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Original Article

Meta-GWAS identifies the heritability of acute radiation-induced toxicities in head and neck cancer



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ABSTRACT

Background and purpose: We aimed to the genetic components and susceptibility variants associated with acute radiation-induced toxicities (RITs) in patients with head and neck cancer (HNC).

Materials and methods: We performed the largest meta-GWAS of seven European cohorts ($n = 4,042$). Patients were scored weekly during radiotherapy for acute RITs including dysphagia, mucositis, and xerostomia. We analyzed the effect of variants on the average burden (measured as area under curve, AUC) per each RIT, and standardized total average acute toxicity ($STAT_{acute}$) score using a multivariate linear regression. We tested suggestive variants ($p < 1.0 \times 10^{-5}$) in discovery set (three cohorts; $n = 2,640$) in a replication set (four cohorts; $n = 1,402$). We meta-analysed all cohorts to calculate RITs specific SNP-based heritability, and effect of polygenic risk scores (PRSs), and genetic correlations among RITs.

Results: From 393 suggestive SNPs identified in discovery set; 37 were nominally significant ($p_{replication} < 0.05$) in replication set, but none reached genome-wide significance ($p_{combined} < 5 \times 10^{-8}$). *In-silico* functional analyses identified “3'-5'-exoribonuclease activity” (FDR = $1.6e-10$) for dysphagia, “inositol phosphate-mediated signalling” for mucositis (FDR = $2.20e-09$), and “drug catabolic process” for $STAT_{acute}$ (FDR = $3.57e-12$) as the most enriched pathways by the RIT specific suggestive genes. The SNP-based heritability (\pm standard error) was 29 ± 0.08 % for dysphagia, 9 ± 0.12 % (mucositis) and 27 ± 0.09 % ($STAT_{acute}$). Positive genetic correlation was $r_g = 0.65$ ($p = 0.048$) between dysphagia and $STAT_{acute}$. PRSs explained limited variation of dysphagia (3 %), mucositis (2.5 %), and $STAT_{acute}$ (0.4 %).

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Conclusion: In HNC patients, acute RITs are modestly heritable, sharing 10 % genetic susceptibility, when PRS explains < 3 % of their variance. We identified numerous suggestive SNPs, which remain to be replicated in larger studies.

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Introduction

Radiotherapy (RT) is often used to treat head and neck cancer (HNC), but it causes acute and late radiation-induced toxicities (RITs). Acute RITs usually appear during treatment and resolve 90 days after commencing RT; the most common acute RITs are dysphagia, mucositis, and xerostomia [1]. Large patient-to-patient variability exists regarding RITs. Although some is due to patient-related characteristics (e.g., age) and other treatments (e.g., chemotherapy), underlying genetic susceptibility probably explains much of the variability [2]. Studies suggested a 58 % to 78 % heritability for cell response to irradiation [3–4]. However, genetic susceptibility, heritability, and predictability of for RITs have not been investigated in HNC patients.

An earlier experimental study showed radiation hypersensitivity in individuals with genetic disorders, such as ataxia-telangiectasia, Nijmegen breakage syndrome, Fanconi anaemia and DNA ligase IV deficiency [5]. Two candidate gene studies showed associations between single nucleotide polymorphisms (SNPs) in genes involved in DNA damage response (*XRCC1*, *RAD51*, *NBN*) with mucositis and dysphagia in HNC patients [6–7]. Several genome-wide association studies (GWASs) recently identified SNPs associated with RITs in breast and prostate cancer patients [8]. For HNC, two GWASs found significant associations between two loci on chromosome five with acute mucositis [9] and xerostomia [10]. A Chinese GWAS found 65 genes suggestive mapped to 50 loci associated with mucositis [11]. These studies were small ($N < 1,500$) bearing insufficient statistical power to detect small effects of SNPs. Here, we aimed to unfold the genetic components of and to identify SNPs associated with acute RITs by performing the first multi-national meta-GWAS of 4,042 HNC patients of European ancestry. We investigated (SNP-based) heritability, the genetic correlations between acute RITs, and built polygenic risk scores (PRSs) for prediction of acute RITs in HNC.

Methods

Study design

We included seven Caucasians prospectively collected cohorts in meta-GWAS (Supplementary Fig. 1), and one East Asian (Singapore) cohort evaluated transferability to an East Asian population (Supplementary Methods & Table 1). The cohorts were split into discovery (UMCG-HANS, $n = 1,279$; DAHANCA, $n = 1,183$; RADIOGEN, $n = 178$) and replication (Ghent, $n = 273$; RAPPER, $n = 457$; Head and Neck 5000, $n = 672$) sets based on the availability of data at the time of conduct. These sets were combined to calculate SNP-based heritability, estimate genetic correlations between RITs, and build PRSs (Supplementary Figure S1). Each contributing study received ethics approval from local institutional review boards, and all participants gave written informed consent.

Study participants

We included 4,042 Caucasians and 180 East Asian HNC patients who received curative-intent RT and were scored prospectively for RITs (see Supplementary Methods).

Assessment of acute RITs

Dysphagia, mucositis, and xerostomia were scored weekly during RT by the treating radio-oncologists. Data were collected before, during and up to six weeks post RT. Physician-rated HNC symptoms were assessed according to the Common Toxicity Criteria of Adverse Events (CTCAE, version 2.0 or 4.0). Patient-rated HNC symptoms were assessed using the EORTC-QLQ-C3020 and LENT SOMA questionnaires in the Head and Neck 5000 cohort (Supplementary Table S1). Supplementary Tables S2 and S3 describe the definition, scoring, and availability of acute RITs per cohort.

Multiple imputations of missing value for toxicity

We applied multiple imputation (MI) as implemented in MICE package [12] (details in Supplementary Methods) to impute missing values (Supplementary Table S4).

Outcome modelling

We applied two different scoring systems. First, since risk of acute RIT generally increases during RT, we used area under curve (AUC) to estimate the average burden of an acute RIT during RT (from week one to week seven) per patient (s for formulas). Second, we used the standardized total average toxicity (STAT) score [13] to achieve a composite score describing the general acute RIT (see Supplementary Method and Supplementary Table S2).

