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BIOLOGY CONTRIBUTION

No Association Between Polygenic Risk Scores for Cancer and Development of Radiation Therapy Toxicity

Gillian C. Barnett, PhD,[*] Sarah L. Kerns, PhD, MPH,[†] Leila Dorling, PhD,[‡] Laura Fachal, PhD,[‡,§] Miguel E. Aguado-Barrera, MD, PhD,[‖,¶] Laura Martínez-Calvo, PhD,[‖,¶] Harkeran K. Jandu,[#] Ceilidh Welsh,[**] Jonathan Tyrer,[‡] Charlotte E. Coles, PhD,[*] Joanne S. Haviland, MSc,[††] Christopher Parker, MD,[‡‡] Antonio Gómez-Caamaño, MD,[§§,‖‖] Patricia Calvo-Crespo, MD,[§§,‖‖] Paloma Sosa-Fajardo, MD,[§§,‖‖] Neil G. Burnet, MD,[¶¶] Holly Summersgill, PhD,[##] Adam Webb, PhD,[#] Dirk De Ruysscher, PhD,[***] Petra Seibold, PhD,[†††] Jenny Chang-Claude, PhD,[†††,‡‡‡] Christopher J. Talbot, PhD,[#] Tim Rattay, PhD,[§§§] Matthew Parliament, MD,[‖‖‖] Kim De Ruyck,[¶¶¶] Barry S. Rosenstein, PhD,[###] Paul D.P. Pharoah, PhD,[‡] Alison M. Dunning, PhD,[‡] Ana Vega, PhD,[§,‖,****] and Catharine M.L. West, PhD[##] on behalf of the RGC and REQUITE Consortia

[*]*Department of Oncology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, United Kingdom;* [†]*Department of Radiation Oncology, University of Rochester Medical Center, Rochester, New York;* [‡]*Centre for Cancer Genetic Epidemiology, University of Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom;* [§]*Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom;* [‖]*Fundación Pública Galega de Medicina Xenómica (FPGMX)-SERGAS, Santiago de Compostela, A Coruña, Spain;* [¶]*Grupo Genética en Cáncer y Enfermedades Raras, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Santiago de Compostela, A Coruña, Spain;* [#]*Department of Genetics and Genome*

Corresponding author: Gillian C. Barnett, PhD; E-mail: gill.barnett@addenbrookes.nhs.uk

The members of the REQUITE steering group are David Azria, Jenny Chang-Claude, Ananya Choudhury, Alison Dunning, Rebecca M. Elliott, Sara Gutiérrez-Enríquez, Tiziana Rancati, Tim Rattay, Barry S. Rosenstein, Dirk De Ruysscher, Petra Seibold, Elena Sperk, R. Paul Symonds, Hilary Stobart, Christopher J. Talbot, Ana Vega, Liv Veldeman, Adam Webb, and Catharine M. West.

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Biology, University of Leicester, Leicester, United Kingdom; [**]Department of Oncology, University of Cambridge, Cambridge, United Kingdom; [††]Clinical Trials and Statistics Unit, Institute of Cancer Research, London, United Kingdom; [‡‡]Institute of Cancer Research & Royal Marsden NHS Foundation Trust, London, United Kingdom; [§§]Department of Radiation Oncology, Complexo Hospitalario Universitario de Santiago, SERGAS, Santiago de Compostela, Spain; [‖‖]Instituto de Investigación Sanitaria de Santiago de Compostela, Spain; [¶¶]Proton Beam Therapy Centre, Christie NHS Foundation Trust, Manchester, United Kingdom; [##]Translational Radiobiology Group, Division of Cancer Sciences, University of Manchester, Manchester Academic Health Science Centre, Christie NHS Foundation Trust, Manchester, United Kingdom; [***]Department of Radiation Oncology (Maastro Clinic), Maastricht University Medical Center, GROW School for Oncology and Developmental Biology, Maastricht, The Netherlands; Radiation Oncology, Katholieke Universiteit Leuven, Leuven, Belgium; [†††]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; [‡‡‡]University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Germany; [§§§]Leicester Cancer Research Centre, University of Leicester, Leicester, United Kingdom; [‖‖‖]Division of Radiation Oncology, Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Canada; [¶¶¶]Departments of Basic Medical Sciences and Radiotherapy, Ghent University Hospital, Ghent, Belgium; [###]Departments of Radiation Oncology and Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York; and [****]Biomedical Network on Rare Diseases (CIBERER), Spain

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Purpose: Our aim was to test whether updated polygenic risk scores (PRS) for susceptibility to cancer affect risk of radiation therapy toxicity.

