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Association between the Peroxisome proliferator-activated receptor-gamma Pro12Ala Variant and Haplotype and Pancreatic Cancer in a High-Risk Cohort of Smokers: A Pilot Study

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Abstract

Objectives—The Pro12Ala variant in the proliferator-activated receptor-gamma (PPARG) gene has been associated with diabetes and several cancers. This pilot study tested for the association between Pro12Ala and pancreatic cancer risk in a high-risk sample of smokers.

Methods—A nested case-control study was conducted in 83 incident cases of pancreatic cancer and 166 matched controls originally recruited into a cohort chemoprevention study of lung cancer. Associations between Pro12Ala and pancreatic cancer risk were measured using conditional logistic regression.

Results—Carriers of the G allele (Ala) of the Pro12Ala variant had a borderline increased relative risk of pancreatic cancer compared to homozygous carriers of the C allele (Pro), with an odds ratio (OR) of 1.79 (95% confidence interval (CI): 0.96–3.33, p-value: 0.06). Among subjects randomized to high-dose vitamin A, the OR was 2.80 (95% CI: 1.16–6.74, p-value 0.02) versus 1.20 (95% CI: 0.45–3.23, p-value 0.71) in the placebo group. A haplotype including Pro12Ala was also significantly associated with pancreatic cancer risk in all subjects and in subjects randomized to vitamin A.

Conclusions—This analysis presents the first evidence that PPARG may be associated with pancreatic cancer risk, and this candidate gene should be investigated in future, larger studies.

Keywords

Pancreatic neoplasms; PPARG; Vitamin A; Cigarette smoking

Introduction

A recent report using data from the Surveillance, Epidemiology, and End Results program and the National Center for Health Statistics estimated that pancreatic cancer will be diagnosed in over 37,000 men and women in the United States during 2007, and will be responsible for over

33,000 deaths.[1] The most well-established risk factor for pancreatic cancer is tobacco use, particularly cigarette smoking.[2] Diabetes, hereditary pancreatitis, and chronic pancreatitis are also associated with increased risk of pancreatic cancer.[3–8] In total, an estimated 5% to 10% of pancreatic cancer is believed to be caused by hereditary factors, including both rare, high-risk mutations and common, low-risk variants.[9]

The relationship between diabetes and pancreatic cancer has spurred many investigations into diabetes as a pancreatic cancer risk factor. Diabetes itself is a major cause of morbidity and mortality in the United States. The CDC and NIH estimate that 7.0% (20.8 million) of the U.S. population has either diagnosed or undiagnosed diabetes, and 20.9% of all individuals aged 60 or older have diabetes.[10] A meta-analysis of twenty case-control and cohort studies found that diabetes was associated with a 2-fold increased risk for pancreatic cancer.[3] A large population-based study found a significant positive trend between risk for pancreatic cancer and increased duration of diabetes.[5] However, a recent retrospective cohort study of over one million U.S. veterans found the greatest pancreatic cancer risk in recently-diagnosed diabetics.[11] These observations suggest that diabetes may be either a precursor or symptom of pancreatic cancer. Another previously unexplored explanation for the observed association between diabetes and pancreatic cancer is that the two diseases could share risk factors in common. This study investigated the latter possibility, and examined whether genetic variants in the PPARG gene, already known to be associated with diabetes, may be important in understanding risk of pancreatic cancer.

The PPARG protein is a member of the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors.[12,13] Two isoforms of PPARG, PPARG1 and PPARG2 are constructed via alternate splicing and alternate transcription start sites. [14] While PPARG1 protein is expressed in breast, colon, liver, pancreas, adipose cells, and other tissue types, PPARG2 protein expression is almost entirely restricted to adipose cells.[15] The PPARG protein is required for adipogenesis, and plays a key regulatory role in adipose cell differentiation.[15] Laboratory studies provide evidence that the PPARG protein could be important in pancreatic cancer as well: ligands that activate the PPARG protein have been shown to inhibit the invasive behavior of pancreatic cancer cells in vitro, suggesting that increased PPARG protein activity may be associated with decreased pancreatic cancer growth. [16]

The PPARG gene is located on chromosome 3p25, and consists of nine exons covering more than 100 kb of genomic DNA. The most commonly studied PPARG gene variant is Pro12Ala (rs1801282), a nonsynonymous SNP in exon B.[17,18] The G allele (coding for Ala) is thought to be associated with reduced PPARG activity.[19] Associations between the G allele (Ala) and reduced risk for types 1 and 2 diabetes have been observed in several ethnic groups.[18, 20–22] In addition, the G allele (Ala) has been associated with decreased risk for renal cell carcinoma [23] and colon cancer[24].

