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Genome Wide Association Study to Identify Single Nucleotide Polymorphisms (SNPs) Associated with the Development of Erectile Dysfunction in African-American Men Following Radiotherapy for Prostate Cancer

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Abstract

Purpose—To identify single nucleotide polymorphisms (SNPs) associated with erectile dysfunction (ED) among African American prostate cancer patients treated with external beam radiation therapy (EBRT).

Methods and Materials—A cohort of African American prostate cancer patients treated with EBRT was followed for development of ED using the five-item Sexual Health Inventory for Men (SHIM) questionnaire. Final analysis included 27 cases (post-treatment SHIM score ≤ 7) and 52 controls (post-treatment SHIM score ≥ 16). A genome-wide association study was performed using ~909,000 SNPs genotyped on Affymetrix 6.0 arrays.

Results—We identified SNP rs2268363, located in the follicle stimulating hormone receptor (FSHR) gene, as significantly associated with ED after correcting for multiple comparisons (unadjusted p-value = 5.46×10^{-8}; Bonferroni p-value = 0.028). We identified four additional

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SNPs that tended toward significant association with unadjusted p-value < 10^{-06}. Inference of population substructure revealed that cases had a higher proportion of African ancestry compared to controls (77% compared to 60%, p=0.005). A multivariate logistic regression model that incorporated estimated ancestry and four of the top-ranked SNPs was a more accurate classifier of ED than a model that included only clinical variables.

Conclusions—To the best of our knowledge, this is the first genome wide association study to identify SNPs associated with adverse effects resulting from radiotherapy. It is important to note that the SNP that proved significantly associated with ED is located within a gene whose encoded product plays a role in male gonad development and function. Another key finding of this project is that the four SNPs most strongly associated with ED were specific to people of African ancestry and would therefore not have been identified had a cohort of European ancestry been screened. This study demonstrates the feasibility of a genome-wide approach to investigate genetic predisposition to radiation injury.

Keywords

radiation injury; prostate cancer; genome-wide association study; African American; admixture

Introduction

Radiotherapy can provide a sustainable cure for prostate cancer and is accepted as a standard treatment option. However, many men develop side effects following radiotherapy, including urinary morbidity, proctitis, and erectile dysfunction (ED), which have a substantial effect on quality of life. Acute symptoms resolve in most patients after a few months, but a subset of patients experience long-term morbidity[1, 2]. A method to predict which patients are at greatest risk for permanent side effects would assist both clinicians and patients in weighing the benefits of radiotherapy versus other options of treating localized prostate cancer. Cohort studies have found that some clinical factors such as treatment type, radiation dose, pre-treatment symptoms, age, and co-morbidities, such as diabetes and vascular disease, are associated with developing side effects, but these clinical factors do not fully explain the variability in outcome[1–4].

Research on radiation induced injury in prostate and breast cancer patients suggest that much of the variation in normal tissue damage can be attributed to patient-specific, possibly genetic variation rather than treatment differences or random effects. An initial study examining development of skin telangiectasia among breast cancer patients treated with radiation therapy estimates that 81–90% of variation in normal tissue damage is due to patient-specific characteristics[5]. Studies among prostate cancer patients have identified single nucleotide polymorphisms (SNPs) in candidate genes, including ATM, LIG4, ERCC2, SOD2, TGFB1, XRCC1, XRCC3, and CYP2D6, among others, that are associated with development of late toxicity in response to radiation therapy[6–10]. SNPs in several of these genes have also been associated with adverse effects in breast cancer patients[5, 11–14].

To date, research attempting to identify genetic variants associated with radiotherapy side effects has taken a candidate-gene approach, mostly screening genes involved in the DNA damage response. However, conflicting results have been obtained in these studies and no single SNP or group of SNPs has proven to exhibit a consistent association with the development of a normal tissue toxicity following radiotherapy[15, 16]. For this project, we took a broader approach and performed a genome-wide association study (GWAS) with ED as a measure of normal tissue damage experienced by prostate cancer patients treated with external beam radiation therapy (EBRT). This study was also designed to address a health disparity in radiogenomics research in that subjects with primarily European ancestry have

constituted the majority of individuals included in these studies. There is growing recognition that the results obtained from one ethnic/racial group are not necessarily applicable to individuals whose ancestry is primarily from a different ethnic/racial group. Thus, the findings in this field of research may not benefit other racial groups if the research is performed primarily with subjects of European background. The research reported in this paper focused on African-American men with the goal to identify the genetic variants specific to this population that may be associated with the development of radiation-induced ED. This study identifies several potentially predictive genetic variants and supports the feasibility of using a genome-wide approach to investigate the genetic predisposition to radiation injury.

