Gene-Environment Interaction of Genome-Wide Association Study-Identified Susceptibility Loci and Meat-Cooking Mutagens in the Etiology of Renal Cell Carcinoma

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BACKGROUND: Meat-cooking mutagens may be associated with renal cell carcinoma (RCC) risk. In this study, the authors examined associations between meat-cooking mutagens, genetic susceptibility variants, and risk of RCC. METHODS: The authors used 659 newly diagnosed RCC cases and 699 healthy controls to investigate the association between dietary intake of meat-cooking mutagens and RCC. They examined whether associations varied by risk factors for RCC and genetic susceptibility variants previously identified from genome-wide association studies. Odds ratios and 95% confidence intervals were estimated using tertiles of intake of dietary polycyclic aromatic hydrocarbons/heterocyclic amines. RESULTS: Dietary intake of the mutagenic compounds 2-amino-3,8-dimethylimidazo(4,5-f) quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP) was found to be significantly associated with an increased risk of RCC (odds ratios across tertiles: 1.00 [referent], 1.28 [95% confidence interval, 0.94-1.74], and 1.95 [95% confidence interval, 1.43-2.66] [P for trend <.001], respectively; and 1.00 [referent], 1.41 [95% confidence interval, 1.04-1.90], and 1.54 [95% confidence interval, 1.14-2.07] [P for trend = .02], respectively). The authors observed evidence of interactions between PhIP and RCC susceptibility variants in 2 genes: inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) (rs718314; multiplicative P for interaction = .03 and additive P for interaction = .002) and endothelial PAS domain-containing protein 1 (EPAS1) (rs7579899; additive P for interaction = .06). CONCLUSIONS: The intake of meat may increase the risk of RCC through mechanisms related to the cooking compounds MeIQx and PhIP. These associations may be modified by genetic susceptibility to RCC. Further research is necessary to understand the biological mechanisms underlying these interactions.

INTRODUCTION
The increase in the incidence of renal cell carcinoma (RCC) in the United States and other developing nations suggests that factors related to a Western lifestyle, such as a diet high in meats, processed foods, and starches, may play an important role in RCC etiology. Although previous studies have linked meat intake with an increased risk of RCC, to the best of our knowledge the underlying mechanism for this association remains unclear. Cooking meat at high temperatures, particularly barbecuing or pan-frying, results in the formation and ingestion of carcinogenic compounds including heterocyclic amines (HCAs) such as 2-amino-1 methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), amino-3,8-dimethylimidazo(4,5-f) quinoxaline (MeIQx), and 2-amino-3,4,8-trimethylimidazo(4,5-f) quinoxaline (DiMeIQx) and polycyclic aromatic hydrocarbons (PAHs), specifically benzo(a)pyrene (BaP). To the best of our knowledge, few epidemiologic studies to date have investigated the association between these carcinogenic compounds and RCC risk.

The kidney is a biochemically active organ that contributes significantly to the metabolism of xenobiotics and therefore is exposed to higher concentrations of carcinogens than other organs. Genome-wide association studies (GWAS) have implicated common variants involved in the cellular response to changes in oxygen, iron, nutrients, or energy as playing an important role in the etiology of RCC. Recently, a single-nucleotide polymorphism (SNP) located near the inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) gene related to lipid metabolism and obesity was found to modulate the association between adherence to a Western dietary pattern and increased risk of RCC. Therefore, genetic variants...
related to RCC susceptibility may play a role in modifying the association between dietary intake of carcinogens and RCC risk.

In the current study, we investigated whether dietary intake of meat-cooking mutagens, such as BaP, MeIQx, DiMeIQx, and PhIP, played a role in RCC risk in a large case-control study of newly diagnosed RCC cases and healthy controls. We examined whether these associations were modified by known or suspected risk factors for RCC, including smoking, and previously identified GWAS-identified RCC genetic susceptibility variants. To the best of our knowledge, the current study is the first to investigate potential interactions between dietary intake of meat-cooking mutagens and RCC susceptibility variants in a case-control study of newly diagnosed RCC cases and healthy controls.

