Genetic Predictors of Severe Skin Toxicity in Patients with Stage III Colon Cancer Treated with Cetuximab: NCCTG N0147 (Alliance)

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ABSTRACT

Background: Cetuximab, an EGFR inhibitor used to treat multiple cancer types, including colon cancer, causes severe skin toxicity in 5%–20% of patients, leading to decreased quality of life and treatment delays. Our understanding of which patients have an increased risk of severe toxicities is limited. We conducted a genome-wide association study to identify germline variants predictive of cetuximab-induced severe skin toxicity.

Methods: Our study included 1,209 patients with stage III colon cancer randomized to receive cetuximab plus 5-fluorouracil and oxaliplatin as part of the NCCTG N0147 (Alliance) clinical trial. Skin toxicity outcomes were collected using the Common Toxicity Criteria for Adverse Events version 3.0. We performed genotyping, evaluating approximately 10 million genetic variants. We used logistic regression to evaluate the association of each genetic variant and severe (grade ≥ 3) skin toxicity, adjusting for age, sex, and genetic ancestry. Genome-wide significance was defined as $P < 5 \times 10^{-8}$.

Results: Participants were predominantly middle-aged white men; 20% ($n = 243$) experienced severe skin toxicity. Two genetic variants in the retinoic acid receptor alpha (*RARA*) gene were significantly associated with severe skin toxicity [OR, 3.93; 95% confidence interval (CI), 2.47–6.25; $P < 7.8 \times 10^{-9}$]. Functional annotations indicate these variants are in the *RARA* promoter. Additional significantly associated variants were identified in chromosome 2 intergenic regions.

Conclusions: Identified variants could represent a potential target for risk stratification of patients with colon cancer receiving cetuximab.

Impact: Retinoids have shown promise in the treatment of cetuximab-induced skin toxicity, so follow-up work could evaluate whether individuals with the *RARA* variant would benefit from retinoid therapy.

Introduction

Cetuximab, a mAb inhibiting the EGFR, is commonly used for treatment of metastatic squamous cell carcinoma of the head and neck and RAS-wild-type colorectal cancer. Skin toxicities, generally in the form of a follicular papulopustular ("acneiform") rash, are a common, dose-dependent side effect of treatment, affecting >90% of patients (1–3). With the high prevalence of rash in cetuximab-treated patients, prophylactic treatment with sunscreen, topical steroids, and oral antibiotics has become an important adjunct therapy (4, 5). This approach has been shown to reduce the severity of skin toxicity without reducing drug efficacy (6–8). Nonetheless, about 5%–20% of patients still develop skin toxicities severe enough to affect quality of life (9–11) and compromise drug delivery by causing treatment delays, dose reductions, or even discontinuation of therapy (1, 6, 12).

Our understanding of why patients develop EGFR-associated skin toxicity and which patients have an increased risk of severe toxicities is limited. It appears to be a class effect related to EGFR inhibition, with both cetuximab and panitumumab (a fully humanized mAb also inhibiting EGFR) capable of generating this rash in patients. Initially, research focused on candidate genetic variants related to EGFR (13–15). However, the clinical utility of such associations are complicated by the finding that rash severity is associated with cetuximab efficacy (1, 16–18). Thus, individuals with the highest risk of rash based on EGFR polymorphisms may also receive the most antitumor benefit from cetuximab therapy. Genome-wide association studies (GWAS) have been successful in identifying novel genetic markers of drug toxicity due to their agnostic and comprehensive approach (19). This approach provides the best opportunity to identify targetable markers of cetuximab-induced skin toxicity that do not impact drug efficacy. While one such GWAS has been conducted for EGFR toxicity, the power and sample size were limited and no variants reached genome-wide significance (20). The goal of our study was to

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

ClinicalTrial registration ID: NCT00079274 (NCCTG N0147).

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Cancer Epidemiol Biomarkers Prev 2021;30:404–11

doi: 10.1158/1055-9965.EPI-20-1274

use a large population to agnostically identify germline genetic variants associated with cetuximab-induced severe skin toxicity among patients with stage III colon cancer.