Genotyping, quality control, and imputation of non-genotyped variants

Germline DNA from the whole blood of 4,042 patients was genotyped using commercially available genome-wide SNP arrays (Supplementary Table S5). Quality control (QC) procedures were performed using standard procedures in each cohort. First, SNPs and samples with call rates below 95 to 98 % and SNPs with $MAF < 1\%$ were removed. We assessed gender mismatch using the actual X chromosome homozygosity index (F) of > 0.8 representing for the male gender, and an F of < 0.2 represents a female gender. Relatedness was evaluated by pairwise identity by descent (IBD) values when duplicate samples were considered by a $pi_{hat} > 0.8$ who were removed, and the remaining pairs were manually checked. We checked the ethnicity of subjects using multidimensional scaling (MDS) clustering of our samples with Hapmap Phase 3 individuals using EIGENSTRAT. Samples that deviated more than 3 SD from the mean of their closest clusters were removed. Furthermore, individuals were assigned to populations based on principal component analysis (PCA). PCA was performed using EIGENSTRAT. The top 10 PCA eigenvectors were included in the final models for all endpoints. Next, strand ambiguous SNPs and duplicate SNPs were removed. Finally, SNPs were imputed either on the Michigan server using the HRC r1.1 2016 reference panel or 1000 Genome Phase 3 with European samples. We performed post-imputation QC involved removing SNPs with an imputation quality (info) score of $R [2] < 0.3$, or with a MAF of < 0.01 or < 0.05 , and SNPs that had a discordant MAF (maximum allowed difference < 0.15) compared to the reference panel, or strand ambiguity AT/CG SNPs, or multi-allelic SNPs (Supplementary Table S5).

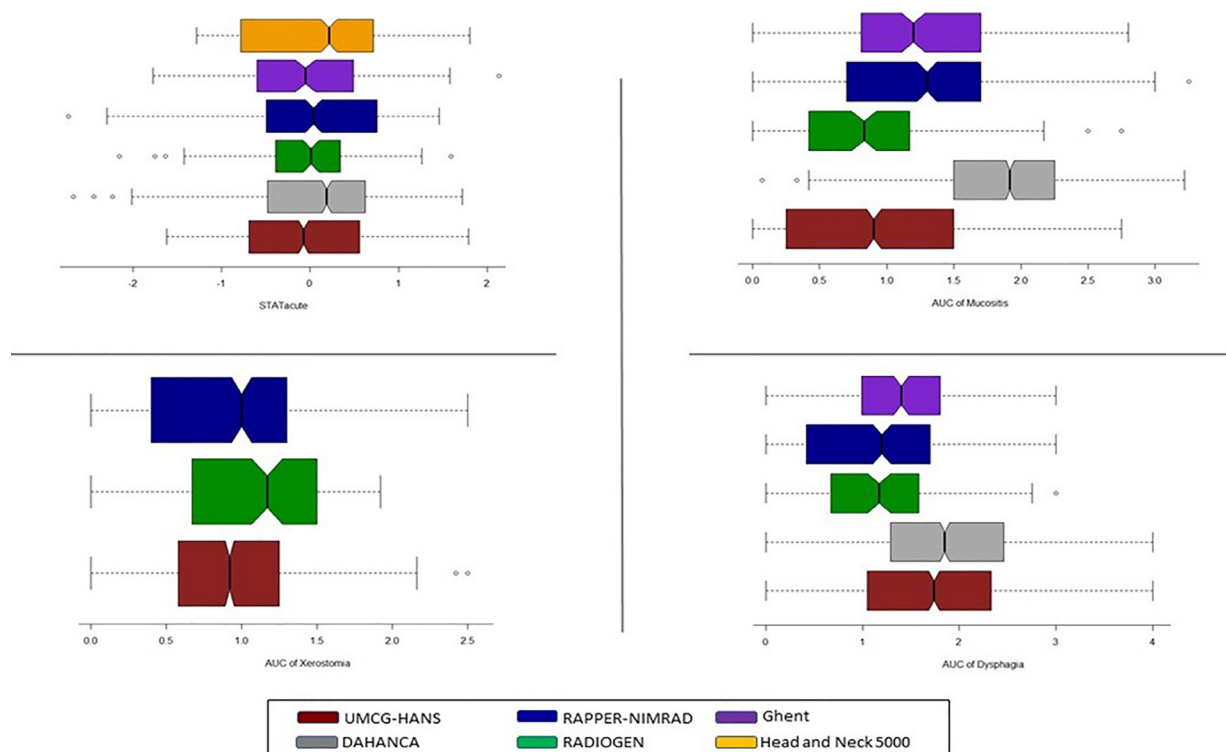


Fig. 1. The dispersion of AUCs and $STAT_{acute}$ of RIT endpoints in HNC patients by discovery and replication cohorts. The Y-axis show the cohorts, and X-axes show the measured value of endpoints. Each color represents a cohort. The lowest line represents the minimum (Q0 or 0th percentile); the top line represents the maximum (Q4 or 100th percentile) data point excluding any outliers; The middle line represents the median (Q2 or 50th percentile); the box represents the interquartile range (IQR) which is the distance between the first quartile (Q1 or 25th percentile; that is the median of the lower half of the dataset) and the third quartile (Q3 or 75th percentile that is the median of the upper half of the dataset).

Clinical factors

Following expert consensus within RgC consortium, variables included in analyses were age, gender, type of RT, use of concomitant chemotherapy, tumour-site, volume surrogate (defined by Volume 1 = T1a-1bN0M0 glottic laryngeal carcinomas, Volume 2 = all other TxN0 sites, and Volume 3 = TxN1-3 carcinomas), total prescribed biologically effective dose (BED), and baseline toxicity (Supplementary Tables S1&S6).

Statistical genetic association analysis

We analysed residual of AUCs (individual RITs) and $STAT_{acute}$ using multivariate linear regression for each cohort including an allele's additive effect while adjusting for covariables and the top 10 PCAs to correct for population stratification (see Supplementary Methods). A regression coefficient presents the effect size for one copy/dose of effect allele of the corresponding SNP. A p-value $< 5.0 \times 10^{-8}$ was considered statistically significant and a $5.0 \times 10^{-8} < p\text{-value} < 1.0 \times 10^{-5}$ as suggestive association. Significance for heterogeneity was denoted when the heterogeneity p-values (H_{etp}) was < 0.05 . Data preparation and statistical analyses were carried out using PLINK/1.90b3.44 [14] and SNPTTEST [15]. Meta-analyses were done using the inverse variance weighted, a fixed-effects method implemented in METAL [16] (version 2011-03-25).

Discovery GWAS meta-analysis

GWAS summary results for each cohort underwent a series of QC checks using the GWASinspector [17] package in R. For each acute RIT, univariate and multivariate GWAS results were meta-

analysed. SNPs with $p_{discovery} < 1.0 \times 10^{-5}$ and no significant test of heterogeneity, which were available in at least two (out of three) discovery cohorts, were selected for replication.

Replication and combined meta-analysis

A similar QC was conducted for Replication set (Supplementary Table S5). We meta-analysed summary results from the four replication cohorts. SNPs were considered replicated when $p_{replication} < 0.05/\text{number of SNPs tested}$. The summary meta-GWAS results from the discovery and replication sets were meta-analysed. SNPs were considered genome-wide significant if $p_{combined} < 5 \times 10^{-8}$.

The entire samples analysis

The entire discovery and replication cohorts were jointly meta-analysed to maximize the study power; hereon called whole-meta.

