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Apolipoprotein E/C1 Locus Variants Modify Renal Cell Carcinoma Risk

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Summary

Lipid peroxidation is considered a unifying mechanistic pathway through which known risk factors induce renal cell carcinoma (RCC). We hypothesized that genes selected *apriori* for their role in lipid peroxidation would modify cancer risk. We genotyped 635 single nucleotide polymorphisms (SNPs) in thirty-eight candidate genes in 777 Caucasian RCC cases and 1035 controls enrolled in a large European case-control study. Top candidate SNPs were confirmed among 718 Caucasian cases and 615 controls in a second study in the United States. Two of the three SNPs (rs8106822 and rs405509) that replicated in the US study were within a regulatory region of the *APOE* promoter. The odds ratio (OR) for rs8106822 A>G variant was 1.22_{AG} and 1.41_{GG} (p-trend=0.01) in the European study, 1.05_{AG} and 1.51_{GG} (p-trend=0.03) in the US study, and 1.15_{AG} and 1.44_{GG} (p-trend=0.001) among 1485 cases and 1639 controls combined. The rs405509 G>T variant was associated with risk in the European ($OR=0.87_{TG}$; $OR=0.71_{TT}$; p-trend=0.02), the US ($OR=0.68_{TG}$; $OR=0.71_{TT}$; p-trend=0.02), and both studies combined (OR_{TG}=0.79; OR_{TT}= 0.71; p-trend=0.001), as was the G-G haplotype (r^2=0.64; $p=4.7 \times 10^{-4}$). This association is biologically plausible as SNP rs405509 was shown to modify protein binding and transcriptional activity of the *APOE* gene *in vitro* and is in LD with key known variants defining the e2, e3, e4 alleles that modify risk of atherosclerosis, Alzheimer's disease risk, and progression to AIDS. In two large case-control studies, our findings further define a functional region of interest at the *APOE* locus that increases RCC susceptibility.

Introduction

The association between kidney cancer and known risk factors such as obesity and hypertension, tobacco use, and suspected occupational risk factors such as chlorinated solvents,

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gasoline, and lead has been supported through several epidemiologic studies (1-4). In humans, evidence that oxidative damage and lipid peroxidation may be important intermediate risk factors underlying kidney carcinogenesis has come from studies reporting higher rates of lipid degradation by-products among cancer cases with the above known risk factors, and related risk factors such as diabetes (5-7). In animal models, by-products that result from lipid peroxidation of the renal tubules can form DNA adducts, leading to alterations relevant to carcinogenesis (8,9). Lipid peroxidation by-products can also cause direct oxidative damage at the DNA, protein, and cellular levels.

To further clarify the role of lipid peroxidation in kidney cancer and specifically renal cell carcinoma (RCC) the most common form of kidney cancer, we selected *a priori* 38 candidate genes for analysis with 635 tagging single-nucleotide polymorphisms (SNPs) that provided comprehensive genomic coverage of each candidate gene and regulatory regions upstream and downstream from coding regions among subjects enrolled in a large hospital-based case-control study of kidney cancer. This study was sufficiently powered to investigate modification of cancer risk associated with common genetic variation. To confirm these findings, we had the opportunity to select three promising markers for rapid replication among cases and frequency-matched population controls from a kidney cancer case-control study conducted in the United States. In total, this study included 1485 genotyped cases and 1639 controls.

Materials and Methods

Study Populations: The Central and Eastern European Renal Cancer Study

This study is a hospital-based case-control study that was conducted in seven centers in Central and Eastern Europe (Moscow, Russia; Bucharest, Romania; Lodz, Poland; and Prague, Olomouc, Ceske-Budejovice and Brno, Czech Republic). Details of the study have been described previously (10). Patients with newly diagnosed and histologically confirmed kidney cancer (ICD-0-2 code C64) between the ages of 20 and 79 years were recruited from August 1999 through January 2003. Trained medical staff reviewed medical records and extracted information on date and method of cancer diagnosis, histological classification and confirmation of the RCC subtype, tumor location, stage and grade. Eligible controls were chosen from among patients admitted to the same hospital as cases for conditions unrelated to smoking or genitourinary disorders (except for benign prostatic hyperplasia) and were frequency-matched to cases on age (within 3 years), sex, and study center. Some controls had been previously recruited from an earlier case-control study of lung and head and neck cancer (11). No single disease made up more than 20% of the control group. Response rates at each center ranged from 90.0 to 98.6% for cases and from 90.3 to 96.1% for controls. Interviews were conducted by trained personnel to collect data on demographic characteristics, education, tobacco smoke exposures, alcohol consumption, dietary practices, medical history, family history, and occupational history. In total, 1097 cases and 1476 controls were interviewed. Blood samples were collected and stored at -80°C. Genomic DNA was extracted from whole blood buffy coat by the standard phenol chloroform method at an NCI designated laboratory from 987 of 1097 (90%) of cases and 1298 of 1476 (88%) of controls. All subjects in this study provided written informed consent. This study was approved by the institutional review boards of all participating centers.

