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PRINCIPAL INVESTIGATOR: Elizabeth A. Platz, Sc.D., M.P.H.

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, MD 21205-2103

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Elizabeth A. Platz, Sc.D., M.P.H.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Johns Hopkins University
Baltimore, MD 21205-2103

E-Mail: eplatz@jhsph.edu

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We are evaluating the association of polymorphisms in genes involved in the generation of reactive oxygen species, the detoxification of reactive oxygen species, and the repair of oxidative DNA damage with prostate cancer progression. After several meetings among the collaborators to define cases and controls, we have selected from among all men who underwent radical prostatectomy for clinically organ-confined prostate cancer at Johns Hopkins between 1993 and 2004 (the PSA era) all men who have progressed to biochemical failure, overt metastases, or death from prostate cancer. From among the cohort of men who underwent prostatectomy we selected controls using incidence density sampling. The total number of eligible cases was 524. We selected an equal number of controls who were closely matched to the cases on age, race, and pathologic stage and grade. We are cataloging those cases and controls for whom DNA has already been extracted and are identifying from our archives frozen seminal vesicles and paraffin-embedded unaffected lymph nodes from which we will extract DNA. Selection of genes and polymorphisms in those genes will be done close in time to genotyping to capitalize on the emerging HapMap. We expect to begin genotyping by late summer; statistical analysis to follow. No results are available yet.

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INTRODUCTION

We are investigating the role of genes involved in oxidation in the etiology of prostate cancer progression. Biochemical failure (re-elevation in PSA) is an important and common clinical problem in men who have undergone radical prostatectomy for organ-confined prostate cancer. Clinicians are unable to predict with certainty, which of these men are likely to fail therapy and subsequently develop metastatic disease and which are not. If susceptible men could be identified, then they could be targeted for effective treatment strategies. Oxidative damage to neoplastic cells is one mechanism by which these cells may develop the potential to grow and disseminate. Therefore, inter-individual variability in genes that encode enzymes involved in the production of reactive oxygen species (ROS), detoxification of ROS, and repair of oxidative DNA damage may help to identify men at risk for prostate cancer progression despite prostatectomy.

BODY

The aims of this proposal were to:

1) using expression data from cDNA microarrays coupled with published information on the functionality of sequence changes, we plan to identify 5 single nucleotide polymorphisms (SNP) in each of 25 genes encoding enzymes involved in production of ROS, detoxification of ROS, and repair of oxidative DNA damage, and then

2) to test whether these SNPs are independently and in combination associated with risk of prostate cancer progression.

We had proposed that these aims be accomplished via the tasks specified below. For each task we provide an update on accomplishments.

Task 1. Select 25 polymorphic genes involved in production of ROS, detoxification of ROS, and repair of oxidative damage, Months 1-2

a. Review cDNA expression data for prostate tumors generated in laboratory of Dr. Isaacs to identify genes involved in oxidation that are expressed above the 80th percentile or below the 20th percentile compared to normal tissue.
b. Conduct searches of public and proprietary databases of the highly or lowly expressed genes to identify one or more single nucleotide polymorphisms with functional consequence.
c. Choose final set of genes using the criteria outlined in the proposal.

The field of genetic epidemiology is advancing rapidly. Publications in the area of genes involved in oxidation and prostate cancer are appearing in the literature almost monthly (e.g., MnSOD Val 16 Ala (1)). The search for new SNPs is ongoing and the

HapMap is emerging. We are conducting our research in parallel with an NCI-funded project in the Johns Hopkins Prostate SPORE on genes involved in the same pathways and prostate cancer incidence in a case-control study and in a cohort study. This work is also being conducted in parallel with a Career Development Award in the Hopkins Prostate SPORE to Dr. Platz on genes involved in the inflammation, including the innate and adaptive immune response, and prostate cancer progression. The latter study will use the same case-control dataset that we are developing in this project.

For all of these reasons, we have opted to delay selecting the final list of genes and SNPs until very soon before we begin genotyping for three reasons: 1) so that we will have the most up to date information on relevant genes and choice of SNPs, 2) so that the data generated from both of these projects together will be able to inform us about whether these pathway are important only in the incidence of prostate cancer, the progression, of prostate cancer, or both, and 3) so that genes that would be expected to interact between the oxidative and inflammatory pathways can be selected together and jointly evaluated.