LD score regression (LDSC), SNP-based heritability, and genetic correlation

LDSC analysis regressed SNP χ^2 estimates from GWAS with LD scores across the genome. An LDSC intercept = 1 suggests no LD related confounding bias, whereas an intercept > 1 suggests a contribution of LD confounding in χ^2 estimates due to cryptic relatedness (Supplementary Methods). We applied cross-trait LDSC to estimate genetic correlations between RITs [18]. In brief, the cross-product of two GWAS test statistics is calculated at each SNP, and this cross-product is regressed on the LD score. The slope of the regression estimates the genetic relationships between two tested RITs.

Table 1
Demographic and clinical characteristics of HNC cohorts.

Characteristic	Discovery Set			Replication Set				Transferability
	UMCG-HANS ^a	DAHANCA ^b	RADIOGEN-HNC ^c	Ghent-HNC ^d	RAPPER-HNC ^e	NIMRAD ^f	Head and Neck 5000 ^g	Singapore ^h
	N = 1279	N = 1183	N = 178	N = 273	N = 187	N = 270	N = 672	N = 180
Gender; Female (%)	411 (32.10)	243 (21)	21 (11.79)	45 (16.5)	22 (11.76)	57 (22)	146 (22)	47 (26.1)
Age Median (range)	65 (19–93)	60 (27–90)	62.93 (35–91.41)	59.7(9.64)	60.5 (39–85)	73 (44–87)	60 (25–94)	52.7 (43.8–58.5)
Tumor site (%)					Not available			
Oral cavity	227(17.70)	0	22(12.36)	43 (15.8)		0	191 (28.4)	0
Oropharynx	274(21.40)	760 (64.5)	77(43.26)	86 (31.5)		211 (78.15)	332 (49.4)	0
Larynx	338(26.40)	419 (35.5)	56(31.46)	54 (19.8)		59 (21.85)	97 (14.4)	0
Others	430(33.60)	0	23(12.93)	90 (32.9)		0	52 (7.2)	180 (100.0)
BED, Mean (SD)	72.97 (7.18)	80.11 (1.31)	78.93 (7.28)	83.29(7.49)	80.43 (3.89)	75.71 (16.05)	74.4 (10.6)	84.8 for all patients
Chemotherapy; No (%)	913 (71.38)	714 (60.4)	67(37.64)	169 (61.9)	152 (81.28)	0	109 (16.2)	35 (19.4)
Radiotherapy;	524 (40.97)	All definitive RT	87(49.15)	76 (27.8)	27(14.43)	0	310 (46.1)	0 (0.0)
Postoperative RT (%)								
Volume Surrogate (%)								
Glottic/laryngeal T1N0M0	148(11.60)	159(13)	7(3.93)	1 (0.4)	1 (0.53)	0	72 (10.7)	0
All other TxN0 sites	440(34.40)	215(18)	47(26.40)	70 (25.6)	110 (58.82)	80 (30.0)	168 (25.0)	0
TxN1-3 carcinomas	608(47.50)	809(68)	124(69.66)	202 (74.0)	76 (40.64)	190 (70.0)	432 (64.3)	180 (100.0)
Average score (SD) of radiation-induced toxicities measure by the AUC ⁱ and STAT ^j acute								
AUC dysphagia: observed	1.84(0.82)	1.85(0.85)	1.12(0.63)	1.37(0.65)	1.15(0.81)		-	1.06(0.52)
AUC dysphagia: residual (adjusted)	0.00(0.69)	0.00(0.65)	0.00(0.56)	0.00(0.52)	0.01(0.70)		-	0.00(0.50)
AUC mucositis: observed	0.94(0.72)	1.85(0.51)	0.83(0.57)	1.23(0.59)	1.21(0.68)		-	1.19(0.48)
AUC mucositis: residual (adjusted)	0.00(0.62)	0.00(0.43)	0.00(0.49)	0.00(0.52)	0.00(0.61)		-	0.00(0.47)
AUC xerostomia: observed	0.91(0.51)	-	1.06(0.54)	-	0.89(0.60)		-	1.29(0.33)
AUC xerostomia: residual (adjusted)	0.00(0.46)	-	0.00(0.47)	-	0.02(0.56)		-	0.01(0.33)
STATacute: observed	0.00(0.78)	0.01(0.80)	0.01(0.77)	0.01(0.82)	0.02(0.74)		0.09(0.88)	0.01(0.76)
STATacute: residual (adjusted)	0.00(0.62)	0.00(0.71)	0.00(0.64)	0.00(0.79)	0.00(0.71)		0.01(0.82)	0.01(0.72)

^a University Medical Center Groningen- Head And Neck Study.

^b Danish Head and Neck Cancer Group.

^c Estudio de la influencia genómica en pacientes sometidos a radioterapia.

^d Ghent University Hospital Radiogenomics studies in head and neck cancer patients.

^e Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy.

^f Randomised placebo-controlled H&N trial of synchronous NIMorazole versus RADiotherapy alone.

^g Head and Neck 5000.

^h Discovery of biomarkers for intrinsic radiation sensitivity in cancer patients.

ⁱ AUC: area under curve, average toxicity per week during RT.

^j STAT: standardized total average toxicity. Fig. 1 shows more details for the distribution of outcomes.

Table 2
Suggestive SNPs ($p < 1.0 \times 10^{-5}$) in discovery stage that showed a nominal association in the replication stage.