Methods and Materials: Analyses included 9,717 patients with breast (n=3,078), prostate (n=5,748) or lung (n=891) cancer from Radiogenomics and REQUITE Consortia cohorts. Patients underwent potentially curative radiation therapy and were assessed prospectively for toxicity. Germline genotyping involved genome-wide single nucleotide polymorphism (SNP) arrays with nontyped SNPs imputed. PRS for each cancer were generated by summing literature-identified cancer susceptibility risk alleles: 352 breast, 136 prostate, and 24 lung. Weighted PRS were generated using log odds ratio (ORs) for cancer susceptibility. Standardized total average toxicity (STAT) scores at 2 and 5 years (breast, prostate) or 6 to 12 months (lung) quantified toxicity. Primary analysis tested late STAT, secondary analyses investigated acute STAT, and individual endpoints and SNPs using multivariable regression.

Results: Increasing PRS did not increase risk of late toxicity in patients with breast (OR, 1.000; 95% confidence interval [CI], 0.997-1.002), prostate (OR, 0.99; 95% CI, 0.98-1.00; weighted PRS OR, 0.93; 95% CI, 0.83-1.03), or lung (OR, 0.93; 95% CI, 0.87-1.00; weighted PRS OR, 0.68; 95% CI, 0.45-1.03) cancer. Similar results were seen for acute toxicity. Secondary analyses identified rs138944387 associated with breast pain (OR, 3.05; 95% CI, 1.86-5.01; $P = 1.09 \times 10^{-5}$) and rs17513613 with breast edema (OR, 0.94; 95% CI, 0.92-0.97; $P = 1.08 \times 10^{-5}$).

Conclusions: Patients with increased polygenic predisposition to breast, prostate, or lung cancer can safely undergo radiation therapy with no anticipated excess toxicity risk. Some individual SNPs increase the likelihood of a specific toxicity endpoint, warranting validation in independent cohorts and functional studies to elucidate biologic mechanisms.

Introduction

An American Society for Radiation Oncology workshop in 2019 highlighted the lack of evidence to support genetic testing to inform clinical decisions and personalize radiation therapy.[1] Studies are identifying the radioresponsive tumor genome[2] and germline variants predisposing for risk of radiotoxicities.[3] Our interests lie in the latter. There is evidence that common germline variants such as single nucleotide polymorphisms (SNP) affect risk of toxicity,[4] but the workshop consensus was that the individual effects are too small to be clinically actionable. The future lies in finding multiple variants and including polygenic risk scores (PRS) in toxicity risk prediction models that incorporate dose and patient factors.[5]

PRS generated by summing risk-allele dosages from cancer susceptibility loci illustrate the potential. Individuals in the top centile of a 300-variant PRS for breast cancer have a 32.6% lifetime risk of the disease.[6] Similarly, a 147-variant PRS was associated with a relative risk for prostate cancer of 0.15 versus 5.71 for those in the lowest and highest centiles compared with the population average.[7] Genetic variants increasing susceptibility to cancer can lie in genes that modulate radiation response. Patients with an inherent compromised ability to repair DNA damage have a higher risk of developing cancer. DNA repair pathways play a central role in cellular response to radiation.[8] It has previously been hypothesized that individuals with an increased genetic predisposition to cancer would have increased risks of radiation therapy toxicity.[9,10] It has been shown that high PRS for breast (90-loci)[11] or prostate (75-loci)[12] cancer did not increase risk of toxicity.

Consortia recently increased the number of breast[13] and prostate[7] cancer variants, and it is now possible to generate a PRS for lung cancer with 25 susceptibility loci identified.[14-18] We expect that cancer predisposition PRS would confer only small increased risks for toxicity. Given the availability of additional cohorts for testing and the

increased number of variants available to generate PRS for breast, prostate, and lung cancer, we decided to revisit the initial hypothesis in a more definitive analysis. Our primary objective was to determine whether PRS for breast, prostate and lung cancer susceptibility are associated with an overall measure of late radiation therapy toxicity. Secondary objectives were to explore associations with early toxicity, and between individual SNPs and toxicity endpoints.