Methods and Materials

Study Population and Case Definition

A cohort of 138 African American males with adenocarcinoma of the prostate were recruited for this study from the Department of Radiation Oncology of the Elmhurst and Queens Hospital Center affiliated with Mount Sinai School of Medicine between 2001 and 2006. Patients were treated with three dimensional conformal RT or IMRT and received 39–42 fractions of 1.8 Gy. Treatments were delivered with 6 MV or 18 MV photons using a Varian 21 EX linac. Men were followed for an average of 2.8 years (minimum of one year) after treatment to assess development of late-phase ED. ED was assessed using the five-item self-administered Sexual Health Inventory for Men (SHIM) questionnaire[17]. We categorized SHIM score such that 16 to 25 indicates optimum sexual function, 8 to 15 indicates moderate dysfunction, and 0 to 7 indicates severe dysfunction. All men enrolled in the study filled out the SHIM questionnaire prior to and at least one year after treatment. Only men with an optimum pre-treatment score (≥ 16) were considered for inclusion. Cases were defined as those men with SHIM ≤ 7 after one year post-treatment. Controls were defined as those men with SHIM ≥ 16 after one year post-treatment. A total of 29 men met the case definition for chronic ED and 53 qualified as control subjects. We obtained sufficient quantity and quality of DNA for 27 of the cases and 52 of the controls. At the time of enrollment in the study, clinical data was collected from patient charts relating to disease stage, radiation dosage, and a number of lifestyle factors and co-morbidities that were explored for association with the outcome. The study was approved by the Institutional Review Board of Mount Sinai Medical Center and all individuals provided informed consent prior to blood collection.

Genotyping and Quality Control

DNA was isolated from lymphocytes using Ficoll separation as described previously18. The DNA was screened for ~909,000 SNPs using Affymetrix6.0 arrays (Affymetrix, Santa Clara, CA). Birdseed v2 was used to make genotype calls[19]. The initial genotype call rate among all individuals was 94.5% (range 87.3% to 98.1%). SNPs were excluded from the analysis if they had no genotype for >5% of individuals, were not in Hardy-Weinberg equilibrium among controls (using threshold $p < 0.001$), or had minor allele frequency <5%. The minor allele was defined as the allele with lower frequency among the total sample of 79 individuals. As all individuals were men, heterozygous calls for SNPs on the X chromosome were set to ‘no call’ prior to filtering. The final dataset contained 512,497 SNPs with genotype rate of 99.0%.

Data Analysis

Quality control filtering, identity by state clustering, and association analysis was carried out using the PLINK genetic analysis software[20]. The main analysis was done using a 2×2 table comparing the frequencies of each of the two alleles (A and a) among cases and controls

with a two-sided chi-square test used to assess the statistical significance of the difference in genotype frequency.

Population sub-structure was assessed using principle components analysis (PCA) performed in R version 2.10.0[21]. PCA included a randomly selected subset of 100,000 SNPs for the 79 study subjects as well as 90 individuals from each of three reference populations from the International HapMap Project: European-Americans from Utah, Yorubans from Nigeria, and a combined population from Tokyo, Japan and Beijing, China22.

Estimation of individual and locus-specific ancestry was performed using the STRUCTURE and MALDsoft programs[23, 24]. A subset of 2,455 ancestry informative markers was selected on the basis of having a greater than 60% minor allele frequency difference between European and African populations from the International HapMap Project[22]. STRUCTURE was run using the admixture model with the number of ancestral populations, K, set to 2. Prior population information from 90 African and 89 European HapMap individuals was included to assist in estimating ancestry. The STRUCTURE run consisted of 10,000 burn-in iterations followed by an additional 10,000 iterations.

General data management and logistic regression modeling was performed using R version 2.10.0[21].

Results

Patient Characteristics

Information on demographic, clinical, and life-style factors that may be related to the outcome was compared between cases and controls (Table 1). A greater proportion of cases reported hypothyroidism compared to controls (11.1% compared to 0; $p = 0.014$). Cases had on average a slightly higher pre-treatment SHIM score than controls (21 compared to 20, $p < 0.001$). This trend is opposite that commonly seen among prostate cancer radiotherapy patients but is likely an artifact of the way patients were selected for this study in that the inclusion criteria required all patients to have a pre-treatment score ≥ 16. A greater proportion of controls reported using hormonal therapy than cases, though this difference was not significant (37.3% compared to 22.2%, $p = 0.176$). A greater proportion of cases had stage III or stage IV prostate cancer compared to controls but again the difference was not statistically significant (7.7% and 11.5% of cases had stage III and stage IV respectively compared to 5.9% and 3.9% of controls, $p = 0.406$). There were 5 patients who reported using 5-phosphodiesterase inhibitors (PDIs) (3 cases and 2 controls).

Genome-Wide Association Test

We performed allelic association tests to compare the allele frequencies for 512,497 SNPs between cases and controls (Figure 1). One SNP was identified, rs2268363 located in the follicle-stimulating hormone receptor (FSHR) gene on chromosome 2, that was significantly associated with ED after correcting for multiple comparisons (unadjusted p-value = 5.46×10^{-8}; Bonferroni corrected p-value = 0.028; Table 2). Men possessing the minor allele of rs2268363 had seven-fold increased odds of developing post-radiotherapy ED compared to men who did not possess this SNP (OR = 7.03; 95% CI 3.4–14.7).