A major obstacle to studies of pancreatic cancer risk factors is the difficulty of recruiting a representative case sample. Median survival for pancreatic cancer is only 4 months,[25] and therefore a large proportion of cases identified die before they can be recruited into a study. If hereditary pancreatic cancer is more quickly fatal than sporadic pancreatic cancer, then cases enrolled in retrospective studies may also under-represent the true number of familial cases, and no genetic association may be identified. The high mortality of pancreatic cancer can be addressed in two ways: using rapid recruitment after diagnosis (along with rapid collection of biospecimens), or identifying incident cases by assembling a large cohort for long-term follow up. The study described here uses the latter approach, and is a matched, nested case-control study within the β -Carotene and Retinol Efficacy Trial (CARET).[26] CARET participants were cancer-free at enrollment, and provided biospecimens at baseline, thereby minimizing

recruitment biases associated with most retrospective study designs. Although this pilot study involves a modest sample size, it provides a starting point for future investigations into the genetic epidemiology of pancreatic cancer.

The objective of this study was to quantify the association between the Pro12Ala PPARG variant and susceptibility to pancreatic cancer in a cohort of individuals at high risk for pancreatic cancer due to their substantial smoking histories. As a comprehensive approach, both the Pro12Ala variant and a haplotype including Pro12Ala were queried for association with pancreatic cancer. To expand our analysis to include the entire PPARG gene, 24 additional tagSNPs located throughout the PPARG gene were investigated for potential association with pancreatic cancer risk.

Materials and Methods

Cases and Matched Controls

Study subjects were participants in the β -Carotene and Retinol Efficacy Trial (CARET), a prospective chemoprevention study described previously.[26] Briefly, CARET was a multicenter, randomized, double-blinded chemoprevention trial aimed at testing the effect of administering a combination of β -carotene and retinyl palmitate (vitamin A) to a cohort of 18,314 men and women at high risk for lung cancer due to smoking and/or asbestos exposure. [26] Covariates were obtained by questionnaire or by direct measurement at baseline or periodic follow-ups. Body mass index (BMI) was computed from height and weight (kg/m^2), which were measured by CARET staff at baseline. Self-reported diabetes status was ascertained either at baseline or during follow-up. Subjects randomized to the intervention study arm (hereafter called “vitamin subjects”) received 30 mg of beta carotene and 25,000 IU of retinyl palmitate daily. Subjects randomized to the control study arm (hereafter called “placebo subjects”) received a placebo. CARET follow-up included annual health questionnaires for all participants and surveillance of state death records. Twenty-one months before the scheduled end of the intervention, the study was stopped due to the observed 28% increased relative risk of lung cancer in the intervention arm (95% CI 1.04 – 1.57).[27] The intervention was also associated with an increased risk of all cause mortality (OR = 1.17, 95% CI 1.03 – 1.33).[28]

All participants who reported a pancreatic cancer diagnosis to CARET as of September 1, 2004 were potentially eligible for this study. Of the 99 subjects meeting these criteria, 14 were excluded due to either non-exocrine tumor histology, lack of medical records confirmation, or non-smoking status. Two cases diagnosed with lung cancer were also excluded, resulting in a sample of 83 confirmed exocrine pancreatic cancer cases.

Two controls with no cancer history were randomly selected from the CARET participant pool for each case, for a total of 166 controls. Controls were matched to cases on age at entry to the study within 5-year groups, race, sex, CARET eligibility criteria (asbestos-exposed smoker vs. smoker), CARET intervention arm, and smoking history using a two-step process. First, cases and controls were matched on a former/current smoking variable. Second, current smokers were matched on number of cigarettes per day (± 10) and former smokers were matched on years since quitting. Due the limited number of controls, the CARET intervention arm matching criterion was relaxed for four cases. For three cases, one matched control had been assigned to a different intervention arm, and for one case, both matched controls were assigned to a different intervention arm.

