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14. ABSTRACT We are evaluating whether polymorphisms in genes involved in the genesis of oxidative species, detoxification of oxidative species, or repair of oxidative DNA damage influence risk of prostate cancer progression in men with clinically organ-confined prostate cancer. We identified 524 men with who underwent radical prostatectomy in 1993-2004 and who subsequently experienced biochemical recurrence, development metastases, or died from their prostate cancer. Using incidence-density sampling, we selected 524 men matched on age, race, and pathological stage and grade who had not progressed by the date of the matched case's progression. Noncancer tissue (either unaffected paraffin-embedded lymph nodes or frozen seminal vesicles) was retrieved from the Hopkins pathology archive from which germline DNA was extracted. For 20 men either tissue could be found or DNA extraction was not successful. We attempted several platforms for genotyping, including a SNP chip that included ~1500 SNPs in relevant genes. We selected the Mass Array system (Sequenom) and identified 100 SNPs in relevant genes. We completed genotyping the 524 pairs for 12 of the 100 SNPs. For 33 of the men, genotyping was not successful. A total of 450 of the 524 pairs had genotype data for both members of the pair for at least on SNP. We used conditional logistic regression to estimate the matched ORs of progression for the 12 SNPs; no associations were observed (all p-trend across number of alleles > 0.15). We are awaiting data for the remainder of the 100 SNPs.					
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INTRODUCTION AND SUMMARY

We are evaluating whether polymorphisms in genes involved in the genesis of oxidative species, the detoxification of oxidative species, or the repair of oxidative DNA damage influence the risk of prostate cancer progression in men with clinically organ-confined prostate cancer who were treated with radical prostatectomy. We hypothesize that men with an inherently greater burden of oxidative stress or inability to repair DNA damage caused by oxidative stress is associated with a higher risk for men. This report is the final report of a project for which funding was awarded January 2004 and ended December 2007.

Since the start of the project, we identified 524 men with clinically organ-confined prostate cancer who underwent radical retropubic prostatectomy at the Johns Hopkins Hospital (Baltimore, MD) in 1993 to 2004 and who subsequently experienced biochemical recurrence, metastasis, or death from their prostate cancer. We used incidence density sampling to select 524 men who also underwent prostatectomy for clinically-organ confined disease during same time span at the same hospital, who were the same age, race, and pathologic stage and grade, but who did not progress by the date of the matched case's progression. The total number of unique men was 724. Note that incidence density sampling involves sampling from risk sets and thus a man may be sampled as a control more than once, if he is present in more than one risk set, and a man may be sampled as a control and then later may be counted as a case. Other control sampling methods were considered. To document that the method we used was unbiased, whereas other suggested methods were biased, we generated a draft manuscript entitled "A Simulation Study of Control Sampling: Methods for Nested Case-Control Studies of Candidate Genes and Prostate Cancer Progression" that compares methods of control sampling for the type of progression study.

Next, we attempted to locate affected paraffin-embedded lymph nodes or frozen seminal vesicles as a source of germline DNA. This step was an unanticipated rate limiting step for this project; nearly 2.5 years were needed to locate the samples (some samples were centrally stored, but others had been checked out for other research projects and not returned to the archives) and to section them for DNA extraction. Ultimately, we located tissue for all but 4 of the 742 unique men.

Because the majority of the tissue source of germline DNA was paraffin-embedded, we tested the amount of tissue needed and the methods of DNA extraction from the paraffin-embedded tissue that would produce a quantity and quality of DNA that was adequate for amplification by PCR. For each subject, we confirmed that the nodes did not contain cancer and we took 10 cores per block. DNA extraction was completed by a commercial laboratory. In addition to the 4 samples for which tissue was not located, 16 men had insufficient tissue for DNA extraction or DNA extraction was not successful.

In the funded proposal we described that we would perform genotyping for 5 SNPs in 25 candidate genes and the selected SNPs would be those for which there was evidence of functionality on the production, stability, or activity of the transcript or protein product