Several other SNPs were associated with ED upon initial statistical testing, but p-values for these associations increased above the threshold of significance after applying the conservative Bonferroni correction for multiple comparisons (Table 2). The second top-ranking SNP, rs10194115, lies within the coding region of the tetratricopeptide repeat domain 7A (TTC7A) gene and is associated with a nine-fold increased odds of developing post-radiotherapy ED (unadjusted p-value = 4.73×10^{-7}, OR = 9.00, 95% CI 3.5–23.2). The

third top-ranking SNP and three additional high-ranking SNPs (p-value less than 10^{-05}), are located within the prostaglandin F2 receptor negative regulator (PTGFRN) gene and are associated with between 4.9- and 6.4-fold increased odds of developing post-radiotherapy ED (Table 2).

Identification of Population Sub-Structure and Association of African Ancestry with Radiotherapy Side Effects

During our initial quality control analyses, we observed that there may be underlying population sub-structure among our patient population. Identity by state (IBS) clustering discerned 6 clusters of individuals who may be more genetically similar to each other than to the rest of the individuals. Permutation testing of IBS distances suggests that cases may be genetically more similar to other cases than to controls ($p = 0.003$). This was not surprising given that this cohort consists of self-identified African Americans, each person with varying degrees of admixture of African and European ancestors. Population stratification (i.e. mismatch of ancestry in cases and controls) is a common confounder in GWAS. To further explore a potential role of ancestry in our association results, we sought to estimate the proportion of each individual's ancestry that came from African versus European progenitors for adjustment in association tests.

To visualize the genetic patterns of our cohort and their expected ancestral populations, we performed principle components analysis (PCA) of our patients together with three groups of individuals genotyped by the International HapMap project (European-Americans, Africans, and Asians)[22]. The PCA plot demonstrates that our patient cohort falls between the European and African clusters, as expected for African American individuals (Figure 2). Interestingly, the cases appear to cluster nearer to the African HapMap population compared to the controls. We found a statistically significant difference in principle components (PC) coordinate values between ED cases and controls ($p = 0.004$ for PC1; $p = 0.003$ for PC2), suggesting an underlying genetic difference between cases and controls.

The program STRUCTURE was used to estimate the proportion of African ancestry for each individual using a set of 2,455 ancestry-informative SNPs. As this cohort has self-identified as African American, we assumed two ancestral populations, African and European. The STRUCTURE analysis accurately grouped the HapMap individuals into two populations with our African American cohort sharing some proportion of their SNPs with both ancestral populations (Figure 3A). In agreement with the principle components analysis, the STRUCTURE estimate suggests that, on average, ED cases have a greater proportion of African ancestry compared to controls. Among ED cases, the average proportion ancestry shared with the African HapMap population is 77% compared with 60% for the ED controls ($p = 0.005$). Using the estimates from the STRUCTURE model, we used the MALDsoft program to calculate the average proportion ancestry among cases or controls at each locus in the genome. The MALDsoft analysis shows that at any given locus, cases have a greater proportion of African ancestry than controls (Figure 3B).

Based on this evidence of population stratification and its association with our outcome, we tested for association between genotype and ED using logistic regression to adjust for individual estimated ancestry (Table 2, column 9). All SNPs with un-corrected p-value less than 10^{-06} were still significantly ($p<0.05$) associated with development of ED, although the p-values did increase for most SNPs after adjusting for proportion of African ancestry.

Classification of Radiotherapy Patients Based on Clinical and Genetic Factors

Logistic regression models were used to investigate the utility of genetic and clinical factors for the classification of radiotherapy patients according to development of ED following

treatment. In a model including clinical covariates for which sufficient data was available, only pre-treatment SHIM score is significantly associated with developing ED (Table 3). After controlling for all other covariates, a one-point higher pre-treatment SHIM score is associated with an odds ratio of 1.3 for developing ED ($p = 0.016$). Again, this association may be an artifact of the inclusion criteria for this study. We did not find any significant association between age at diagnosis, stage, Gleason score, EBRT dose, hormone use, length of follow-up, smoking status, diabetes, hypertension, or asthma with development of ED, either alone or in a multivariate model (Table 3). We should note that given the small sample size of this study, the numbers of individuals reporting many of the covariates investigated in this model are small, even after excluding some covariates (Table 1).

We next developed a logistic regression model including genetic factors to classify patients in terms of developing ED. We included the five SNPs with chi-square association p-values less than 10^{-6} as well as proportion African ancestry. We also included pre-treatment SHIM score, as that was associated with ED in the clinical model. Pre-treatment SHIM score as well as SNP rs7064929 were no longer significantly associated with developing ED once the other SNPs were included ($p = 0.8682$ for rs7064929 and $p = 0.0671$ for pre-treatment SHIM score), so we excluded these variables from the final regression model. Proportion African ancestry was no longer significantly associated with development of ED once the SNPs were included ($p = 0.3584$), however inclusion of ancestry controls for population stratification and modifies the strength of association between the SNPs and ED, so it was retained in the final model. The final model included proportion African ancestry, and four of the most strongly associated SNPs (Table 4). We should note that given the relatively small sample size for this study, the odds ratios associated with the SNPs in this model are imprecise measurements and warrant replication in a larger cohort. Among these four SNPs, a greater percentage of cases were homozygous for more than one risk allele compared to controls (Table 5). Similarly, a greater percentage of cases were heterozygous for more than one risk allele compared to controls. This suggests that it may be informative to use a combination of SNPs to formulate a predictive model.