United States Kidney Cancer Study

This study is a population based case-control study conducted in Detroit, Michigan and Chicago, Illinois, in the United States. Cases included residents of each study area aged 20-79 years who were newly diagnosed with histologically confirmed renal cell carcinoma (ICD-02 C64.9) from February 2002 through January 2007. Controls were frequency-matched to cases by study center, race, age, and sex. Controls aged 65 years and older were identified from

Medicare files, and those under age 65 years were identified from Division of Motor Vehicle records. Written informed consent was obtained from all participants, and institutional review board approvals were obtained from all participating study centers. Participants were interviewed by trained interviewers to collect information on demographic background, consumption of tobacco, alcohol and other diet, occupational history, as well as known or suspected risk factors for kidney cancer such as hypertension and antihypertensive medicines. Buccal and blood samples were collected as a source of genomic DNA. A total of 1568 Caucasians (856 cases and 712 controls) were interviewed. Of these subjects, blood samples were collected from 718 of 856 (83.9%) of cases and 615 of 712 (86.4%) controls. Genomic DNA was extracted using standard phenol chloroform methods at an NCI designated laboratory, and 708 Caucasian cases and 604 controls were successfully genotyped.

Genotyping

We analyzed 635 tagging SNPs spanning regions both upstream and downstream of 38 candidate genes involved in mediation and response to lipid regulation, peroxidation, and metabolism: [gene (number of tagging SNPs)]: *AKRFC3* (13), *ALOX5* (8), *ALOX12* (8), *APOB* (14), *APOE/C1* (5), *ATP1A2* (17), *CAT*(17), *COMT*(34), *CYP17*(9), *CYP19(35)*, *FOXO1*(17), *FOXO3* (9), *GGH* (7), *GPX1*(3), *GPX2* (10), *GPX3*(15), *GPX4* (5), *GSR*(9), *GSTO1* (7), *HOA2* (10), *IL12A* (16), *IL12B* (16), *INS*(12), *INSR(49)*, *LEPR*(27), *MGMT(57,)* *MUTYH*(8) *NOS2A*(19), *NOS3*(11), *NOX1*(8), *PON 1/2/3(48)*, *PPARA*(22), *PPARG*(9), *PTGS* (7), *SOD2*(8), *SOD3*(8), *TXN*(22)) (Suppl T1). SNPs were selected to provide high genomic coverage. Tag SNPs were selected 20 kb 5′ of the start of transcription and 10 kb 3′ of the last exon using HapMap CEU data (http://www.hapmap.org) among SNPs with minor allele frequencies of at least 5% and an $r^2 \geq 0.80$ (12). In addition, nonsynonymous SNPs or those correlated with polymorphisms with potential functional significance were included in the analysis. All SNPs and assay information are reported in the NCI SNP500 Cancer database (http://snp500cancer.nci.nih.gov) (13). Genotyping was conducted at NCI's Core Genotyping Facility where staff was blinded to case/control status. Duplicate quality control samples (5% samples) were interspersed among plates. All genotyping was performed using an Illumina GoldenGate ® Oligo Pool All (OPA) assay, which was designed using publicly available sequencing information. The genotype frequencies among controls showed no deviation from the expected Hardy-Weinberg equilibrium proportions ($p > 0.05$). The genotyping completion rate ranged from 98-100% for all SNPs, except was 97% for rs2240714 tagging *TXNRD2/ COMT*, rs4135182 tagging *TXN* gene region, and rs732498 tagging the 3′ region of the *SOD2* gene.

For the replication study, TaqMan based assays were used. Methods for TaqMan based assays for rs8106922, rs405509, and rs4795067 can be found at http://snp500cancer.nci.nih.gov/home.cfm. Completion rates for each of the SNPs was >99%. Concordance rates were >98%.

