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TITLE: Genome Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy

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14. ABSTRACT

The hypothesis that forms the basis for this research is that patients who possess certain SNPs or CNPs are at a greater risk for developing severe urinary morbidity or ED resulting from radiotherapy for prostate cancer. The specific aim of this project is to identify through a genome wide association study the SNPs and CNPs associated with the development of severe urinary morbidity and ED resulting from the use of radiation to treat prostate cancer. It should be noted that we may also identify SNPs or CNPs that are associated with protection against the development of these forms of radiation injury. The main accomplishment of the first year of the project was the performance of a rigorous review of the subjects to be genotyped in this study to insure that all criteria were met for their designation as cases and controls. In addition, all technical issues were resolved to enable performance of genome wide genotyping with Affymetrix 6.0 arrays as well as establishment of the assays for SNP and CNP genotyping that will be performed with a replication set of subjects.

15. SUBJECT TERMS
Radiation, SNP and CNP genotyping, normal tissue toxicities

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INTRODUCTION:

Radiotherapy can provide a sustainable cure for prostate cancer and has become accepted as a standard treatment option. However, some men develop radiation injury side effects following treatment, including urinary morbidity and erectile dysfunction, which have a substantial effect on quality of life. Development of these side effects varies in duration and severity, and while most patients return to baseline symptom levels after a year, a subset of patients experience more severe and lasting effects. A predictive assay that could identify such patients could be used to help tailor treatments plans. Previous research on radiation induced injury in breast cancer patients suggests that the variation in such side effects is largely due to patient-specific, possibly genetic effects rather than treatment differences or random effects. The purpose of the current study is to identify genetic polymorphisms associated with development of urinary morbidity or erectile dysfunction following radiotherapy for prostate cancer. The medical application of these findings will be to develop a risk assessment genetic test to assist physicians and patients in making informed decisions on the course of therapy for prostate cancer. Physicians and patients could together weigh the benefits of therapy with the individualized risk of developing radiation side effects and could then customize the treatment course.

BODY:

Since a critical aspect of any association study is to insure that the cases and controls rigorously conform to the criteria for their selection, the main effort during the first year of the project was an intensive review of the clinical data for each subject in this study to verify their inclusion in this study. Thus, efforts have been focused on the following tasks: patient follow-up, finalization of inclusion criteria, case definitions and preparation of high quality genomic DNA for microarray analysis. We have completed patient follow-up pertaining to urinary outcomes (International Prostate Symptom Score, IPSS) and erectile dysfunction (Sexual Health Inventory for Men score and Mount Sinai Erectile Function Score) for the minimum time period for all individuals in our database. Case and control definitions have been modified based on clinical characteristics of our patient set and findings in recently published reports.

Our database now includes over 3,000 men treated with brachytherapy and followed-up for a minimum of one year. We have identified two replicate sets of 100 cases and 100 controls for each of the two outcomes required for genotyping analysis using the Affymetrix 6.0 SNP arrays. All patients have been followed with assessment of urinary outcomes using the IPSS questionnaire and erectile dysfunction using the SHIM and MSEF questionnaires as planned. We have collected blood samples and prepared genomic DNA from 726 patients. Demographic and clinical data for the 726 patients for whom we have DNA samples has been analyzed to confirm that cases and controls are similar with respect to potential confounders (Table 1).

The patients included in the study have been selected based on the criteria and case definitions outlined in the initial proposal with minor modifications based on clinical characteristics of the patients in our database and recently published findings regarding radiation injury outcomes. First, we have decreased the minimum follow-up time for inclusion in the study from two years to one year. Our data as well as recent reports tracking the same radiotherapy adverse effects suggest that on average IPS scores and sexual function return to pre-treatment levels by 12 months post-treatment (Keyes, 2009; Aaltomaa, 2009; Tanaka, 2009). Twelve months appears to be sufficient time to separate out those individuals who experience long-term symptoms that may have a genetic basis.

We also increased the number of patients included in the study. We initially planned to use a single set of 100 controls for both outcomes. After closer examination of the clinical characteristics of the patients, we found that it is appropriate to select a separate set of control patients for each outcome. We found that among the patients in our database, many exhibit one form of radiation injury and not the other suggesting that different genetic variants may contribute to the different outcomes, indicating that it is appropriate to study each outcome with a separate set of cases and controls.

Table 1. Demographic and clinical characteristics of cases and controls for urinary morbidity and erectile dysfunction controls.

