Research Article

Cancer Epidemiology, Biomarkers & Prevention

CDKN2A Germline Rare Coding Variants and Risk of Pancreatic Cancer in Minority Populations

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Abstract

Background: Pathogenic germline mutations in the *CDKN2A* tumor suppressor gene are rare and associated with highly penetrant familial melanoma and pancreatic cancer in non-Hispanic whites (NHW). To date, the prevalence and impact of *CDKN2A* rare coding variants (RCV) in racial minority groups remain poorly characterized. We examined the role of *CDKN2A* RCVs on the risk of pancreatic cancer among minority subjects.

Methods: We sequenced *CDKN2A* in 220 African American (AA) pancreatic cancer cases, 900 noncancer AA controls, and 183 Nigerian controls. RCV frequencies were determined for each group and compared with that of 1,537 NHW patients with pancreatic cancer. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for both a case–case comparison of RCV frequencies in AAs versus NHWs, and case–control comparison between AA cases versus noncancer AA controls plus Nigerian controls. Smaller sets of Hispanic and Native American cases and controls also were sequenced.

Results: One novel missense RCV and one novel frameshift RCV were found among AA patients: 400G>A and 258_278del. RCV carrier status was associated with increased risk of pancreatic cancer among AA cases (11/220; OR, 3.3; 95% CI, 1.5–7.1; $P = 0.004$) compared with AA and Nigerian controls (17/1,083). Further, AA cases had higher frequency of RCVs: 5.0% (OR, 13.4; 95% CI, 4.9–36.7; $P < 0.001$) compared with NHW cases (0.4%).

Conclusions: *CDKN2A* RCVs are more common in AA than in NHW patients with pancreatic cancer and associated with moderately increased pancreatic cancer risk among AAs.

Impact: RCVs in *CDKN2A* are frequent in AAs and are associated with risk for pancreatic cancer. *Cancer Epidemiol Biomarkers Prev; 27(11); 1364–70. ©2018 AACR.*

Introduction

Pancreatic cancer, especially pancreatic ductal adenocarcinoma, is a highly lethal cancer with 1-year and 5-year survival rates of 26% and 8%, respectively (1). Long-term survival with pancreatic cancer is generally dependent on resection of an early-stage tumor. However, early detection of pancreatic cancer is uncommon, with only 20% of all patients found to have localized disease at the time of diagnosis (1). African Americans (AAs) consistently have a higher incidence of pancreatic cancer and poorer survival after diagnosis compared with non-Hispanic whites (NHWs; ref. 1). AAs also tend to present with more advanced-stage cancer at diagnosis (2). Reasons for the higher incidence of pancreatic cancer among AAs are not completely clear. Known epidemiologic risk factors, such as obesity and tobacco smoking, do not fully explain the excess risk of pancreatic cancer among AAs (3). It is therefore plausible that the higher incidence of pancreatic cancer among AAs may be due in part to inherited genetic predisposition.

It is well established that risks for pancreatic cancer and melanoma are increased in families of the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*) germline mutation carriers (4–8). In general, melanoma occurs primarily in NHWs, with an annual incidence rate of 32.3 per 100,000 men and 20.0 per

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

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doi: 10.1158/1055-9965.EPI-17-1065

100,000 women in the United States, which is far in excess of that observed among AAs (1.0 per 100,000 in males and females), Hispanics (4.8 per 100,000 males and 4.6 per 100,000 females), or Native Americans (4.1 per 100,000 males and 4.0 per 100,000 females; ref. 9). The prevalence of *CDKN2A* RCVs among NHWs with melanoma is approximately 20% to 57% in melanoma-prone families (10), but the prevalence is only about 1% to 2% among unselected patients with a single melanoma diagnosis in their families (11). The penetrance estimates for melanoma among NHW *CDKN2A* mutation carriers is 28% by age 80 years (12), and for pancreatic cancer approximately 58% by age 80 (13). Somatic mutations and loss of p16 expression are commonly found in cutaneous malignant melanoma (14, 15). Similarly, somatic alterations (including mutations, loss of heterozygosity, and hypermethylation) in *CDKN2A* have been reported in up to 95% of pancreatic tumors, underscoring the importance of this gene in pancreatic tumorigenesis (16).