Endpoint	SNP	A1	Discovery stage		Replication stage		Combined meta-analysis						
			Effect size BETA \pm SE	P _{dis}	BETA \pm SE	P _{rep}	BETA	P _{com}	Q	Het P	N of study	Total N	
Adjusted AUC Dysphagia	rs9885106	C	0.09 \pm 0.02	5.3E-06	0.08 \pm 0.04	0.04	0.09 \pm 0.02	6.2E-07	2.65	0.62	5	2899	
	rs7840941	C	-0.08 \pm 0.02	9.8E-06	-0.09 \pm 0.04	0.03	-0.08 \pm 0.02	8.7E-07	3.93	0.42	5	2899	
	rs10097379	C	-0.08 \pm 0.02	9.8E-06	-0.08 \pm 0.04	0.04	-0.08 \pm 0.02	9.7E-07	4.10	0.39	5	2900	
	rs11787431	G	-0.08 \pm 0.02	9.5E-06	-0.08 \pm 0.04	0.03	-0.08 \pm 0.02	9.3E-07	4.14	0.39	5	2899	
	rs10112474	T	-0.08 \pm 0.02	9.5E-06	-0.08 \pm 0.04	0.04	-0.08 \pm 0.02	9.4E-07	4.11	0.39	5	2899	
	rs6996246	A	-0.08 \pm 0.02	9.5E-06	-0.08 \pm 0.04	0.04	-0.08 \pm 0.02	9.6E-07	4.07	0.40	5	2900	
	rs1061660	C	-0.09 \pm 0.02	9.9E-06	-0.09 \pm 0.05	0.05	-0.09 \pm 0.02	1.3E-07	1.01	0.91	5	2900	
Adjusted AUC Mucositis	rs112997435	T	-0.09 \pm 0.02	1.2E-06	-0.09 \pm 0.05	0.05	-0.09 \pm 0.02	1.7E-07	2.00	0.74	5	2865	
	rs113787224	A	-0.09 \pm 0.02	3.1E-06	-0.09 \pm 0.05	0.05	-0.09 \pm 0.02	4.9E-07	1.36	0.85	5	2865	
	rs73039018	A	-0.10 \pm 0.02	8.5E-07	-0.09 \pm 0.05	0.05	-0.10 \pm 0.02	1.2E-07	1.95	0.74	5	2865	
STAT _{acute}	rs7207494	C	0.12 \pm 0.03	8.7E-06	0.11 \pm 0.05	0.03	0.12 \pm 0.02	8.6E-07	0.77	0.98	6	3437	
	rs74645182	A	0.17 \pm 0.04	6.6E-06	0.16 \pm 0.07	0.02	0.16 \pm 0.03	3.5E-07	3.84	0.57	6	3438	
	rs111794065	A	0.16 \pm 0.04	5.8E-06	0.16 \pm 0.06	0.01	0.16 \pm 0.03	2.1E-07	2.78	0.73	6	3437	
	rs76956887	A	0.17 \pm 0.04	6.1E-06	0.16 \pm 0.07	0.02	0.17 \pm 0.03	3.0E-07	4.07	0.54	6	3438	
	rs76337261	A	0.17 \pm 0.04	7.5E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	8.2E-07	3.53	0.47	5	2981	
	rs76139001	A	0.17 \pm 0.04	5.8E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	6.3E-07	3.54	0.47	5	2981	
	rs79610404	A	0.17 \pm 0.04	7.5E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	8.2E-07	3.53	0.47	5	2981	
	rs80023733	A	0.17 \pm 0.04	7.5E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	8.2E-07	3.53	0.47	5	2981	
	rs112813112	A	0.17 \pm 0.04	4.2E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	4.6E-07	3.92	0.42	5	2981	
	rs112107185	A	0.17 \pm 0.04	5.7E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	6.2E-07	4.12	0.39	5	2981	
	rs41282994	A	0.17 \pm 0.04	5.7E-06	0.18 \pm 0.09	0.04	0.17 \pm 0.03	6.2E-07	4.13	0.39	5	2981	
	rs137992872*	A	0.20 \pm 0.04	2.6E-06	0.26 \pm 0.11	0.02	0.21 \pm 0.04	1.9E-07	1.98	0.74	5	2981	
	Adjusted STAT acute	rs56070086	A	0.18 \pm 0.04	1.0E-05	0.25 \pm 0.11	0.02	0.19 \pm 0.04	8.0E-07	1.32	0.72	4	2475
		rs4349441	T	0.18 \pm 0.04	5.6E-06	0.25 \pm 0.11	0.02	0.19 \pm 0.04	4.4E-07	1.36	0.71	4	2475
		rs4464374	A	0.18 \pm 0.04	5.6E-06	0.25 \pm 0.11	0.02	0.19 \pm 0.04	4.4E-07	1.36	0.71	4	2475
rs8142673		A	0.18 \pm 0.04	9.6E-06	0.25 \pm 0.11	0.02	0.19 \pm 0.04	7.7E-07	1.41	0.70	4	2475	
rs137992872*		A	0.19 \pm 0.04	2.2E-06	0.25 \pm 0.11	0.02	0.20 \pm 0.04	1.7E-07	1.45	0.70	4	2475	
rs17478318		T	0.20 \pm 0.04	6.3E-06	0.24 \pm 0.11	0.03	0.21 \pm 0.04	5.7E-07	1.64	0.65	4	2475	

Abbreviations; SNP: Single nucleotide polymorphism, A1: effect allele, SE: standard error, P: p-value, Q: Cochran's Q, N: number of studies, AUC: area under curve, STAT: standardized total average toxicity, *SNP repeated in results, univariate and multivariate of STAT_{acute}.

Table 3
SNP-based heritability of RITs using whole meta-analysis.

	N	N of SNPs	Lambda GC	Mean Chi ²	Intercept	h2 (se)
AUC Dysphagia	2,354	1,142,016	1.022	1.020	1.015 (0.007)	0.294 (0.096)
Adjusted AUC Dysphagia	1,933	1,139,586	0.998	1.002	1.004 (0.007)	0.040 (0.113)
AUC Mucositis	2,364	1,141,988	1.010	1.004	1.008 (0.007)	0.061 (0.091)
Adjusted AUC Mucositis	1,910	1,145,863	1.004	1.006	1.001 (0.007)	0.097 (0.122)
AUC Xerostomia	1,258	1,161,794	1.022	1.017	1.012 (0.006)	0.506 (0.195)
Adjusted AUC Xerostomia	1,141	1,155,004	1.010	1.011	1.013 (0.007)	0.359 (0.202)
STAT _{acute}	2,292	1,148,120	1.019	1.018	1.005 (0.006)	0.268 (0.088)
Adjusted STAT _{acute}	2,256	1,140,063	1.013	1.009	1.005 (0.006)	0.152 (0.091)

Abbreviations; N: number of analysed subjects, SNP: Single nucleotide polymorphism, GC: Genomic control, h2: SNP-based heritability, se: standard error, intercept protects from bias from population stratification and cryptic relatedness.

Polygenic risk score (PRS) analysis

To evaluate the contribution of common variants on prediction of acute RITs, we repeated the whole-meta while excluding the UMCG-HANS cohort for each RITs. The SNP effect sizes were then used to build PRSs for SNPs below different thresholds of association p-values (Supplementary Methods). These PRSs were tested in the UMCG-HANS cohort (n = 1,279) at two different SNP MAFs (<0.01, <0.1) for their ability to predict their corresponding RIT.

In-silico functional analysis

To understand the plausible functional effects of the suggestive SNPs, we performed an *in-silico* functional analysis using whole-meta summary results (Supplementary Methods). Briefly, we first annotated independent functional genome-wide suggestive SNPs to a set of relevant genes, then we tested the statistical enrichment of these gene sets, and finally we predicted the biological and genetic pathways of significantly enriched gene sets.

Results

We included 4,042 patients (67 % men; age median 60; range 19 to 94 years); mostly treated for oropharyngeal (44.4 %) and larynx (26.5 %) tumours with definitive (74.7 %) or postoperative (25.3 %) RT with 58 % received concomitant chemotherapy (Table 1 and Supplementary Table S1).