MATERIALS AND METHODS

Study Population and Recruitment
Cases were drawn from an ongoing case-control study of RCC initiated in 2002. The study was approved by The University of Texas MD Anderson Cancer Center Institutional Review Board. Procedures for subject recruitment and eligibility criteria have been described previously. Briefly, all case subjects were newly diagnosed and had histologically confirmed RCC. Healthy control subjects without a history of cancer, except nonmelanoma skin cancer, were identified and recruited via random digit dialing. Control subjects were frequency matched to case subjects according to age (±5 years), sex, ethnicity, and county of residence. All participants provided written informed consent before participation in the study.

Exclusion and inclusion criteria for the parent case-control study have been described in detail elsewhere. Of 1516 matched cases and controls, we in addition excluded individuals with outlying total energy intake by excluding men (33 individuals) and women (34 individuals) with values that fell outside the interval delimited by the 25th percentile minus 1.5 times the interquartile range and the 75th percentile plus 1.5 times the interquartile range. Due to the small number of minority participants (91 individuals), we limited the analysis to non-Hispanic whites only, leaving 1358 individuals (659 cases and 699 controls) for inclusion in the current study.

Data Collection
Epidemiologic data were collected by staff interviewers at The University of Texas MD Anderson Cancer Center via in-person interview including history of hypertension (yes/no), physical activity, alcohol use, and smoking status. After the interview, a 40-mL blood sample was collected from each participant and delivered to the laboratory for molecular analysis. Alcohol intake was adjusted for total energy intake using the nutrient density method. An individual who had never smoked or had smoked <100 cigarettes in his or her lifetime was defined as a never-smoker. An individual who had smoked at least 100 cigarettes in his or her lifetime but had quit ≥12 months before diagnosis (for cases) or the interview (for controls) was classified as a former smoker. Current smokers were those who were currently smoking or quit <12 months before diagnosis (for cases) or before the interview (for controls).

Weight at the time of diagnosis (for cases) or recruitment (for control subjects) was recorded. Body mass index (BMI) was derived from height and weight. BMI was categorized according to the standard classifications of the World Health Organization (normal: <25 kg/m²; overweight: 25-29.9 kg/m²; and obese: ≥30 kg/m²). Participants also reported the average frequency they spent on 5 broad groups of physical activity. A metabolic equivalent value (MET) was assigned to each activity group. Energy expenditure from physical activity was calculated as the MET value of each activity multiplied by the frequency of each activity and then summed across all activities.

Dietary Assessment
We used a previously validated and modified version of the National Cancer Institute Health Habits and History Questionnaire. The validity and reliability of this food frequency questionnaire (FFQ) has been documented previously. The questionnaire queried the frequency of intake, method of preparation, and portion size of a wide range of foods and beverages, including cereals, grains, fruits, vegetables, meats, dairy, dessert, fast foods, juices, alcohol, and water. Total energy intake and grams per day of consumption for each food item were estimated using the US Department of Agriculture Food and Nutrient Database for Dietary Studies. Foods items were standardized using the nutrient density method (g/1000 kcal) for total energy intake.

Mutually exclusive categories were created from the meat, poultry, and fish items queried in the modified FFQ. These categories included fresh red meat (beef [hamburger, beefsteak, ground beef, beef stew, roast beef, pot roast, and beef ribs] and pork [ham/ham steak, pork chops, pork roast, and pork ribs]), processed red meat (bacon, sausage, ham hocks, smoked meats, and hot dogs), fresh white meat (poultry [chicken, turkey, and fried...
chicken), processed white meat (low-fat hot dogs, low-fat sausages, and low-fat lunch meat), and fish (canned, smoked, salted, fresh, or fried).