Statistical Analyses

Initial exploratory analyses were based on the 777 cases and 1035 controls from the Central and Eastern European study that provided a sufficient quantity and suitable quality of genomic DNA for genotyping on the Illumina GoldenGate ® platform. Associations were evaluated through a comprehensive sequence of methods that were sensitive to detect significant associations by gene and by region, while controlling for type I error that is inherent in a large study of 38 genes and 635 SNPs. First, subject characteristics having statistically significant ($p<0.05$) differences in distributions between cases and controls were examined for their associations with SNPs and RCC risk using a chi-square test, and were evaluated as potential confounders. Genotypes were evaluated by coding the homozygous common allele as the referent group and separately comparing the heterozygous and homozygous rare allele

genotypes to the most common homozygous referent group. Linear tests for trends were conducted by including a variable coded 0, 1, and 2 corresponding to the number of rare alleles. Unadjusted and adjusted (age, sex, and study center, smoking, BMI, hypertension) single marker odds ratios (OR) and 95% confidence intervals (95% CI) were generated both for single SNPs and SNPs in regions with a high level of signal. Linkage disequilibrium (LD) between markers was assessed by calculating pairwise Lewontin's D′ and r^2 values using the Haploview program among controls applied to genotype data.

For gene based analyses, global p-values were evaluated using the minimum-p value permutation test (MIN-P). This method corrects for multiple testing while also accounting for correlations between SNPs within a gene (14). In addition, a Haplowalk sliding window analysis of three consecutive SNPs was used to identify chromosome regions of interest that remained significant at a false discovery rate (FDR) level of 1% or less; FDR is defined as the expected proportion of falsely rejected hypotheses among the rejected hypotheses (15,16). Given k genotyped SNPs in a gene, the Haplowalk procedure considered a 3-SNP window across each gene from SNP 2 through SNP K-1, resulting in K-2 analyzed overlapping windows. For each window, haplotype frequencies in cases and controls were reconstructed using the EM algorithm, and a Wald test was used to screen for association with case-control status (15,16). The Benjamini-Hochberg FDR procedure was applied to the set of k-2 Wald-test p-values yielding FDR adjusted p-values that control for multiple testing at the gene level (16). In the initial screening phase, no adjustment was made for potential confounders, and the Wald tests used a threshold value of 5%, such that haplotypes in cases and controls with an estimated frequency below the threshold value in controls were combined into a separate “rare haplotype” category for purposes of statistical testing. Genes with a MIN-P value less than or equal to 0.05, and genes in which we identified one or more region(s) using the haplowalk analysis, and with an FDR adjusted p-value less than or equal to 0.05, were reconstructed and associations evaluated using Haplostats (Version 1.3.1) in R (version 2.4.1) adjusting for sex, age, center, smoking status, BMI, and hypertension, using the most common haplotype as the referent.

The replication analysis was conducted among 708 Caucasian cases and 604 controls from the US study. Three SNPs; rs8106922, rs405509, and rs4795067 were selected for replication based on having high minor allele frequencies (between 30-50%) to increase study power to detect the odds ratios observed in the European study. The same statistical methods were used where applicable, without adjustment for multiple comparisons. Therefore, estimates were calculated for both studies independently and combined and adjusted for center, sex, age in quintiles based on combined controls. Additional adjustment for potential confounders (body mass index (BMI), self-reported hypertension, and smoking) did not result in meaningful changes in risk estimates and therefore were not included in models. In addition, we investigated multiplicative interactions between individual SNPs and age, sex, BMI, self-reported hypertension, and smoking using the likelihood ratio test to compare the fit of models with and without interaction terms but we did not find evidence of heterogeneity.

Heterogeneity of genotype frequencies between countries was evaluated by using the likelihood ratio test to compare the fit of models with and without interaction terms. We found no evidence of heterogeneity across study centers. Moreover, no evidence of population stratification was apparent from a principal components analysis of a genome wide association study conducted in this population (17), and the likelihood of this is small among European populations (18).

Results

Among cases and controls in the European study, most of the study population was from the Czech Republic, and a slightly higher proportion of cases were from that country (Table 1). Controls were more likely to be male, but were similar in age distribution. The prevalence of subjects in the lowest (<51 years) and highest age quintiles (>=69 years) were about twice as high in the European study than in the US study. Cases were more likely than controls to have higher BMI, and self-reported hypertension (4). The prevalence of clear cell renal cancer was 83% in both studies.

Ranked results from global gene-based tests of association (MIN-P test), the false discovery rate (FDR)-adjusted minimum p-trend for the additive models, and the smallest FDR-adjusted p-value from the 3-SNP sliding window analysis are presented in Table 2. Based on the MIN-P test, four genes were selected for in-depth analysis after multiple testing correction of single marker associations: *APOE, GPX4, NOS2A, PTGS2*, The overall MIN-P adjusted p-values for these were 0.017, 0.020, 0.055, 0.069 respectively. These genes also had the lowest minimum p-values from a 3-SNP sliding window analysis with a false discover rate (FDR) level<1% (4.8×10^{-4}, 7.4×10^{-4}, and 1.7×10^{-4}, 1.09×10^{-5}, respectively). Individual associations between tagging SNPs and risk associated with these four genes are presented in Suppl Table 3. For the *APOE/C1* gene region[see supplemental Figure 1 to see correlations between *APOE* gene tagging SNPs in haploview], significant inverse associations were observed with two promoter region SNPs, one rare variant located in the *APOC1* gene promoter (rs283813) and the other in the *APOE* gene promoter region (rs405509). In addition, increased risk was observed with the G allele of SNP rs8106922. For the *GPX4/POLR2E* region, increased risk was observed for one of the five tagging SNPs (rs11668388). For the *NOS2A* gene, five of 19 SNPs significantly modified RCC risk. Two SNPs were located in the promoter region (rs2531860, rs2779248), and three were intronic (rs3729508; rs4795067; rs2248814). Three of five *PTGS2* tag SNPs were also significantly associated with risk, more than would be expected by chance.