Thus, our objective was to elucidate the role of pathogenic germline rare coding variants (RCV) of *CDKN2A* in relation to pancreatic cancer risk in minority groups. There are major challenges to the study of germline *CDKN2A* RCVs in pancreatic cancer because of (i) the requirement for rapid case ascertainment to obtain biospecimens suitable for genetic analysis due to the poor prognosis of pancreatic cancer (17), (ii) the anticipated low frequency of deleterious RCVs (13), (iii) the lower absolute numbers of AA, Hispanic, and Native American pancreatic cancer patients (18), and (iv) the perennially low participation rates of minority groups in clinical research (14, 15, 19). To overcome these challenges, we performed a pooled analysis of individual-level data from 12 centers to investigate the role of pathogenic *CDKN2A* RCVs in incident pancreatic cancer.

Materials and Methods

Patient recruitment

This study was reviewed and approved by the Mayo Clinic institutional review board (IRB), as well as IRBs of all collaborating centers. Risk factor questionnaires or medical record surveys were used by each site to solicit self-reported information on participants' race and ethnicity. Lymphocyte DNA or DNA from buccal cells obtained from patients with histologically or clinically documented pancreatic ductal adenocarcinoma were provided by investigators from the following research registries: Mayo Clinic Biospecimen Resource for Pancreas Research (20, 21) at all three Mayo Clinic campuses (MN, AZ, and FL), The University of Texas MD Anderson Cancer Center (MD Anderson Cancer Center; 22), the H. Lee Moffitt Cancer Center, and the Vanderbilt-Ingram Cancer Center (23, 24). Germline DNA was extracted from surgically resected normal tissue of pancreatic cancer patients from Columbia University. Control subjects were identified from (i) deidentified healthy AAs who underwent clinical testing for cystic fibrosis in Rochester, MN (25), (ii) a convenience sample of AAs recruited through a church-based study in Jacksonville, FL (26), (iii) the Mayo Clinic BioBank in Rochester, MN (27), (iv) a large breast cancer control group including Chicago-area AAs (28), (v) Native Nigerians (29), (vi) the MD Anderson Cancer Center (22), (vii) the H. Lee Moffitt Cancer Center, and (viii) the Southern Community Cohort Study at the Vanderbilt-Ingram Cancer Center (23, 24). All cases and controls were recruited prospectively except the Columbia patients and the samples from Mayo Clinic Laboratory Medicine, which were retrospective. The study sample was comprised of pancreatic cancer cases and noncancer controls of NHW, AA, Nigerian, Hispanic, and Native American races/ethnicities.

Compliance with ethical standards. Written informed consent was obtained from all participants. The study was approved by the Mayo Clinic IRB.

Sequencing

All DNA samples were shipped to the Mayo Clinic Genome Analysis Core for analyses. Sanger sequencing was performed as previously described in detail (13, 30). Resequencing of the four exons of the *CDKN2A* gene, including three exons of *CDKN2A* isoform 1 (*NM_000077*) and exon 1 of *CDKN2A* isoform 4 (*NM_058195*), was performed. Primer sets for polymerase chain reactions (PCR) were designed using the web-based design tool Primer 3 software (version 0.4.0). Intronic primers covering sequences of interest were designed at least 30 bp away from the intron–exon boundaries of the gene. PCRs were carried out using AmpliTaq Gold DNA Polymerase (Applied Biosystems) following the manufacturer's protocol. After PCR reactions, the amplicons were treated with the ExoSAP-IT (USB Corp) to degrade unincorporated PCR primers and deoxynucleotide triphosphates. The cleaned products were mixed with 5 picomoles of the forward or reverse PCR primers for sequencing. DNA sequence variants were identified using PolyPhred (31).