The meat portion of the FFQ ascertained cooking methods (eg, pan-fried, broiled, microwaved, baked, grilled, brown-and-serve, other, or do not know). Photographic models were used to help participants determine a level of doneness (inside and outside) of the meat. Meat intake, cooking methods, and level of doneness were linked to the National Cancer Institute’s Computerized Heterocyclic Amines Resource for Research In Epidemiology of Disease (CHARRED) database (http://charred.cancer.gov/) to estimate values (in ng/d) of 3 HCAs (PhIP, MeIQx, and DiMeIQx), and 1 PAH (BaP). The exposure index has previously been described elsewhere.\textsuperscript{18,19}

Genotyping and Selection of SNPs
We selected 6 SNPs (rs12105918 [chromosome 2; ZEB2], rs10054504 [chromosome 5; PDZD2], rs718314 [chromosome 12; ITPR2], rs7579899 [chromosome 2; EPPAS1], rs7105934 [chromosome 11; CCND1], and rs4765623 [chromosome 12; SCARB1])\textsuperscript{20-22} previously identified through GWAS that demonstrated significant or marginally significant associations $P<.000001$ with RCC risk. Genomic DNA was extracted from peripheral blood using the QI Amp DNA extraction kit (Qiagen, Valencia, Calif) and genotyped by Taqman genotyping assays on the ABI PRISM 7900HT Sequence Detection System (Life Technologies, Grand Island, NY) according to the specified protocol. Runs included negative controls (water) and 5% of samples as replicates. All SNPs were in Hardy-Weinberg equilibrium ($P > .05$) and concordance was 100%.

Statistical Analysis
METs of physical activity and alcohol intake were categorized into tertiles based on the distribution in control subjects. Missing data regarding physical activity were consistent between cases and controls and therefore were coded as a separate “unknown” category. All continuous intake variables were categorized into tertiles based on the distribution among the controls and by sex with the reference group comprised of individuals in the lowest category of intake. Quintiles of intake were also assessed for the overall analysis; however, due to limited power for strata and genetic interaction, we limited the analysis to tertiles. Results for the overall analysis using quintiles were consistent and therefore are not presented herein.

Comparisons for case-control characteristics were performed using generalized linear models and Pearson chi-square tests. We evaluated the association between both meat intake and PAH/HCA exposure and risk of RCC using unconditional logistic regression models. Age and sex, as well as multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs), were reported across sex-specific tertiles of intake. Multivariable-adjusted models included the following covariates: age (continuous), sex, BMI (categories), total energy intake (tertiles), total fruit and vegetable intake (tertiles), smoking status (never-smoker and ever-smoker), physical activity (low [<30 METs/week], medium [30-48 METs/week], and intense [$\geq 48$ METs/week]), and history of hypertension (yes/no). Alcohol intake did not appear to contribute significantly to the model and therefore was not included. Risk estimates for the meat consumption models were also adjusted for tertiles of other meat intake so that the sum of all the meat variables in each model represented total meat intake (eg, fresh red meat intake was adjusted for processed red meat intake, total white meat intake, and fish intake). Tests for trend were obtained by including an ordinal exposure variable in the model. Models evaluating processed and fresh red and white meats individually were consistent with the models for overall red, white, and processed meats and therefore are not presented herein. Fish intake was not found to be associated with the risk of RCC and therefore was not included in the current analysis.

We conducted multivariable-adjusted analyses stratified by sex, age (<60 years vs $\geq 60$ years), obesity, smoking, hypertension, and physical activity in addition to 6 previously identified genetic variants using the homozygous major genotype as the reference category. We included the cross-product term of the dichotomous risk factor variables and ordinal mutagen variables in the logistic regression model to test for multiplicative interaction (Wald statistic). Additive interaction was determined using the relative excess risk due to interaction measure.\textsuperscript{23} $P$ values and 95% CIs (bias corrected and accelerated) were determined using 1000 bootstrap samples.\textsuperscript{24} All $P$ values were 2-sided with a significance level of .05. All analyses were performed using Stata statistical software (version 13.0; StataCorp LP, College Station, Tex).

RESULTS
Characteristics of the participants are described in Table 1. Cases were more likely to have a history of hypertension, be obese, and have low levels of physical activity compared with healthy controls. Dietary intake of red, white, and fresh meat and intakes of MeIQx, PhIP, and DiMeIQx were significantly higher in the cases compared
with controls \((P<.03\) for each). Case subjects had overall higher daily total energy intake and lower overall total fruit and vegetable intake.