Patients and Methods

Study population and design

This study included data from the N0147 trial, a NCI-sponsored phase III multicenter randomized clinical trial led by the North Central Cancer Treatment Group (NCCTG). NCCTG is now part of the Alliance for Clinical Trials in Oncology. The details of this efficacy trial have been described previously (21). Briefly, participants with resected stage III colon cancer were recruited from clinical institutions across North America and randomly assigned to a variety of treatment groups. This analysis is limited to those patients who received adjuvant fluorouracil, leucovorin, and oxaliplatin (FOLFOX) every 2 weeks along with weekly cetuximab for a total of 12 cycles, as tolerated. All participants provided written informed consent and the study was approved by the institutional review board of all participating centers.

Classification of adverse events

Detailed information on adverse events was collected by clinicians at each study institution and graded according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 3.0 (22) prior to each chemotherapy dose. Toxicity grade was defined as the maximum grade experienced throughout the treatment period and subsequently dichotomized as grade 0–2 versus grade ≥3 to focus on severe skin toxicity. Skin toxicity was captured as a combined variable including desquamation and acneiform rash.

Genotyping and quality control

DNA was extracted from whole blood collected at study recruitment (n = 1,237 of 1,349 randomized to the cetuximab with FOLFOX treatment arm). As previously described (23), genotyping was performed at the Center for Inherited Disease Research at Johns Hopkins University (Baltimore, MD) using the Illumina HumanOmniExpress + Exome array (HumanOmniExpressExome-8v1–2, BPM annotation version A, genome build GRCh37/hg19), which consisted of a total of 964,193 genetic variants. We included 133 HapMap samples as genotyping controls. Standard quality control (QC) for genetic variants included removing variants with call rates <98%, discordance with technical duplicates, Mendelian errors, and sex differences in allele frequencies and heterozygosity. A total of 873,829 variants passed quality control (QC). Individual-level QC included removing individuals with <98% call rate, chromosomal anomalies, discordance in self-reported versus genetic sex, and relatedness. A total of 1,209 individuals (98%) passed quality control.

Population structure and imputation

Principal components analysis was implemented using PLINK (v1.9) to investigate population structure. The first seven eigenvectors discriminated individuals based on self-identified race and explained 82% of the genetic variation; these were used as covariates in analysis.

We imputed genotypes to infer unobserved genotypes and increase the genetic variant density. Samples were phased using SHAPEIT2 (24) and imputed using IMPUTE2 (25, 26), with the 1000 Genomes Project phase 3 (27) as the imputation reference panel. After imputation, we converted genotype probabilities to allelic dosages. Poorly imputed variants (info metric < 0.3) and variants with a minor allele frequency less than 1% were excluded. A total of 10,574,903 directly genotyped and imputed variants were included in the final analysis. Only autosomal chromosomes were analyzed.

Statistical analysis

Descriptive statistics comparing demographic variables by skin toxicity were calculated using χ^2 tests or t tests, as appropriate. Logistic regression was used to calculate ORs and 95% confidence intervals (CI) for the association of each genetic variant and severe skin toxicity, adjusting for age at diagnosis (continuous), sex, and the first seven eigenvectors of genetic ancestry. Genetic variants were modeled using the log-additive approach, relating genotype dosage (the expected number of risk allele copies) to development of severe skin toxicity. Quantile-quantile (QQ) plots were produced with 95% CIs based on the null distribution of observed P values, as well as genomic control coefficients to assess for possible systemic inflation and bias (28). We produced Manhattan plots and used a threshold of $P < 5 \times 10^{-8}$ to denote genome-wide significance. Analyses were performed using R version 3.5.2 (29).

