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Research Paper

Meta-analysis of Genome Wide Association Studies Identifies Genetic Markers of Late Toxicity Following Radiotherapy for Prostate Cancer

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ABSTRACT

Nearly 50% of cancer patients undergo radiotherapy. Late radiotherapy toxicity affects quality-of-life in long-term cancer survivors and risk of side-effects in a minority limits doses prescribed to the majority of patients. Development of a test predicting risk of toxicity could benefit many cancer patients. We aimed to meta-analyze individual level data from four genome-wide association studies from prostate cancer radiotherapy cohorts including 1564 men to identify genetic markers of toxicity. Prospectively assessed two-year toxicity endpoints (urinary frequency, decreased urine stream, rectal bleeding, overall toxicity) and single nucleotide polymorphism (SNP) associations were tested using multivariable regression, adjusting for clinical and patient-related risk factors. A fixed-effects meta-analysis identified two SNPs: rs17599026 on 5q31.2 with urinary frequency (odds ratio [OR] 3.12, 95% confidence interval [CI] 2.08–4.69, p-value 4.16×10^{-8}) and rs7720298 on 5p15.2 with decreased urine stream (OR 2.71, 95% CI 1.90–3.86, p-value $= 3.21 \times 10^{-8}$). These SNPs lie within genes that are expressed in tissues adversely affected by pelvic radiotherapy including bladder, kidney, rectum and small intestine. The results show that heterogeneous radiotherapy cohorts can be combined to identify new moderate-penetrance

Abbreviations: SNP, single nucleotide polymorphism; GWAS, genome-wide association study; EBRT, external bean radiotherapy; BED, biologic effective dose; MAF, minor allele frequency; STAT, standardized total average toxicity; PCA, principle components analysis; TURP, transurethral resection of the prostate; LD, linkage disequilibrium; ENCODE, encyclopedia of DNA elements; eQTL, expression quantitative trait locus; GTEx, Genotype-Tissue Expression project.

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genetic variants associated with radiotherapy toxicity. The work provides a basis for larger collaborative efforts to identify enough variants for a future test involving polygenic risk profiling.

1. Introduction

Radiotherapy is used in the treatment of up to 50% of cancer patients and around 40% of long-term cancer survivors underwent radiotherapy at some point in their treatment. For example, approximately half of the 1.1 million men diagnosed with prostate cancer worldwide each year receive radiotherapy, and the 5-year relative survival rates approach 100% for non-metastatic disease (Howlader et al., 2013). Although modern treatments minimize radiation doses to surrounding normal tissues, some men develop long-term toxicity (Bentzen et al., 2010). The risk of severe toxicity limits doses, which aim to keep the prevalence below 5%. Mild and moderate effects are common (10–50% of those treated) (Alemozaffar et al., 2011; Dearnaley et al., 2012; Heemsbergen et al., 2006; Kneebone et al., 2004; Resnick et al., 2013; Syndikus et al., 2010), impact negatively on quality-of-life, and are an important factor when men consider treatment options (Davison et al., 2002).

There is a need for a test that reflects a cancer patient's radiosensitivity and predicts the likelihood of toxicity. Many assays have been explored but none proved sufficiently reliable for clinical application. Over the past 15 years interest increased in identifying the genetic variants associated with risk of toxicity. The rationale behind the work is that a future test based on a germline polygenic risk score will not suffer from the poor reproducibility associated with other assays measuring radiosensitivity (Barnett et al. 2015).

Mutations associated with well-characterized radiosensitivity syndromes such as ataxia telangiectasia (Taylor et al., 1975) are rare and do not explain the general inter-individual variation in toxicity following radiotherapy (Safwat et al., 2002). Rather, it is hypothesized that common genetic variants, such as single nucleotide polymorphisms (SNPs) account for most of the heritability of radiosensitivity (West and Barnett, 2011). Studies have begun to identify common variants associated with radiotherapy toxicity. Candidate gene studies showed rs2868371 in *HSPB1* (MIM 602195) (Lopez Guerra et al., 2011; Pang et al., 2013) and rs1800469 in *TGFB1* (MIM 190180) (Guerra et al., 2012) are associated with late effects of lung radiotherapy; and rs1800629 in *TNF* (MIM 191160) (Talbot et al., 2012) and rs1139793 in *TXNRD2* (MIM 606448) (Edvardsen et al., 2013) are risk SNPs for late toxicity following breast radiotherapy. Genome-wide association studies (GWAS) identified a locus on chr11q14.3 associated with rectal bleeding (Kerns et al., 2013b) and a locus on chr2q24.1 within *TANC1* (MIM 611397) associated with overall toxicity (Fachal et al., 2014) following radiotherapy for prostate cancer. Another study showed more associations at the p-value $< 5 \times 10^{-7}$ level than expected by chance, providing the strongest evidence to date that many common genetic variants are associated with risk of toxicity (Barnett et al., 2014).