Age-adjusted and sex-adjusted and multivariable-adjusted models for risk of RCC by meat-related exposures are described in Table 2. Higher intakes of all red meat \((P\text{ for trend }<.001)\), all white meat \((P\text{ for trend }=.01)\), and all fresh meat \((P\text{ for trend }<.001)\) were associated with an increased RCC risk. High dietary intake of MelIQx and PhIP was found to be associated with a significantly increased risk of RCC (Table 2). High intake of MeIQx was associated with an increased risk of RCC (age-adjusted and sex-adjusted OR, 2.61 [95% CI, 1.99-3.44] and multivariable-adjusted OR, 1.95 [95% CI, 1.43-2.66] \([P\text{ for trend }<.001\) for both]). Increased intake of PhIP was significantly associated with RCC risk across all tertiles for both models (highest vs lowest tertile: OR, 1.88 [95% CI, 1.42-2.49] in the age-adjusted and sex-adjusted model \([P\text{ for trend }<.001\) and highest vs lowest tertile: OR, 1.54 [95% CI, 1.14-2.07] in the multivariable-adjusted model \([P\text{ for trend }=.02\)])). Results using a composite HCA value (sum of MeIQx, PhIP, and DiMeIQx) were consistent with these significant findings (data not shown).

The associations between dietary intake of meat-cooking mutagens and RCC risk stratified by smoking status are presented in Table 3. Results suggested that a trend between PhIP intake and risk of RCC may be more profound in ever-smokers \((P=.02\) vs \(P=.18\) in never-smokers). The association between MeIQx and risk of RCC was largely consistent between the 2 groups. Results did not yield significant interactions between intake of meat-cooking mutagens and smoking status. The remaining RCC risk factors did not appear to modify these associations (results not shown).

Age-adjusted and sex-adjusted associations between the selected GWAS SNPs and risk of RCC in this population have been shown previously. \(^{10}\) Only 1 meat-cooking mutagen yielded evidence of a significant interaction with

### Table 1. Characteristics for Cases and Controls

|                     | Cases (N=659) | Controls (N=699) | Chi-square | P       |
|---------------------|--------------|------------------|------------|---------|
| Age, ya             | 59.27 (10.36) | 60.70 (10.50)    | .01        |
| Sex                 |              |                  |            |         |
| Female              | 220 (33.38)  | 237 (33.91)      | .03        |
| Male                | 439 (66.62)  | 462 (66.09)      |            |
| Smoking status      |              |                  |            |         |
| Never-smoker        | 341 (51.75)  | 353 (50.50)      | .65        |
| Ever-smoker         | 318 (48.25)  | 346 (49.50)      |            |
| High blood pressure/hypertension | |                  |            |         |
| Yes                 | 388 (58.88)  | 299 (42.78)      | <.001      |
| No                  | 271 (41.12)  | 400 (57.22)      |            |
| BMI, kg/m\(^2\)     |              |                  |            |         |
| Normal (<25 kg/m\(^2\)) | 171 (25.95)  | 224 (32.05)      | .002       |
| Overweight (25-29 kg/m\(^2\)) | 240 (36.42)  | 276 (39.48)      |            |
| Obese (≥30 kg/m\(^2\)) | 248 (37.63)  | 199 (28.47)      |            |
| Physical activity\(^b\) |        |                  |            |         |
| Intensive (>48 METs/wk) | 94 (15.11)   | 221 (32.50)      | <.001      |
| Medium (30-48 METs/wk) | 196 (31.51)  | 253 (37.21)      |            |
| Low (<30 METs/wk)   | 332 (53.38)  | 206 (30.29)      |            |
| Dietary intake\(^c\) |              |                  |            |         |
| Total energy intake, kcal | 2350.96 (913.19) | 1994.22 (806.73) | .001       |
| Total fruit and vegetable intake, g/d | 246.57 (140.45) | 292.11 (159.00) | .001       |
| Red meat, g/d       | 55.25 (31.47) | 39.03 (22.59)    | .001       |
| White meat, g/d     | 23.03 (16.84) | 21.23 (17.15)    | .01        |
| All fresh meat, g/d | 59.37 (32.03) | 43.53 (25.69)    | .001       |
| Fish, g/d           | 15.14 (13.66) | 13.68 (13.63)    | .46        |
| MeIQx, ng/d         | 16.95 (134.23) | 9.45 (40.68)    | <.001      |
| PhIP, ng/d          | 48.65 (179.54) | 43.94 (153.82)  | .001       |
| DiMeIQx, ng/d       | 1.22 (15.35)  | 0.73 (4.91)      | .03        |
| BaP, ng/d           | 12.54 (42.93) | 13.18 (80.61)    | .06        |