Methods and Materials

Patients

The study design was a retrospective analysis of multiple prospectively recruited trial or observational cohorts. Inclusion criteria were breast, prostate, or lung cancer; radiation therapy alone or as part of a curative-intent treatment; and availability of prospectively collected toxicity. All participants gave written informed consent for use of their data in research. Supplementary Methods provide details of the cohorts and ethical approvals. There were 3074, 5731, and 891 patients with breast, prostate, and lung cancer, respectively, of European descent. Demographic and clinical characteristics are in Table E1.

Radiation therapy toxicity

Overall toxicity was represented by unweighted standardized total average toxicity (STAT) scores.[19] Tables E2 to E4 list the individual toxicity endpoints used to generate STAT scores in breast, prostate, and lung cancer cohorts, respectively. The primary analysis tested late STAT scores ($STAT_{late}$) ~2 and 5 years (breast, prostate) or ~1 year (lung) after radiation therapy. Supplementary Methods and Tables E2 to E4 provide details on the approaches used to collect toxicity data. Secondary analyses used acute STAT scores ($STAT_{acute}$; 3 months after radiation therapy start) and individual toxicity endpoints. STAT scores used the worst grade recorded for each considered endpoint within each timeframe. Before calculating STAT scores in prostate patients, individual toxicity endpoints were adjusted for baseline symptoms (Tables E2-E4).

Genotyping and calculation of PRS

Supplementary Methods describe the genotyping platforms and imputation approaches. Individual patient PRS were calculated by summing the number of risk alleles for breast (n = 352), prostate (n = 136 available out of 147 published), or lung (n = 24) cancer within the respective radiation therapy cohorts. Table E5 lists the SNPs used and where they were identified. In patients with prostate and lung cancers, we generated unweighted and weighted PRS. Weighted PRS were derived by multiplying the allele dosage for each SNP by the published log odds ratio for cancer susceptibility. In patients with breast cancer, only unweighted PRS were calculated as weighting required estrogen receptor status, which was not available. For the breast cohort the analysis was repeated using the SNP list from Mavaddat et al,[6] obtained by a modeling rather than a fine-mapping approach.

Statistical analyses

Supplementary Methods list the clinical covariates used in multivariable analyses. Associations between PRS and $STAT_{late}$ were tested using linear regression analysis of residuals generated after first regressing clinical covariates on $STAT_{late}$. The same approach tested associations with $STAT_{acute}$. To obtain more clinically interpretable effects, STAT scores were dichotomized at the mean plus one standard deviation. Logistic regression tested associations of dichotomized STAT scores with unweighted/weighted PRS. Associations were considered statistically significant if $P < .05$.

Secondary analyses of individual toxicity endpoints involved severity of grade and ordinal regression (breast) or dichotomized grade (0/1 vs ≥2) and logistic regression (prostate and lung), due to the low frequency of high-grade events and/or heterogeneity in grading schema across cohorts. Secondary analyses of individual SNPs followed the same approaches used for the PRS. Associations with individual toxicity endpoints were deemed statistically significant if the false discovery rate, calculated using the Benjamini-Hochberg Procedure, was <0.05.

Results

PRS were normally distributed (Fig. 1). Table 1 shows no statistically significant associations between PRS and $STAT_{late}$ in patients with breast and prostate cancer. This finding remained 5 years after radiation therapy in patients with breast and prostate cancer (Table E6). Increasing lung cancer PRSs were associated with a statistically significant lower $STAT_{late}$ (Table 1) in both univariable and multivariable analyses, indicating a protective effect. The estimated per-allele odds ratios from the multivariable analyses of PRS and $STAT_{late}$ dichotomized at the mean plus one standard deviation were 1.01 (95% confidence interval [CI], 1.00-1.02; $P = .07$), 0.99 (95% CI, 0.98-1.00; $P = .15$), and 0.93 (95% CI, 0.87-1.00; $P = .046$) for breast, prostate, and lung cancer, respectively.