Missing data varied per RIT from 2.6 % in week one to 28 % in week six post-RT (Supplementary Table S4). Supplementary Table S6 presents characteristics per cohort. Fig. 1 and Table 1 describe the distribution of AUC for acute RITs in the discovery and replication cohorts. The adjusted mean AUC of RITs was zero with SD range 0.43–0.82 across RITs.

We found no genome-wide significant SNP associated with any RIT in the discovery stage, nonetheless, identified 393 genome-wide suggestive SNPs spanning 118 loci (Supplementary Tables S7 to S14). In replication analysis, 37 out of 393 suggestive SNPs were nominally associated ($p_{\text{replication}} < 0.05$) with the corresponding acute RITs (Supplementary Tables S7 to S14). No SNP passed genome-wide significance threshold. Supplementary Tables S7 to S14 list the most statistically significant SNPs. Twenty-seven of the 37 SNPs achieved a more significant association in combined-meta (p_{combined}) with concordant directions in effect sizes (i.e., beta's) across the individual cohorts and in meta-analyses (Table 2).

Multivariate GWAS found 37 suggestive SNPs in 15 loci associated with dysphagia in the discovery set (Fig. 2) with rs3770941 being most significant ($\beta = 0.10$; $p_{\text{discovery}} = 5.07 \times 10^{-7}$; Supplementary Table S11). Seven of the 37 SNPs were replicated (Table 2) being rs1061660 the top SNP (-0.09 ; $p_{\text{combined}} = 1.27 \times 10^{-7}$). LocusZoom plot showed the nearest gene was *MTFP1* (Supplementary

Figure S2). None of the replicated SNPs reached genome-wide significance in meta-analysis.

For mucositis, we found 103 suggestive SNPs in 17 genomic regions (Fig. 2), with rs141501282 the most significant (-0.38 ; $p_{\text{discovery}} = 9.47 \times 10^{-8}$). Five suggestive SNPs were replicated ($p_{\text{replication}} < 0.05$, Table 2; Supplementary Table S12) being rs73039018*A the top SNP (-0.10 ; $p_{\text{combined}} = 1.20 \times 10^{-7}$) as *ZNF573* the nearest gene (Supplementary Figure S2).

We found 26 suggestive SNPs across 12 loci associated with xerostomia; the top SNP was rs6458543 with a meta-effect size 0.22 (p_{combined} improved to 9.65×10^{-7} , Fig. 2; Supplementary Table S13) and was mapped to nearest *TNFRSF21* (Supplementary Figure S2).

For STAT_{acute}, out of 31 multivariate suggestive SNPs across 17 genomic regions, rs11712108 was the top SNP (-0.10 ; $p_{\text{discovery}} = 2.70 \times 10^{-7}$; Supplementary Table S14). Six of the suggestive SNPs were nominally replicated (Fig. 2 & Table 2) being rs137992872 the top association (meta-effect size 0.19; $p_{\text{combined}} = 1.66 \times 10^{-7}$) mapped close to *TCF20* (Supplementary Figure S2).

Ten of the 176 available suggestive SNPs in Asian cohort (one for mucositis and nine for xerostomia) showed a nominal association ($p < 0.05$) with the same direction of effect size as the European meta-analysis (Supplementary Tables S7 to S14).

The Q-Q plots for 4,042 HNC patients showed no genomic inflation due to population substructure in whole GWAS. No SNP reached genome-wide significance. There were 525 suggestive SNPs spanning 159 genomic regions (Supplementary Tables S15 to S22; Fig. 2).

For LD score regression, around 1,1 million SNPs were analysed. The LDSC intercept showed minor inflation attributable to confounding bias (Table 3), indicating the observed statistics was due to the polygenicity of RITs. SNP-based heritability was 0.29 (se = 0.09) for dysphagia, 0.09 (se = 0.12) for (adjusted) mucositis and 0.26 (se = 0.08) for STAT_{acute}. The heritability and standard error were too high (up to 0.51, se = 0.19) for xerostomia likely due to few event rates. A strong positive genetic correlation was found between dysphagia and STAT_{acute} (rg 0.65, $p = 0.048$), but not for the others (Supplementary Table S23).

Fig. 3 presents PRSs for acute RITs. The best fit PRS, which explained the highest RIT variance, differed across RITs. In the UMCG-HANS cohort, PRS_{dysphagia} (at MAF > 0.01, p-value < 0.1) explained 3 % of the variance of dysphagia. PRS_{mucositis} (MAF > 0.01) explained 2.5 % of the variance for mucositis. PRS_{dysphagia} (MAF > 0.1, p-value < 0.001) explained a negligible proportion (0.4 %) of the variance in (adjusted) dysphagia, which was similar for PRS_{statacute} for (adjusted) STAT_{acute}. There were insufficient patients to build a meaningful PRS_{xerostomia}.

Knowing our limited power and risk of false-negative results, we performed an *in-silico* functional analysis to understand the potential pathways underlying RITs using whole-meta summary results (see Supplementary Results). Briefly, the top pathways