In silico genomic follow-up

In silico bioinformatics analysis was performed for loci that reached genome-wide significance. The NCI "LDassoc" web tool (https://ldlink.nci.nih.gov) was used to nominate candidate causal variants at each locus by selecting the most statistically significant variant (the lead variant) and all variants in linkage disequilibrium (LD; defined as $R^2 \geq 0.8$ in 1000 Genomes Phase 3 "EUR" population; ref. 27) with the lead variant. HaploReg v4.1 was then used to evaluate the functional annotation and regulatory chromatin states in different cell lines for each locus variant (30). Using SNP NEXUS (31), locus variants were annotated with genome-wide functional prediction scores [CADD (ref. 32; Combined Annotation Dependent Depletion) and Eigen-PC; ref. 33], prioritizing variants with CADD phred score ≥ 20 or Eigen-PC phred scores ≥ 17. Using the University of California Santa Cruz (UCSC, Santa Cruz, CA) genome browser (http://genome.ucsc.edu/; ref. 34), we visually inspected whether any of the locus variants mapped to (i) regions with predicted regulatory function using the GeneHancer (35) trackhub or (ii) DNaseI hypersensitivity or transcription factor ChIP-seq (chromatin immunoprecipitation followed by sequencing) clusters based on the ENCODE (36, 37) trackhub. Tissue-specific gene expression and overlap of locus variants with expression Quantitative Trait Loci (eQTLs) were evaluated using GTEx data (https://www.gtexportal.org). Tissue-specific gene expression at the mRNA and protein level were further examined using Human Protein Atlas data (https://www.proteinatlas.org/humanproteome/tissue; ref. 38).

Results

Baseline demographic and clinical characteristics of the 1,209 patients with stage III colon cancer fulfilling the eligibility criteria for this study are shown in **Table 1**. Overall, 243 individuals (20%) had severe (grade 3–4) skin toxicities. The majority of these were grade 3, with only 7 individuals experiencing grade 4 skin toxicities. Compared with patients without severe skin toxicity, individuals with severe skin toxicity were younger (mean age 55 vs. 59 years, $P < 0.001$), more likely to be male (61% vs. 50%, $P = 0.002$), and less likely to be underweight (21% vs. 30%, $P = 0.032$).

The Manhattan plot for the genome-wide analysis is shown in **Fig. 1** (QQ plot: Supplementary Fig. S1). Variants on chromosomes 2 and 17 reached genome-wide significance ($P < 5 \times 10^{-8}$; **Table 2**). The peak on chromosome 17 contained three variants in strong LD ($R^2 > 0.8$),

Table 1. Characteristics of 1,209 patients with stage III colon cancer treated with cetuximab in the N0147 clinical trial.

	Overall ($n = 1,209$)	Severe skin toxicity ($n = 243$)	No severe skin toxicity ($n = 966$)	*P*
Age at diagnosis, mean years (SD)	58.0 (11.2)	58.7 (11.5)	55.1 (11.0)	<0.001[a]
Male sex	629 (52.0)	148 (60.9)	481 (49.8)	0.002
Race, self-reported				0.200[b]
White	1,035 (85.6)	216 (88.9)	819 (84.8)	
Black or African-American	86 (7.1)	12 (4.9)	74 (7.7)	
Asian	60 (5.0)	9 (3.7)	51 (5.3)	
American Indian or Alaska Native	4 (0.3)	0 (0.0)	4 (0.4)	
Native Hawaiian or other Pacific Islander	6 (0.5)	3 (1.2)	3 (0.3)	
Unknown/refused	18 (1.5)	3 (1.2)	15 (1.6)	
Hispanic ethnicity, self-reported				0.822
Yes	52 (4.3)	11 (4.5)	41 (4.2)	
No	953 (78.8)	188 (77.4)	765 (79.2)	
Unknown/refused	204 (16.9)	44 (18.1)	160 (16.6)	
Body mass index				0.032
<25	338 (28.0)	51 (21.0)	287 (29.7)	
25–29.9	433 (35.8)	95 (39.1)	338 (35.0)	
30+	429 (35.5)	93 (38.3)	336 (34.8)	
Missing	9 (0.7)	4 (1.6)	5 (0.5)	
Tumor stage				0.351
T1	50 (4.1)	9 (3.7)	41 (4.2)	
T2	136 (11.2)	23 (9.5)	113 (11.7)	
T3	891 (73.7)	190 (78.2)	701 (72.6)	
T4	132 (10.9)	21 (8.6)	111 (11.5)	
Node stage N2 (vs. N1)	506 (41.9)	92 (37.9)	414 (42.9)	0.181
Tumor location				0.683[b]
Proximal	631 (52.2)	129 (53.1)	502 (52.0)	
Distal	559 (46.2)	109 (44.9)	450 (46.6)	
Missing	19 (1.6)	5 (2.1)	14 (1.4)	