	Urinary Morbidity		Erectile Dysfunction	
	Cases N = 154	**Controls N = 193**	**Cases N = 200**	**Controls N = 179**
Age*, mean (sd)	64.4 (7.7)	65.2 (7.7)	63.2 (6.2)	60.6 (6.5)
Race, N(%)				
Hispanic	12 (7.8%)	21 (10.9%)	18 (9.0%)	16 (8.9%)
Caucasian	110 (71.4%)	138 (71.5%)	121 (75.5%)	134 (74.9%)
African American	23 (14.9%)	30 (15.5%)	28 (14.0%)	21 (11.7%)
Asian	3 (1.9%)	2 (1.0%)	2 (1.0%)	1 (0.6%)
Not known	6 (3.9%)	2 (1.0%)	1 (0.5%)	7 (3.9%)
Initial PSA, mean (sd)	8.1 (7.8)	8.8 (7.2)	8.3 (8.6)	7.2 (5.0)
Stage, N (%)				
T1a	0	0	1 (0.5%)	0
T1b	0	1 (0.5%)	0	0
T1c	82 (53.2%)	92 (47.7%)	111 (55.5%)	119 (66.5%)
T2a	33 (21.4%)	31 (16.1%)	36 (18.0%)	27 (15.1%)
T2b	26 (16.9%)	41 (21.2%)	37 (18.5%)	21 (11.7%)
T2c	7 (4.5%)	19 (9.8%)	9 (4.5%)	8 (4.5%)
T3a	4 (2.6%)	9 (4.7%)	6 (3.6%)	4 (2.2%)
T3c	1 (0.6%)	0	0	0
Gleason Score, N(%)				
4	1 (0.6%)	1 (0.5%)	1 (0.5%)	0
5	3 (1.9%)	7 (3.6%)	9 (4.5%)	3 (1.7%)
6	93 (60.4%)	100 (51.8%)	129 (64.5%)	133 (74.3%)
7	42 (27.3%)	56 (29.0%)	40 (20.0%)	38 (21.2%)
8	12 (7.8%)	23 (11.9)	15 (7.5%)	3 (1.7%)
9	3 (1.9%)	6 (3.1%)	5 (2.5%)	2 (1.1%)
10	0	0	1 (0.5%)	0
Treatment Type				
Implant Only	85 (55.2%)	83 (43.0%)	119 (59.5%)	125 (69.3%)
Implant + EBRT	69 (44.8%)	110 (57.0%)	81 (40.5%)	55 (30.7%)
Follow-up days, mean (min.,max.)	1707 (379, 4915)	1536 (370, 5064)	1973 (379, 5482)	1658 (370, 4013)
Taking PDIs	74 (48.1%)	76 (39.4%)	113 (56.5%)	95 (53.1%)
Pre-treatment IPSS, mean (sd)	5.4 (4.1)	7.6 (5.3)	6.9 (5.4)	6.9 (5.7)
Pre-treatment SHIM, mean (sd)	15.8 (8.5)	15.8 (8.4)	20.0 (5.4)	22.3 (3.7)
Pre-treatment MSEF				
0	22 (14.5%)	30 (15.8%)	7 (3.5%)	7 (3.9%)
1	17 (11.2%)	26 (13.7%)	5 (2.5%)	0
2	46 (30.3%)	49 (25.8%)	32 (16.1%)	25 (14.0%)
3	67 (44.1%)	85 (44.7%)	155 (77.9%)	147 (82.1%)

We have removed the constraint on ethnicity for inclusion in the study as requested by the DOD Human Research Protection Office (HRPO). We had initially restricted inclusion to white, non-Hispanic patients in an effort to reduce identification of false

positive associations due to population stratification. We have since identified several software programs including STRUCTURE and ADMIXMAP that are designed to determine ancestry based on genetic markers and to cluster individuals by genetic ancestry. Using this methodology we can assign a value for a genetic ancestry variable to each individual and control for population stratification in the tests for association.

We have included patients in the study who have been treated with either I-125 seed implant alone or in combination with external beam radiation therapy. There is no constant evidence in the literature to suggest that the effects on urinary or erectile function are different in the monotherapy versus the combination therapy (Lee, 2006; Hurwitz, 2008). Dosimetric measurements are collected for each patient and only patients whose dose to the prostate (D90) is within the range of 160-180 Gy will be included regardless of treatment type.