of the gene. As time went on, the ability to genotype larger numbers of SNPs increased and the price decreased. Eventually a 1,500 SNP chip, which contained many genes relevant for this project as well as two others in our group, became available. We piloted the chip and expected to have good completion proportions. However, once we began genotyping the 524 pairs, we determined that the DNA concentration was too low for effective genotyping using this approach for many of the samples. This step was the next major stumbling block for this project. We then tested the ability of the Mass Array system (Sequenom, San Diego, CA) to give accurate genotyping calls for these paraffin-embedded samples and moved forward with genotyping. In the meantime, another project, this time in collaboration with investigators at the National Cancer Institute was formed. Adequate funds remained from this project and the others to genotype a total of 100 SNPs using this platform for the 524 pairs (742 unique men). We re-selected SNPs related to oxidation, inflammation, metastasis, and a set specified by NCI based on the most up-to-date literature for prostate cancer incidence, including the data released from CGEMS (<https://caintegrator.nci.nih.gov/cgems/browseSetup.do>) and work conducted by Drs. Platz and Isaacs in the CLUE II cohort. To date, genotyping has been completed for 12 of the 100 SNPs. Of the originally sampled 524 pairs, genotyping was successful for at least one of the 12 SNPs for both members of 450 pairs. Genotyping is ongoing for the remaining SNPs.

We used conditional logistic regression modeling, which takes into account the case-control matching, to estimate the matched ORs of progression separately for each of the 12 SNPs. We entered into the models indicator variables for men who were heterozygotes and for men who were homozygous variants. To assess whether the risk of prostate cancer increased or decreased with each additional variant allele, we ran a model that included a single ordinal variable with values of 0, 1, or 2 alleles, the coefficient for which we evaluated using the Wald test. We did not observe any statistically significant associations between the 12 SNPs and progression (all p-trend > 0.15). We will perform the same analyses when the genotyping for the remaining SNPs is complete.

We are pleased to report that the nested case-control set for prostate cancer progression generated under this DOD funding has been viewed as a valuable resource by the Hopkins basic, translational, and epidemiologic prostate cancer investigators and the set is now being proposed for wide use at Hopkins. This set has been proposed as the basis for several other projects from Dr. Platz's group as well as from other prostate cancer researchers at Johns Hopkins (as detailed in the 2007 interim report).

BODY

The aims of this proposal were:

- 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.

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 by the following tasks. After each task, progress is described.

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.

Because the literature on genetic variation and prostate cancer has blossomed over the time frame of this project, we changed our approach to identifying relevant genes and the SNPs therein. We performed searches of PubMed for SNPs associated with prostate cancer incidence, mortality, or progression; browsed the CGEMS results (<https://caintegrator.nci.nih.gov/cgems/browseSetup.do>); and considered the findings from our own work on genes and prostate cancer incidence.

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 a larger number of case-control pairs (524 pairs) than we originally planned because we were able to combine funds from this project and two others that were subsequently funded. We consulted with a biostatistician and two other statistical epidemiologists to confirm our approach to control sampling and to ensure that planned analytical approach could handle the data structure that would be imposed by the method chosen. When describing the approach to statistical geneticists we encountered a difference of opinion in the optimal approach. We chose to use incidence density sampling of controls. 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. The statistical geneticists and urologists suggested that we sample controls from among the men who did not progress by the end of the follow-up period and who were still under follow-up. Their thought was that genes dictate progression and thus need a pure group of men who were never to progress. We disagreed and suspected that the latter approach would generate a distorted allele frequency from that in the population that gave rise to the cases. To resolve the controversy, we conducted

a simulation study in which we sampled controls using 3 methods: incidence density sampling with replacement (meaning a man's person-time experience may be sampled more than once), sampling without replacement, and sampling from the end of follow-up from among the men who were still under follow-up. We compared the estimates of the association of genes and prostate cancer progression from these simulated nested case-control studies to what would be observed if the entire cohort had been studied (gold standard). As we hypothesized, incidence density sampling with replacement was the least biased approach and sampling from the end of the interval was the most biased approach. Sampling without replacement performed only slightly more poorly than sampling with replacement. A manuscript has been prepared and will be submitted for publication: Wang MS, Shugart YY, Zarfes K, Cole SR and Platz EA. "A Simulation Study of Control Sampling: Methods for Nested Case-Control Studies of Candidate Genes and Prostate Cancer Progression". This work forms one aim of MS Wang's doctoral dissertation; he is pursuing a degree in genetic epidemiology at the Johns Hopkins Bloomberg School of Public Health. Dr. Shugart is a statistical geneticist and Dr. Cole is a statistical epidemiologist who collaborated with us.

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.

As we described in the Introduction and Summary, the rate limiting step for this project was locating the tissue blocks. Ultimately, tissue for all but 4 men was located. Many of the case's blocks had been checked out of the Hopkins pathology archive and not returned or had been returned but misplaced. Our pathology colleague Dr. De Marzo had his laboratory technician, Ms. Helen Fedor, track down these samples. We are grateful to them for their efforts on our behalf.

