Radiotherapy and Oncology 107 (2013) 372–376

Contents lists available at SciVerse ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

GWAS in prostate cancer RT

Genome-wide association study identifies a region on chromosome 11q14.3 associated with late rectal bleeding following radiation therapy for prostate cancer ☆

Sarah L. Kerns [a,b], Richard G. Stock [a], Nelson N. Stone [a,c], Seth R. Blacksburg [a], Lynda Rath [a], Ana Vega [d] Laura Fachal [d], Antonio Gómez-Caamaño [e], Dirk De Ruysscher [f,g], Guido Lammering [g], Matthew Parliament [h], Michael Blackshaw [h], Michael Sia [i], Jamie Cesaretti [j], Mitchell Terk [j], Rosetta Hixson [j], Barry S. Rosenstein [a,k,l,m,*,1], Harry Ostrer [b,n,1]

[a] Department of Radiation Oncology, Mount Sinai School of Medicine, New York; [b] Department of Pathology, Albert Einstein College of Medicine, Bronx; [c] Department of Urology, Mount Sinai School of Medicine, New York, United States; [d] Fundación Pública Galega de Medicina Xenómica-SERGAS, Grupo de Medicina Xenómica-USC, CIBERER, IDIS; [e] Department of Radiation Oncology, Complexo Hospitalario Universitario de Santiago, SERGAS, Santiago de Compostela, Spain; [f] Department of Radiation Oncology, University Hospital Leuven, KU Leuven, Belgium; [g] Department of Radiation Oncology (Maastro Clinic/GROW), Maastricht University Medical Center, The Netherlands; [h] Division of Radiation Oncology, Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Canada; [i] Department of Radiation Oncology, Tom Baker Cancer Center, University of Calgary, Canada; [j] Florida Radiation Oncology Group, Jacksonville; [k] Department of Radiation Oncology, New York University School of Medicine; [l] Department of Dermatology; [m] Department of Preventive Medicine, Mount Sinai School of Medicine, New York; and [n] Department of Genetics, Albert Einstein College of Medicine, Bronx, United States

ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 8 April 2013
Accepted 1 May 2013
Available online 26 May 2013

Keywords:
Radiogenomics
Prostate cancer
Rectal toxicity
Genome-wide association study

ABSTRACT

Background and purpose: Rectal bleeding can occur following radiotherapy for prostate cancer and negatively impacts quality of life for cancer survivors. Treatment and clinical factors do not fully predict rectal bleeding, and genetic factors may be important.

Materials and methods: A genome-wide association study (GWAS) was performed to identify SNPs associated with the development of late rectal bleeding following radiotherapy for prostate cancer. Logistic regression was used to test the association between 614,453 SNPs and rectal bleeding in a discovery cohort (79 cases, 289 controls), and top-ranking SNPs were tested in a replication cohort (108 cases, 673 controls) from four independent sites.

Results: rs7120482 and rs17630638, which tag a single locus on chromosome 11q14.3, reached genome-wide significance for association with rectal bleeding (combined p-values 5.4×10^{-8} and 6.9×10^{-7} respectively). Several other SNPs had p-values trending toward genome-wide significance, and a polygenic risk score including these SNPs shows a strong rank-correlation with rectal bleeding (Sommers' $d = 5.0 \times 10^{-12}$ in the replication cohort).

Conclusions: This GWAS identified novel genetic markers of rectal bleeding following prostate radiotherapy. These findings could lead to the development of a predictive assay to identify patients at risk for this adverse treatment outcome so that dose or treatment modality could be modified.

 Radiotherapy and Oncology 107 (2013) 372–376

Prostate cancer is one of the most common cancers in the world, with approximately one million new cases diagnosed per year worldwide [1]. Prostate cancer can be successfully treated when detected early, by radiotherapy (brachytherapy or intensity-modulated radiation therapy (IMRT)) and/or surgery, and the risk of side effects becomes a deciding factor when choosing among treatment options [2]. Even with improvements in precision of radiation delivery, some men experience late adverse effects of radiotherapy for prostate cancer, including rectal bleeding. The incidence of rectal bleeding is dependent, in part, on dosimetry or underlying medical conditions. For example, a multivariable analysis of clinical and patient-specific factors among approximately 780 men treated under the UK Medical Research Council RT01 trial found that prescribed dose and age were significantly associated with risk of rectal toxicity [3]. But, variability in dose and demographic factors does not fully explain the variability in rectal bleeding, and prior studies suggest there are underlying genetic risk factors [4–5].