Variant calling and *in silico* analysis

Each potential coding variant identified was investigated and classified as polymorphic (nonpathogenic) or high impact (deleterious or probably damaging), affecting protein coding of p16 or p14ARF, excluding known polymorphisms (e.g., A148T). We used available online databases for determination of variant frequency in populations, along with identification of prior reports of variants, including exome sequencing project (ESP; ref. 32), the catalog of somatic mutations in cancer (COSMIC; ref. 33), the University of Vermont *CDKN2A* gene database (UVM Biodesktop; ref. 34), dbSNP (35), the gnomAD database (36), the genoMEL paper (10), the *CDKN2A* LOVD database (August 31, 2016, version; ref. 37), and ClinVar (38). Using the cDNA position and amino acid change, a thorough literature search was performed to determine whether variants had previously been reported in cancer kindreds, in melanoma or pancreatic cancer patients, or in functional studies of *CDKN2A* (10, 39–48). *In silico* descriptive analyses were performed with SIFT (49) and PolyPhen2 (50) for the variants identified (insertions/deletions assumed deleterious by those tools are not annotated) when available but were not used for the final determination of variant status due to their imperfect specificity (51).

Statistical analysis

The pancreatic cancer patients and noncancer controls were classified based on whether they carried at least one nonsynonymous or frameshift RCV in *CDKN2A*. Race/ethnicity was determined by self-report. Variants previously determined to be polymorphic ($\geq$1%) in the above-cited publicly available databases were excluded from the analysis. Differences in demographic characteristics were compared among the racial/ethnic groups using Kruskal–Wallis test for continuous variables and Fisher exact test for categorical variables. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by comparing the

proportion of RCV carriers among the pancreatic cancer cases with the proportion of carriers among the noncancer controls in each minority group (i.e., AAs only, AAs plus Nigerians, Hispanics, and Native Americans). We also performed case–case comparison by comparing proportion of carriers among NHW cases (referent groups; ref. 13) versus proportion of carriers among cases in each of the minority groups. All statistical tests were two sided and were considered significant at the $\alpha = 0.05$ level. Analyses were performed in SAS version 9.4 (SAS Institute). Population-attributable risk was estimated as the difference in incidence rates between the AAs and NHWs divided by incidence in the AAs. Approximations from previously published studies of prevalence and SEER incidence rates were used.

Results

Biospecimens and epidemiologic and sequencing data used in the present study originated from 12 hospital-based and population-based studies. Table 1 presents the design, source population, and participant characteristics, including age, sex, and race or ethnicity, for each of the participating centers. In total, the study included 220 AA pancreatic cancer cases and 900 healthy AA controls, 183 healthy Nigerian controls, 119 Hispanic cases and 58 healthy Hispanic controls, 11 Native American cases and 20 healthy Native American controls, and 1,537 NHW pancreatic cancer cases.

Supplementary Table S1 summarizes the RCVs found in each race or ethnic group. Some variants are not reported, if they appeared to be polymorphisms, defined *a priori* as presence in 1% or more in publicly available databases. The RCVs were classified as deleterious, probably damaging, or possibly damaging/tolerated based on SIFT and PolyPhen designation. We found five novel RCVs: 3 missense variants exon 1B:c.116A>G, exon 2: c.192G>C, exon 2:c.400G>A, and two frameshift variants on exon 2:(c.258_278del) and exon 2:c.280dupC. Two of these RCVs were unique to the 220 AA pancreatic cancer patients only: c258_278del and c.400G>A, two were RCVs found in our 900 AA controls only: 116A>G and 280dupC. One other novel RCV was found in the 1,537 NHW pancreatic cancer cases. Among the pancreatic cancer cases, RCV frequencies were highest among Native Americans (1/11; 9.1%), followed by AAs (11/220; 5.0%) and Hispanics (4/119; 3.4%), and lowest among NHWs (6/1,537; 0.4%; Table 2). Among the healthy controls, RCV frequencies were highest in AAs (16/900; 1.8%) followed by Nigerians (1/183; 0.5%). No RCV was found among healthy controls of Hispanic and Native American ancestry (Table 2). Two AA cases and two AA controls carried multiple RCVs. Phase was not determined.