We tested the amount of tissue needed and the methods of DNA extraction from the paraffin-embedded tissue that would produce a quantity and quality of DNA that was adequate for amplification by PCR. 10 cores proved to be adequate. For each subject, we confirmed that the nodes did not contain cancer and we took 10 cores per block. DNA extraction was performed by Bioserve. We had originally planned to perform DNA extraction in the laboratory of Dr. Isaacs (co-I), but because of the increased sample size, we decided to use a company with high through-put technology and experience with extraction from paraffin-embedded samples.

Genotyping is being performed in the laboratory of our collaborator Dr. Jianfeng Xu at Wake Forest. His group uses Sequenom's (San Diego, CA) high-throughput MassARRAY system. We tested the ability of the Mass Array system to give accurate genotyping calls for a small number of these paraffin-embedded samples. We concluded that this approach would be successful.

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

Data management and the interim analysis were performed for the first 12 SNPs as described in the Introduction and Summary. No associations were observed.

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

The Introduction and Methods sections of a manuscript detailing the findings of this project are currently being drafted.

KEY RESEARCH ACCOMPLISHMENTS

- Showed that our method of control sampling is the least biased based on a simulation study.
- In initial analysis, observed no association between 12 SNPs and prostate cancer recurrence. Although null, this work showed that genotyping could be performed using these samples. We estimate that the results for the remaining SNPs will be available in the next two months.
- Generated a resource for other studies on genetic variation and gene expression in the etiology of prostate cancer progression
- Accomplishments of Dr. Platz related to this New Investigator Award
 - o Since 2004, she heads the cancer epidemiology, prevention and control training program for pre- and post-docs and in 2006 the T32 grant supporting the training program was refunded by the National Cancer Institute
 - o In 2006, she was appointed as a Staff Investigator in the Cancer Prevention and Control Program at the Sidney Kimmel Comprehensive Cancer Center.
 - o She continues to conduct research on genes and prostate cancer incidence. Her group recently presented findings on genes involved in inflammation and obesity and prostate cancer incidence in CLUE II at the 2007 AACR Frontiers in Cancer Prevention meeting in Philadelphia, PA:

B79 Association of immune response- and obesity-related genes with prostate cancer in CLUE II. M. Wang,¹ K. J. Helzlsouer,² M. W. Smith,³ J. A. Hoffman-Bolton,¹ S. C. Hoffman,¹ V. Grinberg,³ A. M. De Marzo,⁴ W. B. Isaacs,⁴ C. G. Drake,⁴ Y. Y. Shugart,¹ E. A. Platz¹. ¹Johns Hopkins University School of Public Health, Baltimore, MD, ²Mercy Medical Center, Baltimore, MD, ³SAIC-Frederick, National Cancer Institute, Frederick, MD, ⁴Johns Hopkins Medical Institutions, Baltimore, MD.

Background: Chronic intra-prostatic inflammation and obesity are thought to play a role in the pathogenesis of prostate cancer. Thus, polymorphisms in genes related to the host innate or adaptive immune response or in energy regulation, including insulin secretion and sensitivity, could alter the risk of prostate cancer.

Methods: 17 common single nucleotide polymorphisms (SNPs) in RNASEL, TLR4, IL1B, IL6, IL8, IL10, TNF, CRP, ADIPOQ, LEP, PPARG and TCF7L2 were genotyped in 264 prostate cancer cases and 264 matched controls nested in the prospective CLUE II cohort of Washington County, MD. Single-locus association analyses were conducted by conditional logistic regression. TagSNPs were selected (exclusive of the candidate SNPs) in IL10 (n=7), CRP (n=4), and TLR4 (n=8) and haplotype analyses were conducted.

Results: Carrying the variant allele of IL10 -1082G>A (rs1800896), a known lower producer of the inflammation-inhibitory cytokine IL-10, was associated with an increased risk of prostate cancer when compared with the homozygous wild type (AG: OR, 1.69; 95% CI 1.10-2.60, AA: OR, 1.81; 95% CI, 1.11-2.96, $P_{\text{trend}}=0.02$). The direction of the association did not vary by stage or grade, although it was strongest among cases that were both organ-confined and low stage (AA/AG vs GG: OR, 2.67, 95% CI, 1.04-6.82). The IL10 -1082G>A (rs1800896) association with total prostate cancer was stronger among users of non-steroidal anti-inflammatory drugs (OR, 3.02; 95% CI, 1.34-6.78) than nonusers (OR, 1.40; 95% CI, 0.88-2.23) and stronger among men who were lean (OR, 2.35; 95% CI, 1.16-4.79) than men who were overweight (OR, 1.49; 95% CI, 0.92-2.40), although the interactions were not statistically significant. Several IL10 haplotypes were associated with total prostate cancer when compared with the most common haplotype. However, these associations were due to high linkage disequilibrium between two tagSNPs (rs1800890 and rs3024496) and IL10 -1082G>A. A candidate SNP (rs4986790; ≥ 1 variant allele vs. 0: OR, 0.60; 95% CI 0.33-1.08, $P_{\text{trend}}=0.09$) and a tagSNP (rs10116253, OR, 3.05; 95% CI, 1.11-8.41) in TLR4 were weakly associated with prostate cancer risk. None of the other candidate SNPs, nor the TLR4 and CRP haplotypes, were statistically significantly associated with prostate cancer overall.