Task 2. Select 200 cases (progressors) and 200 matched controls (nonprogressors) Months 3-5

a. Link the Hopkins Pathology Tissue Core database to electronic hospital records to identify prostate cancer patients treated with radical prostatectomy and who experienced biochemical failure.
b. From the total set of eligible patients, select 200 men who had biochemical failure and 200 men who still had undetectable PSA at the date of the case's failure, same follow-up time, and who are similar on demographic and tumor characteristics.

We identified 4,860 men who underwent prostatectomy in 1993 or later at the Brady Urological Institute. Of these men, we excluded 365 because they received hormonal or radiation therapy prior to prostatectomy (46.9%), had positive surgical margins or we could not confirm organ confined disease (26.8%), or follow-up was incomplete (17.0%). Of the 4,495 eligible men, 524 subsequently experienced biochemical failure (73.8%), local recurrence (7.8%), local and/or distant metastasis (15.7%), or death from prostate cancer (2.6%).

In discussions with our colleagues in the Departments of Urology and Pathology, we recognized that the prostate cancer progression case-control set that we planned to prepare would have great utility for other investigators at our institution who are studying other markers, inherent or somatic, that predict prostate cancer progression. Thus, Dr. Angelo De Marzo, who is the director of Hopkins Pathology Tissue Core, convened a meeting of such investigators, including other another pathologist and a two statisticians. We discussed in detail the matching criteria for stage and grade to minimize the likelihood of residual confounding, but also to ensure that matching would be successful, and considered whether other matching should be performed.

In addition, subsequently we also consulted with two statistical epidemiologists to confirm the approach to sampling and to ensure that planned analytical approach could handle the data structure that would be imposed by the method chosen. We decided to use the approach to the selection of matched controls from among men who underwent radical prostatectomy called incidence density sampling. In this method, a man's person-time at risk is sampled and thus, a man may be sampled more than once represent different person-years at risk and a man who goes on to recur may be sampled as a control prior to failure and then also be counted as a case. Because stage and grade are such strong predictors of recurrence and because our goal is to study genetic variants that influence recurrence independent of stage and grade at diagnosis, we matched the cases and controls very closely on stage and grade. We also matched the men on age at diagnosis and race.

For each case then, we sampled another man who had not yet recurred, who was still alive and under follow-up, and who had the same stage, grade, and age at diagnosis and was the same race as the case in question. We used a SAS macro (Tassoni et al. "One-to-one matching of case/controls using SAS software") to optimize the closeness of the matching.

The final set contains 524 cases and 524 controls (see Table), of which the controls are 326 unique men (204 men selected 1 time, 74 men selected 2 times, etc.). Of the 326 unique controls, 108 later became cases. Thus, the total number of unique men is 742. 95% of the matches are exact on stage, grade, and race and are within 10 years of age.

Characteristics of 524 cases and 524 controls matched on age, race, pathologic stage, and pathologic Gleason sum among men who underwent radical prostatectomy, Johns Hopkins Hospital 1993 – 2004

	Cases	Controls
Mean age (years)	58.9 ± 6.3	59.1 ± 5.9
Race (%)		
White	85.9%	88.6%
Black	9.2%	7.6%
Hispanic	1.3%	0.6%
Asian	0.4%	0%
Other	3.2%	3.4%
Mean PSA at prostatectomy (ng/mL)*	12.3 ± 10.2	11.0 ± 8.3
Mean pathologic Gleason sum	7.2 ± 0.8	7.2 ± 0.8
Pathologic stage (%)		
T2	13.6%	13.7%
T3a	51.7%	51.7%
T3b or N1	34.7%	34.5%
Mean time since prostatectomy to progression or last follow-up (years)*	2.5 ± 1.8	5.9 ± 2.4

* Not a matching factor

Task 3. Genotyping, Months 6-12

a. Pull samples for the 400 patients from Hopkins Pathology Tissue Core archive and review for normal regions.
b. Extract genomic DNA in laboratory of Dr. Isaacs.
c. Ship samples to laboratory of Dr. Xu and perform high throughput genotyping.