Receiver-operating characteristic (ROC) curves comparing the clinical and the genetic models show that the genetic model is more accurate in classifying patients according to development of post-radiotherapy ED among this cohort of prostate cancer patients (AUC for clinical model = 0.749, AUC for genetic model = 0.983; Figure 4).

Discussion

To the best of our knowledge, this is the first study to use a genome-wide approach to identify genetic variants associated with development of long-term side effects of radiation therapy. Out of 512,497 SNPs, we have identified rs2268363, located in the FSH receptor gene, which is significantly associated with development of ED after using Bonferroni correction for multiple testing. The FSH receptor is expressed in Sertoli cells of the testis and is involved in testis development and function. Severe disruption of the FSH signaling pathway can lead to abnormal spermatogenesis, small testis size, and infertility in humans[25–27]. Mutations in the FSH gene in mice result in improper gonad development as well as hypothyroidism[27]. Interestingly, three of the ED cases in this study reported hypothyroidism, whereas none of the control patients reported this co-morbidity (Table 1). Two of these men are heterozygous and one is homozygous for the associated risk allele of rs2268363.

We have identified additional SNPs, which although not significantly associated after Bonferroni correction, map to genes that have evidence of a biological role in erectile function and warrant further investigation. The second-ranked SNP, rs10194115 (p-value =

4.73×10^{-07}) and another high-ranked SNP, rs5965182 (p-value = 6.25×10^{-06}), reside within the TTC7A gene and near the HEPH gene respectively. The proteins encoded by TTC7A and HEPH are involved in regulating blood iron levels, and excessively high blood iron levels are clinically associated with low testosterone and gonadotrophin levels and erectile dysfunction[28–31]. Four other highly ranked SNPs (p-value < 10^{-5}) lie within the prostaglandin F2 receptor negative regulator gene. Prostaglandin F2 signaling is involved in vascular smooth muscle constriction and increase in blood pressure; physiological processes involved in erectile function[32]. A limitation of our study is the small sample size, and it will be necessary to investigate these SNPs (or other SNPs within these genes) in a larger cohort of radiation therapy patients.

The SNPs identified in this study lie in or near genes that appear to be biologically involved in erectile function. This raises the issue that different types of radiotherapy side effects may have distinct genetic predictors, and it will be important to examine each phenotype separately. In contrast, previous studies using a candidate gene approach focused mainly on genes involved in DNA damage repair and cellular radiation response6–10. We did find some evidence for association between DNA damage repair genes and ED in this study. For example, two SNPs in the DNA ligase 4 gene (LIG4), rs7995376 and rs7489413, had p-values of 0.001 and 0.009 respectively. Similarly, SNPs rs6927534 and rs7771621 in the superoxide dismutase 2 gene (SOD2) had p-values of 0.007 and 0.01 respectively. These are similar findings to those previously reported by candidate gene studies6, 10. However, it is difficult to compare this study directly to previous studies for several reasons. First, this study looked for SNPs among African American men whereas previous studies included largely Caucasian populations. Second, this study only looked at ED as a measure of radiation injury whereas some studies looked at urinary morbidity and proctitis as well. For example, Burri et al. found a SNP in SOD2 to be associated with proctitis but not ED10. Finally, because several hundred thousand comparisons are made in a genome-wide study there is a high likelihood of detecting false positive associations, and so SNPs with p-values on the order of 0.001 to 0.01 (as seen for the DNA repair genes) are below the threshold of significance. However, the broader approach taken here was able to identify potentially important genes and pathways that would not have otherwise been investigated using a candidate gene approach.

Another key finding of this project is that 19 of the 30 SNPs with p-values $<10^{-5}$, including the top four SNPs (Table 2), exhibit minor allele frequencies in the African HapMap population that are at least five-fold greater than the frequencies seen in the European HapMap population. Thus, if this study had been conducted using exclusively subjects of European ancestry, it is unlikely that the highest-ranking SNPs associated with ED in our African-American cohort would have been identified. Hence, our work addresses the existing health disparity in radiogenomics research, which has involved studies composed primarily of European-ancestry patients.

The observation that the cases in our study shared more genetic variants with African ancestors compared to controls raises an interesting hypothesis that ancestry may be linked to radiation adverse response. It is well established that African ancestry is associated with increased risk of developing prostate cancer (http://seer.cancer.gov/csr/1975_2006/), and admixture mapping studies have identified 8q24 as a risk locus for prostate cancer among African Americans[33–35]. All of the patients in this study came from the same general population, and to the best of our knowledge there was no selection bias towards cases having a greater proportion of African ancestry than controls. It will be interesting to explore this observation in a larger cohort and to investigate the potential role of African ancestry in development of other prostate cancer radiotherapy side effects such as urinary morbidity and proctitis.

