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ORIGINAL ARTICLE

Genetic variation in radiation and platinum pathways predicts severe acute radiation toxicity in patients with esophageal adenocarcinoma treated with cisplatin-based preoperative radiochemotherapy: results from the Eastern Cooperative Oncology Group

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Abstract
Purpose Germline genetic variations may partly explain the clinical observation that normal tissue tolerance to radiochemotherapy varies by individual. Our objective was to evaluate the association between single-nucleotide polymorphisms (SNPs) in radiation/platinum pathways and serious treatment-related toxicity in subjects with esophageal adenocarcinoma who received cisplatin-based preoperative radiochemotherapy.

Methods In a multicenter clinical trial (E1201), 81 eligible treatment-naïve subjects with resectable esophageal adenocarcinoma received cisplatin-based chemotherapy concurrent with radiotherapy, with planned subsequent surgical resection. Toxicity endpoints were defined as grade ≥3 radiation-related or myelosuppressive events probably or definitely related to therapy, occurring during or up to 6 weeks following the completion of radiochemotherapy. SNPs were analyzed in 60 subjects in pathways related to nucleotide/base excision- or double stranded break repair, or platinum influx, efflux, or detoxification.
Results Grade ≥3 radiation-related toxicity (mostly dysphagia) and myelosuppression occurred in 18 and 33% of subjects, respectively. The variant alleles of the *XRCC2* 5′ flanking SNP (detected in 28% of subjects) and of *GST-Pi* Ile-105-Val (detected in 65% of subjects) were each associated with higher odds of serious radiation-related toxicity compared to the major allele homozygote (47% vs. 9%, and 31% vs. 0%, respectively; $P = 0.005$). No SNP was associated with myelosuppression.
Conclusions This novel finding in a well-characterized cohort with robust endpoint data supports further investigation of *XRCC2* and *GST-Pi* as potential predictors of radiation toxicity.

Keywords Chemoradiation · Esophageal cancer · Radiation toxicity prediction · Single nucleotide polymorphism · Trimodality

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Introduction

Esophageal adenocarcinoma (EAC) is one of the fastest rising cancers in the West [1] and, despite the use of aggressive therapy, remains highly fatal [2]. One standard for

treating locally advanced disease, the most common stage at presentation, involves concurrent radiochemotherapy (RCT) followed by surgery [3, 4]. Cisplatin often forms the base of the chemotherapy regimen [5]. RCT causes serious toxicity in many—but not all—patients. The ability to predict which patients will experience serious RCT-related toxicity may aid clinical decision-making. This is partly because the administration of perioperative chemotherapy alone, without radiotherapy, is another valid approach for cure [4, 6, 7]. In its simplest form, patients who are predicted to have serious radiotherapy-related toxicity may be selected for perioperative chemotherapy alone, rather than combined RCT. In the actual clinical setting, such information may be integrated into a risk–benefit predictive model, which could be used as a clinical decision-making tool.

It is currently unknown how to predict serious dose-limiting toxicity in EAC patients receiving combined RCT. The field of developing biomarkers which predict RCT-related toxicity is in its early phases—i.e., the identification of candidate markers which can subsequently be validated [8–10]. In the last decade, interest has grown in the concept that individual genetic profiles, particularly genetic polymorphisms, may contribute to therapy-related normal tissue toxicity [11]. That platins and radiotherapy function mainly by damaging DNA (by forming platinum–DNA adducts and oxygen radicals, respectively) has brought attention to polymorphisms in DNA repair pathways as a source of variance in normal tissue tolerance [11, 12]. Interest in single-nucleotide polymorphisms (SNPs) has been supported by links between SNPs in DNA repair pathways and varying levels of DNA repair capacity [13]. Pathways involving drug disposition have also been studied in relation to chemotherapy-specific toxicity. For example, genetic polymorphisms in genes involved in irinotecan metabolism (*UGT1A*) or in folate (*MTHFR*) and fluoropyrimidine (*DPYD*) metabolism have been associated with neutropenia [14] and with hand-foot syndrome and diarrhea [15, 16], respectively.

In EAC, studies are emerging which evaluate the association of SNPs with cancer risk and survival outcome. However, toxicity has not been well examined. All three endpoints may be related. It is hypothesized that deficient DNA repair increases risk for developing cancer by lowering the normal cell's ability to repair carcinogen-induced damage [17]. On the other hand, in patients with cancer, deficient DNA repair may increase tumor response to radio- or cytotoxic chemotherapy, by lowering the tumor cell's capacity to repair radiation or chemotherapy lesions [18]. By contrast, lowering the ability of normal bystander cells to repair therapy-related genetic lesions may lead to toxicity [12].