There was insufficient evidence for an association between PRS and increased $STAT_{acute}$ (Table 2) or individual toxicity endpoints (Table 3) in patients with breast, prostate, and lung cancers on multivariable analysis. On the contrary, the prostate weighted PRS was associated with lower $STAT_{acute}$ (beta, −0.032; 95% CI, −0.056 to −0.0088). The significant association between the lung cancer PRS and $STAT_{acute}$ on univariable analysis was lost after adjusting for covariates. $STAT_{acute}$ scores dichotomized at the

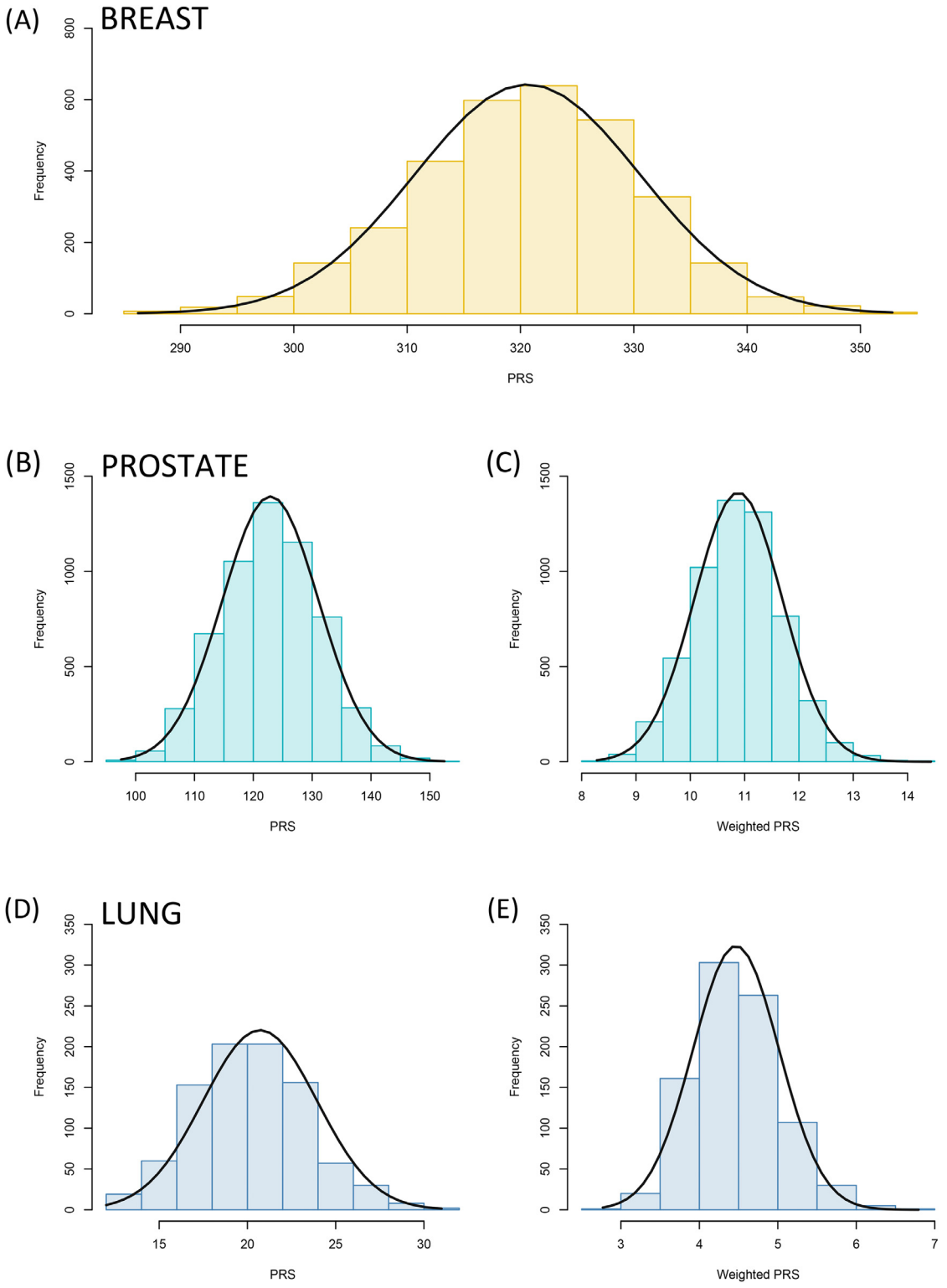

Fig. 1. Histograms for the nonweighted PRS in (A) breast cohorts, (B) prostate cohorts, and (D) lung cohorts and for the weighted PRS in (C) prostate cohorts and (E) lung cohorts. *Abbreviation*: PRS = polygenic risk scores.