Acknowledgments

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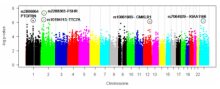

Figure 1.
Plot of -$\log_{10}$ p-values from allelic chi-square tests for association of each of 512,497 SNPs with development of post-radiotherapy ED.

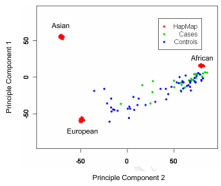

Figure 2.

Principle Components Analysis using 100,000 SNPs randomly selected from the 512,497 SNPs used in the association test. Includes 27 ED cases, 52 controls, and 90 HapMap individuals from each of three reference populations: Asian, African, and European.

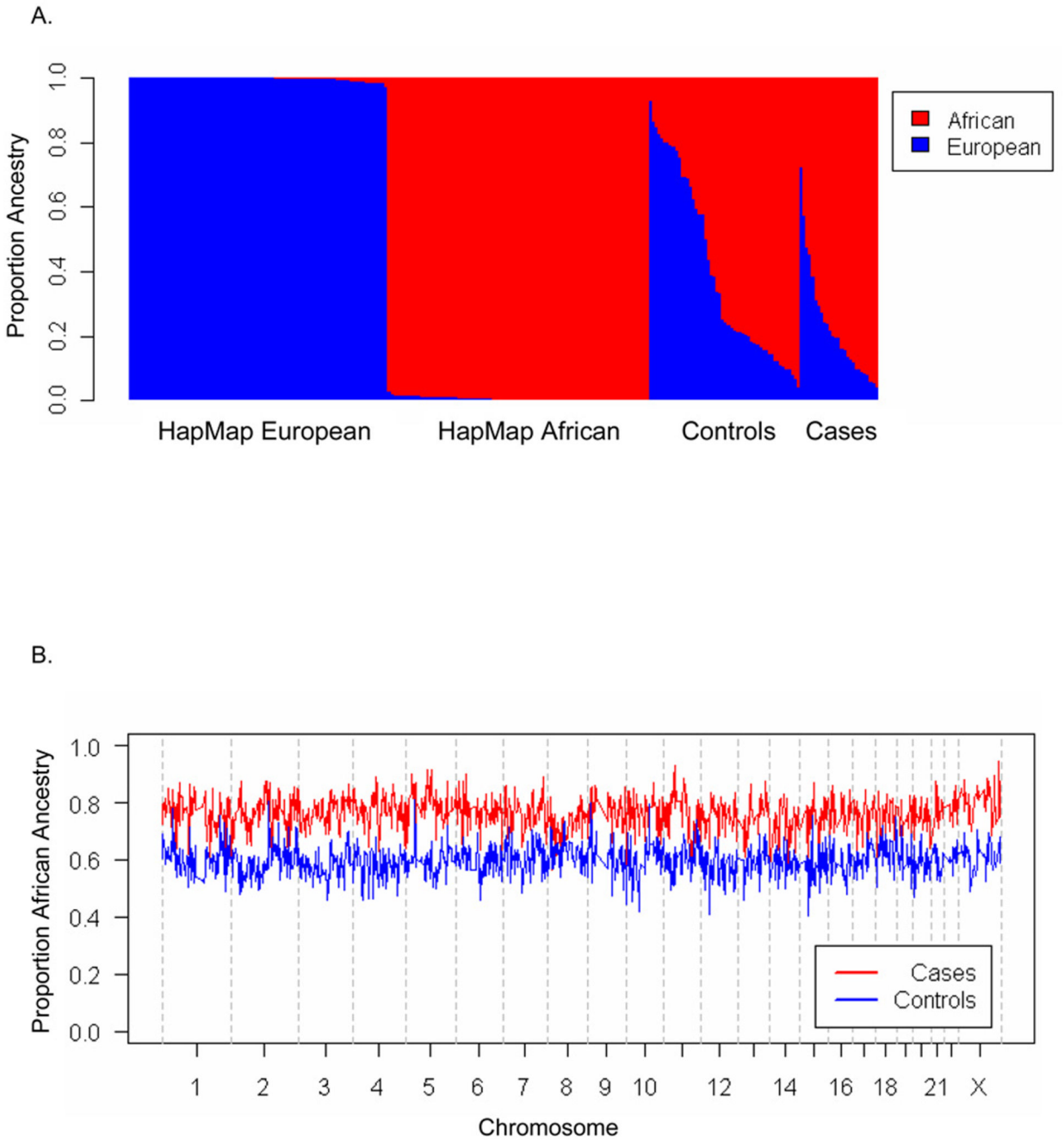

Figure 3.
(A) STRUCTURE analysis showing average individual ancestry using 2,455 ancestry-informative markers (AIMs), and (B) MALDsoft locus-specific average ancestry among cases and controls.