Identifying markers of toxicity poses considerable challenge partly due to the difficulty of collecting clinical toxicity data. Whereas some efficacy endpoints (e.g., death) can be retrieved retrospectively with reasonable precision and thoroughness, toxicity events may be more vulnerable to incomplete ascertainment and variable toxicity criteria. A prospective cohort that emphasizes the collection of toxicity data—e.g., a clinical drug trial—may address these issues more reliably.

Our objective was to evaluate the association between 21 SNPs (18 genes) across 7 radiation/platin pathways and serious treatment-related toxicity in EAC patients who received cisplatin-based RCT and surgery as part of a multicenter trial of the Eastern Cooperative Oncology Group (ECOG). We focused on two toxicity endpoints: (1) acute radiation-related, and (2) myelosuppression. We assessed genes in both radiation and platin pathways for each endpoint because radiation and platins have synergistic effects when administered concurrently [19]. We chose genes involved in platinum influx, efflux, and detoxification, as well as those involved in repairing cisplatin lesions through nucleotide excision repair (NER) or mismatch repair (MMR) and repairing radiation lesions through double-stranded break repair (DSBR) and base excision repair (BER).

Methods

Subjects

Germline DNA was obtained from subjects enrolled in ECOG trial E1201. Briefly, E1201 was a multicenter, randomized phase II trial (2002–2004) which enrolled treatment-naïve subjects with newly diagnosed adenocarcinoma of esophagus or gastroesophageal junction (tumor extension $\leq$2 cm into gastric cardia) [20]. Other eligibility criteria included: locally advanced stage (i.e., $T_{2-3}N_0M_0$, $T_{1-3}N_1M_0$ or $T_{1-3}N_{0-1}M_{1a}$), surgically resectable disease (T_{1-3} but not T_4), ECOG performance status 0–1, and staging by endoscopic ultrasound with esophagogastroduodenoscopy and computed tomography (CT) chest and abdomen. Subjects received in both arms radiotherapy to 45 Gy administered at 1.8 Gy per day, 5 days a week for 5 weeks, concurrent with chemotherapy. One arm received cisplatin 30 mg/m² days 1, 8, 22, 29, and irinotecan 65 mg/m² days 1, 8, 22, 29. The other arm received cisplatin 30 mg/m² days 1, 8, 15, 22, 29, and paclitaxel 50 mg/m² (1 h) days 1, 8, 15, 22, 29. Subjects in both arms underwent surgical resection about 5 weeks after the completion of RCT.

Toxicity data collection and categorization

Preoperative toxicity data were collected as part of E1201 during and up to 4–6 weeks following the completion of

RCT. At the time of each toxicity event, site investigators assigned a grade of 1, 2, 3, 4, or 5 corresponding to severity (Common Terminology Criteria for Adverse Events version 2.0 [21]) and a relationship to therapy of "possible," "probable," or "definite." For the current analysis, we included only grade 3–5 events of "probable" or "definite" relation to therapy. We focused on 2 toxicity endpoints: (1) radiation-related—events designated by site participants during clinical data collection as specifically resulting from radiotherapy; and (2) myelosuppressive (i.e., neutropenia, leukopenia, thrombocytopenia, anemia leading to red cell transfusion). Analysis was performed using worst-grade method [22]: that is, a subject was counted as having an event if he/she experienced at least one grade 3–4 event within each endpoint.

Retrieval and processing of specimens

Stored paraffin-embedded tissue specimens were obtained from the ECOG Pathology Coordinating Office. Both pretreatment biopsies and posttreatment resection samples were obtained for each subject whenever available. Fresh hematoxylin and eosin-stained sections (H&Es) were generated, then marked by an esophageal anatomic pathologist (E.A.M.) for areas containing only histologically nonmalignant tissue. (Tumor tissue was stored for other use.) Areas of Barrett's metaplasia and dysplasia were avoided. These H&Es were used as references for macrodissection of unstained slides. DNA was extracted from macrodissected specimens using the Qiagen QIAamp DNA FFPE Tissue Kit (Valencia, CA) following manufacturer's instructions.