Table 1 Association between PRS and $STAT_{late}$ in patients with breast, prostate, and lung cancers

	Univariable analysis		Multivariable analysis[*,†,‡]	
	Beta (95% CI)	*P*	Beta (95% CI)	*P*
Breast cancer n = 3133	PRS: −0.0002 (−0.0021, 0.0017)	.83	PRS:−0.0005 (−0.0026, 0.0016)	.67
Prostate cancer n = 4861	PRS: −0.0003 (−0.0018, 0.0012)	.70	PRS: −0.0002 (−0.0016, 0.0013)	.82
	wPRS: −0.0074 (−0.0220, 0.0071)	.31	wPRS: −0.0060 (−0.0203, 0.0083)	.38
Lung cancer n = 621	PRS: −0.0123 (−0.0245, −0.0001)	.05	PRS: −0.0139 (−0.0259, −0.002)	.02
	wPRS: −0.0739 (−0.1453, −0.0025)	.04	wPRS: −0.0847 (−0.1544, −0.015)	.02

$STAT_{late}$ was calculated using 2-year toxicity in patients with breast and prostate cancers, and 1-year toxicity in patients with lung cancer. Results for the wPRS are shown for the prostate and lung cohorts in which this score was available.

Abbreviations: CI = confidence interval; PRS = polygenic risk scores; wPRS = weighted polygenic risk scores.

[*] In patients with breast cancer, covariates included in multivariable analysis (MVA) were breast volume, patient age, presence of cardiovascular disease, smoker status, weight of the surgical specimen after surgery, cosmesis assessed after surgery but before radiation therapy, presence of postoperative hematoma or infection, breast volume receiving >107% of the prescribed dose, delivery of a radiation therapy boost, acute toxicity, tamoxifen, and chemotherapy use.

[†] In the prostate cancer cohorts, covariates included in MVA were age at radiation therapy, total biologic effective dose, use of androgen deprivation therapy, and prior prostatectomy.

[‡] In the lung cancer cohorts, covariates included in MVA: Study, sex, age, smoking status, concurrent chemotherapy, radiation therapy technique, FEV_1, V20 lung, and V35 esophagus.

Table 2 Association between PRS and $STAT_{acute}$ in patients with breast, prostate, and lung cancers

	Univariable analysis		Multivariable analysis[*,†,‡]	
	Beta (95% CI)	*P*	Beta (95% CI)	*P*
Breast cancer n = 2755	PRS 0.0033 (−0.0002, 0.0067)	.06	PRS 0.0022 (−0.0011, 0.0055)	.20
Prostate cancer n = 3947	PRS: −0.0026 (−0.0050, 0.0002)	.04	PRS: −0.0022 (−0.0044, 0.0001)	.06
	wPRS: −0.0333 (−0.0575, 0.0091)	.01	wPRS: −0.0324 (−0.0560, −0.0088)	.01
Lung cancer n = 619	PRS: 0.0190 (0.0018, 0.0361)	.03	PRS: 0.0034 (−0.0099, 0.0167)	.62
	wPRS: 0.0882 (−0.0126, 0.189)	.09	wPRS: 0.0138 (−0.0642, 0.0918)	.73

Results for the wPRS are shown for the prostate and lung cohorts in which this score was available.

Abbreviations: CI = confidence interval; PRS = polygenic risk scores; wPRS = weighted polygenic risk scores.

[*] In patients with breast cancer, covariates included in multivariable analysis (MVA) were age, body mass index, smoker status, cardiovascular disease, axillary surgery, chemotherapy, breast volume, radiation therapy boost, and postoperative infection.

[†] In the prostate cancer cohorts, covariates included in MVA were age at radiation therapy, total biologic effective dose, use of androgen deprivation therapy, and prior prostatectomy.