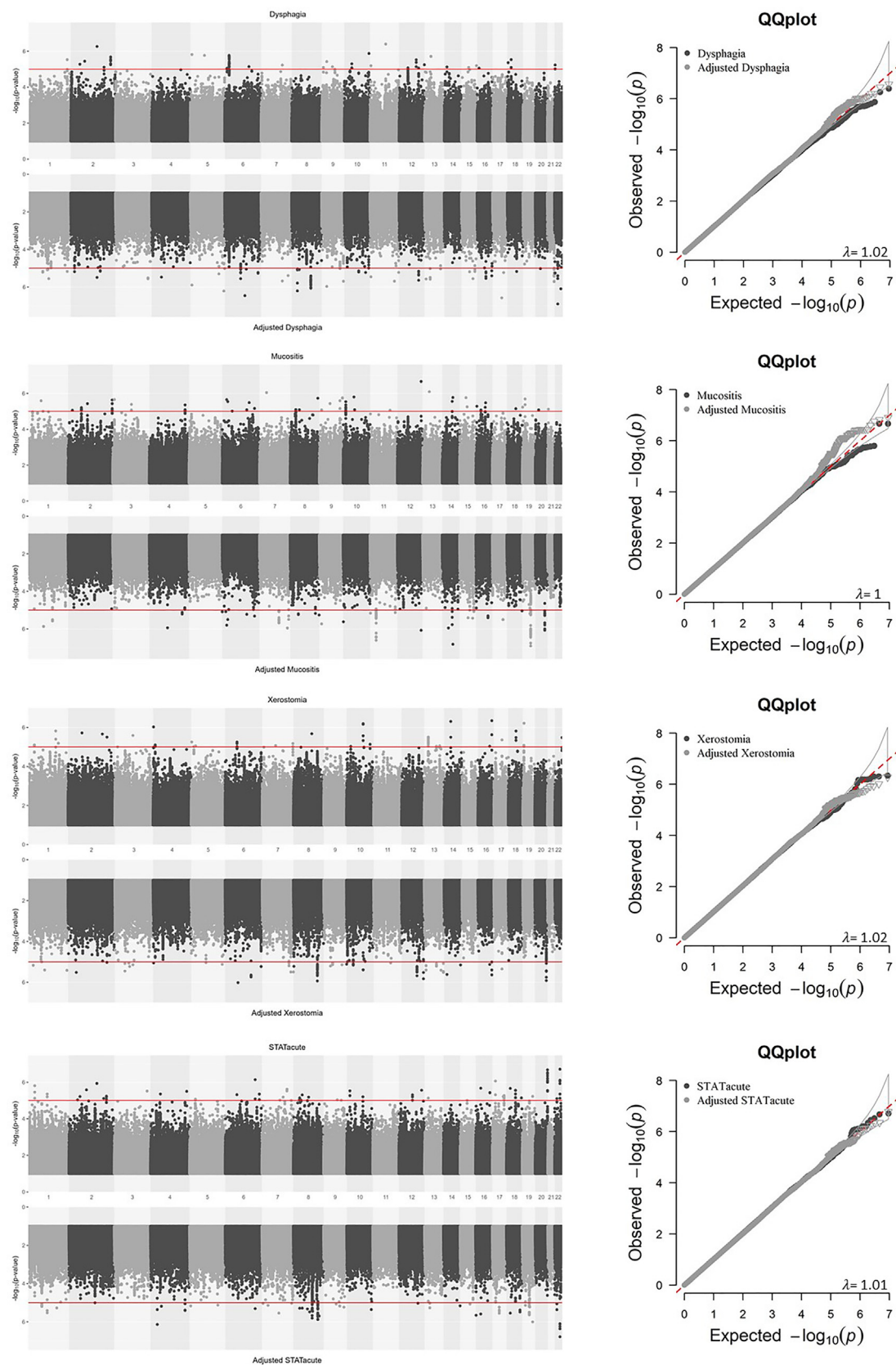


Fig. 2. Manhattan (right) and QQ (left) plots of whole meta-analysis for the tested acute RITs in HNC patients. Mirror Manhattan plot: GWAS and Adjusted results are shown in the upper and the lower panel, respectively. The X axis shows location in the genome. Each SNP is plotted as a dot. The Y axis shows $-\log_{10}$ P-values for the association of each of the SNPs to the desired outcome. The red line shows the threshold for genome-wide suggestive (P -value $< 1 \times 10^{-5}$). QQ plot: The Y axis shows observed $-\log_{10}$ P-values, and the X axis shows the expected $-\log_{10}$ P-values. Each SNP is plotted as a dot, and the red line shows null hypothesis of no true association. Deviation from the expected P-value distribution is clear only in the tail area, and along with the estimated lambda coefficients, suggesting that population stratification was adequately controlled.

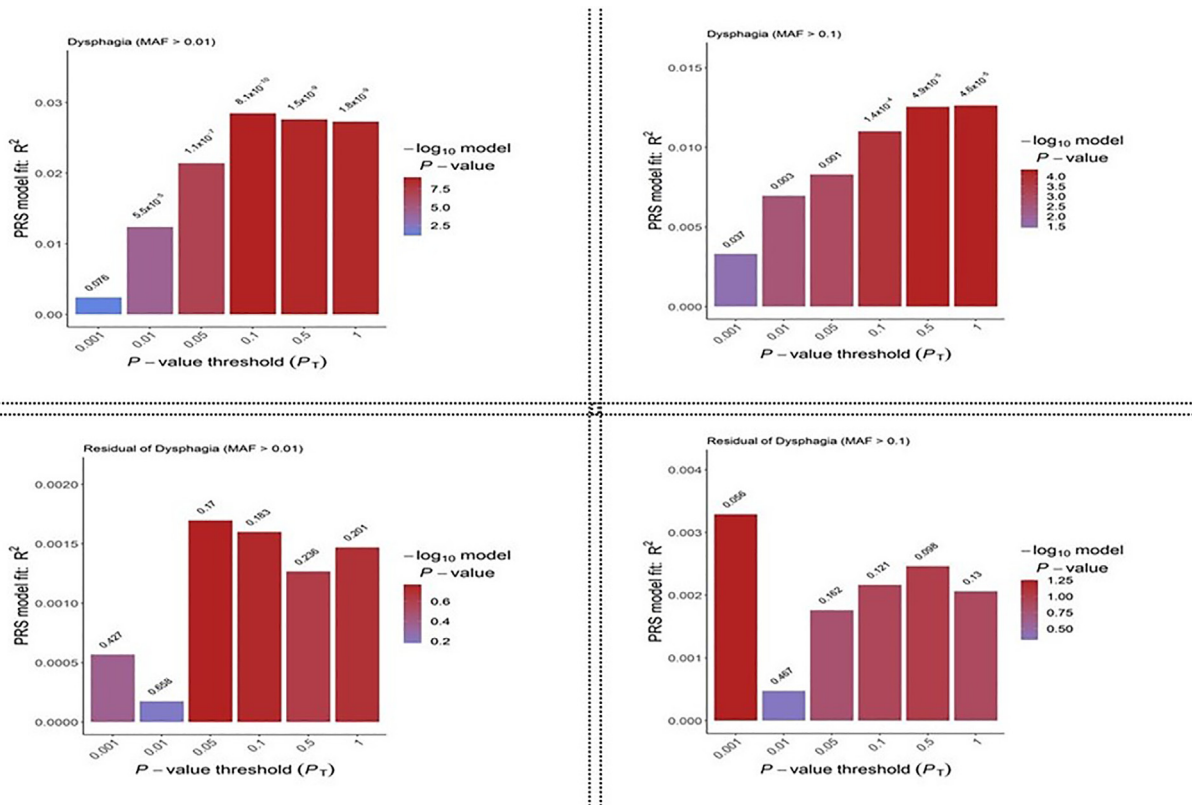


Fig. 3. The polygenic risk score corresponding to a range of p-value thresholds for RIT endpoints by two MAF levels of 0.01 & 0.1. The X-axis show the p-value thresholds, and Y-axes show the variance of toxicity endpoints which is explained by PRSs.