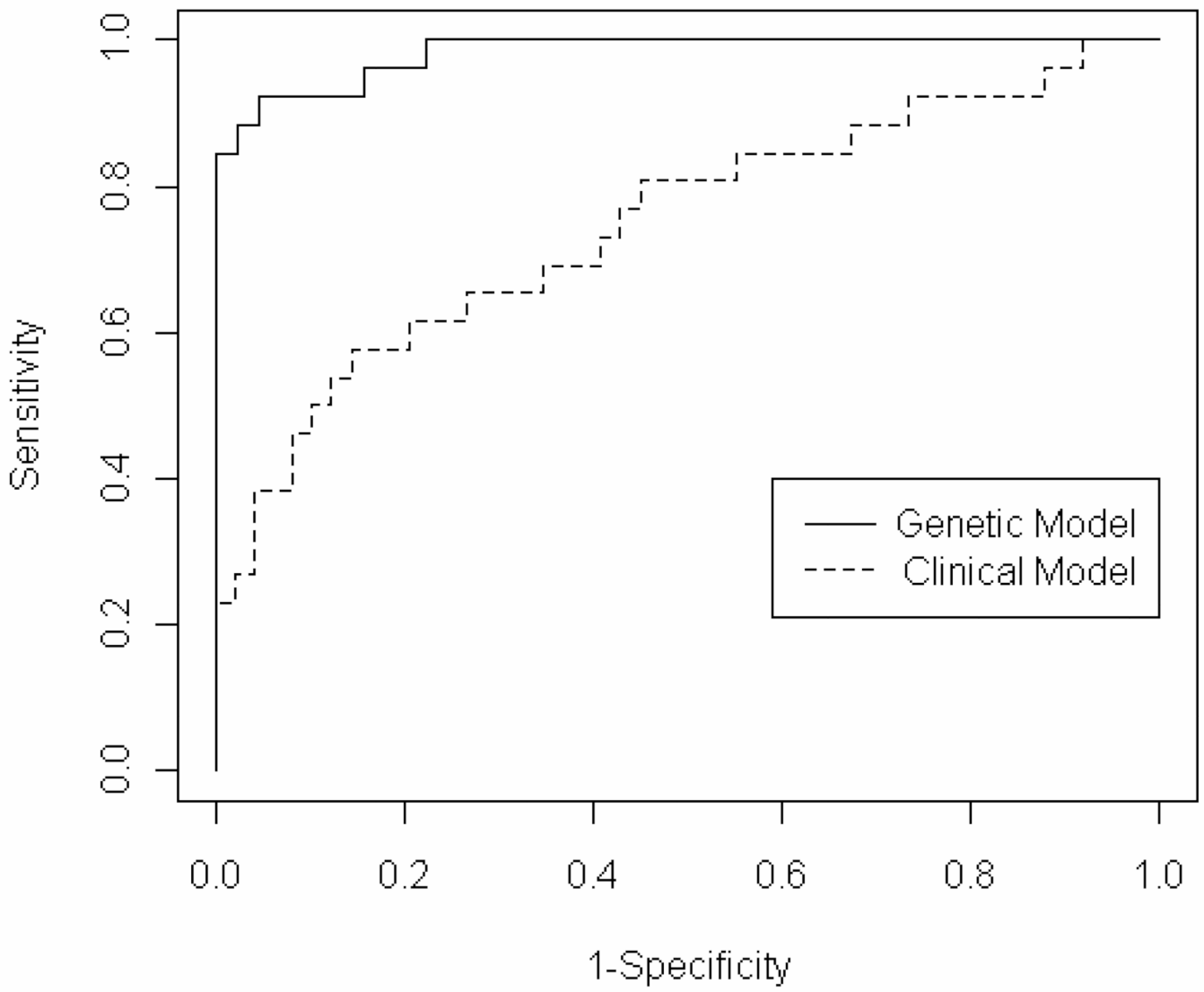

Figure 4.
ROC curves for the clinical model and the genetic model.
The genetic model includes rs2268363, rs10194115, rs2806864, rs10861905, and African ancestry. The clinical model includes age at diagnosis, stage, Gleason score, EBRT dose, hormone use, pre-treatment SHIM score, follow-up time, diabetes, hypertension, asthma, and smoking status.

Table 1

Clinical information for patients.