This study was approved by the Institutional Review Board of the University of Washington. Written informed consent for blood and serum banking was obtained for research purposes by

the CARET study, and was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

DNA Extraction, SNP Selection, and Genotyping

Constitutional DNA was obtained from whole blood (N=216) or serum (N=33) obtained as part of CARET study procedures. DNA was extracted using QIAamp DNA Blood Mini Kit/protocol (Qiagen, Inc, Valencia, CA) and was quantified using a SpectraMax Plus (Molecular Devices, Sunnyvale, CA). Serum samples were sent to QIAGEN REPLI-g Services (Hilden, Germany) for DNA extraction and whole-genome amplification. The mean DNA yield from 200 μ l whole blood was 25.8 μ g per subject, and the mean DNA yield from 1.7 ml serum was 224.5 μ g per subject post amplification.

The Pro12Ala nonsynonymous SNP was identified from the literature. To construct a haplotype containing Pro12Ala, PPARG tagSNPs were selected from a pool of SNPs identified by the SeattleSNPs variation discovery resource.[29] The LDSelect algorithm was used to cluster these PPARG SNPs into “bins”.[29] LDSelect uses pairwise correlation (r^2) calculations to define bins containing at least one tagSNP by minimizing the pairwise r^2 between SNPs in different bins. Selection was limited to SNPs with a rare allele frequency of at least 0.05 in the European HapMap population, and an r^2 threshold of 0.64 was used. The algorithm identified 27 bins in PPARG, including rs1801282 (Pro12Ala). One tagSNP was selected from each bin for genotyping. TaqMan[™] assays were used to genotype 21 SNPs, two SNPs were analyzed by PCR Restriction Fragment Length Polymorphism (RFLP), and four SNPs were characterized using DNA sequencing assays. At least 5% of subjects were randomly selected for repeat genotyping for each SNP, and any discrepant genotypes were reanalyzed until concordance was reached.

Statistical Analysis

The χ^2 test was used to confirm consistency of the PPARG variants with Hardy-Weinberg equilibrium in controls. The Pearson correlation between minor allele frequencies observed in CARET controls and expected frequencies reported by Seattle SNPs and the HapMap Project was calculated to identify overall deviation of study frequencies from expected values. For association analyses, conditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) to estimate the association between PPARG minor alleles and risk of pancreatic cancer using STATA 9.0 (Stata Corp. College Station, TX). Models adjusted for BMI and diabetes status were also determined to control for potential confounding of these covariates.

Conditional logistic regression of the Pro12Ala minor allele and risk for pancreatic cancer was performed in all subjects, then stratified by CARET intervention arm assuming a dominant genetic model (i.e., odds of pancreatic cancer in heterozygous and homozygous carriers of the minor allele compared to homozygous carriers of the major allele). One case matched to controls on the opposite intervention arm was excluded from stratified analyses.

Haplotype analysis was performed in 82 case-control trios. One trio was excluded due to a case with more than 75% missing genotype data. All remaining subjects had less than 5% missing genotype data. Four SNPs with a minor allele frequency less than 5% were excluded from haplotype analyses. A haplotype block containing the Pro12Ala variant was identified in Haploview version 3.32 using the Solid Spine of Linkage Disequilibrium (LD) method with a D' threshold of > 0.80 to define blocks.[30]

Block-specific haplotypes were estimated for each subject using the expectation-maximization algorithm as implemented by the Haplo.stats module in R.[31] Haplotype estimations were

transformed into a design matrix using the haplo.design program in R.[32] The design matrix for the Pro12Ala haplotype block was exported into STATA to calculate the association between each block and pancreatic cancer using conditional logistic regression. A global p-value of association was calculated for the Pro12Ala haplotype block by performing a likelihood ratio test comparing a full model containing all haplotypes modeled as continuous variables with a reduced model containing no independent variables. The global null hypothesis is that the risk of disease is equal for all haplotypes in the block, and the alternative hypothesis is that the risk of disease is different for at least one haplotype.[33]

The false positive report probability (FPRP) was calculated for all single-SNP associations with a p-value less than 0.10.[33] The FPRP estimates the probability that an observed association is a false positive, based on the prior probability of a true association, the power to detect an association, and the observed OR and 95% CI. A prior probability of 0.10 and a “notworthiness” cutoff of 0.50 (i.e., 50% likelihood that an observed association is a false positive) were used to identify potential true associations.