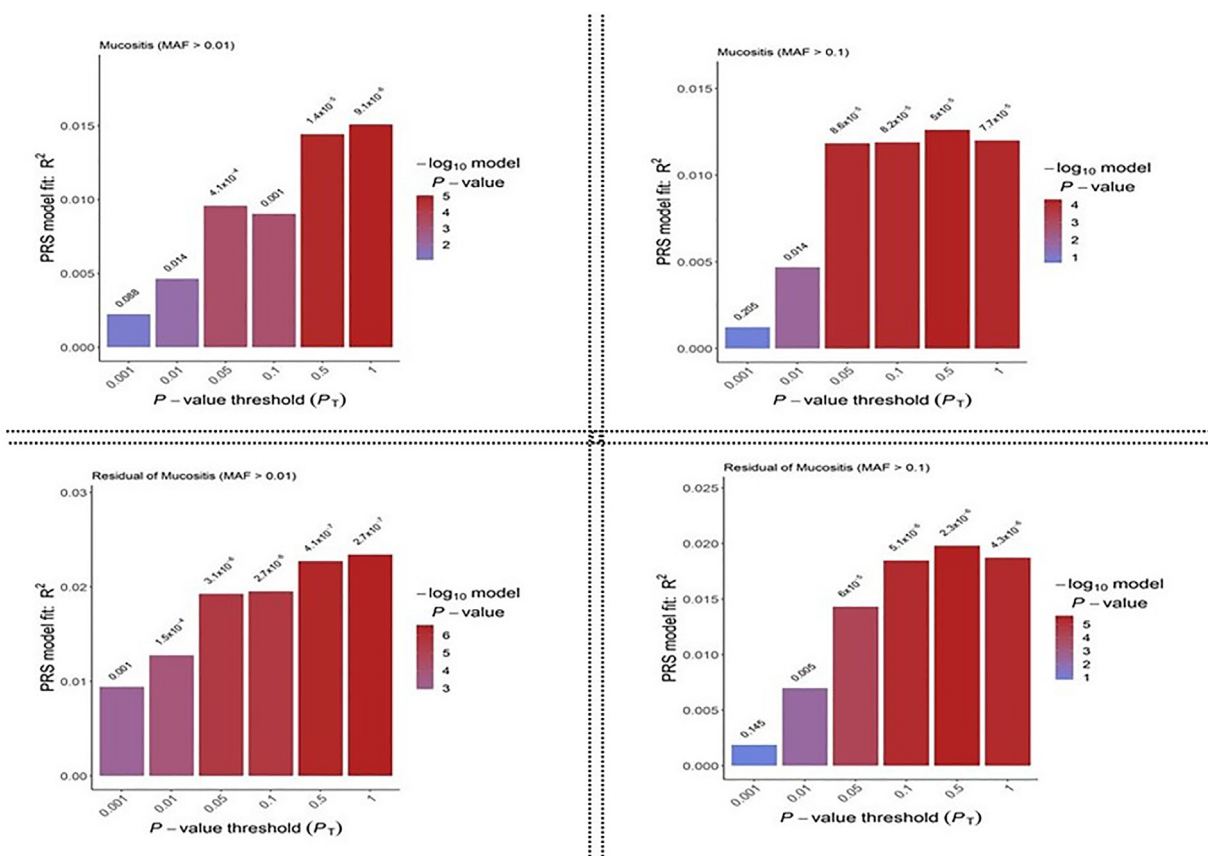


Fig. 3 (continued)

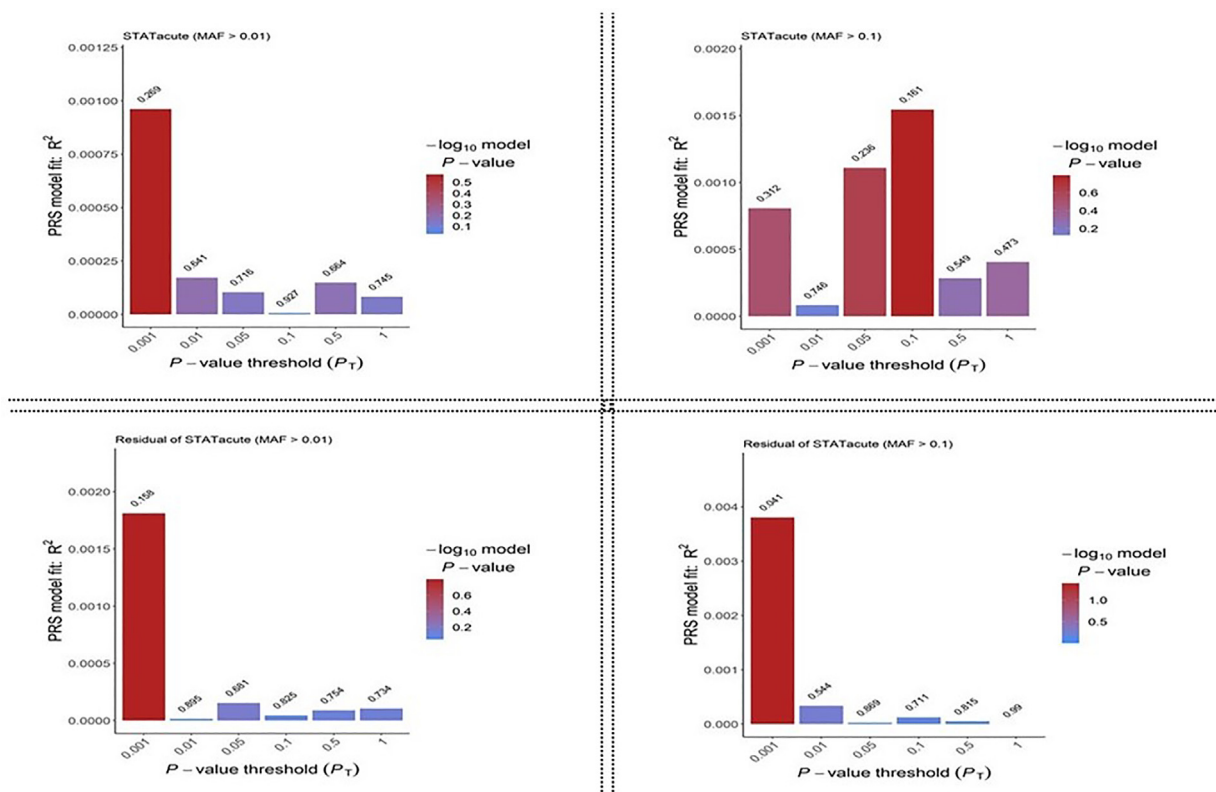


Fig. 3 (continued)

identified were “3'-5'-exoribonuclease activity” (for dysphagia, FDR = 1.64e-10), “inositol phosphate-mediated signalling” (mucositis, FDR = 2.20e-09), and “drug catabolic process” (STAT_{acute}, FDR = 3.57e-12).

Discussion

Our study is the first multicentre meta-GWAS investigating the genetic component of acute RITs in HNC patients. We identified 393 suggestive SNPs associated with RITs, of which 27 maintained the effect size direction and became more significant in the replication stage. Whole Meta-analysis identified 525 suggestive SNPs spanning 159 genomic regions. We estimated heritability of acute RIT as 29 % for dysphagia and 26 % for STAT_{acute}. We also showed that genetic susceptibility to RITs (dysphagia, mucositis, and xerostomia) is likely to be independent, as we found no significant genetic correlation between the three studied RITs. We built specific PRS for RITs, which predicted its corresponding RITs. In addition, *in-silico* functional analysis found several novel genes and genomic pathways not previously associated with acute RITs.

