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PRINCIPAL INVESTIGATOR: Andrew K. Godwin, Ph.D.

CONTRACTING ORGANIZATION: Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

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Andrew K. Godwin, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

E-Mail: A_Godwinr@fccc.edu

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Familial breast cancer accounts for 15 to 35% of all breast cancers. Mutations in a number of genes are now known to cause susceptibility to breast cancer; the most notorious are the *BRCA1* and *BRCA2* genes. However, it has become evident that not all (and not even the majority) of familial breast cancer families can be attributed to mutations in *BRCA1* and *BRCA2*. In a recent study by the Breast Cancer Linkage Consortium, only one third of families with four or five cases of female breast cancer and no cases of ovarian cancer carry mutations in either *BRCA1* or *BRCA2*. Because smaller familial clusters are much more common than families with large numbers of cases, the indication from these and other studies is that a substantial proportion of familial clustering is not accounted for by mutations in *BRCA1* and *BRCA2*; therefore, there is a great need to discover other genes that contribute to this disease. Recently, it was reported that germline *CHK2* mutations were found in two families with Li-Fraumeni syndrome and a third case with multiple primary cancers. The two families with Li-Fraumeni syndrome had diverse cancers, including early-onset breast cancers at ages 37, 41, and 45 years. The third proband developed breast cancer at age 47, malignant melanoma at 53 and primary lung cancer at 58, but had no family history of malignancies. These data suggest that germline *CHK2* mutations predispose to breast cancer, similar to other inherited mutations in *BRCA1*, *BRCA2*, *TP53* and perhaps *ATM*. However the extent of *CHK2* involvement in hereditary breast cancer is not fully known. Our objective was to determine the frequency of germline mutations in *CHK2/CDS1* in breast cancer-prone kindreds that have previously tested negative for mutations in *BRCA1* and *BRCA2*.

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INTRODUCTION:

Susceptibility genes presently account for only 20-25% of the hereditary risk for breast cancer (Lichtenstein et al., 2000). The majority of this risk can be attributed to the two breast cancer susceptibility genes, *BRCA1* and *BRCA2* (Miki et al., 1994; Wooster et al., 1995). Mutations in a third gene, *TP53*, appear to be responsible for a minor additional fraction of predisposition to breast cancer (reviewed in Easton, 1999). In recent studies, *TP53* changes occurred exclusively in those breast cancer families also displaying a Li-Fraumeni syndrome (LFS) or Li-Fraumeni-like syndrome (LFL) (Huusko et al., 1999). This syndrome is described by a cancer background within a family consisting of sarcomas, breast cancer, leukemia, and tumors of the central nervous system and adrenal cortex (Garber et al., 1991). These observations indicate that other breast cancer susceptibility genes must be involved to account for hereditary breast cancer risk.

Bell *et al.* (1999) identified germline *CHK2* mutations in *TP53*-negative LFS and LFL families (13.6%). It was suggested that mutations in *CHK2*, a gene that encodes a protein kinase that activates p53 by phosphorylation in a DNA damage dependent and ATM dependent manner (reviewed in Prives and Hall, 1999), may contribute to predisposition to sarcoma, breast and brain tumors. However, one of four alterations documented in the Bell *et al.* publication (1422delT) has subsequently been located to a homologous fragment in that case and in 5% of a control sampling (Sodha et al., 2000). Upon characterization of the other Bell *et al.* mutations one missense alteration (I157T) appears to have wild type protein kinase activity in the assays used and the other (R145W) appears to have basal activity, thought perhaps due to a shortened half-life (Wu et al., 2001). A recent evaluation of 79 hereditary breast cancer families (21 characterized as LFL) found 8.9% positive for the I157T missense alteration (Allinen et al., 2001). Four of the positive families were classified as LFL.

The association of *CHK2* alterations with LFS/LFL families and its identification as a regulator of *BRCA1* (Lee et al., 2000) makes *CHK2* a valid candidate gene to contribute to hereditary breast cancer. Further evaluation of hereditary breast cancer families may confirm the present suspicion that *CHK2* alterations do not alone predispose to cancer, but are contributory on a cancer predisposing genetic background. Recent evaluation of the sporadic colon cancer cell line HCT15 containing the R145W *CHK2* missense alteration on the selectively expressed allele provide evidence that *CHK2* and p53 have cell cycle

checkpoint roles in non-overlapping pathways (Falch et al., 2001). This theory lends support to the notion that mutations of *CHK2* can provide some additional selective advantage even to cells with deleted or mutant *TP53*.