Attributable risk percent (AR%) was calculated to measure the proportion of pancreatic cancer attributable to a genetic exposure (tagSNPs or haplotypes) in the study sample. The AR% was calculated from the relative risk (RR) associated with genetic exposure, as estimated by odds ratios obtained from conditional logistic regression:[34]

$$AR\% = \frac{RR - 1}{RR} \times 100\%$$

Results

Case and Control Characteristics

Table I compares characteristics of cases and controls. Matching resulted in very similar distributions of age, race, gender, and smoking status between cases and controls. Overall, 94% of subjects were white, 74.7% were male, and the median age at baseline was approximately 61 years. Diabetes and increased BMI were slightly more frequent among cases. When stratified by CARET study arm, vitamin subjects were less likely to be current smokers than placebo subjects, and vitamin cases were less likely to self-report diabetes than placebo cases (8.7% versus 24.3%, respectively).

Single SNP Analyses

Table II shows allele and genotype frequencies for the Pro1Ala variant and two other SNPs (rs11715073 and rs4135247) included in the Pro12Ala haplotype block, as well as p-values for test for deviation from Hardy-Weinberg equilibrium in controls. Results for the 24 tagSNPs not included in the Pro12Ala haplotype are not shown. All three SNPs were polymorphic and did not deviate from Hardy-Weinberg expectations. Among the 24 additional PPARG tagSNPs, 23 were polymorphic and none deviated from Hardy-Weinberg equilibrium. Allele frequencies for all 27 tagSNPs in CARET controls were similar to expected frequencies listed in the SeattleSNPs and HapMap databases, with a calculated correlation (r^2) of 0.94 (data not shown).

Table III shows the results of adjusted conditional logistic regression testing for associations between three PPARG SNP minor alleles and risk for pancreatic cancer assuming a dominant genetic model. The ORs in Table III represent the risk of disease in heterozygous and homozygous carriers of the minor allele compared to homozygous carriers of the major allele (i.e., reference group), adjusted for diabetes and BMI.

In this analysis, the Pro12Ala variant was associated with a borderline significant adjusted OR of 1.82 (95% CI: 0.97 – 3.41, $p=0.06$) in all subjects, and met FPRP criteria for noteworthiness. After stratification on CARET study arm, the overall association between the Pro12Ala (rs1801282) minor allele and pancreatic cancer appeared to be driven by vitamin subjects, who had an adjusted OR of 2.80 (95% CI: 1.16 – 6.74, $p=0.02$), which met FPRP criteria for noteworthiness.

As shown in Table III, the rs4135247 tagSNP was associated with a 44% reduction in relative risk (OR = 0.56, 95% CI: 0.32 – 0.98, $p=0.04$), which was significant and met FPRP criteria for noteworthiness. This inverse association between SNP rs4135247 and pancreatic cancer appeared to be driven by placebo subjects, who had an adjusted OR of 0.39 (95% CI: 0.13 – 1.17, $p=0.09$). However, this association did not meet FPRP criteria for noteworthiness in the stratified analysis.

Of the 24 additional PPARG tagSNPs, a significant association was observed for one tagSNP (rs17029006), which was associated with a reduced risk of pancreatic cancer (OR = 0.41, 95% CI: 0.19 – 0.90) in vitamin subjects only.

The attributable risk percent (AR%) estimates the proportion of disease attributable to an exposure in the study sample. Because the most consistent association was found for the Pro12Ala variant, the AR% was calculated for rs1801282. Using the OR calculated for placebo subjects, the AR% for one or two copies of the G allele (coding for Ala) was 16.7%. Among vitamin subjects, the AR% for the G allele was 64.3%.

Haplotype Analyses

As shown in Table IV, Haploview identified a haplotype block containing Pro12Ala and two other SNPs in PPARG. Table IV also shows estimated haplotype frequencies in all subjects as a percentage of total subject chromosomes. These frequencies are estimated based on the probability that a subject's chromosome carries a particular haplotype. The GGA haplotype (which includes the Pro12Ala minor allele) was associated with increased pancreatic cancer risk, with an adjusted OR of 2.09 (95% CI: 1.06 – 4.11, $p=0.03$) compared to the most frequent haplotype (CCG). This association appeared to be driven by vitamin subjects, who had an adjusted OR of 3.32 (95% CI: 1.27 – 8.66, $p=0.01$).