We performed case–control analyses within each race/ethnicity and found higher RCV prevalence among AA pancreatic cancer cases compared with AA controls (OR, 2.9; 95% CI, 1.3–6.4, $P = 0.005$). The RCV prevalence estimate among the AA cases increased slightly when the Nigerian controls were combined with AA controls and used as the comparison group (OR, 3.3; 95% CI, 1.5–7.1, $P = 0.004$; Table 2A). After exclusion of variants predicted by SIFT or PolyPhen to be benign/tolerated, the association remained significant (10/220 AA cases vs. 15/1083 AA controls; OR, 3.4; 95% CI, 1.5–7.6, $P = 0.005$). Adjustment for age, smoking status (ever/never), and diabetes (yes/no) further increased the observed association (OR, 4.3; 95% CI, 1.5–12.2,

Table 1. Sources of case and control subjects in study, race and ethnicity, median age, and sex

Center	Type of study; case status	Origin	Subjects (*N*)	Median age (range)	Sex (% male)
African/African American					
Columbia University Medical Center	Case series	Hospital—U.S.	7	69 (36–86)	29
H. Lee Moffitt Cancer Center	Case–control; cases	Hospital—U.S.	15	59 (38–82)	40
Mayo Clinic Pancreas Biospecimen Resource	Case–control; cases	Clinic—U.S.	48	62.5 (38–89)	42
MD Anderson Cancer Center	Case–control; cases	Hospital—U.S.	52	61.5 (37–80)	48
Vanderbilt University	Cohort; cases	Population-based—southern U.S.	98	58 (40–79)	48
H. Lee Moffitt Cancer Center	Case–control; controls	Hospital—U.S.	15	60 (37–79)	40
Mayo Clinic Laboratory Medicine	Clinical biorepository; controls	Referral lab	191	–	–
Mayo Clinic BioBank	Cohort; controls	Olmsted County, MN	80	48 (21–82)	44
Mayo Clinic MGUS Study	Cohort; controls	Jacksonville, FL	118	57.5 (37–91)	28
MD Anderson Cancer Center	Case–control; controls	Hospital—U.S.	25	55 (36–82)	48
University of Chicago	Case–Control; controls	Chicago, IL	185	56 (27–95)	8
University of Chicago	Case–control; controls	Nigeria	183	59 (37–100)	0
Vanderbilt University	Cohort; controls	Population-based—southern U.S.	286	58 (40–79)	49
Hispanic					
Columbia University Medical Center	Case series	Hospital—U.S.	20	62 (53–83)	45
H. Lee Moffitt Cancer Center	Case–control; cases	Hospital—U.S.	6	53 (39–65)	50
Mayo Clinic Pancreas Biospecimen Resource	Case–control; cases	Clinic—U.S.	31	60 (20–88)	58
MD Anderson Cancer Center	Case–control; cases	Hospital—U.S.	62	61 (40–79)	53
H. Lee Moffitt Cancer Center	Case–control; controls	Hospital—U.S.	6	53.5 (39–64)	50
MD Anderson Cancer Center	Case–control; controls	Hospital—U.S.	49	53 (38–78)	61
Mayo Clinic MGUS Study	Cohort; controls	Jacksonville, FL	3	50 (46–56)	100
Native American					
Mayo Clinic Pancreas Biospecimen Resource	Case–control; cases	Clinic—U.S.	9	53 (44–69)	56
MD Anderson Cancer Center	Case–control; cases	Hospital—U.S.	2	62.5 (62–63)	50
Mayo Clinic BioBank	Cohort; controls	Olmsted County, MN	20	57.5 (25–85)	40
Non-Hispanic white					
Mayo Clinic Pancreas Biospecimen Resource	Case–control; cases	Clinic—U.S.	1,537	65.5 (28–91)	56

Abbreviation: MGUS, monoclonal gammopathy of undetermined significance.