BODY:

Progress Report

Objective: The objective of this proposal is to determine the frequency of germline mutations in *CHK2/CDS1* in breast cancer-prone kindreds that have previously tested negative for mutations in *BRCA1* and *BRCA2*.

BRCA1 and *BRCA2* negative individuals selected for *CHK2* evaluation are members of hereditary breast/ovarian cancer families. Li-Fraumeni syndrome (LFS) families and Li-Fraumeni like (LFL) families were chosen by pedigree analysis and according to the following criteria. Clinical criteria for diagnosing a family as having LFS are the combination of (i) proband with sarcoma diagnosed under age 45, (ii) first-degree relative with an LFS component tumor (sarcoma, breast cancer, brain tumor, leukemia, or adrenal cancer) diagnosed under age 45, and (iii) first- or second-degree relative with any cancer diagnosed under age 45 or with sarcoma diagnosed at any age. Clinical criteria for LFS-variant are an individual with three separate primary cancers, with the first cancer diagnosed under age 45, or the combination of (i) proband with childhood cancer or LFS component tumor diagnosed under age 45, (ii) first- or second-degree relative with LFS component tumor diagnosed at any age, and (iii) first- or second-degree relative with any cancer diagnosed under age 60 (Birch et al., 1994; Eng et al., 1997).

A total of 34 individuals were screened for alterations in the *CHK2* gene. One individual was from a LFS classified family while the remaining individuals were from LFL families that reported a history of breast cancer. All the individuals evaluated had been diagnosed with some type of cancer; 28 individuals were diagnosed with breast cancer and 4 were diagnosed with ovarian cancer. Two of the individuals were males; one diagnosed with melanoma at age 21 and another diagnosed with sarcoma at age 33. Eight of the individuals reported to be of Ashkenazi Jewish heritage and were screened for founder mutations only. All of the participants had previously tested negative for *BRCA1* and *BRCA2* germline mutations.

Evaluation of the *CHK2* gene by PCT amplification and direct sequencing is complicated by the duplication of *CHK2* exons 10, 11, 12, 13, and 14 on multiple human chromosomes. A PCR strategy was

designed (Bell et al., 1999) to specifically amplify these exons from chromosome 22 only, where the intact *CHK2* gene is located, by initially performing a primary long range PCR spanning exons 10-14 (~10kb) and subsequently performing nested PCR's for each of exons 10-14. Direct sequencing of the PCR fragments failed to detect any of the previously reported *CHK2* mutations or any other mutation in the individuals screened.

In addition, in collaboration with Dr. Daniel Haber (Massachusetts General Hospital), we have recently reported the identification of *CHK2* missense mutations in three variant-LFS families (Lee et al., 2001). Ten additional cases of LFS and 49 cases of variant-LFS were screened for germline mutations in *CHK2*. Three missense mutations were detected, R145W, R3W, and I157T. None of these missense changes were detected in 400 chromosomes from healthy donors who were ethnically matched with the patient population. The R145W mutation was shown to destabilize the encoded protein, reducing its half-life from >120 min to 30 min. We also report that this effect is abrogated by treatment of cells with a proteasome inhibitor, suggesting that *CHK2*^{R145W} is targeted through the degradation pathway. The R145W germline mutation, but not the R3W or the I157T missense variants in *CHK2* was associated with loss of the wild-type allele in the corresponding tumor specimens. Interestingly, the R145W bearing tumor did not harbor a somatic *TP53* mutation. Our observations support the functional significance of a missense *CHK2* mutation in rare cases of LSF, and suggest that such mutations may substitute for inactivation of *TP53*.

KEY RESEARCH ACCOMPLISHMENTS:

- Identified LFS/LFL individuals for *CHK2* gene evaluation.
- Found that 34 of the probands tested to date are negative for *CHK2* mutations.
- Have reported that the R145W *CHK2* mutation functionally destabilizes the encoded protein (in collaboration with D. Haber, see below).