Haplotype blocks constructed from 24 additional PPARG tagSNPs were not significantly associated with pancreatic cancer risk.

The AR% was calculated for the GGA haplotype of the Pro12Ala block, the only haplotype significantly associated with pancreatic cancer risk in any subject subgroup. Using the OR calculated placebo subjects, the AR% for one or two copies of the GGA haplotype was 21.3%. Among vitamin subjects, the AR% for the GGA haplotype was 69.9%.

Discussion

The results of this study in the CARET high risk cohort of smokers suggest that the Pro12Ala variant and haplotype in the PPARG gene may play an important role in susceptibility to pancreatic cancer. The Pro12Ala variant causes an amino acid change in the PPARG protein, and could therefore be directly (i.e., causally) associated with pancreatic cancer.

Proline to alanine substitutions affect protein structure stability, and the Pro12Ala variant could therefore alter PPARG function by influencing its folding behavior.[35] Although not definitive, there is some evidence that the Ala residue results in reduced PPARG activity[19], and that activated PPARG may inhibit pancreatic tumor invasion[16]. This implies that the

reduced PPARG activity associated with the Ala residue could encourage pancreatic tumor growth, and is consistent with our finding that the Pro12Ala G allele (coding for Ala) is associated with an increased relative risk for pancreatic cancer. Alternatively, the observed associations between variants in PPARG and risk for pancreatic cancer could also be attributable to linkage disequilibrium with an unmeasured causal variant, or a false positive result.

Haplotype analyses identified a haplotype including the Pro12Ala variant possibly associated with pancreatic cancer. The GGA haplotype was associated with an increased relative risk for pancreatic cancer in all subjects and in vitamin subjects. The GGA haplotype contains the Pro12Ala variant, and was associated with a greater odds ratio for pancreatic cancer than this variant considered alone (OR = 3.32 versus OR = 2.80, respectively). The greater magnitude of risk associated with the GGA haplotype could be attributable to the combined effects of SNPs in this block, or to linkage disequilibrium between SNPs in this block and an unmeasured causal variant.

While the molecular mechanism driving the association between PPARG and pancreatic cancer is unknown, these analyses indicate that vitamin A may be an important effect modifier of this association. CARET subjects randomized to the vitamin arm ingested 5 times the U.S. RDA for vitamin A, and the risk of pancreatic cancer associated with the Pro12Ala variant was most pronounced in subjects randomized to the CARET vitamin arm. This result could be attributable to vitamin A-modulated PPARG gene expression and protein activity. The PPARG gene product is inactive until it forms a heterodimer with the Retinoid X Receptor (RXR), forming a complex that activates transcription by binding to PPARG-response elements in the promoter regions of target genes.[36] RXR cannot bind to PPARG without first binding with metabolites of vitamin A, suggesting that vitamin A may modulate PPARG transcriptional activity.[37]

In addition to regulating PPARG function, Vitamin A has also been shown to regulate PPARG gene expression. In a mouse feeding study, a diet high in vitamin A was associated with increased RXR and PPARG2 expression in adipose tissue.[38] Additional studies are needed to determine if vitamin A alters PPARG1 expression in pancreas tissue, and if differences in PPARG1 expression might be associated with pancreatic cancer.

In this sample of high-risk smokers, the Pro12Ala variant G allele (coding for Ala) accounted for 16.7% of pancreatic cancer among placebo subjects, and 64.3% of pancreatic cancer among vitamin subjects. Given that substantial smoking exposure and high-dose vitamin A are uncommon exposures outside of the CARET subject group, these AR% estimates are probably not applicable to the general population.

This study has several strengths and limitations that should be considered. The major strength of this study is its use of prospectively identified incident pancreatic cancer cases, minimizing selection bias due to mortality that have limited previous case-control studies of pancreatic cancer.[39,40] Because pancreatic cancer is relatively rare, this study relied on cases identified via a lung cancer cohort study. Although the CARET study enrolled over 18,000 participants, only 83 were diagnosed with pancreatic cancer over more than a decade of follow up. This exemplifies the difficulty of assembling a large group of incident pancreatic cancer cases, and the necessary compromise between statistical power and selection bias.