Table 2. Frequency of *CDKN2A* RCV carriers among population samples of pancreatic cancer cases and controls by race and ethnicity, and RCV frequencies

A	**Pancreatic cancer cases**	**Healthy controls**	**Cases vs. controls**	
Group	***N* carriers/*N* tested (%)**	***N* carriers/*N* tested (%)**	**OR (95% CI)**	***P* value**
African American and African Nigeria controls (combined)	11/220 (5.0)	17/1,083 (1.6)	3.3 (1.5–7.1)	0.004
• African American	11/220 (5.0)	16/900 (1.8)	2.9 (1.3–6.4)	0.005
• African—Nigeria[a]	—	1/183 (0.5)	—	
Hispanic	4/119 (3.4)	0/58 (0)	4.6 (0.2–86.1)	0.30
Native American	1/11 (9.1)	0/20 (0)	5.9 (0.2–156.6)	0.35
B		**Pancreatic cancer cases**	**Minority cases vs. NHW cases**	
Group		***N* carriers/*N* tested (%)**	**OR (95% CI)**	***P* value**
NHW		6/1,537 (0.4)	Reference	–
African American		11/220 (5.0)	13.4 (4.9–36.7)	<0.001
Hispanic		4/119 (3.4)	8.9 (2.5–31.9)	0.004
Native American		1/11 (9.1)	25.5 (2.8–231.8)	0.048

NOTE: ORs, 95% CIs, and *P* values are reported for (**A**) case–case comparison between NHW cases versus cases in the racial minority groups, and (**B**) comparisons between pancreatic cancer cases versus controls by in each race or ethnic group.
[a]Data were not available for Nigerian pancreatic cancer cases.

P, 0.006). ORs for comparison of RCV prevalence between Hispanic cases and controls (OR, 4.6; 95% CI, 0.2–86.1, $P = 0.30$) and between Native American cases and controls (OR, 5.9; 95% CI, 0.2–156.6, $P = 0.35$) did not differ significantly, likely due to the small numbers of cases and controls in these groups.

We further performed a case–case comparison of RCV frequencies among pancreatic cancer cases in the NHW sample (referent group) with RCV frequencies among AA cases, Hispanic cases, and Native American cases. Compared with NHW pancreatic cancer cases, AA pancreatic cancer cases had higher RCV prevalence (OR, 13.4; 95% CI, 4.9–36.7, $P < 0.001$), as did Hispanic cases (OR, 8.9; 95% CI, 2.5–31.9, $P = 0.004$), and Native American cases (OR, 25.5, 95% CI 2.8–231.8, $P = 0.048$; Table 2B). Because of the known potential contributions of splice-site and upstream variants to disease risk, we also performed an ancillary analysis comparing frequency of these RCVs among NHW pancreatic cancer patients (0.9%) with that of pancreatic cancer patients in the minority groups. We found higher RCV prevalence among the AA (5.9%, OR, 6.8; 95% CI, 3.2–14.7, $P < 0.001$), Hispanic (6.7%, OR, 7.8; 95% CI, 3.2–19.1, $P < 0.001$), and Native American (9.1%, OR, 10.9; 95% CI, 1.3–90.8, $P = 0.006$) pancreatic cancer patients, although no statistically significant differences were observed in comparisons by minority group (Supplementary Table S2).

By assuming (i) a 1.8% prevalence of CDKN2A RCVs in AAs; (ii) a 0.1% prevalence of CDKN2A RCVs in NHWs (assuming a lower prevalence than the 0.4% reported in NHW cases); (iii) a pancreatic cancer incidence rate about 5% to 7% higher in AAs than in NHWs; and (iv) the current SEER pancreatic cancer rates for AAs (15.5/100,000) and NHWs (12.7/100,000) or 22% higher for AAs, we estimate that the CDKN2A RCVs may account for approximately one fourth of the excess risk of pancreatic cancer in AAs.

We had previously reported that 4 of 9 (44%) and 2 of 9 (22%) of NHW carriers had a family history of pancreatic cancer and malignant melanoma, respectively (13). Among 11 AA pancreatic cancer cases who carried an RCV in *CDKN2A*, 7 had family history information available. One carrier (14.3%) reported pancreatic cancer diagnosis in a first-degree relative compared with 6.3% of 111 AA cases without a RCV detected who had family history data available ($P = 0.40$). No family history of melanoma was reported among the 7 AA RCV carriers, and one family history of melanoma was reported among the 88 noncarriers. The mean age at diagnosis of pancreatic cancer was similar among AA *CDKN2A* RCV carriers and noncarriers (58.5 years vs. 60.6, $P = 0.66$), and these ages are similar to those reported for NHW (13). We also found in our Hispanic pancreatic cancer cases that none of the 4 carriers and 3 of 112 noncarriers had a positive family history of pancreatic cancer and no cases reported a family history of melanoma. Among our Native American cases, no family history of either pancreatic cancer or melanoma was reported.