Genotyping

A SNP was selected if it was: (1) located in one of the following pathways: NER, BER, DSBR, MMR, platinum influx/efflux/detoxification; and (2) previously associated with functional activity, cancer risk, outcome, radio- or chemotherapy response, or toxicity; or nonsynonymous. Twenty-one SNPs were chosen in 18 genes: multidrug resistance protein 1 (*MDR1*) [23]; ATPase, Cu ++ transporting, beta polypeptide (*ATP7B*) [24]; ATP-binding cassette, subfamily G, member 2 (*ABCG2*) [25]; glutathione S-transferase pi 1 (*GSTPi*) [26, 27]; mutS homolog 6 (*MSH6*); xeroderma pigmentosum, complementation group A (*XPA*) [28]; xeroderma pigmentosum, complementation group C (*XPC*); excision repair cross-complementing rodent repair deficiency, complementation group 2 (*XPD*) [18, 29–32]; cyclin H (*CCNH*) [33]; excision repair cross-complementing rodent repair deficiency, complementation group 1 (*ERCC1*) [30]; excision repair cross-complementing rodent repair deficiency, complementation group 6 (*ERCC6*), RAD23 homolog B (*RAD23B*) [34], X-ray repair complementing defective repair in Chinese hamster cells 2 (*XRCC2*) [35], ligase IV, DNA, ATP-dependent (*LIG4*) [32]; breast cancer 1, early onset (*BRCA1*); X-ray repair complementing defective repair in Chinese hamster cells 1 (*XRCC1*) [18, 31]; APEX nuclease 1 (*APEX*); poly (ADP-ribose) polymerase 1 (*ADPRT*) [36]. PCR and extension primers were designed on the Sequenom Assay Designer 3.1 Software (San Diego, CA) based on sequences available through the National Center for Biotechnology Information (dbSNP; http://www.ncbi.nlm.nih.gov/projects/SNP). Genotyping was performed using mass spectrometry-based genotyping software and matrix-assisted laser desorption ionization-time-of-flight spectrometry (MassArray System; Sequenom, San Diego, CA) following the manufacturer's instructions [37]. All samples were run in triplicate. The analysis of the spectra was done using the Sequenom Allelotyping Software Typer Version 4.0 (San Diego, CA; http://www.sequenom.com).

Analytic approach and statistical considerations

Data were pooled across both arms of the parent study and analyzed at ECOG. Lab investigators remained blinded to individual subject data throughout the study, including during data analysis. Genotypes were dichotomized a priori into 2 groups: (1) major homozygote or (2) variant allele group (heterozygote or variant-allele homozygote) [38]. Univariate comparisons were made between each SNP and toxicity endpoint. A pathway "dose" analysis was performed to assess whether subjects who had a greater number of molecular pathways "affected" (i.e., containing at least one variant allele in that pathway) experienced greater toxicity compared to subjects with fewer affected pathways [38]; a maximum of 7 molecular pathways (i.e., cisplatin influx, efflux, detoxification, MMR, DSBR, NER, and BER) could be affected. The analysis was done using a trend test from a logistic regression model with the number of affected pathways as the independent variable. Exact logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). All *P*-values and CIs are two-sided. Analyses were done in SAS version 8.2 (Cary, NC) for UNIX (SunOS). Using R^2 correlation (Haploview 4.2, broadinstitute.org), linkage was assessed between SNPs on 7q (*MDR1, XRCC2*), 9q (*XPA, RAD23B*), 13q (*LIG4, ATP7B*), 11 (*CD44, GST-Pi*), and 19q (*XPD, ERCC1, XRCC1*). $R^2 \geq 0.80$ were considered significant.

The study was approved by the ECOG Laboratory Science Committee, ECOG Gastrointestinal Committee, and the Institutional Review Boards of Johns Hopkins University and Mayo Clinic. Informed consent was obtained from the subject(s) and/or guardian(s) for enrollment in the parent study and for use of biospecimens in correlative laboratory studies.

Results

Assembly and description of cohort

Of 89 subjects in E1201 who consented to correlative lab use, baseline or follow-up tissue was available in 73 subjects. Sufficient germline DNA was extracted in 68 subjects. Subsequent review revealed that 8 of these subjects were ineligible for the parent study. Therefore, 60 subjects are included in this analysis, representing 74% of the 81 subjects ultimately deemed eligible for the parent study. Baseline clinicopathologic traits of the ancillary cohort were: median age 57 years (range 38–76); male 88%; white 93%, black 2%, Hispanic 2%, Asian 3%; node-negative ($T_{2-3}N_0M_0$) 28%, node-positive ($T_{1-3}N_1M_0$ or $T_{1-3}N_{0-1}M_{1a}$) 72%; ECOG performance status 0 (65%) and 1 (35%); mid- (2%) and lower thoracic (45%) esophagus, gastroesophageal junction (48%), esophagus not otherwise specified (3%), and unknown (2%).