	Cases N=27	Controls N=52	p-value*	Total N=79
Age in years, mean(sd)	64.1 (6.6)	64.9 (7.4)	0.643	64.6 (7.1)
Stage, n(%)				
II	21 (80.8%)	46 (90.2%)		67 (87.0%)
III	2 (7.7%)	3 (5.9%)	0.406	5 (6.5%)
IV	3 (11.5%)	2 (3.9%)		5 (6.5%)
Gleason Score, n(%)				
5–6	17 (63.0%)	31 (59.6%)		48 (60.8%)
7	4 (14.8%)	13 (25.0%)	0.507	17 (21.5%)
8–10	6 (22.2%)	8 (15.4%)		14 (17.7%)
EBRT dose (Gy), mean (sd)	72.6 (4.3)	71.2 (5.9)	0.308	71.7 (5.4)
Follow-up days, mean (sd)	981 (288)	1068 (279)	0.194	1038 (284)
Hormone therapy†, n(%)	6 (22.2%)	19 (37.3%)	0.176	25 (31.6%)
PD inhibitor use, n(%)	3 (11.1%)	2 (3.8%)	0.208	
Smoking status, n(%)				
Current or former smoker	5 (18.5%)	13 (25.0%)	0.515	18 (22.8%)
Never smoker	22 (81.5%)	39 (75.0%)		61 (77.2%)
Diabetes, n(%)	6 (22.2%)	10 (20.2%)	0.819	16 (20.8%)
Heavy alcohol use, n(%)	2 (7.4%)	4 (7.7%)	0.964	6 (7.6%)
Arthritis, n(%)	0	5 (9.6%)	0.096	5 (6.3%)
Asthma, n(%)	3 (11.1%)	4 (7.7%)	0.612	7 (8.9%)
Heart Disease, n(%)	1 (3.7%)	7 (13.5%)	0.173	8 (10.1%)
Hypertension, n(%)	16 (59.3%)	31 (59.6%)	0.976	47 (59.5%)
Stroke, n(%)	1 (3.7%)	2 (3.8%)	0.975	3 (3.8%)
Emphysema, n(%)	0	1 (1.9%)	0.468	1 (1.3%)
Gout , n(%)	1 (3.7%)	0	0.163	1 (1.3%)
Hypothyroidism, n(%)	3 (11.1%)	0	0.014	3 (3.8%)
Glaucoma, n(%)	2 (7.4%)	2 (3.8%)	0.493	4 (5.1%)
Pre-treatment SHIM score, mean(sd)	21 (2.7)	20 (2.8)	< 0.001	20 (2.8)

*Chi-square test was used for categorical variables and one-way ANOVA was used for continuous variables

†22 individuals reported taking leuprolide, 1 individual reported taking leuprolide and bicalutamide, 1 individual reported taking nilutamide, 1 individual was missing details of drug type.

Table 2

SNPs showing the strongest association (p-value < 10^{-05}) with development of ED following radiotherapy. Minor allele identity is based on all 79 individuals. Chr, chromosome; BP, base pair; MAF, minor allele frequency; CEU, European-American HapMap population; YRI, African HapMap population.

SNP	Chr	BP Position	Nearest Gene	Distance to Gene (BP)	MAF Cases	MAF Controls	p-value	Odds Ratio	Bonferroni p-value	Ancestry adjusted p-value	MAF CEU	MAF YRI
rs2268363	2	49054832	FSHR	0	0.611	0.183	5.46×10^{-08}	7.03	0.028	1.98×10^{-04}	0.1	0.592
rs10194115	2	47093516	TTC7A	0	0.404	0.07	4.73×10^{-07}	9.00	0.242	9.39×10^{-05}	0.033	0.242
rs2806864*	1	117271304	PTGFRN	0	0.537	0.153	5.86×10^{-07}	6.42	0.300	7.15×10^{-04}	0.017	0.533
rs7064929	23	64283744	KIAA1166	170683	0.741	0.173	6.88×10^{-07}	13.65	0.352	8.57×10^{-05}	0	0.971
rs10861905	12	107291463	CMKLR1	34247	0.259	0.010	8.33×10^{-07}	33.95	0.427	0.00145	0.033	0.1
rs1527243	2	123007492	TSN	765594	0.167	0.57	1.39×10^{-06}	0.15	0.713	0.00141	0.85	0.233
rs12336160	9	33273582	CHMP5	2066	0.407	0.087	1.46×10^{-06}	7.26	0.746	1.36×10^{-04}	0	0.25
rs2716734	2	39801225	TMEM178	2618	0.482	0.135	1.98×10^{-06}	5.97	1	7.35×10^{-04}	0.025	0.375
rs10210358	2	141512090	LRP1B	0	0.704	0.308	2.01×10^{-06}	5.34	1	3.36×10^{-04}	0.15	0.658
rs16861326	1	18167074	IGSF21	139753	0.259	0.019	2.10×10^{-06}	17.85	1	4.36×10^{-04}	0	0.108
rs5925696	23	22801998	DDX53	126010	0.815	0.26	3.08×10^{-06}	12.52	1	1.24×10^{-04}	0.7	0.817
rs7552382	1	117324524	PTGFRN	0	0.611	0.235	3.53×10^{-06}	5.11	1	3.51×10^{-04}	0.283	0.512
rs6741148	2	38131336	FAM82A1	0	0.722	0.333	3.64×10^{-06}	5.2	1	0.00198	0.042	0.733
rs3802458†	9	96781095	C9orf3	0	0.296	0.039	3.78×10^{-06}	10.53	1	3.23×10^{-04}	0.008	0.192
rs6862844	5	124393866	ZNF608	285162	0.389	0.087	4.31×10^{-06}	6.72	1	9.08×10^{-04}	0	0.233
rs10993429†	9	96779286	C9orf3	0	0.315	0.048	4.36×10^{-06}	9.10	1	0.00107	0.008	0.192
rs2901964	1	15665013	ELA2A	0	0.241	0.625	4.60×10^{-06}	0.19	1	2.34×10^{-04}	0.783	0.225
rs11122834	2	121417759	GLI2	0	0.667	0.289	4.83×10^{-06}	4.93	1	0.00205	0	0.6
rs9948	2	96864527	CNNM3	0	0.685	0.308	5.69×10^{-06}	4.90	1	0.00133	0.083	0.583
rs5965182	23	65523418	HEPH	119463	0.615	0.12	6.25×10^{-06}	11.73	1	4.28×10^{-04}	0.183	0.55
rs17005499	2	121425911	GLI2	0	0.596	0.231	6.70×10^{-06}	4.92	1	0.00316	0	0.492
rs943371*	1	117269213	PTGFRN	0	0.558	0.202	7.17×10^{-06}	4.98	1	0.00167	0.017	0.533