Note: *n* (%) shown unless otherwise specified. *P* values are χ^2 unless otherwise specified.
[a]*P* value from *t* test.
[b]*P* value from Fisher exact test.

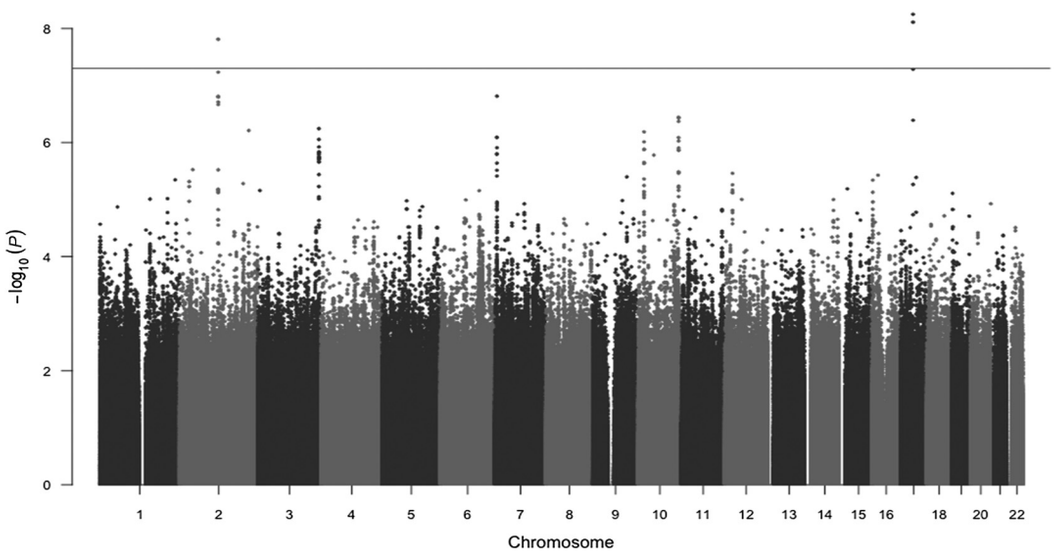

Figure 1.
Manhattan plot for the association of cetuximab-induced severe (grade ≥3) skin toxicity.

Table 2. Association of variants with cetuximab-induced severe skin toxicity, including variants reaching genome-wide significance and nearby variants in linkage disequilibrium ($R^2 \geq 0.8$; locus variants).

