

BRIEF COMMUNICATION

A Rare Truncating BRCA2 Variant and Genetic Susceptibility to Upper Aerodigestive Tract Cancer

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Abstract

Deleterious BRCA2 genetic variants markedly increase risk of developing breast cancer. A rare truncating BRCA2 genetic variant, rs11571833 (K3326X), has been associated with a 2.5-fold risk of lung squamous cell carcinoma but only a modest 26% increase in breast cancer risk. We analyzed the association between BRCA2 SNP rs11571833 and upper aerodigestive tract (UADT) cancer risk with multivariable unconditional logistic regression adjusted by sex and combinations of study and country for 5942 UADT squamous cell carcinoma case patients and 8086 control patients from nine different studies. All statistical tests were two-sided. rs11571833 was associated with UADT cancers (odds ratio = 2.53, 95% confidence interval = 1.89 to 3.38, $P = 3 \times 10^{-10}$) and was present in European, Latin American, and Indian populations but extremely rare in Japanese populations. The association appeared more apparent in smokers (current or former) compared with never smokers ($P_{\text{het}} = .026$). A robust association between a truncating BRCA2 variant and UADT cancer risk suggests that treatment strategies orientated towards BRCA2 mutations may warrant further investigation in UADT tumors.

Received: July 1, 2014; Revised: September 5, 2014; Accepted: January 29, 2015

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Upper aerodigestive tract (UADT) cancers, predominantly squamous cell in origin, comprise the oral cavity, larynx, and esophagus. UADT cancers are the fourth most common cause of cancer death worldwide (1). Consumption of tobacco and alcohol are established UADT cancer risk factors (2), but genetic epidemiology (3) and familial studies (4) provide evidence for a role of genetic susceptibility in the pathogenesis of this disease.

Exposure to tobacco and alcohol leads to cell damage and DNA alterations that, in the absence of repair, may cause cell cycle deregulation and cancer development (5,6). DNA repair genes, like BRCA2, play an important role in repair of DNA lesions (7,8), and germline genetic variation affecting gene function may modulate disease risk. A recent imputation-based genome-wide association study of 21597 lung cancer case patients and 54 083 control patients identified a rare truncating BRCA2 genetic variant, rs11571833 (K3326X), to be robustly associated with lung cancer. While present in lung adenocarcinomas

(odds ratio [OR] = 1.47, $P = 5 \times 10^{-4}$), the association was more pronounced in lung squamous cell carcinomas (OR = 2.47, $P = 10^{-20}$) (9). As squamous cell carcinomas (SCCs) of different anatomical sites share many phenotypic and molecular characteristics (10), the present study investigated rs11571833 in the context of genetic susceptibility to UADT cancers.

We explored rs11571833 in nine UADT cancer case-control studies (study designs and population characteristics have been described previously [3,11,12]) totalling 5942 UADT cancer case patients and 8086 control patients (Table 1). Study participants underwent informed consent and approval by local and International Agency for Research on Cancer institutional ethics (IRB) review. rs11571833 was genotyped using Taqman (C_27537307_30 Applied Biosystems, Carlsbad, CA), with operators blinded to case-control status, as described elsewhere (9). Regentyping samples of known genotype obtained by orthogonal genotyping techniques (90 Hapmap CEU samples where

Table 1. Demographic characteristics of the case patients and control patients included in the genetic susceptibility study of BRCA2 rs11571833 genetic variant*