REPORTABLE ACCOMPLISHMENTS:

Lee, S.B., Kim, S.H., Bell, D.W., Wahrer, D.C.R., Schiripo, T.A., Jorczak, M.M., Sgroi, D., Garber, J.E., Li, F.P., Nichols, K., Varley, J.M., Godwin, A.K., Shannon, K.E., Harlow, E., Haber, D.A. Destabilization of *CHK2* by a missense mutation associated with Li-Fraumeni Syndrome. *Cancer Research*, accepted, 2001

CONCLUSIONS:

Recent studies have confirmed the presence of *CHK2* germline mutations in familial breast cancer families. However, the contribution of *CHK2* to hereditary breast cancer appears to be minimal. Vahteristo and colleagues recently found a frameshift mutations (1100delC) in one family with breast cancer. The proband was diagnosed with breast cancer at age 41 years, and her mutation was inherited from the father diagnosed with prostate cancer at 76 years. A second mutation, in *BRCA1* in the maternal lineage was also found, but not in the proband. The recent study by Allinen and colleagues failed to identify a deleterious *CHK2* mutation in 79 Finnish breast cancer families. Base on ours and other recent studies, it appears that germline mutations in *CHK2* do not contribute significantly to the hereditary breast cancer or LFL-associated breast cancer risk.

REFERENCES:

- Allinen M, Huusko P, Mantyniemi S, Launonen V, and Winqvist R. (2001). Mutation analysis of the *CHK2* gene in families with hereditary breast cancer. *Br J Can* 85: 209-212.
- Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DCR, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, and Haber DA. (1999). Heterozygous germline h*CHK2* mutations in Li-Fraumeni syndrome. *Science* 286: 2528-2531.
- Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, Harris M, Jones PH, Binchy A, Crowther D, et al. (1994). Prevalence and diversity of constitutional mutations in the *p53* gene among 21 Li-Fraumeni families. *Cancer Res* 54: 1298-1304.
- Easton DF. (1999) How many more breast cancer predisposition genes are there? *Br Cancer Res* 1: 14-17.
- Eng C, Schneider K, Fraumeni JF, Li FP. (1997). Third international workshop on collaborative interdisciplinary studies of *p53* and other predisposing genes in Li-Fraumeni syndrome. *Cancer Epidemiol Biomark Prev* 6: 379-383.
- Falck J, Lukas C, Protopopova M, Lukas J, Selivanova G, and Bartek J. (2001). Functional impact of concomitant versus alternative defects in the *Chk2-p53* tumour suppressor pathway. *Oncogene* 20: 5503-5510.

Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF, and Li FP. (1991). Follow-up study of twenty-four families with Li-Fraumeni syndrome. *Cancer Res* 51: 6094-6097.

Huusko P, Paakkonen K, Launonen V, Poyhonen M, Blanco G, Kauppila A, Puistola U, Kiviniemi H, Kujala M, Leisti J, and Winqvist R. (1998) Evidence of founder mutations in Finnish BRCA1 and BRCA2 families. *Am J Hum Genet* 62: 1544-1548.

Lee J-S, Collins, KM, Brown AL, Lee C-H, and Chung JH. (2000). HCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response, *Nature* 404: 201-204.

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, and Hemminki K. (2000) Environmental and heritable factors in the causation of cancer. *N Engl J Med* 343: 78-85.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PD, Harchman K, Tavtigian S, Liu Q, Cochran C, Bennett JM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow BK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgr D, Wiseman R, Kamb A, and Skolnick MH. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266:66-71.

Prives C and Hall PA. (1999). The p53 pathway. *J Pathol* 187: 112-126.

Sodha N, Williams R, Mangion J, Bullock SL Yuille MR, and Eeles RA. (2000). Screening hCHK2 for mutations, 289: 359a.

Vahteristo, P. Bartek, J., Barkova, J., Ojala, S., Eerole, H., Kononen, J., Heikkila, P., Kallioniemi, O.P., Nevanlinna, H. CHK2 in familial breast cancer. (2001) *Amer. J. Hum. Genetics*, 69:427a.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G, Barfoot R, Hamoudi R, Patel S, Rice C, Biggs P, Hashim Y, Smith A, Connor F, Arason A,

Gudmundsson J, Ficenec D, Kelsell D, Ford D, Tonin P, Bishop DT, Spurr NK, Ponder BAJ, Eeles R, Peto J, Devilee P, Cornelisse C, Lynch H, Narod S, Lenoir G, Egilsson V, Barkardottir RB, Easton DF, Bentley DR, Futreal PA, Ashworth A, and Stratton MR. (1995) Identification of the breast cancer susceptibility gene BRCA2. *Nature* 378: 789-792.

Wu X, Webster SR, and Chen J. (2001) Characterization of tumor-associated CHK2 mutations, *J Biol Chem* 276: 2971-2974.

APPENDICES: None