Subsequently, genomic regions identified from the sliding window analysis were reevaluated after adjustment for potential confounders in R using Haplostats (Suppl Table 4). Two variant haplotypes in the *APOC1* regulatory region were inversely associated with risk (T-T-A, OR=0.76; 95% CI:0.61-0.94; p=0.01) and A-C-A (OR=0.56; 95% CI:0.40-0.79; $p=9\times10^{-4}$) when compared to the common referent haplotype T-C-A (P-global=0.002). This association was similar in magnitude to that observed for the rare *APOC1* (rs283813) AA variant that was found in only 3% of cases and 8% of controls ($OR=0.69_{TA}$, $OR=0.51_{AA}$; p-trend =0.004). In contrast, when SNPs (rs8106822, rs405509) tagging the *APOE* promoter region were considered as a haplotype, increased risk was observed for the G-G haplotype compared to the common haplotype A-T (OR=1.21; 95% CI:1.05-1.40, p=0.01). Analysis of the *GPX4* gene region revealed increased risk associated with the haplotype A-G-A compared to the common referent T-A-A haplotype (OR=1.22; 95% CI:1.05-1.42, p=0.01), although the rs11668388 variant appeared to be driving the association (data not shown). Additional adjusted haplotype analysis of the *NOS2A* and *PTGS2* gene regions that were significantly associated with risk are presented in Suppl Table 4.

Subsequently, three loci (rs8106922, rs405509, and rs4795067) were selected for rapid replication within regions of interest having high minor allele frequencies (between 30-50% to increase study power). SNPs were analyzed among 708 RCC cases and 604 frequency-matched population Caucasian controls from the US Renal Cancer Study (Table 1). SNPs rs8106922 and rs405509 in the *APOE* promoter region were significantly associated with risk in the US study (Table 3). As observed in the European Study ($OR=1.22_{AG}$; $OR=1.41_{GG}$; p-trend=0.01), increased risk was associated with the G allele of rs8106922 in the US study

(OR=1.05_{AG}; OR=1.51_{GG}; p-trend=0.03), and when the two studies were combined among 1485 cases and 1639 controls (OR=1.15_{AG}; 1.44_{GG}; p-trend=0.001). Similarly, as observed in the European study (OR=0.87_{TG}; OR=0.71_{TT}; p-trend=0.02), decreased risk was associated with the T allele of rs405509 in the US study (OR=0.68_{TG}; OR=0.71_{TT}; p-trend=0.02). When data from the two studies were combined, the results for rs405509 were strengthened (OR_{TG}=0.79; OR_{TT}=0.71; p-trend=0.001), as were the results from the combined G-G haplotype (r^2=0.64) when compared to the A-T referent (OR=1.22; 95% CI: 1.09-1.36, $p=4.7\times10^{-4}$; p-global=0.003). The *NOS2A* SNP rs4795067 that was selected for replication based on the results from the European study (OR_{TC}=1.33; 95% CI:1.08-1.63, OR_{TT}=1.50; 95% CI:1.11-2.04, p-trend=0.002) was not significantly associated with risk in the US study (OR_{TC}=1.05; 95% CI:0.83-1.32, OR_{TT}=1.15; 95% CI:0.79-1.67, p-trend=0.48). When genotyping data from the two studies were combined, risk was significantly elevated among subjects with at least one variant allele (OR_{TC}=1.17; 95% CI:1.01-1.37 and OR_{CC}=1.34; 95% CI:1.06-1.37; p-trend=0.006). Results were similar when analyses were restricted to include clear cell renal cancer cases only (Table 3). No interactions between our significant SNPs and potential and risk factors/environmental exposures were detected (data not shown).