Toxicity outcomes

Toxicity data were available in all subjects (Table 1). Grade 3 radiation-related toxicity occurred in 12 subjects: 10 subjects had dysphagia, 1 had pain, and 1 had both dysphagia and pain. No subject experienced grade 4 radiation-related toxicity. Grade 3 or 4 myelosuppression occurred in 20 subjects: 18 subjects had only neutropenia or leukopenia, 1 subject had thrombocytopenia and leukopenia, and 1 subject had anemia and neutropenia. No grade 5 events were reported.

Table 1 Toxicity outcomes

Toxicity	No. of toxicity events[a]		
	Grade 3	Grade 4	Total
Radiotherapy-related			
Dysphagia	11	0	11
Pain	2	0	2
Mucositis	0	0	0
Subtotal	13	0	13
No. unique subjects			12
Myelosuppression			
Neutropenia	9	3	12
Leukopenia	13	6	19
Thrombocytopenia	1	0	1
Anemia	1	0	1
Subtotal	24	9	33
No. unique subjects			20

[a] Probably or definitely related to therapy

Genotype and outcome

All subjects were successfully genotyped for all evaluated SNPs. For *GST-Pi* Ile-105-Val, 0 of 21 subjects with AA (major homozygote) genotype had grade 3–4 radiation-related toxicity, compared to 12 (31%) of 39 patients with GG or GA (variant) genotypes (Table 2, $P = 0.005$). For the *XRCC2* 5′ flanking SNP, 4 (9%) of 43 subjects with AA (major homozygote) genotype had grade 3–4 radiation-related toxicity, compared to 8 (47%) of 17 subjects with GG or GA (variant) genotypes ($P = 0.005$). No SNP was associated with myelosuppression (data not shown). Variant alleles were found in 2 molecular pathways in 1 subject, 3 pathways in 4 subjects, 4 pathways in 12 subjects, 5 pathways in 28 subjects, and 6 pathways in 15 subjects. Possessing a variant allele in a greater number of pathways was not associated with either toxicity endpoint (data not shown). No significant linkage was noted for the genotyped SNPs.

Discussion

RCT is commonly used to cure locally advanced EAC, yet causes substantial dose-limiting toxicity in a significant portion of patients. The a priori identification of patients who would experience serious radiation-related toxicity would aid clinical decision making, with the goal of individualizing therapy and minimizing adverse effects. In this study, using a multicenter trial cohort in which robust toxicity data was available, we found that variant alleles at 2 SNP loci—*XRCC2* or *GST-Pi*—were each linked to serious acute radiation-related toxicity that was probably or definitely related to cisplatin-based RCT. While numerous studies have evaluated biomarkers predicting therapeutic efficacy or prognosis in EAC, to our knowledge this is the first published report of genetic or molecular predictors of *toxicity* in EAC subjects receiving concurrent RCT. We did not find associations with grade 3–4 myelosuppression. In addition, under the hypothesis that chemotherapeutic agents and radiotherapy exert their effects through multiple steps and genes, we evaluated the possibility that possessing a variant allele across a greater number of radiation/platin pathways may influence toxicity [38]; however, we did not identify any associations. The fact that we did not detect such an association may be related to our smaller sample size.

In other tumor types, DNA-repair SNPs have been studied in their potential relationship to normal tissue complications caused by radiotherapy in gastrointestinal organs [39, 40], skin [11, 41], or unspecified anatomic locations [42]. Clear candidate markers which predict radiotherapy toxicity have yet to be identified. It is important to study