Conclusions: Our prospective study suggests that genetic polymorphisms in IL10 and possibly TLR4 may be associated with risk of prostate cancer. Although none of the candidate SNPs in the genes related to obesity was statistically significantly associated with prostate cancer, this does not rule out the complex role of obesity and its metabolic perturbations in the etiology of prostate cancer.

- o Dr. Platz has become well-known for her research on genes and prostate cancer and has been asked to collaborate with groups outside of Hopkins:

Michaud DS, Daugherty SE, Berndt SI, **Platz EA**, Yeager M, Crawford ED, Hsing A, Huang WY, Hayes RB. Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. *Cancer Res.* 2006;66:4525-30.

Daugherty SE, **Platz EA**, Shugart YY, Fallin MD, Isaacs WB, Chatterjee N, Welch R, Huang WY, Hayes RB. Variants in the alpha-Methylacyl-CoA racemase gene and the association with advanced distal colorectal adenoma. *Cancer Epidemiol Biomarkers Prev.* 2007;16:1536-42.

Daugherty SE, Shugart YY, **Platz EA**, Fallin MD, Isaacs WB, Pfeiffer RM, Welch R, Huang WY, Reding D, Hayes RB. Polymorphic variants in alpha-methylacyl-CoA racemase and prostate cancer. *Prostate.* 2007;67:1487-97.

Mikhak B, Hunter DJ, Spiegelman D, **Platz EA**, Hollis BW, Giovannucci E. Vitamin D receptor (VDR) gene polymorphisms and haplotypes, interactions with plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, and prostate cancer risk. *Prostate.* 2007;67:911-23.

Daugherty SE, Hayes RB, Yeager M, Andriole GL, Chatterjee N, Huang WY, Isaacs WB, **Platz EA**. RNASEL Arg462Gln polymorphism and prostate cancer in PLCO. *Prostate.* 2007;67:849-54.

Danforth KN, Rodriguez C, Hayes RB, Sakoda LC, Huang WY, Yu K, Calle EE, Jacobs EJ, Chen BE, Andriole GL, Figueroa JD, Yeager M, **Platz EA**, Michaud DS, Chanock SJ, Thun MJ, Hsing AW. TNF polymorphisms and prostate cancer risk. *Prostate.* 2008 Jan 14; [Epub ahead of print]

Danforth KN, Hayes RB, Rodriguez C, Yu K, Sakoda LC, Huang WY, Chen BE, Chen J, Andriole GL, Calle EE, Jacobs EJ, Chu LW, Figueroa JD, Yeager M, **Platz EA**, Michaud DS, Chanock SJ, Thun MJ, Hsing AW. Polymorphic variants in PTGS2 and prostate cancer risk: results from two large nested case-control studies. *Carcinogenesis.* 2007 Nov 13; [Epub ahead of print] PMID: 17999989

Dr. Platz was also asked to write an editorial on a genetic locus and prostate cancer risk:

Platz EA. Genetic variation at 8q24 as a susceptibility factor for prostate cancer: definitive results from epidemiologic studies? *Cancer Res.* 2007;67:2905-7. No abstract available.

REPORTABLE OUTCOMES

- Generated a manuscript for publication” A Simulation Study of Control Sampling: Methods for Nested Case-Control Studies of Candidate Genes and Prostate Cancer Progression”, as described above. Manuscript will be submitted after the first author’s dissertation defense.
- No association between 12 SNPs and prostate cancer progression on initial analysis. Additional SNPs and detailed analysis to follow.

CONCLUSIONS

- In genetic epidemiology studies of prostate cancer progression, incidence density sampling with replacement is the least biased approach to control sampling.
- Select candidate SNPs that are associated with prostate cancer incidence are not associated with the risk of prostate cancer recurrence in men surgically treated for clinically organ-confined disease.

REFERENCES

- None to date