Discussion

Although still relatively rare, RCC incidence has increased rapidly in the U.S. over the past few decades (19-21). Several well-established lifestyle risk factors such as BMI, hypertension, and smoking have been identified and are thought to explain approximately 50% of cases (3). Causes for the remaining half of cases remain unknown. Genetic susceptibility to sporadic kidney cancer is a promising area of research, and has not been fully investigated. To our knowledge, this is the first evaluation of variation in lipid metabolism/peroxidation genes in the two largest kidney cancer case-control studies with genomic DNA conducted to date. These findings are important as lipid peroxidation has emerged as a unifying mechanism through which several known and suspected risk factors are thought to modify kidney cancer risk. Many kidney cancer genetic susceptibility studies conducted to date have been under-powered, and have focused primarily upon common variation in xenobiotic metabolism (reviewed in 20,21), micronutrient metabolism (20,21,22), in addition to genes associated with familial cancers such as von Hippel-Lindau (*VHL*) (20,21) and did not employ a tagging approach across genes selected *a priori* for their role in lipid metabolism/peroxidation. Our findings suggest that SNPs in the *APOE/C1* gene region are associated with renal cancer. In two studies, we found that the high risk G-G haplotype, that included SNPs rs8106922 and rs405509, was common among controls in both studies (39%), and conferred a significant 20% increased risk above that observed for the most prevalent low-risk genotype, found in 49% of controls. The *APOE* protein plays an important role in the cellular uptake of lipoproteins through ligand-receptor interactions with the low-density lipoprotein receptor (LDL) and chylomicron remnant receptors (23). Through this interaction, *APOE* mediates the uptake and metabolism of lipoproteins and is thought to be a major determinant of blood lipid levels in humans, a precursor to risk factors for renal cancer. In the *NOS2A* gene, three high-risk haplotypes were identified across introns 5 through 12. Within this region, we selected SNP rs4795067 for replication although it alone was not driving the association observed. Additional genotyping is warranted to identify other causal variants across this region. Moreover, only the combined analysis was sufficiently powered to detect the OR (1.34) observed.

Variation in *APOE* was previously evaluated for associations with risk factors such as hypertension and body mass index (BMI), and diseases such as biliary tract cancer (24), gall stones (25,26) and Alzheimer's disease (27,28). In a recent study the *APOE* variant (IVS1 +69C>G; rs440446), the G allele was significantly associated with biliary stones, bile duct cancer, and cancer of the ampulla of Vater (24). The *APOE* gene has three major isoforms encoded by three alleles (e2, e3, e4) resulting from the presence of two non-synonymous SNPs

located within coding regions for amino acids 112 and 158. The allele distribution in most Caucasian populations for the most prevalent e3 allele (Cys112; Arg158) ranges from 70-85%, the e4 allele (Arg112; Arg 158) from 10-20%, and the e2 allele (Cys112; Cys158) from 5-10%. The *APOE* e4 allele has been associated with increased levels of total and LDL serum cholesterol, whereas the e2 allele was associated with the reverse effect (23). Using these alleles as markers, one study showed an increased risk of gallstones associated with the e4 allele, compared to subjects that had the e3 and e2 alleles (29). In addition to lipid metabolism, this protein also modulates proinflammatory and inflammatory responses (23,29). It has also been shown to modify risk of other malignancies including breast, colon/rectum, and prostate (30-32). In our study, *APOE* SNPs were selected to tag regions upstream from and including the SNP rs405509 which is located within the *APOE* gene promoter. The functional SNP rs405509 that was associated with reduced risk in our studies was recently shown to modify the differential protein binding and transcriptional activity of the *APOE* gene in HEPG23 liver cells and astrocytoma cells (33,34). This region is in high LD with other SNPs in the *APOE* gene promoter and is tightly correlated with the location of known codon 112 and 158 variants which define the e2, e3, e4 alleles, that are also thought to be under regulatory control by the regions tagged in this analysis (34).

To the best of our knowledge, this is the first large scale evaluation of key candidate genes involved in lipid metabolism/peroxidation and RCC susceptibility that provide evidence to support that common functional variation at the *APOE* gene locus may increase susceptibility to RCC. Validation studies in additional independent study populations will be necessary to confirm these findings to exclude the possibility of chance findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Subjects genotyped in the European and US renal cancer case-control studies