		Case patients	Control patients	MAF control patients	MAF case patients
Study name/population characteristics	Study setting				
ARCAGE	Europe - multicenter	1401	1505	0.006	0.017
Central-Europe	Europe - multicenter	741	1652	0.005	0.015
Rome (HNI)	Roma - Italy	309	231	0.009	0.024
ACTREC	India	220	358	0.010	0.002
Japan	Aichi Cancer Center Hospital	556	1203	0.000	0.001
SA	Latin America - multicenter	1493	1207	0.006	0.011
Oral cancer (ORC)	Europe - multicenter	419	457	0.005	0.013
Oral cancer (ORC)	India	401	457	0.004	0.012
Poland	Szczecin - Poland	402	1016	0.003	0.011
Sex					
Male		4584	5966	0.004	0.012
Female		1358	2120	0.005	0.013
Age group					
<50 y		1091	1629	0.005	0.013
≥50 y		4447	6456	0.004	0.013
Missing		404	1	0.000	0.011
Smoking status					
Never smokers		847	2632	0.006	0.005
Ever smokers		4686	4433	0.005	0.014
Former		1145	1981	0.003	0.013
Current		3485	2338	0.005	0.014
Missing		56	114	0.009	0.000
Missing		409	1021	0.003	0.013
Alcohol intake status					
Never drinkers		915	1927	0.004	0.010
Ever drinkers		4614	5141	0.005	0.013
Former		720	508	0.007	0.017
Current		2575	2514	0.004	0.011
Missing		1319	2119	0.006	0.014
Missing		413	1018	0.003	0.013
Site of tumor					
Oral cavity		2230			0.011
Oropharynx		856			0.013
Larynx/hypopharynx		2195			0.014
Esophagus		635			0.012
Missing		26			0.019

* ACTREC = Advanced Centre for Treatment, Research and Education in Cancer oral cancer study; ARCAGE = Alcohol Related Cancers and Genetic susceptibility in Europe; HNI = Rome (Italy) head and neck cancer study; MAF = minor allele frequency; ORC = IARC multicentre oral cancer case control study; SA = South America.

one individual was heterozygous) and direct DNA sequencing of Taqman-defined heterozygote individuals ($n = 10$) confirmed the fidelity of the Taqman assay. Internal study duplicate (approximately 8% from each study) concordance was over 99%. rs11571833 minor allele (T) was rare in Europe and Latin America, with a minor allele frequency in control patients of 0.50% and 0.62%, respectively, less prominent in the Indian subcontinent (0.27%) and extremely rare in Japan (only one single heterozygote observed in case patients and none in the 1203 control individuals). rs11571833 homozygote individuals were not observed, although none were expected given the variant frequency and genotype distributions expected under Hardy-Weinberg equilibrium. We used multivariable unconditional logistic regression with variables for sex and combinations of study and country included in the model as covariables. All statistical tests were two-sided. rs11571833 was strongly associated with UADT cancers (OR = 2.53, 95% CI = 1.89 to 3.38, two sided $P = 3 \times 10^{-10}$), with the minor allele carriers of BRCA2 rs11571833 having an important 2.5-fold increased risk of UADT cancers (Figure 1). Within the Central-European and alcohol-related cancers and genetic susceptibility in European head and neck cancer studies where genome-wide genotyping data were available, this association did not appear sensitive to cryptic population structure, as adjusting for genotype inferred genetic ancestry had little effect (Supplementary Table 1, available online).

rs11571833 has been previously associated with increased risk of squamous cell esophageal cancers (13,14), a finding we replicated in an independent study population (OR = 3.30, $P = 3 \times 10^{-4}$) and extended to other UADT cancer subsites ($P_{\text{het}} = .58$) (Figure 1). Serological HPV16E6 status has been defined for 514/856 of the oro-pharyngeal cancers presented here (15,16); however, only two oro-pharyngeal cancers and one control patient were both HPV16E6-positive and rs11571833 carriers. The association appeared consistent across the various studies ($P_{\text{het}} = .33$) and by drinking status ($P_{\text{het}} = .63$), although it appeared to vary somewhat by smoking status ($P_{\text{het}} = .026$), with little evidence for association within never smokers. Where survival information was available (the ARCAGE study), there was a tendency for decreased overall survival in allele carriers compared with noncarriers (hazard ratio = 1.72, $P = .05$), although this result should be interpreted with caution as it is based on low numbers (Supplementary Figure 1, available online).