Abbreviations: BaP, benzo(a)pyrene; BMI, body mass index; DiMeIQx, 2-amino-3,4,8-trimethylimidazo(4,5-f) quinoxaline; MelIQx, amino-3,8-dimethylimidazo(4,5-f) quinoxaline; MET, metabolic equivalent value; PhIP, 2-amino-1-methyl-6-phenyl-imidazo(4,5-b)pyridine.

\(^a\) Shown as the mean (standard deviation), with the \(P\) value derived using the Student \(t\) test.

\(^b\) Individuals with missing physical activity data were not included (N=56).

\(^c\) Daily energy-adjusted (g/1000 kcal) values for dietary intake. Intake values are shown as the mean (standard deviation); \(P\) values were derived from nonparametric Kruskal-Wallis test.
GWAS susceptibility loci for RCC (Table 4). Significant interactions were observed between PhIP and rs718314 (highest vs lowest tertile: OR, 1.14 [95% CI, 0.73-1.76] among individuals with no minor alleles and OR, 2.19 [95% CI, 1.37-3.49] for individuals with at least 1 copy of the minor allele; multiplicative $P$ for interaction = .03 and additive $P$ for interaction = .002) and rs7579899 (highest vs lowest tertile: OR, 1.25 [95% CI, 0.75-2.08] among individuals with no minor alleles and OR, 1.89 [95% CI, 1.25-2.85] among individuals with at least 1 copy of the minor allele; additive $P$ for interaction = .06).

No interactions were observed between the remaining SNPs and meat-cooking mutagens (see Supporting Information Tables (1 and 4)).

### DISCUSSION

In the current case-control study, we observed a nearly 2-fold increase in RCC risk associated with dietary MeIQx intake and a 54% increased risk associated with PhIP intake, suggesting that the intake of meat cooked at high temperatures may impact the risk of RCC through mechanisms related to mutagenic cooking compounds. To the best of our knowledge, the current study is the first to investigate potential interactions between known RCC susceptibility variants and dietary intake of meat-cooking mutagens. We found a significant interaction between PhIP and rs718314 located downstream of the $ITPR2$ gene, and evidence of a marginal synergistic interaction between PhIP and rs7579899 located near $EPAS1$.
Previous studies of RCC risk have suggested positive associations with red meat and poultry intake, whereas others have found null associations with RCC risk.\textsuperscript{1,6,25-27} A large prospective investigation of meat intake, related mutagens, and risk of RCC also found a significant association between red meat and RCC risk (fifth vs first quintile of consumption: hazard ratio, 2.02 [95% CI, 1.11-3.08]).\textsuperscript{1} In addition, this study found significant associations between meat mutagen intake and RCC risk, specifically for BaP and PhIP.\textsuperscript{1} The results of the current study provide additional evidence of the role of red meat, white meat, and PhIP in RCC etiology and to our knowledge is the first study of dietary intake of mutagenic compounds and RCC risk to suggest an association with MeIQx, which is one of the most abundant HCAs commonly created in the grilling, barbecuing, and pan-frying of meats at high temperatures.\textsuperscript{6,28} Further adjustment for total fresh meat intake (data not shown) yielded slightly attenuated, but consistent, associations between meat-cooking mutagens and risk of RCC. This suggests an independent effect of meat-cooking mutagens on RCC risk. However, other potentially tumorigenic components of meat including heme iron and N-nitroso compounds exposure, which were not measured in the current study, also may play a role in RCC etiology.\textsuperscript{6,29} The results of the current study yielded a similar increase in RCC risk associated with the intake of white meat, which is generally low in heme iron, thereby suggesting that some of these other mechanisms may not play a large role in increasing the risk of RCC.