	Central European Study				US Study			
	Case		Control		Case		Control	
	N	%	N	%	N	%	N	%
Total	**777**	**42.9**	**1035**	**57.1**	**708**	**54.0**	**604**	**46.0**
Center								
Bucharest, Romania	68	8.8	120	11.6	-		-	
Lodz, Poland	80	10.3	197	19.0	-		-	
Moscow, Russia	242	31.1	368	35.6	-		-	
Czech Republic	387	49.8	485	46.9	-		-	
Chicago	-		-		98	13.8	80	13.2
Detroit	-		-		610	86.2	524	86.8
Sex								
Male	472	60.7	648	62.6	405	57.2	382	63.2
Female	305	39.3	387	37.4	303	42.8	222	36.8
Age Quintile (Q)								
Q1 <51yrs	188	24.2	252	24.3	89	12.6	75	12.4
Q2 51-<56yrs	116	14.9	186	18.0	151	21.3	129	21.4
Q3 56-<63yrs	174	22.4	205	19.8	221	31.2	175	29.0
Q4 63-<69yrs	141	18.1	205	19.8	177	25.0	166	27.5
Q5 ≥69yrs	158	20.3	187	18.1	70	9.9	59	9.8
Body Mass Index								
0-<25	256	28.8	423	36.2	44	16.6	110	28.2
25-<30	384	43.2	487	41.6	97	36.6	152	39.0
30-<35	199	22.4	204	17.4	63	23.8	67	17.2
≥35	51	5.7	52	4.4	58	21.9	58	14.9
	0	0.0	4	0.3	3	1.1	3	0.8
Ever Diagnosed with Hypertension								
Never	494	55.5	732	62.6	309	44.4	376	62.3
Ever	395	44.4	437	37.4	377	54.2	224	37.1
Unknown	1	0.1	1	0.1	10	1.4	4	0.7
Smoking								
Never	421	47.4	477	40.8	261	37.5	251	41.6
Occasional	1	0.1	1	0.1	29	4.2	19	3.2
Former	192	21.6	277	23.7	308	44.3	252	41.7
Current	274	30.9	414	35.4	98	14.1	82	13.6
RCC Histology								
Clear Cell	639	83.5	-	-	467	83.4	-	-
Other RCC	126	16.5	-	-	93	16.6	-	-

Table 2

Lipid peroxidation gene-based global, trend, and haplowalk minimum p-values for associations with kidney cancer risk

Gene Symbol/Direction	Name	Function	Chromosome	Number of Tag SNPs	Gene-based MINP-adj	mininum p-trend[1]	FDR-adjusted[2]	Haplowalk[3]
APOE	Apolipoprotein E	Lipid metabolism	19q13.2	5	0.017	4.00E-03	0.02	4.84E-04
GPX4	Glutathione peroxidase 4	Antioxidant response	19p13.3	5	0.020	4.56E-03	0.02	7.40E-04
NOS2A	Nitric oxide synthase 2A	Oxidative damage, inflammation	17q11.2-q12	19	0.055	1.75E-03	0.03	1.65E-04
PTGS2	Prostaglandin-endoperoxide synthase 2	Lipid peroxidation, inflammation	1q25.2-q25.3	7	0.069	5.08E-03	0.04	1.09E-05
INSR	Insulin Receptor	Glucose uptake	19p13.3-p13.2	49	0.110	4.76E-03	0.23	0.05
LEPR	Leptin receptor	Lipid metabolism	1p31	27	0.246	0.02	0.54	0.04
HAO2	Hydroxyacid oxidase 2	Lipid Peroxidation	1p13.3-13.1	10	0.195	0.05	0.47	0.08
GSTO1	Glutathione S-transferase omega 1	A cellular redox, oxidative stress	10q25.1	7	0.248	0.06	0.42	0.20