Heritability of acute RITs

Recent studies showed the total variance explained by all SNPs is larger for complex traits and diseases compared to using only limited genome-wide significant SNPs [19]. Our heritability estimates (up to 29 %) were comparable with those from other complex traits like coronary artery disease (40 % twin-study heritability [20], 4.69 % SNP-based heritability), schizophrenia (81 % twin-study heritability [21], 25.9 % SNP-based heritability), autism spectrum disorder (80 % twin-based heritability [21], 11.7 % SNP-based heritability [22]), and chronic kidney disease (family-heritability up to 75 % [23], 53 % SNP-based heritability).

To control for potential confounders, we applied strict criteria on sample selection, quality controls for genotyping. While adjusting for ancestry components, and confounding co-factors, We running conservative meta-GWAS analyses (i.e., a double genomic control, and controlling for heterogeneity). We did additional statistical checks to find any source of statistical inflation in GWAS effect estimates, and post-GWAS analyses. Furthermore, we applied the LDSC method, which is robust to confounding due to cryptic population stratification [24]. Lastly, we used narrow-sense heritability, i.e., the proportion of a trait's phenotypic variance attributable to additive genetic variance. Therefore our analyses might missed part of heritability due to limited samples size, the presence of rare variants with large effects not tagged by SNPs, or imputation and non-additive genetic variation and/or epigenetic factors [25]. We, therefore, estimated a valid but limited proportion of the heritability for RITs. Whole-genome or -exome sequencing are the next likely approach to estimate the large effect of rare variants in heritability estimates for RITs.

PRS for RITs and its predictability

As the first study, we showed the predictability of RITs by SNPs. Though it seems the percent of explained variance was modest, they were similar to findings for other traits and diseases. For example, even using a large sample size to generate PRS for autism spectrum disorder, the best PRS model explained only 2.5 % of the variation in autism [26] and this was 3 % for schizophrenia [27]. By expanding the high-quality RITs data, the performance of PRS can be optimised for RIT, as done for other complex traits.

The first suggestive genome-wide association to RITs

Dysphagia We found 37 suggestive SNPs in 15 genomic regions associated with dysphagia in HNC patients. *In-silico* annotations,

identified chr22q12, and chr8p22 (out of 12 regions), were enriched by the prioritised genes related to dysphagia. The top hit, rs1061660, mapped in chr22q12 containing *GATSL3*, *TBC1D10A*, *SF3A1*, *CCDC157*, *RNF215*, *SEC14L2* genes, while *MTFP1* is the nearest gene. The other enriched region, chr8p22, contains *ZDHHC2*, *CNOT7*, *VPS37A* and *MTMR7* genes. The nearest gene, mitochondrial fission process 1 (*MTFP1*), is a nuclear-encoded protein that promotes mitochondrial fission [28]. The details of functional results are presented in Supplementary Discussion. Our analyses concluded exonuclease activity mechanisms as a potential mechanism in cell response and hypersensitivity to radiation, and in incidence of acute RT induced dysphagia.

Mucositis We found 103 suggestive SNPs in 17 genomic regions associated with mucositis in HNC patients. The top SNP was rs73039018, and the nearest gene was *ZNF573* (Zinc Finger Protein 573) which involves nucleic acid binding and DNA-binding transcription factor activity. Our functional analyses (Supplementary Discussion) suggested the dysregulation of the inositol phosphate-mediated signalling due to the radiation is likely involved in mucositis.

Xerostomia We found 54 suggestive SNPs in 22 genomic regions associated with xerostomia. The top SNP was rs6458543 mapped to *TNFRSF21* (TNF Receptor Superfamily Member 21), also known as DR6, encodes a member of the tumour necrosis factor (TNF) receptor superfamily and induces apoptosis upon overexpression [29]. Our functional analyses highlighted the complexity of mechanism of xerostomia which requires larger studies to be unravelled.

STAT_{acute} is a clinical indicator of accumulated toxicities for which we identified 31 suggestive SNPs in 17 genomic regions. The top SNP was rs137992872, mapped to *TCF20* (Transcription Factor 20) that encodes a widely expressed transcriptional coregulator, and *TCF20* mutations are associated with autism and intellectual disability [30]. Our functional analysis (see Supplementary Discussion) highlighted chromosome 22q13 region and the arachidonic acid metabolic process are associated with an average of RITs (*STAT_{acute}*).

Clinical Implications and future perspectives

The risk of RIT is assessed using normal tissue complication probability (NTCP) models. NTCPs are multivariable prediction models built on radiation dose metrics, clinical factors, and patients' characteristics [31]. One immediate impact is including genetic risk scores in forming a prediction model that explains patients' sensitivity to RT, which may eventually allow a more individualized RT treatment. By this multicentre meta-GWAS, we showed heritability for RIT in HNC patients and adds a piece to the increasing evidence that an individual genetic predisposition is a contributory factor in the development of acute RITs. We found limited but significant predictability for RITs using genetic risk scores.

Limitations

We did not reach to genome-wide significant threshold for top hits, though this is common in GWASs. Generally, testing each SNP an association with the trait in GWAS needs to account for the large number of statistical tests carried out; thus, a very stringent p-value is used. This reduces false positives, but it may mask real associations too, especially if individual SNPs have a negligible effect on the trait. *In-silico* functional analysis added additional evidence by linking our suggestive regions to interesting relevant biological functions. Future studies should be conducted on extended sample size and define better phenotypes for RITs. We tested common SNP with MAF of more than 1 %; however, exploring the rare variants will be highly instructive.

Conclusion

We showed acute RIT is heritable and predicably by PRS. We identified 393 suggestive SNPs associated with the four RITs, of which 27 SNPs maintained the direction of their effect sizes and showed an improvement in the significance of associations in the replication stage. Furthermore, we showed that the genetic susceptibility of acute RITs is likely independent. In addition, *in-silico* functional analysis identified exonuclease activity, inositol phosphate-mediated signalling, and drug catabolic process with potential roles in RITs. Our work extends the field of radiogenomics by combining cohorts of HNC patients with reliable data for RIT would enable the identification of genetic variants with lower penetrance, possibilities for validation, and eventually, to enrich NTCP models with genetic factors.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Data sharing

This study was done using HNC cohorts involved in radiogenomics consortium. Data are available from the corresponding author (Elnaz Naderi) on request. Summary statistics for GWAS results will be made available to download from the GWAS Catalog.

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Conflicts of interest

The authors declare no conflicts of interest. No one was paid to write this article by a pharmaceutical company or agency.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.radonc.2022.09.016>.

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