				Alt allele frequency						
Chr	Position	rs	(ref/alt)	Severe skin toxicity	No severe skin toxicity	OR (95% CI)	*P*	R^2	Imputation quality	Variant type
2	119,942,096	**rs7601541**	(C/G)	5.4%	1.3%	6.78 (3.49–13.15)	**1.54E-08**	1.00	0.87	Intergenic
2	119,942,097	**rs7562256**	(A/G)	5.4%	1.3%	6.78 (3.49–13.15)	**1.54E-08**	1.00	0.87	Intergenic
2	119,942,324	rs7557444	(G/T)	5.0%	1.3%	5.76 (2.99–11.09)	1.57E-07	0.85	0.87	Intergenic
2	119,943,540	rs114991178	(C/A)	5.0%	1.3%	5.76 (2.99–11.09)	1.57E-07	0.85	0.87	Intergenic
2	119,944,168	rs4277535	(G/C)	5.0%	1.3%	5.76 (3.00–11.09)	1.56E-07	0.85	0.87	Intergenic
2	119,945,757	rs114109299	(T/A)	4.9%	1.2%	5.72 (2.97–11.04)	1.95E-07	0.85	0.88	Intergenic
2	119,946,838	rs7600179	(C/T)	5.4%	1.6%	5.89 (3.10–11.18)	5.82E-08	0.85	0.88	Intergenic
17	38,433,225	**rs78201730**	(A/G)	9.1%	3.4%	3.98 (2.50–6.33)	**5.62E-09**	1.00	0.84	Intronic: *WIPF2*
17	38,468,692	rs117011100	(C/T)	9.1%	3.4%	3.93 (2.47–6.25)	**7.76E-09**	0.88	0.83	Intronic: *RARA*
17	38,470,142	rs74453681	(G/C)	9.1%	3.4%	3.93 (2.47–6.25)	**7.73E-09**	0.88	0.83	Intronic: *RARA*

Note: Bolded rs numbers indicate the lead SNP for that locus. Bolded *P* value indicates genome-wide significance ($P < 5 \times 10^{-8}$). ORs are adjusted for age at diagnosis, sex, and the first seven principal components.
Abbreviations: alt, alternate allele; ref, reference allele.

located at 38,433,225–38,470,142 bp (genome build GRCh37/hg19). The lead variant (rs78201730: OR, 3.98; 95% CI, 2.50–6.33; $P = 5.6 \times 10^{-9}$) was within the intronic region of the *WIPF2* gene (WAS/WASL-interacting protein family member 2). The other two variants (rs117011100: $P = 7.76 \times 10^{-9}$; rs74453681: $P = 7.73 \times 10^{-9}$; both OR, 3.93; 95% CI, 2.47–6.25) were within the intronic region of the *RARA* gene (retinoic acid receptor alpha).

The peak on chromosome 2 contained two neighboring intergenic variants (rs7601541 and rs7562256 at 119,942,096–119,942,097bp) in complete LD with each other ($R^2 = 1.0$) (**Fig. 2**). These were rare variants, with a 5% minor allele frequency (MAF) among affected individuals (OR, 6.78; 95% CI, 3.49–13.15; $P = 1.54 \times 10^{-8}$). Five additional intergenic variants at 119,942,324–119,946,838bp were in LD with the lead variants ($R^2 > 0.8$) but did not reach genome-wide significance.

In silico genomic annotation

On chromosome 17, the *RARA* intronic variants (rs117011100 and rs74453681) had higher predicted importance (CADD phred score 20.9 and 12.8, respectively) than the *WIPF2* intronic variant (rs78201730; CADD phred score 0.04; Supplementary Table S1). According to HaploReg, these variants overlapped with active enhancer (H3K27ac) and promoter (H3K9ac) histone marks in multiple cell lines, including skin (enhancer only). GeneHancer functional annotations indicated both *RARA* variants are in a promoter. In addition, these variants are located in regions consistent with transcription factor–binding sites and with active regulatory elements for several genes. Evaluation of gene expression and tissue specificity using GTEx and the Human Protein Atlas showed that both *RARA* and *WIPF2* have widespread expression and low tissue specificity, with moderate expression in the skin.

All variants on the chromosome 2 locus were intergenic with low predicted importance (CADD phred score < 1) with the exception of rs7557444 (CADD phred score 15.5; Supplementary Table S1). None of the variants were in active promoters or enhancers for skin cell lines according to HaploReg. Minimal functional annotations were identified using GeneHancer and eQTL data were not available for these loci. ENCODE annotations suggested rs7557444 may be in a transcription factor motif for FOS and MYC, two proto-oncogenes.

Discussion

Our GWAS identified novel genetic loci within the intronic regions of *WIPF2* and *RARA* that reached genome-wide significance for the association of severe skin toxicity in patients receiving cetuximab for stage III colon cancer treated within the NCCTG (Alliance) N0147 clinical trial. Intergenic variants on chromosome 2 also reached genome-wide significance.