PPARD	Peroxisome proliferator receptor delta	Lipid metabolism	6p21.1-21.1	9	0.329	0.05	0.46	0.40
FOXO3	Forkhead box O3	Oxidative stress, inflammation	6q21	9	0.345	0.05	0.49	0.07
NOX1	NADPH oxidase 1	Oxidative stress	Xq22	8	0.354	0.03	0.27	0.29
CAT	Catalase	Oxidative stress	11p13	17	0.378	0.05	0.80	0.22
TXN	Thioredoxin	Oxido-reductase	9q31	22	0.412	0.04	0.79	0.12
CYP19A	Cytochrome p450, family19 A	Estrogen biosynthesis	15q21.1	35	0.477	0.02	0.53	0.43
GGH	Gamma-glutamyl hydrolase	Hydrolase	8q12.1	7	0.512	0.03	0.20	0.05
APOB	Apolipoprotein B	Lipid Metabolism	2p24-p23	14	0.551	0.07	1.00	0.78
FOXO1	Forkhead box O1	Lipid Metabolism	13q14.1	17	0.580	0.04	0.74	0.93
GPX2	Glutathione peroxidase 2	Antioxidant response	14q24.1	10	0.582	0.05	0.50	0.94
AKR1C3	Aldo-keto reductase family 1, member C3	Inflammation	10p15-p14	16	0.589	0.05	0.86	0.31
NOS3	Nitric oxide synthase 3	Oxidative damage, inflammation	7q36	11	0.596	0.06	0.69	0.46
COMT/TXNRD2	Catechol-O-methyltransferase	Oxido-reductase	22q11.21	34	0.610	0.03	1.00	0.18
SOD3	Superoxide dismutase 3, extracellular	Oxidative stress	4p16.3-q21	8	0.639	0.07	0.58	0.56
GSR	Glutathione reductase	Antioxidant response	8p21.1	9	0.664	0.05	0.46	0.40
PON1,2,3	Paraoxonase 1/2/3	Lipid peroxidation	7q21.3	48	0.707	0.05	1.00	0.64
IL12A	Interleukin 12A	Inflammatory response	1q12q13.23	16	0.741	0.17	1.00	0.91
ALOX5	Arachidonate 5-lipoxygenase	Lipid metabolism,	10q11.2	15	0.747	0.09	1.00	0.64
IL12B	Interleukin 12B	Inflammatory response	5q31.1-q33.1	18	0.748	0.14	1.00	0.15
GPX1	Glutathione peroxidase 1	Antioxidant response	3p21.3	3	0.751	0.32	0.97	0.73
ATP1A2	ATPase	Osmoregulation, hypertension	1q21-23	17	0.753	0.11	1.00	0.79
SOD2	Superoxide dismutase 2, mitochondrial	Oxidative stress	6p25.3	8	0.754	0.20	1.00	0.63
PPARG	Peroxisome proliferator receptor gamma	Lipid metabolism	10q25	24	0.772	0.07	1.00	0.36
INS	Insulin	Glucose uptake	11p13.3	12	0.818	0.11	1.00	0.36
MUTHY	MutY homolog	Oxidative damage & repair	1p34.3-32.1	8	0.855	0.24	1.00	0.91
CYP17A	Cytochrome p450, family17A	Lipid metabolsim	10q	9	0.871	0.19	1.00	0.66
GPX3	Glutathione peroxidase 3	Antioxidant response	5q2.3	15	0.882	0.13	1.00	0.24
PPARA	Peroxisome proliferator receptor alpha	Lipid metabolism, inflammation	22q13.31	22	0.933	0.11	1.00	0.08
MGMT	Methylguanine-DNA methyltransferase	DNA repair, metal binding	10q26	57	0.968	0.07	1.00	0.97
ALOX12	Arachidonate 12-lipoxygenase	Lipid metabolism, inflammation	17p13.1	8	0.990	0.41	1.00	0.95