[‡] In the lung cancer cohorts, covariates included in MVA were study, sex, age, smoking status, concurrent chemotherapy, radiation therapy technique, FEV_1, V20 lung, and V35 esophagus.

mean plus one standard deviation yielded estimated PRS per-allele odds ratios of 1.00 (95% CI, 0.99-1.02; $P = .95$), 1.00 (95% CI, 0.99-1.01; $P = .44$), and 1.01 (95% CI, 0.94-1.08; $P = .79$) for breast, prostate, and lung cancer, respectively. There were no associations between PRS and individual acute toxicity endpoints in patients with lung cancer (Table E7) for whom data on individual acute toxicity endpoints was available across all cohorts. Repeating the breast analyses using the modelled PRS developed by Mavaddat et al[6] identified no associations in multivariable analyses (Table E8).

Multivariable analysis of individual SNPs identified rs138944387 associated with breast pain at 2 years (beta, 1.12; 95% CI, 0.62-1.61; $P = 1.09 \times 10^{-5}$) and rs17513613 associated with breast edema at 2 years (beta, −0.059; 95% CI, −0.086 to −0.033; $P = 1.14 \times 10^{-5}$) (Table 4). rs17513613 was not significant at 5 years, but the SNP remained protective (beta, −0.06; 95% CI, −0.18 to 0.048; $P = .25$). We found no statistically significant associations between individual SNPs and prostate or lung toxicity endpoints.

Discussion

Patients with cancer with a higher polygenic predisposition to breast, prostate, or lung cancer calculated using an updated polygenic risk score do not have an increased risk of radiation therapy toxicities. It is possible that an individual with an increased genetic risk of cancer incidence may

Table 3 Association between PRS and individual toxicity endpoints assessed at 2 years after radiation therapy in patients with breast and prostate cancers and 1 year after radiation therapy in patients with lung cancer

		Univariable analysis[†]		Multivariable analysis[‡,§,‖,¶]		
Toxicity endpoint	Total n (toxicity[*])	Beta (95% CI)	*P*	Beta (95% CI)	*P*	FDR *P*
Breast cancer						
Telangiectasia	2858 (191/58/19)	PRS: −0.0071 (−0.014, −0.00026)	.04	PRS: −0.0003 (−0.0014, 0.0008)	.20	.70
Edema	2858 (461/131/33)	PRS: 0.0069 (0.0064, 0.0073)	<.01	PRS: 0.0007 (−0.0011, 0.0025)	.44	.73
Shrinkage (photos)[#]	934 (285/56)	PRS: 0.0052 (−0.0086, 0.019)	.46	PRS: 0.0011 (−0.0053, 0.0076)	.73	.73
Induration	2902 (1022/329/77)	PRS: −0.0010 (−0.010, −0.0097)	<.01	PRS: −0.0032 (−0.0057, −0.0007)	.012	.08
Pigmentation	2690 (380/71)	PRS: 0.00091 (0.00051, 0.0013)	<.01	PRS: 0.0005 (−0.0013, 0.0024)	.56	.73
Pain	834 (362/45/11)	PRS: 0.0057 (−0.0075, 0.019)	.40	PRS: 0.0027 (−0.0041, 0.0094)	.44	.73
Oversensitivity	836 (278/41/10)	PRS: 0.0018 (−0.012, 0.015)	.80	PRS: 0.0016 (−0.0054, 0.086)	.66	.73
Prostate cancer						
Rectal bleeding	4104 (583)	PRS: −0.0035 (−0.0146, 0.0076)	.54	PRS: −0.0003 (−0.0016, 0.0010)	.68	.88
		wPRS: −0.0555 (−0.1649, 0.0538)	32	wPRS: −0.0051 (−0.0183, 0.0081)	.45	.88
Increased urinary frequency	4447 (999)	PRS: −0.0014 (−0.0108, 0.0079)	.76	PRS: −0.0002 (−0.0017, 0.0012)	.77	.88
		wPRS: 0.0318 (−0.0606, 0.1242)	.50	wPRS: 0.0046 (−0.0099, 0.0190)	.54	.88
Decreased urinary stream	4194 (600)	PRS: 0.0163 (−0.0022, 0.0349)	.09	PRS: 0.0015 (−0.0002, 0.0033)	.09	.56
		wPRS: 0.1220 (−0.0660, 0.3099)	.20	wPRS: 0.0142 (−0.0049, 0.0333)	.14	.56
Hematuria	4483 (123)	PRS: 0.0083 (−0.0147, 0.0314)	.48	PRS: 0.0001 (−0.0005, 0.0007)	.75	.88
		wPRS: 0.0593 (−0.1674, 0.2859)	.61	wPRS: 0.0001 (−0.0057, 0.0059)	.97	.97
Lung cancer						
Cough	623 (61)	PRS: −0.1648 (−0.253, −0.0765)	<.001	PRS: −0.1751 (−0.2862, −0.0639)	.002	.33
		wPRS: −0.8254 (−1.3417, −0.3091)	.002	wPRS: −0.9496 (−1.6002, −0.2991)	.004	.47
Dyspnoea	621 (144)	PRS: −0.0626 (−0.1213, −0.004)	.036	PRS: −0.1484 (−0.2874, −0.0093)	.04	.69
		wPRS: −0.3221 (−0.6653, 0.021)	.07	wPRS: −0.8250 (−1.6381, −0.012)	.047	.69
Pneumonitis	607 (48)	PRS: −0.0897 (−0.1833, 0.004)	.06	PRS: −0.0748 (−0.1763, 0.0267)	.15	.74
		wPRS: −0.5029 (−1.0557, 0.0499)	.07	wPRS: −0.3891 (−0.982, 0.2038)	.20	.80
Dysphagia	623 (18)	PRS: −0.0975 (−0.2472, 0.0522)	.20	PRS: 0.0186 (−0.1389, 0.1761)	.82	1.00
		wPRS: −0.5886 (−1.4771, 0.2999)	.19	wPRS: −0.0374 (−0.9582, 0.8834)	.94	1.00