We had initially defined a urinary morbidity case as a patient whose pre-treatment IPSS was less than 7 and post-treatment score greater than or equal to 20. We have modified this definition to be less dependent on the pre-treatment score and rather more dependent on the individuals change in score following treatment. Controls are patients whose pre-treatment IPSS was less than or equal to 19 and experienced less than a 7 point increase in score following treatment. Cases are patients whose pre-treatment IPSS was also less than or equal to 19 but experienced at least a 10 point increase in IPSS score post-treatment. This definition allows for inclusion of individuals who report a less severe long-term response but, relative to their pre-treatment status, still experience a substantial decline in urinary symptoms. It also includes those patients who already had urinary problems prior to treatment but who still developed significant additional symptoms following treatment. This case definition better accounts for the subjective nature of the IPSS test and the variability in long- term urinary morbidity from moderate to severe.

With regard to erectile dysfunction, we had initially planned to exclude from the study patients who have taken phosphodiesterase inhibitors (PDIs) to treat erectile dysfunction as that may itself be associated causally with the outcome. Upon closer examination of the data we found that a substantial percentage of patients reported using PDIs, and if we included patients who reported using PDIs, there was only a small difference in usage between cases and controls. Rather than exclude these patients and reduce our sample size, we can include these patients and control for PDI usage in the test for association.

During the first year of the project we have run a pilot set of 5 Affymetrix 6.0 microarrays to confirm the quality of our DNA samples and check the protocol for the arrays. We achieved over 99% call rates with these 5 pilot samples. We had previously run 83 Affymetrix 6.0 arrays on a separate patient set and were able to use the quality control results from this set to make adjustments to our protocol, resulting in the high DNA quality and genotyping call rates for the pilot samples from the current study.

We have also established assays in our laboratory for the validation of the SNPs and CNPs that appear significantly associated with either urinary morbidity or erectile dysfunction in the initial training set and have successfully SNP and CNP genotyped patient samples. Through this work, we discovered that the SNPlex assay was not optimal for the genotyping to be performed and determined that more robust results were obtained using the TaqMan assay which also has an important advantage in that over 4.5 million assays are available. Since we have been successful with the use of TaqMan for SNP genotyping, we also decided to use TaqMan copy number assays for CNP analysis. TaqMan copy number assays consist of a TaqMan minor groove binding probe labeled with FAM dye and unlabeled PCR primers. The assays are run simultaneously with a copy number reference assay. The copy number assay detects the target genomic sequence of interest while the reference assay detects a sequence that is known to be present in two copies in the diploid genome. Relative quantitation analysis is performed using either a known calibrator sample.

KEY RESEARCH ACCOMPLISHMENTS:

- Refined and finalized inclusion criteria and case definitions for patients to be included in the study
- Verified IPSS and SHIM/MSEF scores for a minimum of one year for all patients included in the study
- Analyzed demographic and clinical characteristics of patients for whom we have blood collected to ensure similarity of cases and controls for each outcome
- Established assays in our laboratory for the validation of the SNPs and CNPs that appear significantly associated with either urinary morbidity or erectile dysfunction in the initial training set and have successfully SNP and CNP genotyped patient samples.

Reportable Outcomes

None

Conclusions

We have rigorously verified the clinical characteristics for each of the subjects whose DNA samples will be subjected to genotyping with Affymetrix 6.0 arrays and have set up the assays for SNP and CNP genotyping.

References

Aaltomaa SH, Kataja VV, Lahtinen T, Palmgren JE, Forsell T. Eight years experience of local prostate cancer treatment with permanent I125 seed brachytherapy--morbidity and outcome results. *Radiother Oncol.* 2009 May;91(2):213-6.

Hurwitz M D, Halabi S, Ou SS, McGinnis LS, Keuttel MR, Dibiase SJ, Small EJ . Combination external beam radiation and brachytherapy boost with androgen suppression for treatment of intermediate- risk prostate cancer: an initial report of CALGB 99809. *Int J Radiat Oncol Biol Phys.* 2008 Nov 1;72(3):814-9.

Keyes M, Miller S, Moravan V, Pickles T, Liu M, Spadinger I, Lapointe V, Morris WJ. Urinary symptom flare in 712 125I prostate brachytherapy patients: long-term follow-up. *Int J Radiat Oncol Biol Phys.* 2009 Nov 1;75(3):649-55.

Lee WR, DeSilvio M, Lawton C, Gillin M, Mo rton G, Firat S, Baikad i M, Kuettel M, Greven K, Sandler H. A phase I I study of external bea m radiotherapy combined wit h permanent source brachytherapy for inte rmediate-risk, clinically localized adenocarcinoma of the prostate: prelim inary results of RTOG P-0019. *Int J Radiat Oncol Biol Phys.* 2006 Mar 1;64(3):804-9.

Appendices

None