[1] Minimum p-value of all tagging SNPs for each gene region using additive model

[2] FDR adjusted minimum p-value of all tagging SNPs per gene using additive model

[3] Minimum p-value for 3- SNP haplowalk sliding window analysis

Table 3

Association between *APOE* and *NOS2A Snps* and *APOE* haplotype in all renal cancer cases and restricted to the clear cell subtype

		Kidney Cancer Study								
		Central Euorpean Case-Control Study			US Case-Control Study (Caucasians only)			Combined		
APOE gene region		**Cases (%)/Controls (%)**	**OR (95% CI)**	**p-value**	**Cases (%)/Controls (%)**	**OR (95% CI)**	**p-value**	**Cases (%)/Controls (%)**	**OR (95% CI)**	**p-value**
rs8106922-All RCC	AA	228 (29.3) / 357 (34.5)	1.00		248 (35.4) / 227 (38.5)	1.00		476 (32.4) / 584 (36.0)	1.00	
	AG	408 (52.5) / 523 (50.6)	1.22 (0.98-1.51)	0.06	312 (45.9) / 282 (47.8)	1.05 (0.82-1.34)	0.69	720 (49.1) / 805 (49.6)	1.15 (0.98-1.35)	0.09
	GG	141 (18.1) / 154 (14.6)	1.41 (1.06-1.88)	0.02	131 (18.7) / 81 (13.7)	1.51 (1.08-2.10)	0.01	272 (18.5) / 235 (14.5)	1.44 (1.16-1.78)	0.001
	p-trend		***0.01***			***0.03***			***0.001***	
ccRCC only	AA	162 (28.7)/ 357 (34.5)	1.00		165 (35.8)/227 (38.5)	1.00		327 (31.9)/ 584 (36.0)	1.00	
	AG	296 (52.5)/ 523 (50.6)	1.26 (0.99-1.60)	0.06	207 (44.9)/282 (47.8)	1.13 (0.83-1.56)	0.41	503 (49.1)/ 805 (49.6)	1.21 (1.00-1.46)	0.05
	GG	106 (18.8)/154 (14.6)	1.46 (1.07-2.00)	0.002	89 (19.3)/ 81 (13.7)	1.74 (1.15-2.64)	0.008	195 (19.0)/ 235 (14.5)	1.56 (1.21-2.00)	0.001
	p-trend		***0.01***			***0.01***			***1×10^{-4}***	
rs405509-All RCC	GG	219 (28.2) / 252 (24.4)	1.00		222 (32.0) / 146 (24.8)	1.00		441 (30.0) / 398 (24.5)	1.00	
	TG	411 (53.0) / 537 (51.9)	0.87 (0.69-1.09)	0.32	328 (47.3) /314 (53.3)	0.68 (0.52-0.88)	0.004	739 (50.3) / 851 (52.4)	0.79 (0.67-0.94)	0.008
	TT	146 (18.8) / 245 (23.7)	0.71 (0.54-0.94)	0.01	144 (20.8) / 129 (21.9)	0.71 (0.52-0.98)	0.04	290 (19.7) / 374 (23.0)	0.71 (0.58-0.87)	0.001
	p-trend		***0.02***			***0.02***			***0.001***	
ccRCC only	GG	168 (29.8)/ 252 (24.4)	1.00		145 (31.6)/146 (24.8)	1.00		313 (30.6)/398 (24.5)	1.00	
	TG	297 (52.8)/537 (51.9)	0.81 (0.63-1.04)	0.09	220 (47.9)/314 (53.3)	0.73 (0.52-1.03)	0.08	517 (50.6)/ 851 (52.4)	0.78 (0.64-0.96)	0.02
	TT	98 (17.4)/245 (23.7)	0.59 (0.43-0.81)	0.001	94 (20.5)/314 (53.3)	0.75 (0.49-1.14)	0.17	192 (18.8)/ 374 (23.0)	0.65 (0.50-0.83)	0.001
	p-trend		***0.001***			0.14			***0.001***	
Haplotype rs8106922-rs405509		**Haplotype Frequency** (Case/Control)			**Haplotype Frequency** (Case/Control)			**Haplotype Frequency** (Case/Control)		
All RCC	A-T	45%/49%	1.00		44%/48%	1.00		45%/49%	1.00	
	G-G	44%/40%	1.21 (1.05-1.42)	0.01	41%/37%	1.23 (1.04-1.46)	0.02	43%/39%	1.22(1.09-1.36)	4.7×10^{-4}
	A-G	10%/10%	1.09 (0.87-1.38	0.45	14%/15%	1.08 (0.85-1.37)	0.52	12%/12%	1.09 (0.92-1.28)	0.33
	p-global		***0.08***			***0.07***			***0.003***	
ccRCC only	A-T	45%/49%	1.00		44%/48%	1.00		45%/49%	1.00	
	G-G	44%/40%	1.20 (1.03-1.40)	0.02	41%/37%	1.20 (1.00-1.43)	0.04	43%/39%	1.20 (1.07-1.35)	0.002
	A-G	10%/10%	1.09 0.86-1.39)	0.48	14%/15%	1.07 (0.83-1.37)	0.59	12%/12%	1.08 (0.91-1.27)	0.37
	p-global		***0.13***			0.20			***0.01***	
***NOS2A gene region*rs4795067-All RCC**		Cases (%)/Controls (%)	OR (95% CI)	p-value	Cases (%)/Controls (%)	OR (95% CI)	p-value	Cases (%)/Controls (%)	OR (95% CI)	p-value
	TT	292 (37.6) / 457 (44.2)	1.00		278 (39.8) / 244 (41.4)	1.00		570 (38.6) / 701 (43.1)	1.00	
	TC	374 (48.2) / 462 (44.6)	1.33 (1.08-1.63)	0.02	341 (48.8) / 284 (48.1)	1.05 (0.83-1.32)	0.70	715 (48.5) / 746 (45.9)	1.17 (1.01-1.37)	0.04
	CC	110 (14.2) / 116 (11.2)	1.50 (1.11-2.04)	0.01	80 (11.4) / 62 (10.5)	1.15 (0.79-1.67)	0.48	190 (12.9) / 178 (11.0)	1.34 (1.06-1.37)	0.01
	p-trend		***0.002***			***0.48***			***0.006***	
ccRCC only	TT	222 (39.4)/457 (44.2)	1.00		178 (38.6)/244 (41.4)	1.00		400 (39.1)/701 (43.1)	1.00	
	TC	264 (46.9)/462 (44.6)	1.18 (0.95-1.48)	0.04	235 (51.0)/ 284 (48.1)	1.16 (0.86-1.57)	0.31	499 (48.7)/746 (45.9)	1.18 (0.99-1.41)	0.07
	CC	77 (13.7)/116 (11.2)	1.38 (0.98-1.92)	0.06	48 (10.4)/62 (10.5)	1.08 (0.66-1.76)	0.76	125 (12.2)/178 (11.0)	1.27 (0.97-1.67)	0.09
	p-trend		***0.04***			***0.48***			***0.04***	

[1] All analyses adjusted for age, sex, and study center