(*Continued*)

Table 3 (*Continued*)

		Univariable analysis[†]		Multivariable analysis[‡,§,‖,¶]		
Toxicity endpoint	**Total n (toxicity[*])**	**Beta (95% CI)**	***P***	**Beta (95% CI)**	***P***	**FDR *P***
Esophagitis	606 (24)	PRS: −0.0395 (−0.1664, 0.0874)	.54	PRS: −0.0542 (−0.1861, 0.0776)	.42	.90
		wPRS: −0.3554 (−1.1101, 0.3993)	.36	wPRS: −0.3681 (−1.1378, 0.4017)	.35	.89

Abbreviations: CI = confidence interval; FDR = false discovery rate; PRS = polygenic risk score; wPRS = weighted polygenic risk score.
[*] In the breast cancer cohorts, numbers with toxicity are grade 1, grade 2, and grade 3 respectively; in prostate and lung cohorts, the numbers represent those with grade 2 or worse toxicity combined. All the available samples for each individual endpoint were included.
[†] Results are from ordinal logistic regression (breast) or binary logistic regression (prostate, lung) models of toxicity as the dependent variable with PRS or wPRS (prostate and lung only) as the independent variable.
[‡] Results are from linear regression models of residuals generated after first regressing clinical covariates on the given toxicity outcome and cohort.
[§] Photographic assessment of late shrinkage and distortion was graded as none/minimal, mild or marked.
[‖] In patients with breast cancer, covariates included in multivariable analysis (MVA) were breast volume, patient age, presence of cardiovascular disease, smoker status, weight of the surgical specimen after surgery, cosmesis assessed after surgery but before radiation therapy, presence of postoperative hematoma or infection, breast volume receiving >107% of the prescribed dose, delivery of a radiation therapy boost, acute toxicity, tamoxifen, or chemotherapy use.
[¶] In the prostate cancer cohorts, covariates included in MVA were age at radiation therapy, total biologic effective dose, use of androgen deprivation therapy, and prior prostatectomy.
[#] In the lung cancer cohorts, covariates included in MVA: Study, sex, age, smoking status, concurrent chemotherapy, radiation therapy technique, FEV_1, V20 lung, and V35 esophagus.