Table 2 Association between SNPs and grade 3–4 radiation toxicity

SNP and genotype	Toxicity/ Total (no.)	OR	95% CI	*P* value
Platinum uptake, efflux, and detoxification				
GST-Pi I105 V:rs1695				
GG or GA	12/39	NA	NA	0.005[a]
AA	0/21			
MDR1 I1145I:rs1045642				
CC or CT	9/44	1.11	0.23–7.37	1.0
TT	3/16			
MDR1 S893A:rs2032582				
TT, TG, or TA	7/38	0.77	0.18–3.58	0.93
GG	5/22			
ATP7B S406A:rs1801243				
TT or TG	7/39	0.70	0.16–3.28	0.82
GG	5/21			
ABCG2 Q141 K:rs2231142				
AA or AC	4/16	1.49	0.28–6.88	0.80
CC	8/44			
Mismatch repair				
MSH6 G39E:rs1042821				
TT or TC	3/24	0.43	0.07–2.03	0.40
CC	9/36			
Nucleotide excision repair				
XPA 5′ flank:rs1800975				
AA or AG	9/36	2.30	0.49–14.87	0.40
GG	3/24			
XPC K939Q:rs2228001				
CC or CA	5/31	0.61	0.13–2.60	0.65
AA	7/29			
XPD K751G:rs13181				
GG or GT	7/37	0.84	0.20–3.90	1.0
TT	5/23			
XPD D312 N:rs1799793				
AA or AG	7/35	1.0	0.23–4.61	1.0
GG	5/25			
XPD D711D:rs1052555				
TT or TC	7/33	1.18	0.28–5.43	1.0
CC	5/27			
CCNH V270A:rs2266690				
CC or CT	2/25	0.22	0.02–1.21	0.095
TT	10/35			
ERCC1 C8092A:rs3212986				
TT or TG	6/22	1.98	0.45–8.75	0.46
GG	6/38			
ERCC6 M1097 V:rs2228526				
GG or GA	6/25	1.52	0.35–6.63	0.74
AA	6/35			
RAD23B A249 V:rs1805329				
TT or TC	4/19	1.10	0.21–4.93	1.0
CC	8/41			
Double stranded break repair				
XRCC2 5′flank:rs6464268				
GG or GA	8/17	8.26	1.77–46.47	0.005
AA	4/43			
LIG4 D568D:rs1805386				
CC or CT	4/17	1.34	0.25–6.12	0.92
TT	8/43			
BRCA1 P871L:rs799917				
TT or TC	9/37	2.12	0.45–13.69	0.47
CC	3/23			
Base excision repair				
XRCC1 R399Q:rs25487				
AA or AG	7/31	1.39	0.33–6.40	0.85
GG	5/29			
APEX D148E:rs1130409				
TT or TG	9/45	1.0	0.20–6.67	1.0
GG	3/15			
ADPRT V762A:rs1136410				
CC or CT	1/17	0.19	0.004–1.49	0.16
TT	11/43			

CI confidence interval, *NA* not applicable, *OR* odds ratio, *SNP* single nucleotide polymorphism

[a] Fisher's exact test

RCT tolerance specifically in EAC, because EAC patients may tolerate multimodal treatment differently than patients with other cancers due to differences in radiation dose and schedule, comorbidities, nutritional status, and chemotherapy regimen.

In esophageal cancer, potential efforts to identify biomarkers which predict toxicity face numerous challenges. One major obstacle is the retrospective (likely incomplete) ascertainment of toxicity events; this may lead to the conflation or insufficient description of important factors, such as the timing of the tissue reaction (acute vs. late), the dose of radiation administered, or the likelihood that a particular clinical event was caused by radiotherapy [11].

Our study overcomes key limitations through the use of a prospective, interventional clinical trial. Our cohort was well-defined and homogenous in histopathology, staging, and therapeutic approach. Based on the predominance of adenocarcinoma histology, and the distribution of stage

(mostly locally advanced), age, and anatomic subsite (distal esophagus and GEJ) within the cohort [6, 43], and therapeutic approach received (platin-based neoadjuvant chemoradiotherapy) [3, 4, 44], this EAC study population is generalizable to other EAC patients in Western countries. Moreover, toxicity endpoints were clinically meaningful (i.e., grade 3 or 4), likely related to therapy (i.e., probably or definitely), and robustly and uniformly collected specifically to capture *acute* toxicity. An additional strength of our study is that lab investigators were blinded to clinical data, even during analysis.

Our SNPs were selected using a priori knowledge of biologic plausibility. We chose genes in radiation/platin pathways under the hypothesis that normal tissue tolerance may be affected by an altered ability to repair radio-/platin therapy-induced DNA lesions or by altered platin levels. Within these genes we chose SNPs previously linked to cancer risk, outcome, or radio- or chemotherapy response, as well as linked to toxicity itself, partly under the hypothesis that the genetic basis of cancer risk and the response of tumor and normal tissue to DNA-damaging agents, may be related.

