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Mitochondrial DNA copy number in peripheral blood leukocytes and the risk of clear cell renal cell carcinoma

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Abstract

Variation of mitochondrial DNA copy number (mtDNAcn) in peripheral blood leukocytes has been associated with the risk of various cancers, including renal cell carcinoma (RCC). We assessed the association between mtDNAcn and clear cell RCC (ccRCC) risk in 608 cases and 629 controls frequency-matched on age and gender. Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) adjusting for age, gender, body mass index, smoking status, history of hypertension, total energy intake and physical activity. Our results suggest an association between low mtDNAcn and ccRCC risk (OR = 1.28, 95% CI: 0.97–1.68, $P = 0.09$). Lower mtDNAcn was associated with increased ccRCC risk in younger individuals (age <60, OR = 1.68, 95% CI: 1.13–2.49, $P = 0.01$), women (OR = 1.66, 95% CI: 1.03–2.73, $P = 0.04$), individuals without history of hypertension (OR = 1.62, 95% CI: 1.09–2.41, $P = 0.02$) and individuals with low physical activity levels (OR = 1.55, 95% CI: 1.02–2.37, $P = 0.05$). We observed significant and marginally significant interactions between both age and history of hypertension and mtDNAcn in elevating ccRCC risk (P for interaction = 0.04 and 0.07, respectively). Additionally, low mtDNAcn was associated with ccRCC risk in younger individuals with low levels of physical activity [ORs and 95% CI for medium and low physical activity levels, respectively, 2.31 (1.18–4.52) and 2.09 (1.17–3.75), P interaction = 0.04]. To our knowledge, this is the first report to investigate the role of mtDNAcn in the ccRCC subtype and the first to suggest that this association may be modified by risk factors including age, gender, history of hypertension and physical activity.

Introduction

Mitochondria are the primary organelle for cellular energy supply and metabolism. They play an important role in cellular activities such as calcium regulation and apoptosis and are a primary source and target of intracellular reactive oxygen species (1,2). Each mitochondrion has 2–10 copies of its own genome, which consists of a 1.6kb double-stranded DNA molecule (3,4). The mutation rate of mitochondrial DNA (mtDNA) is ~10-fold that of nuclear DNA due to its limited DNA repair mechanisms, chromatin structure and high exposure to reactive oxygen species (2,5,6).

Several studies have suggested that abnormal cellular mtDNA copy number (mtDNAcn) may be a marker for mitochondrial dysfunction which is a suspected contributor to

carcinogenesis (7). Studies of various cancers, including renal cell carcinoma (RCC), have measured mtDNAcn in peripheral blood leukocytes (PBLs) as a suspected marker of DNA damage and mitochondrial dysfunction (1,3,8–13). Both positive and inverse associations have been observed between mtDNAcn and risk of RCC (1,8,13). These inconsistencies could be due to factors such as study design and heterogeneity in study samples or histologic subtypes.

Kidney cancers are estimated to account for 3% of all newly diagnosed adult cancers in the USA in 2013 (14). Clear cell RCC (ccRCC) is the most prevalent histologic subtype of RCC and constitutes 75–85% of all RCCs (15). Established risk factors for ccRCC include smoking, obesity, hypertension, occupational

Abbreviations

BMI	body mass index
ccRCC	clear cell renal cell carcinoma
CI	confidence interval
MET	metabolic equivalent
mtDNAcn	mitochondrial DNA copy number
OR	odds ratio
PBL	peripheral blood leukocyte.

exposure to carcinogens, family history and genetic susceptibilities (16,17). Other suspected risk factors which may contribute to RCC include physical inactivity and other energy balance-related factors such as high caloric intake (16,17). Here, we aim to investigate the role of mtDNAcn on ccRCC risk, and the potential modification of this association via known or suspected risk factors in a case-control study in non-Hispanic whites.

Materials and methods

Study population and recruitment

ccRCC patients were drawn from an ongoing case-control study of RCC initiated in 2002 (18). The study was approved by the MD Anderson Cancer Center Institutional Review Board. The procedures for subject recruitment and eligibility criteria have been described previously (18). All case subjects were newly diagnosed and with histologically confirmed ccRCC recruited within 1 year of diagnosis. Healthy control subjects without a history of cancer, except non-melanoma skin cancer, were identified and recruited via random digit dialing during the same time period as the cases (19). Control subjects were frequency-matched to case subjects according to age (± 5 years), gender, ethnicity and county of residence. All participants provided written informed consent prior to participation in the study. Epidemiologic data were collected by MD Anderson Staff interviewers in an in-person interview. After the interview, a 40 ml blood sample was collected from each participant and delivered to the laboratory for molecular analysis.

Data collection

Epidemiologic data included information regarding history of hypertension (yes/no), physical activity, alcohol use and smoking status. We used a previously validated and modified version of the National Cancer Institute Health Habits and History Questionnaire (20) to assess usual dietary intake. The validity and reliability of this questionnaire has been previously documented (21). Total energy intake and grams per day of consumption for each food item were estimated using the USDA Food and Nutrient Database for Dietary Studies (22).

Alcohol use was also adjusted for total caloric 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 at least 12 months prior to diagnosis (for cases) or interview (for controls) was classified as a former smoker. Current smokers were those who were currently smoking or quit <12 months prior to diagnosis (for cases) or before the interview (for controls).