Table 4 Multivariable analyses of the association between individual SNPs and late toxicity in patients with breast cancer

Genetic Variant	Late toxicity endpoint[*]	Minor allele	EAF[†]	OR (95% CI)	Beta (95% CI)	*P* value[‡]	FDR *P*
rs138944387	Pain at 2 y	T	0.011	3.05 (1.86, 5.01)	1.12 (0.62, 1.61)	1.09×10^{-5}	.004
rs17513613	Edema at 2 y	T	0.33	0.94 (0.92, 0.97)	−0.060 (−0.086, −0.033)	1.08×10^{-5}	.004

Abbreviations: EAF = effect allele frequency; FDR = false discovery rate; OR = odds ratio; SNP = single nucleotide polymorphism.
[*] Breast pain was not measured at 5 years.
[†] EAF in Oncoarray BCAC cohorts.
[‡] Covariates included in multivariable analysis were breast volume, patient age, presence of cardiovascular disease, smoker status, weight of the surgical specimen after surgery, cosmesis assessed after surgery but before radiation therapy, presence of postoperative hematoma or infection, breast volume receiving >107% of the prescribed dose, delivery of a radiation therapy boost, acute toxicity, tamoxifen, or chemotherapy use.

also have an increased risk of a radiation-induced second malignancy. There is a need to look for an association between polygenic risk scores for cancer incidence and risk of second cancer after radiation therapy. SNPs included in the different PRS may be specific to cancer incidence at that site. For example, 8 variants included in the PRS for breast cancer patients were located near the *Estrogen receptor 1* (*ESR1*) gene (Table E5). It would be interesting to study SNPs which are associated with cancer incidence at more than one site, to look for an association with radiation therapy toxicity in all patients.

The associations between individual SNPs and late toxicity in the breast cohorts may point to novel radiobiological mechanisms pending validation in independent cohorts. These individual SNP data are hypothesis-generating and should be investigated for validation for their potential to include in PRS for radiation therapy toxicity.

It is not currently known whether all the individual SNPs found in GWAS studies regulate phenotypes or are merely accidently associated with them, radiogenomics GWAS have progressed to identify credible causal variants.[3,20] Recent genotyping studies of cancer incidence have incorporated gene expression data, chromatin interaction and functional annotations to prioritize genes as targets of these causal variants.[13] Known cancer drivers, transcription factors and genes in the developmental, apoptosis, immune system, and DNA integrity checkpoint gene ontology pathways are among the highest-confidence target genes.

A high PRS appeared protective in the lung cancer cohorts. Smoking has been shown to reduce the risk of radiation pneumonitis in some but not all studies.[21] Studies have shown chronic cigarette smoke exposure leads to systemic hypoxia[22] and cigarette smoking decreases tissue oxygen acutely.[23,24] Therefore, it is plausible that smoking may reduce the risk of pulmonary toxicity due to decreased DNA damage occurring in the normal lung tissue in the presence of hypoxia. Radiation-induced pulmonary toxicity is a multifactorial process, which is not fully elucidated. Besides biologic processes that are influenced by smoking, it is possible that current smokers have more pulmonary comorbidities such as COPD with therefore more nonfunctional space in the lungs, resulting in less active lung tissue

irradiated.[21] Some of the lung cancer incidence SNPs are associated with nicotine dependence, and therefore may be associated with smoking status, which might indirectly explain the protective effect.

Unfortunately, the low proportion of nonsmoking patients in our study (>95% smokers/ex-smokers) prevented testing using a stratified analysis. Larger cohorts are required to test whether SNPs associated with nicotine dependence in smokers with lung cancer confers a protective effect for developing radiation toxicity.

The STAT score combines the toxicity endpoints weighted equally. However, endpoints may not be equally important. In view of the fact that the STAT score was developed to facilitate the analysis of toxicity from multiple trials or centers using different endpoints, it was felt on balance weighting would not be possible if certain key endpoints are missing in some data sets but not others.[19]

The strengths of our study include the large cohort sizes, prospective collection of toxicity data, and analysis of PRS rather than just a few cancer susceptibility SNPs. The limitations include inevitable heterogeneity between treatment and toxicity assessment between cohorts. Lack of functional data continues to be a significant limitation in radiogenomic studies. Because the PRS were developed in largely European ancestry cohorts; we limited our analysis of radiation toxicity to Europeans. As PRS are updated following multiethnic analyses of cancer susceptibility, it will be important to revisit our hypothesis in multiethnic radiation therapy cohorts. Our overall conclusion is that PRS for cancer susceptibility have no clinically meaningful role in the development of risk models for radiation therapy toxicity.

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