Discussion

We report the first collaborative study of germline *CDKN2A* variation among samples of subjects who are non-white. We discovered that high-impact *CDKN2A* RCVs are more common in persons of African descent and are associated with increased risk for pancreatic cancer. The frequencies of RCVs are in striking contrast to that those NHW pancreatic cancer patients, among whom we had previously identified only 0.4% as mutation carriers (13). The ORs of 2.9 to 3.3 seen in AA subjects are much less than the relative risk of 46.6 (95% CI, 24.7–76.4) of pancreatic cancer reported for the highly penetrant Leiden *CDKN2A* founder mutation (52), but more similar in magnitude to the moderate 2- to 4-fold risk for breast cancer in NHW conferred by mutations in *CHEK2*, *ATM*, *PALB2*, and *NBS1*, all with allele frequencies in the general population of ∼1% (53).

The aggregate high frequency of RCVs identified in AA pancreatic cancer cases and controls may potentially be explained by evolutionary/population genetic considerations. First, *CDKN2A* is thought primarily to function as a melanoma tumor suppressor gene. It is well established that individuals with darker skin (due to higher melanin concentration), including AAs, have lower risk of developing skin cancer and melanoma in the presence of ultraviolet radiation (54, 55). It stands to reason that any selection against variation would be minimized in populations with low incidence of melanoma (i.e., relaxed selection in African populations vs. whites). In contrast, any potential selection pressure through pancreatic cancer is unlikely to affect reproductive success, given its median age of onset above 70 years. Secondly, African populations are evolutionarily the most ancestral among humans (56, 57); therefore, one might postulate that the existence

of any given gene variation may be expected to be higher in this population than others. However, an exome sequencing study of 1,351 persons of European ancestry and 1,088 persons of African ancestry suggested that most RCVs are evolutionarily recent. Further, among likely functional SNVs, the proportions of rare and intermediate frequency variants per individual are higher among African ancestry individuals compared with those of European ancestry (58).

In our study, five identified RCVs of *CDKN2A* are novel, which may reflect the understudied nature of this gene in non-white populations. Interestingly, the higher frequencies seen in AAs are comparable with a recent report of high RCV frequencies identified in 225 Italian pancreatic cancer families (31%) and sporadic (5.7%) patients (59). Similarly, in a study among Greek melanoma patients, germline RCVs were identified in 3.3% of 304 sporadic melanoma patients and 22% of familial melanoma kindreds (60). We observed a relatively high frequency of RCVs in Native Americans and Hispanics with pancreatic cancer, but not among corresponding controls. We acknowledge this may be an artifact because of smaller sample sizes, but they are suggestive of high *CDKN2A* RCV frequencies in these groups and require validation in larger samples.

Our results permit a limited estimate of the impact of the *CDKN2A* gene on risk of pancreatic cancer among AAs. Given our observed 1.8% prevalence of *CDKN2A* RCVs in the general population of AAs, and inferring a prevalence of 0.1% in the general population of NHWs (prevalence of 0.4% was found among NHW pancreatic cancer cases in this study), and a 3- to 4-fold increase in pancreatic cancer risk associated with *CDKN2A* RCVs, AAs would be expected have a pancreatic cancer incidence rate about 5% to 7% higher than NHWs due to variation in the *CDKN2A* gene. Furthermore, with the current SEER pancreatic cancer incidence rates of 15.5 per 100,000 for AAs and 12.7 per 100,000 for NHWs (i.e., 22% higher for AAs; ref. 61), *CDKN2A* may account for about one fourth of the excess pancreatic cancer risk in AAs.

CDKN2A is a cell-cycle gene that encodes two different proteins, p16 and p14ARF (62–64). P16 (exons 1B, 2, and 3) regulates progression through the G_1 cell-cycle checkpoint by inhibiting CDK4/6 and subsequently preventing downstream phosphorylation of the retinoblastoma protein (pRb), which affects downstream inhibition of E2F, a transcription factor (62). P14ARF (exons 1A and 2) inhibits mdm2, which stabilizes p53 (63), and exerts a downstream regulatory effect on transcription of genes involved in the G_1–S checkpoint (64). Both p16 and p14 appear to suppress tumorigenesis (62, 63).

