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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, proctitis 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 second year was the completion of the discovery phase of the project. Specifically, case and control samples for each of the outcomes were run on Affymetrix SNP6.0 arrays, checked with quality control steps, and analyzed for association of genetic variants. We identified approximately 700 SNPs associated with one or more of the outcomes that we will investigate in the validation cohort.				
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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 side effects following treatment, including urinary morbidity, proctitis and erectile dysfunction, which have a substantial effect on quality of life. These side effects vary 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 treatment 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 proctitis, 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:

Efforts in the second year of funding have been focused on completion of the discovery phase of the genome-wide association study. Genomic DNA from the 386 prostate cancer patients identified as cases or controls for one or more of the three outcomes of the study was assayed using Affymetrix SNP6.0 arrays.

A considerable amount of effort was spent on quality control checks to ensure sample identity and to assess and minimize risks of batch effects and population stratification. The 386 samples were run in 5 batches (i.e. 5 96-well plates). We incorporated two types of controls for each batch: an external control set comprising a HapMap trio (two parents and an offspring) and an internal control set comprising three duplicates of randomly selected prostate cancer patient samples. Initial overall genotyping rate among all 411 samples (study samples plus controls) was > 97%. We were able to confirm >99% reproducibility of genotype calls among the four batches by comparing the HapMap samples across batches. We also calculated identity-by-descent (IBD) and identity-by-state (IBS) measures to confirm the identity of the control samples and identify any patient samples with greater-than-expected similarity. We obtained expected IBD and IBS values for all controls: approximately 50% IBD sharing between the offspring and each parent of the HapMap trios, and >98% IBS sharing between identical pairs for all duplicate samples. Several prostate cancer samples were excluded based on greater-than-expected IBD sharing (8 pairs of samples) or low call rate (<90%; 2 samples). The final dataset contained 367 samples with call rate >98%.

Because the study involved a multi-ethnic patient population, the genetic population structure was assessed using principal components analysis and ancestry estimation using the program STRUCUTRE v 2.1. As expected, based on self-reported race/ethnicity, approximately 78% of patients share ancestry primarily with Caucasian populations, approximately 4% share ancestry with Asian (Chinese and Japanese) populations, and approximately 18% are admixed with ancestry shared between African and Caucasian populations. For several patients with missing data on race/ethnicity, estimation of ancestry using SNP genotypes allowed us to accurately assign proportion shared ancestry and include those individuals in the analysis. We found no statistically significant differences in ancestry between cases and controls for any of the three outcomes suggesting that despite using a multi-ethnic cohort, we were able to adequately match cases and controls on race/ethnicity, thereby minimizing confounding by this variable in our association tests. To further minimize potential confounding by race/ethnicity, we used the estimated proportion ancestry as a variable in logistic regression models to check that any significant associations identified by chi-square tests were not strictly due to population stratification.

Association tests were carried out for each outcome using a allelic and genotypic models as well as logistic regression models adjusting for ancestry. Analysis included 125 urinary morbidity cases and 106 controls, 135 ED cases and 121 controls, and 76 proctitis cases and 291 controls. As outlined in our proposal, we set a fairly liberal cut-point of $p < 10^{-4}$ for inclusion in the validation study. This two-stage study design allows

us to capture most true positive associations and then filter out false positive associations through the validation study. Using the lowest p-value between the allelic and genotypic models for each SNP, we have identified 157 SNPs associated with urinary morbidity (p-values 6×10^{-7} to 10^{-4}), 167 SNPs associated with ED (p-values 2×10^{-7} to 10^{-4}), and 365 SNPs associated with proctitis (p-values 6×10^{-14} to 10^{-4}) that will be investigated further in the validation study.

The next steps will be to undertake the validation phase of the study. Over the last year, approximately 200 additional prostate cancer patients have been consented and added to our clinical database. We have investigated improved technology for genotyping in the validation set and found that custom SNP arrays will allow us to interrogate the approximately 700 SNPs identified as well as several thousand additional SNPs within the regions or genes surrounding identified SNPs. We are on track to begin genotyping the validation set in the first few months of 2011.

Once we have a validated set of associated SNPs we will be able to build predictive models for each outcome. In preparation for this, we have identified a number of treatment and patient specific variables that have been found to be associated with one or more of the outcomes being investigated. Treatment related variables include total biologically effective dose, isotope used for brachytherapy, and whether the patient received external beam RT in addition to brachytherapy. Patient related variables include age, pre-treatment symptoms, use of hormone therapy, hypertension, diabetes, and smoking status. Among our patient cohort, logistic regression models including these variables are marginally predictive with area under the curve for receiver operator characteristic curves in the range of 0.574 to 0.766. The predictive ability of these models will be improved upon by incorporating significant SNPs identified through the validation study.

KEY RESEARCH ACCOMPLISHMENTS:

- Ran SNP/ CNP genotyping arrays for 411 patient samples and controls in the discovery cohort
- Achieved >98% call rate in final set of 367 patients after performing QC steps
- Confirmed cases and controls were matched on race/ethnicity for all three outcomes and obtained ancestry estimates for each patient to include in logistic regression models for SNP association
- Identified approximately 700 SNPs associated with ED, urinary morbidity or proctitis to be investigated in the validation cohort
- Completed patient recruitment to fulfill the sample size requirement for the validation study

Reportable Outcomes

We have identified 157 SNPs associated with urinary morbidity (p-values 6×10^{-7} to 10^{-4}), 167 SNPs associated with ED (p-values 2×10^{-7} to 10^{-4}), and 365 SNPs associated with proctitis (p-values 6×10^{-14} to 10^{-4}) that will be investigated further in the validation study.

Conclusions

Our results to date support the feasibility of a genome-wide association study to identify genetic variants associated with radiotherapy adverse response. The results of this study would provide the basis for development of a clinically relevant predictive test to identify patients at increased risk for development of adverse events following radiotherapy. Such a tool could be used to aid clinicians in personalizing dosage to improve the therapeutic index of radiotherapy treatment for prostate cancer.

References

None

Appendices

None