Factors underlying cetuximab-induced skin toxicity occurrence and severity are poorly understood. It is believed that skin toxicity is caused by direct inhibition of EGFR in the skin, as EGFR is normally expressed in epidermal keratinocytes (39). To minimize dose-limiting toxicities while maintaining maximal drug efficacy, management of cetuximab-induced skin toxicity involves treatment with corticosteroids and/or antibiotics, escalating from topical to systemic based on rash severity (4, 5). Recommendations include cetuximab dose reduction or interruption with grade ≥3 rash. Recent randomized clinical trials have shown prophylactic therapy with systemic tetracycline antibiotics, prophylactic use of sunscreens, and low potency topical steroids can reduce rash occurrence and severity (40, 41). However, because this nonspecific approach can be associated with substantive adverse sequelae, it would be beneficial to identify individuals at higher risk of severe rash and tailor prophylactic therapy accordingly. Prior studies have identified EGFR polymorphisms (13–16), gene copy-number variants (18), and number of CA repeats within the *EGFR* gene are associated with skin toxicity, but this is not currently applied to clinical practice.

Rash severity is strongly associated with cetuximab efficacy (1, 16–18), highlighting a need to identify predictors of rash that can be targeted through alternative pathways. The agnostic GWAS approach has previously been successful in identifying genetic variants involved in chemotherapeutic toxicity for many cancer types, identifying potentially targetable mechanistic and regulatory pathways (19). With only 282 patients with colon cancer, the only prior GWAS to evaluate germline variants predictive of cetuximab-induced skin toxicity did not identify any variants reaching genome-wide significance (20). Ours is the first investigation to identify genetic variants associated with cetuximab-induced severe skin toxicity that are unrelated to EGFR.