While less common than cancers of the prostate, pancreas, and ovary, some excess of laryngeal and pharyngeal cancers have also been reported in BRCA2 mutation-positive families (17,18), implying a potential role for deleterious BRCA2 mutations in UADT cancer genetic susceptibility. Germline mutations in the BRCA2 gene have also been implicated in Fanconi Anemia (19), which includes UADT cancers within its disease spectrum (20,21). We did not identify any additional deleterious germline

rs11571833/BRCA2 (T)	ca	co	p	OR	95%CI
Log-additive	5942	8086	3.5e-10	2.53	1.89 to 3.38
Heterozygous	149	75	3.5e-10	2.53	1.89 to 3.38
By subgroup ($p_{\text{het}} = .583$)					
Oral cavity	2230	8086	2e-04	2.12	1.44 to 3.13
Oropharynx	856	8086	6e-04	2.50	1.48 to 4.20
Larynx/Hypopharynx	2195	8086	6.6e-08	2.93	1.98 to 4.34
Esophagus	635	8086	3e-04	3.30	1.74 to 6.26
By study ($p_{\text{het}} = .333$)					
Arcage	1401	1505	5.3e-05	3.12	1.80 to 5.41
Central Europe	741	1652	.0013	3.14	1.56 to 6.31
SA (Latin America)	1493	1207	.0971	1.69	0.91 to 3.16
HNI	309	231	.0567	3.04	0.97 to 9.55
Poland	402	1016	.0104	4.01	1.39 to 11.59
ORC	419	457	.0856	2.55	0.88 to 7.43
Japan	556	1203	NA	NA	NA
ORC India	401	457	.0882	2.77	0.86 to 8.91
ACTREC India	220	358	.1222	0.19	0.02 to 1.57
By sex ($p_{\text{het}} = .721$)					
Male	4584	5966	4.7e-08	2.58	1.83 to 3.62
Female	1358	2120	.0047	2.28	1.29 to 4.04
By age ($p_{\text{het}} = .643$)					
Young (age ≤ 50 y)	1091	1629	.0167	2.15	1.15 to 4.02
Old	4447	6456	1.1e-07	2.55	1.80 to 3.59
By smoking status ($p_{\text{het}} = .026$)					
Never smokers	847	2632	.6892	0.84	0.37 to 1.93
Former smokers	1209	2010	3e-04	3.52	1.78 to 6.95
Current smokers	3421	2309	1e-04	2.51	1.59 to 3.97
By smoking intensity ($p_{\text{het}} = .021$)					
Never smokers	847	2632	.6892	0.84	0.37 to 1.93
Light smokers	1441	2126	1.5e-05	3.23	1.90 to 5.50
Heavy smokers	3127	2082	2e-04	2.83	1.63 to 4.94
By alcohol status ($p_{\text{het}} = .626$)					
Never drinkers	915	1927	.0391	2.06	1.04 to 4.10
Ever drinkers	4614	5141	1.8e-07	2.50	1.77 to 3.52

