

AD _____

Award Number: DAMD17-98-1-8535

TITLE: Genetic Susceptibility to Prostate Cancer Among Ashkenazi
Jews

PRINCIPAL INVESTIGATOR: Harry Ostrer, M.D.
Carole Oddoux, Ph.D.

CONTRACTING ORGANIZATION: New York University Medical Center
New York, New York 10016

REPORT DATE: December 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030623 020

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE December 2002	3. REPORT TYPE AND DATES COVERED Annual (15 Sep 01 - 14 Sep 02)	
4. TITLE AND SUBTITLE Genetic Susceptibility to Prostate Cancer Among Ashkenazi Jews			5. FUNDING NUMBERS DAMD17-98-1-8535	
6. AUTHOR(S): Harry Ostrer, M.D. Carole Oddoux, Ph.D				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) New York University Medical Center New York, New York 10016 E-Mail: ostreh01@med.nyu.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) This project will test the basic hypothesis that a given microsatellite marker allele occurs with greater frequency among the individuals affected with prostate cancer than among the controls. These studies will take advantage of the fact that two populations of Ashkenazi Jewish men are readily available for a case-control study. The first is a group of men at high heritable risk based on their having early-onset prostate cancer. The second is a group of men at low heritable risk who have no personal or family history of prostate cancer. Thus, we expect to observe predisposition alleles in the men at high risk that are not present in the men at low risk. The predisposition genes are likely to be within chromosomal regions in which loss of heterozygosity has occurred. Because these regions have remained identical by descent since the high-risk mutations occurred, they can be recognized by the presence of specific alleles of microsatellite markers in the high-risk group that are not present in the low-risk group.				
14. SUBJECT TERMS: prostate cancer			15. NUMBER OF PAGES 6	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	6
Appendices.....	-

INTRODUCTION

This study uses several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been endogamous causing them to have more genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population's endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). Ashkenazi Jews represent such a population.

BODY

Task 1. Subject identification.

Samples from 200 high-risk subjects were identified during the first year. To increase the power of the study, samples from an additional 100 cases were identified during the second year. The medical histories of each of these subjects were reviewed, confirming ethnicity and diagnosis of prostate cancer, and noting family history, age at diagnosis and Gleason score at time of diagnosis. For each subject, tissue blocks were obtained from non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections were cut. DNA was purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized. This technique reproducibly provides approximately 50-fold amplification of the DNA samples. From the pool of low-risk subjects, an additional 200 samples were chosen and added to the 200 samples already obtained for subsequent analysis. These were amplified using PEP for subsequent analysis. Methods of drying down these DNAs in 96-well microtiter plates for subsequent use were developed.

Task 2. Development of markers.

A. Markers from regions associated with loss of heterozygosity (LOH) in prostate cancer will be identified and fluorochrome-labeled primers will be synthesized. We have identified microsatellite markers for each of the following chromosomal regions - 8p21-p22, 10q23-q25, 13q14, 16q22, 17p, 17q21-q22, . We added markers to our analysis. Because of uncertainties about relative map positions, we have refined our selected markers from those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (<http://www.ncbi.nlm.nih.gov/GeneMap99>) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998)

B. Standard PCR conditions will be developed for each of these markers. The primer sequences for each of these markers was identified using standard databases (<http://www.gdb.org>). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). The net effect of this grouping is that multiple markers can either be amplified simultaneous and/or pooled from separate amplifications to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions have been optimized.

The methods for multiplex analysis were developed to enhance the throughput of marker analysis. For each fluorochoime, three markers (small, medium, and large) were coamplified in the same well. In turn, these multiplex amplifications for each of the fluorochoimes were pooled for subsequent fragment length analysis. This led the analysis of up to 9 markers simultaneously in a single run.

Task 3. Data analysis

Data analysis was performed for ~30% of the controls. Data analysis was delayed by turnover in technical staff and recurring technical issues with the major analytical instruments (ABI 310 DNA analyzers). These have been remedied.

KEY RESEARCH ACCOMPLISHMENTS:

Development of DNA databases from cases and controls for genomic analysis.

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

REPORTABLE OUTCOMES:

Proposal, "Genetic Susceptibility to Prostate Cancer in the Netherlands Cohort Study," (PC99-1496) was funded by USARMC.

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), was funded.

CONCLUSIONS

This works demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It creates the foundations for the analyses that will be performed in the remainder of this study.

REFERENCES

Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Woehr G, Latil A, Millasseau P, Mellah I, Cohen N, Blanche H, Bellane-Chantelot C, Demenais F, Teillac P, Le Duc A, de Petriconi R, Hautmann R, Chumakov I, Bachner L, Maitland NJ, Lidereau R, Vogel W, Fournier G, Mangin P, Cussenot O (1998) Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2-43 *Am J Hum Genet* 62:1416-1424

Cooney KA, McCarthy JD, Lange E, Huang L, Miesfeldt S, Montie JE, Oesterling JE, Sandler HM, Lange K. (1997) Prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. *J. Natl Cancer Inst.* 89:955-959.

Gronberg H, Xu J, Smith JR, Carpten JD, Isaacs SD, Freije D, Bova GS, Danber JE, Bergh A, Walsh PC, Collins FS, Trent JM, Meyers DA, Isaacs WB. (1997) Early age at diagnosis in families providing evidence of linkage to the hereditary prostate cancer locus (HPC1) on chromosome 1. *Cancer Res* 57:4707-4709.

McIndoe RA, Stanford JL, Gibbs M, Jarvik GP, Brandzel S, Neal CL, Li S, Gammack JT, Gay AA, Goode EL, Hood L, Ostrander EA. (1997) Linkage analysis of 49 high-risk families does not support a common familial prostate cancer-susceptibility gene at 1q24-25. *Am J Hum Genet* 61:347-353.

Smith JR, Freije D, Carpten, JD, et al. (1996) Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. *Science* 274:1371-1374.

Xu J, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, et al. (1998) Evidence for a prostate cancer susceptibility locus on the X chromosome. *Nat Genet* 20:175-9.