Another drawback of using CARET participants is that all cases and controls had substantial smoking histories. As a result, some of the pancreatic cancer cases were probably caused by smoking, rather than genetic variants. However, this type of misclassification would be expected to attenuate the association between PPARG variants and pancreatic cancer towards the null, thus our results may have underestimated the true pancreatic cancer risk associated

with the Pro12Ala variant. Another strength of this study was that 94% of the subject pool was white, reducing the possibility of false conclusions due to population stratification. However, the homogenous ethnicity and substantial smoking exposures of CARET subjects may limit the generalizability of these results to non-smoking and/or ethnically diverse populations.

This analysis included adjustment for diabetes as a potential confounding factor. In this analysis, a greater percentage of placebo cases were diabetic compared to vitamin cases (24.3% versus 8.7%, respectively). This difference must be considered because diabetes is associated with both PPARG variants and pancreatic cancer risk, and could affect the results of these analyses. We repeated all analyses with diabetic subjects omitted, and found no substantial difference in the results (data not shown). Therefore, differences in diabetes prevalence among vitamin versus placebo cases had little effect on the conclusions of this analysis.

The results of this analysis must be replicated in other populations, and followed up with laboratory investigations. Human gene expression studies could confirm if vitamin A exposure induces PPARG overexpression, and in which tissues. A careful gene expression study could quantify PPARG1 and PPARG2 expression levels in the pancreas, as well as vitamin A induced PPARG expression changes. Studies of PPARG and PPARG-RXR protein structure and biological activity could elucidate a mechanism for the Pro12Ala variant.

Conclusion

This analysis presents the first evidence that the PPARG Pro12Ala variant and a haplotype including this variant may be associated with pancreatic cancer risk. This risk may be limited to heavy smokers exposed to high levels of vitamin A, and future studies of PPARG and pancreatic cancer should strive to include non-smokers. Understanding the risk associated with the Pro12Ala variant is important, because the allele coding for Ala could account for enhanced susceptibility to pancreatic cancer, especially among smokers and/or individuals exposed to high levels of vitamin A.

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Table 1

Characteristics of cases and controls, overall and stratified by CARET intervention arm

	All Cases (N = 83)		All Controls (N=166)		Placebo Cases (N = 37)		Placebo Controls (N = 71)		Vitamin Cases (N = 46)		Vitamin Controls (N = 95)	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Baseline age (years)</u>												
45-49	2	2.4	4	2.4	0	0.0	0	0.0	2	4.3	4	4.2
50-54	12	14.5	25	15.1	4	10.8	8	11.3	8	17.4	17	17.9
55-59	21	25.3	40	24.1	12	32.4	21	29.6	9	19.6	19	20.0
60-64	21	25.3	42	25.3	12	32.4	22	31.0	9	19.6	20	21.1
65-69	27	32.5	55	33.1	9	24.3	20	28.2	18	39.1	35	39.1
Mean (std.dev.)	61.30	(5.54)	61.08	(5.85)	61.03	(4.77)	61.23	(5.13)	61.53	(6.14)	60.97	(6.35)
<u>Race</u>												
White	78	94.0	156	94.0	33	89.2	67	94.4	45	97.8	89	93.7
Black	3	3.6	6	3.6	2	5.4	2	2.8	1	2.2	4	4.2
Other	2	2.4	4	2.4	2	5.4	2	2.8	0	0.0	2	2.1
<u>Gender</u>												
Male	62	74.7	124	74.7	26	70.3	51	71.8	36	78.3	73	76.8
Female	21	25.3	42	25.3	11	29.7	20	28.2	10	21.7	22	23.2
<u>Smoking Status</u>												
Current Smoker	54	65.1	108	65.1	26	70.3	50	70.4	28	60.9	58	61.1
Former Smoker	29	34.9	58	34.9	11	29.7	21	29.6	18	39.1	37	38.9
<u>Diabetes</u>												
Self-reported "yes"	13	15.7	21	12.7	9	24.3	8	11.3	4	8.7	13	13.7
<u>Baseline BMI</u>												
<18.5	0	0.0	2	1.2	0	0.0	1	1.4	0	0.0	2	2.1
18.5 - 24.9	22	26.5	60	36.1	10	27.0	28	39.4	12	26.1	31	32.6
25 - 29.9	38	45.8	67	40.4	17	45.9	30	42.3	21	45.7	37	38.9
30 - 34.9	17	20.5	28	16.9	8	21.6	9	12.7	9	19.6	19	20.0
>35	6	7.2	9	5.4	2	5.4	3	4.2	4	8.7	6	6.4
Mean (std.dev.)	27.95	(4.52)	26.77	(4.67)	27.88	(3.98)	26.20	(4.36)	28.01	(4.96)	27.19	(4.86)