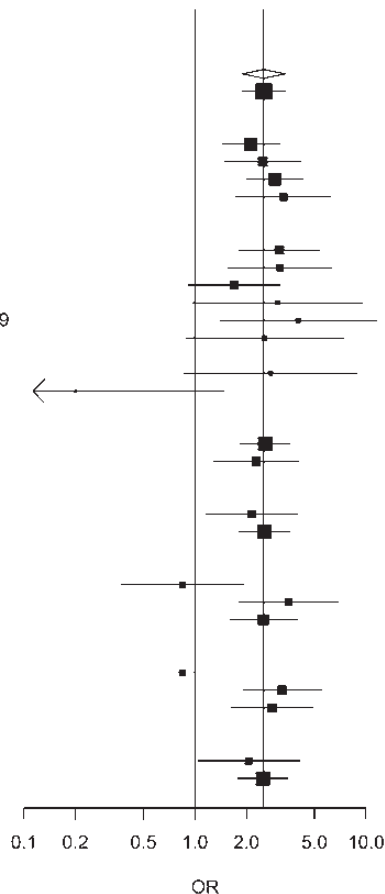


Figure 1. Association between BRCA2 SNP rs11571833 and upper aerodigestive tract cancer risk. Squares represent odds ratios, size of the square represents the inverse of the variance of the log odds ratios; horizontal lines represent 95% confidence intervals. The solid vertical line indicates an odds ratio of 1 and the dashed vertical line the overall odds ratio. The arrow indicates that the confidence interval of this particular estimate exceeds the scale of the plot. Results derived from a two-sided multivariable unconditional logistic regression adjusted by sex and study-specific country. All statistical tests were two-sided. ACTREC = Advanced Centre for Treatment, Research and Education in Cancer oral cancer study; CI = confidence interval; HNI = Rome (Italy) head and neck cancer study; OR = odds ratio; ORC = IARC multicentre oral cancer case control study; SA = South America.

BRCA2 mutations in our genetic analysis of the germline exome sequences of 10 head and neck squamous cell (HNSC) carcinomas in carriers of rs11571833 identified by The Cancer Genome Atlas (TCGA) initiative. Although numbers are small, these observations, together with similar ones made in 24 rs11571833 lung cancer carriers (9), suggest that linkage disequilibrium (LD) with additional BRCA2 mutations appears unlikely to explain the association. rs11571833 encodes a truncated form of the BRCA2 protein, resulting in the loss of the final 93 amino acids and is not considered a high-risk breast cancer allele (22). Consistent with this, in vitro studies suggest that K3326X does not have comparable functional consequences to the gene product as do the deleterious BRCA2 breast cancer susceptibility alleles (23). Somatic loss (somatic mutation or loss of heterozygosity) of the wild-type allele of the BRCA2 gene was not observed in the 10 TCGA HNSC rs11571833 carriers: This is similar to lung cancer (9), whereas this loss is common in BRCA2 germline mutation-positive breast tumors.

The limitations of this study are the rarity of rs11571833, which constrains our statistical power to investigate the heterogeneity of the association across substrata or correlations with factors such as tumor loss of heterozygosity. Nor was it possible to rule out that rs11571833 is an LD proxy for a variant in another gene, although rs11571833 is itself a noteworthy variant in a relevant gene.

rs11571833 has been associated with pancreatic cancer (24) and a modest but statistically significantly increased risk (26%) of breast cancer (OR = 1.26) (25). The 2.5-fold increase in genetic risk noted for UADT cancer here and squamous cell lung cancers (9) is markedly higher. The difference in risk, together with the ambiguous functional consequence of this variant, suggests an alternate susceptibility mechanism for K3326X compared with the highly deleterious BRCA2 breast cancer susceptibility alleles. Nevertheless, although the population-attributable fraction remains modest (0.7%), a robust association between a truncating BRCA2 variant and UADT cancer risk suggests that treatment strategies like PARP1 inhibitors or synthetic lethality approaches targeting RAD52 and BRCA2 (26,27) may warrant further consideration in UADT tumors.

Funding

This work was supported the National Institutes of Health (R01 CA092039 05/05S1) and the National Institute of Dental and Craniofacial Research (1R03DE020116).

Notes

The authors thank all of the participants who took part in this research and the funders and technical staff who made this study possible. We acknowledge and thank Simone Benhamou (INSERM, France) for sample contributions. We also acknowledge and thank The Cancer Genome Atlas initiative, whose data contributed heavily to this study.

The authors have no conflicts of interest to declare.