Recently published GWAS have limitations that we aimed to overcome by using a meta-analysis approach. The published studies used a multi-stage approach, where a small first-stage cohort was analyzed for a genome-wide panel of SNPs and only the most significant SNPs were genotyped in validation datasets. Thus, true positive SNPs were likely missed because they were not tested in the full set of individuals. Here, the Radiogenomics Consortium (West and Rosenstein, 2010) undertook a meta-analysis of four GWAS in order to maximize statistical power (Cohn and Becker, 2003) to discover additional risk variants. It is known that risk factors for late toxicity include not only genetics but also dosimetric parameters, co-morbidities, and patient demographics (Barnett et al. 2009). The latter factors can vary between cohorts as can the treatment (e.g. in prostate cancer: external beam or brachytherapy; type of fractionation – large or small doses per fraction; variable use of hormone therapy; variable use of surgery) and scales used to assess toxicity. There were concerns, therefore, whether the heterogeneity across cohorts might limit our ability to identify variants.

This study is important because our ability to identify enough SNPs for a risk profile for clinical implementation is dependent on combining multiple heterogeneous cohorts. The aim was to show that multi-center radiotherapy cohorts could be harmonized and analyzed to identify risk SNPs by increasing the number of individuals analyzed in a single stage (Skol et al., 2006). STROGAR guidelines (Kerns et al., 2014) for reporting radiogenomic studies, which build on the STREGA and STROBE guidelines (Little et al., 2009; von Elm et al., 2007), were followed throughout.

2. Subjects & Methods

2.1. Participants

The four cohorts (RAPPER, RADIOGEN, Gene-PARE, and CCI) comprised individuals with adenocarcinoma of the prostate treated with radiotherapy with curative intent. Table 1 shows the number of individuals in each cohort the number with genome-wide SNP data available, and the final number included in the GWAS meta-analysis after excluding samples for quality control or due to missing data. Informed consent was obtained from all study participants and all studies conform to standards indicated by the Declaration of Helsinki.

RAPPER (UKCRN1471; n = 727) (Burnet et al., 2006) was approved by the Cambridge South Research Ethics Committee (05/Q0108/365). Individuals received neoadjuvant androgen suppression and external beam radiotherapy, (EBRT): 233 from MRC RT01 (ISRCTN47772397) (Sydes et al., 2004) and 494 from CHHiP (ISRCTN97182923) (Dearnaley et al., 2012).

RADIOGEN (n = 741) was approved by the Galician Ethical Committee. Individuals received conformal radical or post-prostatectomy EBRT at the Clinical University Hospital of Santiago de Compostela, Spain (Fachal et al., 2012), and 511 individuals had hormone therapy.

Gene-PARE (n = 895) (Ho et al., 2006) was approved by the Mount Sinai Medical Center Institutional Review Board. Individuals had

Table 1
Number of individuals in each cohort.

	RAPPER	RADIOGEN	Gene-PARE	CCI
Total in cohort	727	741	895	155
Genotyped via genome-wide SNP chip	672	741	381	155
Excluded: >5% SNPs missing	18	1	8	1
Excluded: cryptic relatedness	14	19	16	0
Excluded: excess heterozygosity	19	–	–	–
Excluded: PCA outlier	–	68	–	–
Excluded: non-European ancestry based on PCA w/ HapMap samples	21	1	67	3
Excluded: lacking all 2 yr toxicity or essential covariate data	73	55	0	1
Number included in analysis of at least one toxicity endpoint	527	597[a]	290[b]	150[c]

[a] Additional follow-up of the RADIOGEN cohort increased the number with late toxicity data available from the 417 reported previously (Fachal et al., 2014). Of the 597 participants in RADIOGEN, one lacked data on rectal toxicity and seven data on rectal volume and were excluded from the analysis of rectal bleeding. 120 participants had no data on baseline urinary frequency and 119 were missing data on decreased urine stream and were excluded from the respective analyses.

[b] Of the 290 participants in Gene-PARE, 55 were lacking data for rectal volume and were excluded from analysis of rectal bleeding. 35 participants did not complete the urinary questionnaire and were excluded from analysis of urinary frequency and decreased urine stream.

[c] Of the 150 participants in CCI, 15 were lacking data for rectal volume or diabetes and were excluded from analysis of rectal bleeding.