We have previously shown that the ITPR2 gene interacts significantly with an American/Western dietary pattern to confer an increased risk of RCC.\textsuperscript{10} The American/Western dietary pattern consists largely of red and processed meats,\textsuperscript{10,30} and the results of the current study suggest that the association between this dietary pattern and cancer may be in part explained by exposure to meat-cooking mutagens. The ITPR2 gene encodes inositol-1,4,5-triphosphate receptor type 2, which is involved in nutrient and lipid metabolism and is suspected to play an important role in carcinogenesis via cell migration, proliferation, and apoptosis.\textsuperscript{31,32}

We also found evidence of a marginal synergistic interaction between PhIP and the variant located near the EPAS1 gene on chromosome 2. This gene has been implicated in RCC susceptibility and encodes the hypoxia-inducible factor 2α (HIF-2α), a key gene in the Von Hippel-Lindau-HIF pathway.\textsuperscript{21} A previous study of colorectal neoplasia suggests that genetic variation in the

### Table 3

| Dietary Intake of HCA From Meat, ng/d | Never-Smoker (N=694) | Ever-Smoker (N=664) | P for Interaction |
|-------------------------------------|----------------------|---------------------|------------------|
|                                     | Cases/Controls OR (95% CI) | Cases/Controls OR (95% CI) |               |
| MeIQx (overall)                     | T1 60/118 Referent T2 101/114 1.75 (1.09-2.81) T3 180/121 2.51 (1.54-4.11) | T1 69/116 Referent T2 75/114 1.06 (0.66-1.70) T3 174/116 1.94 (1.22-3.08) | .001 .003 .54 |
|                                    | P for trend <.001 | .003 | .54 |
| PhIP (overall)                      | T1 66/105 Referent T2 133/118 1.62 (1.01-2.57) T3 142/130 1.45 (0.90-2.36) | T1 73/134 Referent T2 114/105 1.59 (1.02-2.50) T3 131/107 1.77 (1.12-2.79) | .18 .02 .43 |
|                                    | P for trend | .18 | .02 | .43 |
| DiMeIQx (overall)                   | T1 111/107 Referent T2 91/117 0.78 (0.51-1.20) T3 139/129 0.93 (0.62-1.40) | T1 110/137 Referent T2 80/104 0.83 (0.54-1.26) T3 128/105 1.15 (0.77-1.72) | .77 .5 .44 |
|                                    | P for trend | .77 | .5 | .44 |
| Dietary intake of PAH from meat, ng/d | BaP (overall) | | |
|                                    | T1 95/107 Referent T2 137/116 0.65 (0.39-1.04) T3 108/127 1.16 (0.76-1.76) | T1 99/125 Referent T2 90/106 0.99 (0.58-1.37) T3 128/113 1.19 (0.78-1.80) | .08 .4 .09 |
|                                    | P for trend | .08 | .4 | .09 |

Abbreviations: 95% CI, 95% confidence interval; BaP, benzo[a]pyrene; DiMeIQx, 2-amino-3,4,8-trimethylimidazo(4,5-f) quinoxaline; HCA, heterocyclic amines; MeIQx, amino-3,8-dimethylimidazo(4,5-f) quinoxaline; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; RCC, renal cell carcinoma.

a The multivariable logistic regression model was adjusted for age, sex, body mass index, history of hypertension, smoking status, total energy intake, and total fruit and vegetable intake.

b Missing physical activity was included as “unknown.”

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Bioactivation pathway PAHs and HCAs, in conjunction with interacting molecules such as HIF-2α, form heterodimeric complexes that mediate cellular responses to environmental toxins such as PAHs and HCAs. Further research is necessary to investigate the role of the EPAS1 gene in modifying the impact of PAH/HCA exposure on the risk of RCC.

To the best of our knowledge, the current study is the first to evaluate the impact of RCC susceptibility variants, identified via GWAS, on the association between intake of mutagenic compounds and RCC risk. The use of histologically confirmed RCC cases; a validated FFQ with a meat-cooking module; a wide range of dietary intake values; and the inclusion of several important potential confounders for this association, including history of hypertension, smoking status, total energy intake, and total fruit and vegetable intake, allowed us to investigate possible mechanisms underlying the association between meat intake and RCC etiology.