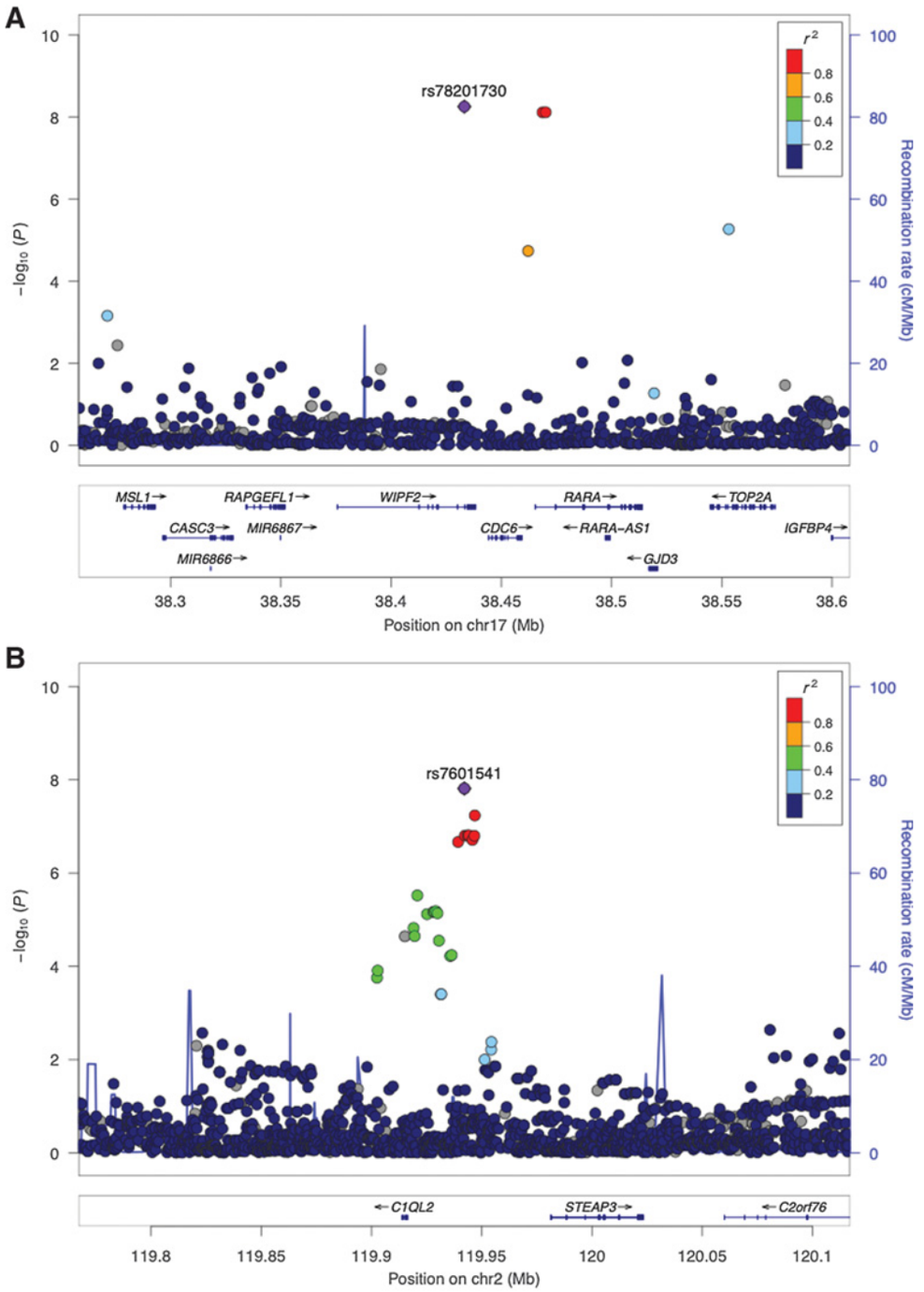

Figure 2.
Regional LocusZoom plot showing the chromosome 17 (**A**) and chromosome 2 (**B**) region of interest using hg19 genome build. Lead variants are depicted as purple diamonds, and color labeling indicates pairwise LD (R^2) in relation to the lead variant.

On the basis of CADD and functional annotation, the two *RARA* variants (rs117011100 and rs74453681) have biologically plausible effect. The *RARA* gene is responsible for producing retinoic acid receptor alpha (RARα), a transcription factor important in retinoid signaling (42). Both the retinoid isotretinoin and topical retinoids, such as adapalene, tazarotene, and tretinoin, have high clinical efficacy for the treatment of acne vulgaris (43). While cetuximab-associated skin toxicity appears visually similar to acne vulgaris, it is histopathologically distinct (44). However, a few case reports have evaluated the treatment of cetuximab-induced skin toxicity with either systemic or

topical retinoids (45–49). In general, these reports have shown promising results, with substantial reduction of skin lesions without requiring cetuximab dose reduction, albeit with limited sample sizes. There has been no apparent effect on cetuximab efficacy, but rigorous assessment was not conducted. However, a randomized clinical trial of prophylactic tazarotene in patients with stage IV colorectal cancer receiving cetuximab found no observed clinical benefit and noted 33% of patients discontinued therapy due to significant skin irritation (8). This could be due to differences in formulation, dosing (once vs twice daily), or prophylactic versus reactive use. More research is needed to determine which patients undergoing cetuximab treatment may benefit from retinoid therapy and to establish the most effective formulation and dosing regimen. Currently, low-dose isotretinoin is only recommended for refractory grade ≥3 rash (12).

The mechanism of action of isotretinoin is unknown. While it does not directly bind RARs, it may act as a prodrug that is converted to metabolites that act as agonists for retinoic acid nuclear receptors (50). Adapalene and tazarotene are retinoid prodrugs which bind to all three members of the retinoic acid receptor family (α, β, and γ), but appear to have selectivity for RARβ and RARγ (51, 52). It is unclear how the identified *RARA* variants may influence rash severity and whether these variants influence response to retinoid therapy. Because of the design of GWAS platforms, the variants identified are likely surrogates for the causal variants and additional sequencing is warranted to gain a detailed understanding of the locus. However, it is plausible that the minority of patients with these variants might benefit from retinoid therapy. Further work is needed to identify the causal variants, determine the utility of retinoids in patients with these germline variants, and ensure there is no effect of retinoid therapy on cetuximab efficacy.