We provided the total list of cases and controls to Dr. De Marzo. Under his direction, two sources of tissues were linked to list of cases and controls: 1) frozen seminal vesicles, and 2) archived tissue blocks. Drs. De Marzo has now recommended that the archived tissue blocks that we should use to extract DNA should be lymph nodes negative for metastasis that were removed at the time of prostatectomy, rather than the tumor blocks. Lymph nodes are replete with lymphocytes, an excellent source of genomic DNA and they have not been previously pulled from the archive and thus, are more likely to be available and locatable. These lymph node blocks are currently being pulled. DNA extraction will take place in the laboratory of Dr. Isaacs. Dr. De Marzo has reported to us that DNA has previously been made for some of the frozen tissue samples for other projects in the past and that this DNA is available to us for this project.

The absolute number of unique men sampled is 742, which is nearly twice what we had proposed (200 cases and 200 controls). However, until we have the final counts of men for whom samples will be located, we will not know the final available sample size. Because DNA has already been extracted from some of the frozen tissue, the price of genotyping continues to decline, and because funds are now available for additional DNA extraction from Dr. Platz's Prostate SPORE Career Development Award, we may be able to increase the sample size, which would increase the power to detect associations. Once Dr. De Marzo's core finishes pulling all of the samples and provides a final count on availability and number with DNA already extracted, we will contact the Grants Officer with a proposal to increase the sample size by a particular amount and we will then seek an amendment from the DOD IRB to increase the sample size. In anticipation of this possibility, we have already sought and received approval to increase the sample size from the local IRB.

High throughput genotyping will be done in the laboratory of Dr. Jianfeng Xu, our consultant at Wake Forest. Dr. Platz is in contact with Dr. Xu periodically and he is still committed to this project despite the delay.

Task 4. Data management and interim analysis, Months 13-18

a. Evaluate quality control data for assay reliability and make decisions about need for re-genotyping plates with imperfect reliability.
b. Perform interim analyses, including conditional logistic regression modeling of associations between specific alleles and biochemical progression and EM reconstruction of haplotypes followed by omnibus likelihood ratio testing for differences in the distribution of haplotypes between cases and controls.

Task 5. Final analyses and report/manuscript preparation, Months 19-24

a. Perform final analyses of the association of genes involved in oxidation and biochemical failure following radical prostatectomy for prostate cancer.
b. Prepare final report.
c. Prepare manuscripts.

No work has been done for tasks 4 or 5. We expect to begin genotyping by late summer; QC checks and statistical analysis and manuscript preparation to follow.

We have identified a doctoral student in the Human Genetics/Genetic Epidemiology Area of Concentration in the Department of Epidemiology at the Johns Hopkins Bloomberg School of Public Health who will be working with Dr. Platz on the statistical analysis of these data, Dr. Ming Hsi. He will be taking the place of Dr. Sabine Rohrmann, the post-doctoral fellow, who has left the School to take a research position at the German Cancer Research Center. Dr. M. Daniele Fallin will still be providing her expertise on haplotype analysis. The approach to statistical analysis of alleles/genotypes and haplotypes/diplotypes has progressed exponentially since this proposal was submitted. We are fortunate to be located in a department with faculty, such as Dr. Fallin at the cutting edge of these methods development.

KEY RESEARCH ACCOMPLISHMENTS

- None to date.

REPORTABLE OUTCOMES

- The development of a standardized prostate cancer progression case-control set that is available to other Hopkins prostate cancer investigators through Dr. De Marzo's Hopkins Pathology Tissue Core.

CONCLUSIONS

- None to date. However, we anticipate that the findings from the work that we are conducting on genes involved in oxidation and prostate cancer progression in men treated for prostate cancer by prostatectomy may provide insights into the etiology of this problem and provide possible preventive and therapeutic interventions for men with this disease.

REFERENCES

1. Li H, Kantoff PW, Giovannucci E, Leitzmann MF, Gaziano JM, Stampfer MJ, Ma J. Manganese superoxide dismutase polymorphism, prediagnostic antioxidant status, and risk of clinical significant prostate cancer. Cancer Res 2005;65:2498-504.

APPENDICES

- None