Previous studies have investigated associations between SNPs in candidate genes, mainly genes involved in DNA damage response and inflammation, and rectal toxicity in prostate cancer

☆ Collaboration developed under the framework of the Radiogenomics Consortium.

* Corresponding author. Address: Department of Radiation Oncology, Box 1236, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, United States.
E-mail address: barry.rosenstein@mssm.edu (B.S. Rosenstein).

[1] Equal contributions as senior authors to this manuscript.

0167-8140/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.radonc.2013.05.001

patients treated with radiotherapy [6]. However, most of these SNPs have either been investigated in only one study or have shown conflicting results across studies. Few, if any, of these associations have been replicated. A large validation study was recently published in which 92 SNPs in 46 genes were investigated in 637 prostate radiotherapy patients [7]. Five rectal endpoints (bleeding, proctitis, sphincter control, stool frequency, and tenesmus) were evaluated in patients two years after radiotherapy. None of the previously reported associations for candidate gene SNPs could be confirmed in this project.

In order to take a broader, unbiased approach, we carried out a two-stage GWAS to identify SNPs associated with late rectal bleeding following radiation therapy for prostate cancer.

Materials and methods

Patient characteristics

The study included men treated with definitive radiation for biopsy proven adenocarcinoma of the prostate (low, intermediate and high risk). Patients were recruited from the Mount Sinai Medical Center in the USA (MSMC), the Clinical University Hospital of Santiago de Compostela in Spain (CHUS), the Maastricht Radiation Oncology Clinic in the Netherlands (MAASTRO), the Cross Cancer Institute and Tom Baker Cancer Centre in Canada (CCI), and the Florida Radiation Oncology Group in the USA (FROG). The MSMC cohort served as a discovery set and the pooled CHUS, MAASTRO, CCI and FROG cohorts served as a replication set. The study was approved by the Institutional Review Boards of the respective study sites, and all men provided informed consent.

The MSMC discovery cohort included 764 patients, of which 368 were included in the GWAS (all cases and a randomly selected set of 289 controls). Unselected controls were similar to selected controls with respect to clinical and demographic variables (Table 1). Treatment included brachytherapy (206; 56%), brachytherapy with external beam radiation therapy (EBRT) (160; 43.5%), or EBRT alone (2, 0.5%). ^{125}I (160 Gy; TG-43) was used in patients who received brachytherapy alone and ^{103}Pd (124 Gy) in patients who also received EBRT. The EBRT prescription dose was 45 Gy in 25 fractions. All implants were performed using a real time ultrasound guided technique previously described [8–9]. 194 patients (52.7%) received hormonal therapy in addition to radiotherapy.

In the pooled replication set, the CHUS cohort consisted of 403 patients from the RADIOGEN trial treated with EBRT [10]. Total delivered dose ranged from 50 to 76 (median 74) Gy with 1.8–2.0 Gy per fraction. 311 (77.2%) patients also received hormonal therapy. The MAASTRO cohort consisted of 203 patients treated with EBRT alone (144 patients; 70.9%) prescribed to 68–72 Gy in 2.0 Gy fractions or with brachytherapy alone (59 patients; 29.1%) prescribed to 145 Gy. 111 patients (54.7%) also received hormonal therapy. The CCI cohort consisted of 153 patients treated with EBRT. Hypo-fraction regimens were given to 33 (21.6%) patients and consisted of 68 Gy in 25 fractions or 55 Gy in 16 fractions. Standard fraction was delivered to 120 (78.4%) patients with 72–82 Gy delivered in 1.8–2 Gy per fraction. Hormonal therapy was given to 77 (50.3%) patients. The FROG cohort consisted of 52 patients. The treatment regimen and technique were similar to the MSMC group. Treatment included ^{125}I brachytherapy (5 patients; 9.6%), ^{103}Pd brachytherapy with EBRT (38 patients; 73.1%), or EBRT alone (9 patients; 17.2%). 28 patients (53.8%) received hormonal therapy as part of their treatment. EBRT patients were all treated with IMRT technique. Total doses ranged from 64.8 to 81 Gy using 1.8 Gy fractions. Total biologically effective dose was calculated among all cohorts using previously published methods with an α/β of 2 [11].