On the basis of the low overall minor allele frequency and minimal predicted biologic effect using *in silico* bioinformatics, we have less confidence in the significance of our GWAS findings associated with the chromosome 2 locus (rs7601541 and rs7562256). These variants are more common in African populations (MAF 9% vs. 2% in European based on 1000 Genomes phase 3; ref. 27) and our study population was primarily of European descent. Validation of this finding in another ethnically diverse population and in larger sample sizes could help differentiate whether this may be a spurious finding or instead a biologically important rare variant not well represented in our current trial sample set.

To date, this is the largest GWAS evaluating variants associated with cetuximab-induced skin toxicity. In addition to the large sample size, strengths of our study include standardized treatment protocols, uniform assessment of skin toxicity, and controlled treatment administration. Our study also has some limitations. Our analysis was unable to account for the timing of skin toxicity, dose cycle at which the greatest toxicity was reported, total number of skin toxicities experienced, or dose density of cetuximab. Despite randomization, we noted some differences in the age and sex of those experiencing severe skin toxicity, so these variables were adjusted for in our analysis. We also noted some differences in BMI for those experiencing severe skin toxicity but were unable to account for this in our analysis due to low sample sizes within each BMI category. Further research is needed to determine whether BMI influences the observed association between *RARA* variants and severe skin toxicity, as some laboratory research has shown a link between RARs and obesity (53). We had limited racial and ethnic diversity in our study population and did not assess patients treated with alternate systemic anti-EGFR antibodies (i.e., panitumumab), which may restrict generalizability of our findings.

In conclusion, we identified novel loci in the *RARA* gene that were associated with severe cetuximab-induced skin toxicity. This finding could represent a potential therapeutic target for prophylactic or reactive treatment in a subset of patients undergoing treatment with this agent. If validated, a precision medicine strategy using these variants could risk stratify cancer patients undergoing cetuximab treatment to direct management of skin toxicity.

Authors' Disclosures

Q. Shi reports other from Amgen, Merck & Co., and Johnson & Johnson; personal fees from Yiviva Inc.; grants from Celgene and Roche/Genentech; and personal fees from Boehringer Ingelheim Pharmaceuticals, Inc outside the submitted work. G. Yothers reports grants from NCI during the conduct of the study. F.A. Sinicrope reports a patent for Roche Tissue Diagnostics issued and with royalties paid. R.M. Goldberg reports grants from NCI during the conduct of the study and personal fees from Amgen, Bayer, AstraZeneca, Merck, Novartis, Genentech, and Taiho outside the submitted work. T.J. George reports other from BMS, Merck, AstraZeneca, Genentech, Tesaro/GSK, Ipsen, and Seattle Genetics outside the submitted work. A.T. Chan reports grants and personal fees from Bayer Pharma AG and personal fees from Pfizer Inc. and Boeringer Ingelheim outside the submitted work. No disclosures were reported by the other authors.

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Acknowledgments

The authors thank all study participants and staff of the NCCTG N0147 (Alliance) clinical trial. This work was supported by the NCI of the NIH under Award Numbers U10CA180821, U10CA180882, and U24CA196171 (to the Alliance for Clinical Trials in Oncology); UG1CA233180 and UG1CA233339 (https://acknowledgments.alliancefound.org); UG1CA189858 (Southeast Clinical Oncology Research Consortium; and R01CA176272 (to P.A. Newcomb and A.T. Chan). It was also supported in part by funds from Genentech, Sanofi, Pfizer, and Eli Lilly and Company. Fred Hutchinson Cancer Research Center investigators were also supported by NIH T32 CA094880 and NIH K05 CA152715 (to J.D. Labadie and P.A. Newcomb).

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Received August 27, 2020; revised October 16, 2020; accepted November 12, 2020; published first November 17, 2020.

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