This is the largest study of *CDKN2A* gene RCVs and pancreatic cancer risk conducted to date among minority groups, an important strength of a multicenter consortium effort. However, our study has some limitations, including the potential heterogeneity of sample sets from diverse centers. Given the relative rarity of minority patients, ascertainment was limited to convenience samples, including controls. Because not all centers contributed data and biospecimens on both cases and controls, we were unable to adjust for other established risk factors such as smoking, family history, diabetes, and obesity in the logistic regression analyses; this should be considered in the interpretation of findings. Genetically, there is always a difficulty with determining the functional role of missense variants such as those identified in this study. We excluded all known polymorphic variants, but the challenges of *in silico* analysis of RCVs are well known (51). Our findings merit further study concerning quantifying the absolute risk for cancer among single and compound RCV carriers, not only for pancreatic cancer, but also for other malignancies such as melanoma, head/neck cancer, and bladder cancer. The clinical application of *CDKN2A* RCV status will require more comprehensive studies of risk and outcomes. The underlying biologic implications of relaxed or even positive selection for *CDKN2A* germline variants and the role of environmental factors, such as sun exposure, vitamin D receptor status and deficiency, will be vital to further our understanding of any disease role of *CDKN2A* among different populations. Moreover, *CDKN2A* RCVs may have therapeutic implications. Commonly mutated in somatic pancreatic tumors (16), *CDKN2A*'s transcript p16 inhibits CDK4, a key function of cell-cycle regulation in pancreatic cancer (65) and CDK4/6 inhibitors have demonstrated strong activity in breast cancer (66), with recent FDA approvals of palbociclib and ribociclib, and some early evidence of activity of CDK4/6 inhibition in pancreatic cancer is emerging (67). Whether this or other screening or treatment strategies emerge related to *CDKN2A* RCVs, biological differences among diverse populations may have a great impact on precision medicine.

Conclusions

RCVs in *CDKN2A* are substantially more common among AAs than among NHW. RCVs among persons of African descent are of moderate penetrance, conferring a 3.3-fold increased risk for pancreatic cancer and may partially account for some excess risk of pancreatic cancer among AAs.

Disclosure of Potential Conflicts of Interest

L. Raskin is a senior manager at Amgen. O.I. Olopade has ownership interest (including stock, patents, etc.) in CancerIQ and Tempus. G. Colon-Otero reports receiving a commercial research grant from Novartis. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

This study was supported by NIH grants P50 CA102701 (G.M. Petersen), R01 CA97075 (G.M. Petersen), R01 CA208517 (G.M. Petersen), R25T CA92049

(G.M. Petersen), P30 CA076292 (J. Permuth), CA98380-05 (D. Li), K07 116303 (R.R. McWilliams,), R01 CA092447 (G.M. Petersen), and U01 CA202979 (G.M. Petersen), and the Sheikh Ahmed Center for Pancreatic Cancer Research Funds, MD Anderson Cancer Center.

The authors thank the participants in this study and project team members Ryan Wuertz, Jodie Cogswell, Bridget Eversman, Traci Hammer, Megan Reichmann, Mary Karaus, Ryan Frank, Que Luu, William Bamlet, MS, Ann Oberg, Ph.D., Monica Albertie, M.H.A. (MD Anderson Cancer Center, Columbia University, and University of Chicago staff). The Mayo Clinic BioBank (principal investigators: Janet Olson, Ph.D., and James Cerhan, Ph.D.) is supported by the Mayo Clinic Center for Individualized Medicine. H. Lee Moffitt Cancer Center specimens and data were collected through the Total Cancer Care Protocol, and work was supported in part by the Information Shared Services and Tissue Core Facilities. The authors honor the memory of the late W. Edward Highsmith Jr, PhD.

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Received November 22, 2017; revised February 13, 2018; accepted July 11, 2018; published first July 23, 2018.

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