Several limitations of the current study also need to be addressed. Residual confounding by unmeasured or unknown risk factors is possible, despite the inclusion of several important potential confounders for this association, including history of hypertension, BMI, smoking status, and physical activity, allowed us to investigate possible mechanisms underlying the association between meat intake and RCC etiology.

Several limitations of the current study also need to be addressed. Residual confounding by unmeasured or unknown risk factors is possible, despite the inclusion of important a priori confounders in the current analysis. Due to the highly correlated nature of the individual components and nutrients in whole foods, isolating the impacts of the mutagenic compounds remains difficult. The current study has a retrospective case-control design and is potentially limited by recall bias and nondifferential misclassification. In addition, analyses stratified by genotype may be limited by sample size. The current study does not address the issue of multiple testing, and therefore some of the more modestly significant findings may be attributable to chance. Finally, because we limited the analysis to non-Hispanic white individuals, these results may not be generalizable to other population subgroups.

The findings of the current study provide further evidence of the role of meat intake and mutagenic cooking compounds, specifically PhIP and MeIQx, in the etiology of RCC. The results of the current study also suggest that interindividual variation in RCC risk may be due to both genetic susceptibility and modifiable dietary risk factors such as mutagenic compounds caused by various methods of cooking meat. Because of the limited epidemiologic evidence linking carcinogenic compounds such as MeIQx to RCC risk, these findings will need to be replicated in future studies of diet and cancer using similar assessments of meat intake and cooking methodologies. Future studies with greater power are needed to simultaneously examine combinations of relevant genetic polymorphisms, intake of meat-cooking mutagens, and risk of RCC.

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### TABLE 4. Risk Estimates for the Association Between Meat-Related Mutagen Exposure and RCC Risk Stratified by Genotypea,b

| Dietary Intake of HCA From Meat, ng/d | Cases/Controls OR (95% CI) | Cases/Controls OR (95% CI) | Multiplicative P for Interaction | Additive P for Interaction |
|--------------------------------------|---------------------------|---------------------------|-------------------------------|---------------------------|
|                                      | AA (N = 736)              | AG/GG (N = 622)           |                               |                           |
| PhIP                                 |                           |                           |                               |                           |
|                                      |                           | rs718314                  |                               |                           |
|                                      |                           | GG (N = 499)              |                               |                           |
|                                      |                           | GA/AA (N = 859)           |                               |                           |
|                                      | T1 72/134  Referent 67/105 Referent 1.37 (0.89-2.11) 1.96 (1.25-3.08) | .03 .002                     |                           |
|                                      | T2 120/132  1.14 (0.73-1.76) | 145/87  2.19 (1.37-3.49) | .65                           |                           |
|                                      | T3 128/150  1.45 (0.89-2.31) | 147/87  2.23 (1.39-3.52) |                               |                           |
|                                        | P for trend                           | .85                           |                               |                           |
|                                      | rs7579899                  | GA/AA (N = 859)           |                               |                           |
|                                      | GG (N = 499)              |                             |                               |                           |
|                                      | T1 84/79  1.66 (1.00-2.76) | 163/144  1.60 (1.08-2.78) | .15 .06                       |                           |
|                                      | T2 83/101  1.25 (0.75-2.08) | 190/136  1.89 (1.25-2.85) |                               |                           |
|                                        | P for trend                           | .5                           |                               | .003                      |

Abbreviations: 95% CI, 95% confidence interval; HCA, heterocyclic amines; OR, odds ratio; PhIP, 2-amino-1-methyl-6-phenyl-imidazo(4,5-b)pyridine; RCC, renal cell carcinoma.

a The multivariable logistic regression model was adjusted for age, sex, body mass index, history of hypertension, smoking status, total energy intake, and total fruit and vegetable intake.

b Missing physical activity was included as "unknown."

Additional supporting information may be found in the online version of this article.

Supplementary Information

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CONFLICT OF INTEREST DISCLOSURES
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