SNP	Chr	BP Position	Nearest Gene	Distance to Gene (BP)	MAF Cases	MAF Controls	p-value	Odds Ratio	Bonferroni p-value	Ancestry adjusted p-value	MAF CEU	MAF YRI
rs5944185	23	25763535	MAGEB18	302849	0.667	0.16	7.26×10^{-06}	10.50	1	2.46×10^{-04}	0.25	0.667
rs219553	2	21431248	APOB	310798	0.204	0.582	7.30×10^{-06}	0.18	1	0.00151	0.783	0.233
rs6049375	20	24006407	GGTLC1	88991	0.648	0.276	7.68×10^{-06}	4.84	1	0.00124	0.058	0.717
rs5971305	23	28142974	WDR42B	233487	0.407	0.02	7.74×10^{-06}	33.69	1	0.00117	0.3	0.233
rs2806863*	1	117271036	PTGFRN	0	0.537	0.192	8.86×10^{-06}	4.87	1	0.00208	0.017	0.533
rs872690	23	37739671	SYTL5	0	0.259	0.029	9.29×10^{-06}	11.78	1	0.02662	0	0.1
rs13408245	2	122183723	MKI67IP	17301	0.482	0.154	9.80×10^{-06}	5.10	1	0.00327	0	0.342
rs6432484	2	14944498	FAM84A	250600	0.596	0.235	9.88×10^{-06}	4.80	1	0.00490	0.125	0.558

*and

†SNPs in linkage disequilibrium with $r^2>0.8$

Table 3

Logistic regression model of effects of clinical variables on post-radiotherapy ED.

	Univariate Analysis		Multivariate Analysis[#]	
	Odds Ratio	p-value	Odds Ratio	p-value
Age at diagnosis	0.98	0.639	1.02	0.732
Prostate cancer stage	1.74	0.201	2.16	0.164
Gleason Score	1.06	0.849	1.05	0.910
EBRT Dose	1.00	0.316	1.00	0.360
Hormone use	0.48	0.180	0.43	0.228
Pre-treatment SHIM score	1.20	0.040	1.29	0.016
Follow-up time	1.00	0.195	1.00	0.157
Diabetes	1.14	0.819	1.68	0.471
Hypertension	0.99	0.976	0.62	0.453
Asthma	1.50	0.614	2.14	0.413
Smoking status	0.68	0.516	0.92	0.905

[#] adjusting for all other covariates listed

Table 4

Logistic regression model of effects of genetic variables on post-radiotherapy ED.

	Multivariate Analysis[#]	
	Odds Ratio	**p-value**
rs2268363	20.01	0.006
rs10194115	33.12	0.013
rs2806864	11.09	0.014
rs10861905	107.45	0.006
African ancestry	0.459	0.765

[#] adjusting for all other covariates listed

Table 5

Prevalence of risk alleles among cases and controls.

	Cases N = 27* N(%)	Controls N = 52* N(%)
Homozygous for high-risk allele:		
1 SNP	14 (51.9%)	3 (5.8%)
2 SNPs	4 (14.8%)	0
3 SNPs	1 (3.7%)	0
4 SNPs	0	0
Heterozygous for high-risk allele:		
1 SNP	9 (33.3%)	22 (42.3%)
2 SNPs	10 (37.0%)	7 (13.5%)
3 SNPs	6 (22.2%)	0
4 SNPs	0	0
Homozygous or Heterozygous for high-risk allele:		
1 SNP	2 (7.4%)	23 (44.2%)
2 SNPs	9 (33.3%)	8 (15.4%)
3 SNPs	12 (44.4%)	0
4 SNPs	4 (14.8%)	0

* One case and two controls are missing data for rs10194115; three controls are missing data for rs2806864, three controls are missing data for rs10861905. Missing genotypes were conservatively counted as being homozygous for the low-risk (common) allele.