Table II

PPARG tagSNP allele and genotype frequencies, p-values for test for deviation from Hardy-Weinberg equilibrium (HWE) in controls.

SNP	Alleles		Case Genotype Frequencies				Control Genotype Frequencies				HWE p-value
	Major	Minor	Case MAF ¹	Major/Major	Major/Minor	Minor/Minor	Control MAF	Major/Major	Major/Minor	Minor/Minor	
rs11715073	C	G	0.25	0.54	0.43	0.04	0.20	0.63	0.35	0.02	0.25
rs1801282 ²	C	G	0.14	0.73	0.26	0.01	0.08	0.84	0.16	0.00	0.52
rs4135247	A	G	0.39	0.41	0.41	0.18	0.47	0.28	0.50	0.22	0.99

¹MAF: minor allele frequency.

²Pro12Ala nonsynonymous variant.

Results of adjusted¹ conditional logistic regression of SNPs and risk for pancreatic cancer in all subjects and stratified by CARET arm assuming a dominant genetic model for the minor allele.

Table III

SNP	All subjects		Placebo subjects		Vitamin subjects	
	OR ²	95% CI ³	OR	95% CI	OR	95% CI
rs11715073	1.38	(0.8 – 2.39)	1.51	(0.62 – 3.67)	1.45	(0.70 – 3.02)
rs1801282 ⁴	1.82 ^{***}	(0.97 – 3.41) [*]	1.20	(0.45 – 3.23)	2.80 ^{****}	(1.16 – 6.74) ^{**}
rs4135247	0.56 ^{***}	(0.32 – 0.98) ^{**}	0.39	(0.13 – 1.17) [*]	0.59	(0.29 – 1.21)

¹ Adjusted for BMI and diabetes.
² OR: odds ratio,
³ 95% CI: 95% confidence interval,
^{*} p-value < 0.10;
^{**} p-value < 0.05,
^{***} meets false positive report probability criteria for noteworthiness at 0.5 level,
⁴ Pro12Ala nonsynonymous variant.

Table IV

Estimated frequencies for haplotype block composed of rs11715073, rs1801282, and rs4135247 and results of adjusted¹ conditional logistic regression of this haplotype and risk for pancreatic cancer in all subjects and stratified by CARET intervention arm.

Haplotype	All Subjects				Placebo Subjects				Vitamin Subjects			
	Estimated Frequency		Association with Pancreatic Cancer		Estimated Frequency		Association with Pancreatic Cancer		Estimated Frequency		Association with Pancreatic Cancer	
	Cases	Controls	OR ²	95% CI ³	Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI
CCG	0.37	0.34	1.00	(reference)	0.42	0.52	1.00	(reference)	0.35	0.42	1.00	(reference)
CCA	0.38	0.46	1.19	(0.77 – 1.83)	0.37	0.29	1.33	(0.67 – 2.64)	0.37	0.37	1.22	(0.66 – 2.24)
GCA	0.11	0.12	1.17	(0.6 – 2.27)	0.09	0.08	1.86	(0.53 – 6.44)	0.12	0.15	1.03	(0.44 – 2.4)
GGA	0.13	0.07	2.09	(1.06 – 4.11)*	0.11	0.10	1.27	(0.4 – 4.05)	0.15	0.05	3.32	(1.27 – 8.66)*
Rare ⁴	0.01	0.01	global p-value: 0.10		0.01	0.01	global p-value: 0.17		0.01	0.01	global p-value: 0.10	

¹ Adjusted for BMI and diabetes.
² OR: odds ratio,
³ 95% CI: 95% confidence interval,
* p-value < 0.05,
⁴ Rare: haplotype frequency < 0.05,