All men were followed prospectively for the development of rectal bleeding. Included patients had at least one year of follow-up, and late (>90 days post-RT) rectal bleeding was considered. Rectal bleeding was assessed as follows: in the MSMC and FROG patients using the Radiation Therapy Oncology Group (RTOG) late radiation morbidity scoring schema; in the CHUS and CCI patients using National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 3; and in the MAASTRO patients using a patient self-report questionnaire, which was harmonized to the RTOG grading scheme (see Appendix for detailed descriptions). For all cohorts, rectal bleeding grade was assigned by a physician based on patient-reported description of symptoms. Care was taken to ensure comparability in case/control definitions across cohorts. Patients with grade 2 or higher rectal bleeding were considered cases and patients with grade 0 or 1 were considered controls.

Genotyping and quality control

Genomic DNA was isolated from lymphocytes. DNA from men in the discovery cohort was genotyped for ~900,000 SNPs using Affymetrix v6.0 genome-wide arrays, and genotypes were called using Genotyping Console (Affymetrix, Santa Clara, CA). DNA from men in the CHUS, MAASTRO and FROG replication cohorts was genotyped using Illumina iSelect custom SNP arrays, and genotypes were called by GenomeStudio (Illumina, San Diego, CA). DNA from men in the CCI cohort was genotyped using Affymetrix v6.0 genome-wide arrays, but these samples were only analyzed for SNPs included in the replication study and present on the Illumina array. SNPs were excluded from the analysis if missing in >5% of samples (137,589 SNPs in the discovery dataset; 14 SNPs in the replication dataset), if they had minor allele frequency (MAF) <5%

Table 1
Patient characteristics for the discovery and replication cohorts. BED, biologically effective dose; rectum V100, rectal volume receiving 100% of the prescription dose.

	Discovery cohort			Replication cohort	
	Cases = 79	Controls = 289	Unselected controls = 395	Cases = 108	Controls = 674
Age (yrs), mean(sd)	65 (7.3)	64 (7.3)	65 (7.3)	70 (7.0)	69 (6.8)
Follow-up (months), mean (sd)	49.8 (11.0)	47.6 (12.8)	46.0 (11.1)	40 (14.6)	41 (19.0)
Gleason, N (%)					
⩽6	51 (64.6%)	189 (65.4%)	231 (58.5%)	45 (55.6%)	324 (61.2%)
7	23 (29.1%)	73 (25.3%)	110 (38.1%)	23 (28.4%)	143 (27.0%)
⩾8	5 (6.3%)	27 (9.3%)	54 (13.7%)	13 (16.0%)	62 (11.7%)
Hormones, N (%)	42 (53.2%)	152 (52.6%)	208 (52.7%)	75 (69.4%)	427 (63.6%)
Treatment, N (%)					
Implant Only	39 (49.4%)	167 (57.8%)	221 (55.9%)	4 (3.7%)	59 (8.9%)
Implant + EBRT	38 (48.1%)	122 (42.2%)	164 (41.5%)	5 (4.6%)	33 (5.0%)
EBRT Only	2 (2.5%)	0	10 (2.5%)	99 (91.7%)	573 (86.2%)
Total BED (Gy2), mean (sd)	201.3 (23.6)	203.9 (22.1)	200.5 (28.0)	148.2 (36.2)	148.7 (30.8)
Rectum V100, mean (sd)	1.00 (0.76)	0.91 (0.71)	0.89 (0.87)	NA	NA