We found that 65% of EAC subjects possessed a variant allele in the *GST-Pi* SNP, and that 31% of these subjects experienced acute serious radiation-related dysphagia or pain, compared to zero subjects without a variant allele ($P = 0.005$). The variant allele (i.e., GA or GG) at this loci codes for a different amino acid (Val instead of Ile at codon 105). Our clinical finding is consistent with mechanistic data regarding this SNP. GST-Pi is one of 7 main classes of human cytosolic glutathione S-transferases, which aid detoxification by catalyzing S-conjugation of glutathione to electrophilic compounds, including chemoagents and carcinogens [45]. The *GST-Pi* genotype has been associated with differences in chemotherapy response and cancer susceptibility [45]. In the *GST-Pi* locus (exon 5) which we studied, the G and A alleles code for valine and isoleucine, respectively, which reside in an electrophile-binding active site of the GST-Pi peptide. Computer modeling of the deduced crystal structures of the encoded peptides show significant deviations in the interatomic distances of critical electrophile-binding active site amino acids as a consequence of the amino acid changes [26]. Variant cDNAs of these 2 polymorphic GST-Pi genes were isolated in malignant glioma cells, and functional analysis of encoded proteins showed the G-allele form (valine) had lower substrate activity compared to the A-allele form (isoleucine) [26, 45]. This report is consistent with our hypothesis that the allele associated with higher toxicity (G) would be linked to reduced ability to conjugate toxins such as chemoagents. Our corollary hypothesis—that this allele (G) would be linked to higher chemotherapy response, due to reduced ability to conjugate chemoagents—is supported by links between the G-allele and lower risk of disease progression and death in colorectal cancer patients receiving oxaliplatin and 5-FU chemotherapy [27]. This SNP has not associated with efficacy or toxicity in other studies, but these other studies involved other tumor types and radiotherapy was not administered [46–48]. The ability of a cell to conjugate a toxin such as a chemoagent is relevant when studying radiation-related toxicity in patients receiving RCT, because chemoagents such as a cisplatin are known to be radiosensitizers [19].

We found that 28% of EAC subjects had a variant allele (i.e., GA or GG) in the *XRCC2* 5′ flanking SNP, and that 47% of these subjects had serious acute radiation-related dysphagia or pain, compared to 9% of subjects without the variant allele ($P = 0.005$). The *XRCC2* gene (7q36.1) is an essential part of the homologous recombination repair pathway, one of the main mechanisms of repairing double-stranded breaks (DSBs) [49]. DSBs may arise in numerous settings, including during physiologic DNA replication or after exposure to ionizing radiation or cytotoxic chemotherapy [49, 50]. Failure to repair DSBs can lead to mutations, apoptosis, tumor predisposition, and carcinogenesis [50]. Nonsynonymous SNPs in *XRCC2* have been associated with cancer risk [51] and outcomes [52], but not with skin complications due to radiotherapy [41, 53]. A recent case–control study found that the variant allele of the SNP in the 5′ flanking sequence of *XRCC2*, analyzed in the current study, was linked to reduced bladder cancer risk [35]. This association, together with the link we found between the variant (G) allele and higher toxicity after RCT, does not support the hypothesis that alleles leading to higher toxicity (presumably through reduced DNA repair) increase cancer risk. Further elucidation of the clinical and functional significance of this SNP may reveal more complex interactions which clarify its roles in cancer risk and therapeutic outcomes.

Despite the strengths of our study, our modest sample size lowers our ability to detect smaller differences. We may have missed other SNPs linked to radiation-related toxicity. However, while identifying smaller associations may refine predictive modeling, associations with large effect size may be most clinically meaningful. In addition, because the two arms of the study were pooled, the study does not account for the potential differential effects of the second chemoagent of each arm. Both possibilities are important, and our study provides rationale for further investigation in larger cohorts. Our study also cannot fully address whether the association between the SNPs and toxicity reflects the effects of cisplatin-radiotherapy versus radiotherapy alone. Only analysis of a large 2-arm study (comparing cisplatin-radiotherapy vs. radiotherapy alone) could answer this relevant question.

In conclusion, using a multicenter well-defined study population with robust endpoint ascertainment, we found

two SNPs involved in radiation/platin pathways to be associated with serious radiation toxicity probably/definitely related to radiotherapy. The results of this exploratory study point to the importance of further evaluating these markers in predicting radiation-related toxicity in this highly fatal and increasingly common disease.

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Conflict of interest The authors have declared no conflicts of interest.

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