References

1. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127(12):2893–2917.
2. Stewart B, Kleihues P. *World Cancer Report*: IARC Press; 2003.
3. McKay JD, Truong T, Gaborieau V, et al. A genome-wide association study of upper aerodigestive tract cancers conducted within the INHANCE consortium. *PLoS Genet*. 2011;7(3):e1001333.
4. Negri E, Boffetta P, Berthiller J, et al. Family history of cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Int J Cancer*. 2009;124(2):394–401.
5. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature*. 2001;411(6835):366–374.
6. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN): 1. Carcinogen metabolism, DNA repair and cell cycle control. *Oral Oncol*. 2000;36(3):256–263.
7. Thacker J. The role of homologous recombination processes in the repair of severe forms of DNA damage in mammalian cells. *Biochimie*. 1999;81(1–2):77–85.
8. Sung P, Klein H. Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nat Rev Mol Cell Biol*. 2006;7(10):739–750.
9. Wang Y, McKay JD, Rafnar T, et al. Rare variants of large effect in BRCA2 and CHEK2 affect risk of lung cancer. *Nat Genet*. 2014;46(7):736–741.
10. Yan W, Wistuba, II, Emmert-Buck MR, et al. Squamous Cell Carcinoma - Similarities and Differences among Anatomical Sites. *Am J Cancer Res*. 2011;1(3):275–300.
11. Anantharaman D, Chabrier A, Gaborieau V, et al. Genetic variants in nicotine addiction and alcohol metabolism genes, oral cancer risk and the propensity to smoke and drink alcohol: a replication study in India. *PLoS One*. 2014;9(2):e88240.
12. Oze I, Matsuo K, Hosono S, et al. Comparison between self-reported facial flushing after alcohol consumption and ALDH2 Glu504Lys polymorphism for risk of upper aerodigestive tract cancer in a Japanese population. *Cancer Sci*. 2010;101(8):1875–1880.
13. Akbari MR, Malekzadeh R, Nasrollahzadeh D, et al. Germline BRCA2 mutations and the risk of esophageal squamous cell carcinoma. *Oncogene*. 2008;27(9):1290–1296.
14. Akbari MR, Malekzadeh R, Lepage P, et al. Mutations in Fanconi anemia genes and the risk of esophageal cancer. *Hum Genet*. 2011;129(5):573–582.
15. Anantharaman D, Gheit T, Waterboer T, et al. Human papillomavirus infections and upper aero-digestive tract cancers: the ARCAE study. *J Natl Cancer Inst*. 2013;105(8):536–545.
16. Ribeiro KB, Levi JE, Pawlita M, et al. Low human papillomavirus prevalence in head and neck cancer: results from two large case-control studies in high-incidence regions. *Int J Epidemiol*. 2011;40(2):489–502.
17. van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, et al. Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J Med Genet*. 2005;42(9):711–719.
18. Breast-Cancer-Linkage-Consortium. Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst*. 1999;91(15):1310–1316.
19. Howlett NG, Taniguchi T, Olson S, et al. Biallelic inactivation of BRCA2 in Fanconi anemia. *Science*. 2002;297(5581):606–609.
20. Alter BP. Cancer in Fanconi anemia, 1927–2001. *Cancer*. 2003;97(2):425–440.
21. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood*. 2003;101(3):822–826.
22. Mazoyer S, Dunning AM, Serova O, et al. A polymorphic stop codon in BRCA2. *Nat Genet*. 1996;14(3):253–254.
23. Wu K, Hinson SR, Ohashi A, et al. Functional evaluation and cancer risk assessment of BRCA2 unclassified variants. *Cancer Res*. 2005;65(2):417–426.
24. Martin ST, Matsubayashi H, Rogers CD, et al. Increased prevalence of the BRCA2 polymorphic stop codon K3326X

- among individuals with familial pancreatic cancer. *Oncogene*. 2005;24(22):3652–3656.
25. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat Genet*. 2013;45(4):353–361, 361e1–361e2.
26. Lok BH, Carley AC, Tchang B, et al. RAD52 inactivation is synthetically lethal with deficiencies in BRCA1 and PALB2 in addition to BRCA2 through RAD51-mediated homologous recombination. *Oncogene*. 2013;32(30):3552–3558.
27. Cramer-Morales K, Nieborowska-Skorska M, Scheibner K, et al. Personalized synthetic lethality induced by targeting RAD52 in leukemias identified by gene mutation and expression profile. *Blood*. 2013;122(7):1293–1304.
28. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38(8):904–909.
29. Yu K, Wang Z, Li Q, et al. Population substructure and control selection in genome-wide association studies. *PLoS One*. 2008;3(7):e2551.