Weight at diagnosis (for cases) or recruitment (for control subjects) was recorded. Body mass index (BMI; kg/m²) 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; obese = ≥ 30 kg/m²). Participants also reported the average frequency they spent on five broad groups of physical activities in the year before the interview. A metabolic equivalent (MET) value was assigned to each activity group (23). The 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.

Exclusions and eligibility

Exclusion and inclusion criteria for the parent case-control study have been described in detail elsewhere (18). We additionally excluded

individuals with outlying total energy intake by excluding men ($N = 26$) and women ($N = 21$) 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. Individuals missing smoking status ($N = 11$) were also excluded from the analysis. Due to small number of minority participants, we limited the analysis to non-Hispanic whites only, leaving 1237 individuals (608 cases and 629 controls) for inclusion in the present study.

mtDNAcn ascertainment

The relative mtDNAcn was measured by a quantitative reverse transcription-PCR-based method as described previously (13,24). Briefly, two pairs of primers were used in the two steps of relative quantification of mtDNAcn. One primer pair (ND1-R and ND1-F) was used for the amplification of the ND1 gene in mtDNA. Another primer pair was used for the amplification of the single-copy nuclear gene human globulin (HGB).

In the first step, the ratio of the copy number of ND1 gene in mtDNA to the HGB copy number was determined for each sample from standard curves. This ratio is proportional to the mtDNAcn in each cell. The ratio for each sample was then normalized to a calibrator DNA in order to standardize between different runs. A genomic DNA sample from a healthy control was used as the calibrator DNA to compare results of different independent assays. The PCR mixture, a total volume of 14 μ l, contained 1 $\times$ SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 215 nM ND1-R (or HGB-1) primer, 215 nM ND1-F (or HGB-2) primer and 4 ng of genomic DNA. The thermal cycling conditions for the mtDNA (MT-ND1 gene) amplification were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min; for the HGB amplification, the cycling conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 56°C for 1 min. Each real-time PCR plate contained DNA samples from cases and controls randomly arranged and plated in duplicate on a 384-well plate with an Applied Biosystems 7900HT Sequence Detection System. The PCRs for mtDNA and HGB were performed on separate 384-well plates with the same samples in the same well positions to avoid possible position effect. For this study, the intra-class correlation is 0.956 [95% confidence interval (CI): 0.952–0.960] for ND1 assay and 0.971 (95% CI: 0.968–0.973) for HGB. The intra-assay coefficient of variation for all samples varies from 0.1 to 34% with an overall mean of 10.3%.

A standard curve of a serially diluted reference DNA, one negative control and one calibrator DNA were included in each run. For each standard curve, one reference DNA sample was serially diluted 1:2 to produce a seven-point standard curve between 0.3125 and 20 ng of DNA. The R^2 for each standard curve was 0.99 or greater. Standard deviations for the cycle of threshold value were accepted at 0.25. If the result was out of the acceptable range, the test was repeated. All the lab technicians were blinded to the case-control status of the DNA samples.

Statistical analyses

Comparisons for case-control characteristics were performed using the Pearson χ^2 test for categorical variables and Student's t -tests for continuous variables. Differences in the relative mtDNAcn across different groups of selected characteristics among control participants were examined using analysis of covariance. The Wilcoxon rank-sum test was used to evaluate the difference in mtDNAcn as a continuous variable by case-control status by each patient characteristic. We estimated the association between mtDNAcn and ccRCC risk using unconditional logistic regression models. Gender and age-adjusted, as well as fully adjusted odds ratios (ORs) and 95% CIs were reported for median mtDNAcn. Potential confounders, including age (<60, 60+), gender, smoking status (ever/never), history of hypertension (yes/no), total energy intake (tertiles based on the distribution by gender), physical activity (low: <30 METs/week, medium: 30–48 METs/week and intense: 48+ METs/week) and BMI (categories) were included in the model. Smoking status was also evaluated using never/former/current and pack-years (never, <20 pack-years and ≥ 20 pack-years); however, results were qualitatively similar and are not shown here. Alcohol use did not contribute meaningfully to the model and was therefore not included in the analysis. The median value of mtDNAcn in controls was used as the cut-off point for dichotomization. mtDNAcn was also categorized using tertiles and splines; however, these results were

qualitatively similar to the findings of the analyses using the median cut-point and are therefore not presented here.

We assessed the impact of known or suspected ccRCC risk factors, including age, gender, BMI, history of hypertension, total energy intake, physical activity and smoking status on modifying the association between mtDNAcn and ccRCC risk. We tested for multiplicative interaction by including the cross-product term of the dichotomous mtDNAcn variable and the dichotomous or ordinal risk factor variable in the logistic regression model. Significance of the interactions was determined using the Wald statistic for the cross-product term. Analyses were repeated for population subgroups based on the results stratified by possible confounders.

All statistical tests were two-sided with a significance cut-off at 0.05. All analyses were conducted using Stata 10.1 statistical software package (StataCorp, College Station, TX).

Results

Participant characteristics are described in Table 1. The overall population was largely male (~66% in cases and controls) with an average age of 59. Compared with the controls, RCC cases were more likely to have a history of hypertension (60 versus 40%), be obese (41 versus 33%), had lower levels of physical activity (57 versus 28% low physical activity) and higher total caloric intake (60 versus 38% high energy intake).

Overall, we observed a marginally significant difference in mean mtDNAcn between cases and controls (mean $\pm$ SD: 1.22 ± 0.37 versus 1.26 ± 0.37 , respectively $P = 0.06$) (Table 2). After stratification for various known and suspected ccRCC risk factors, significant case-control differences in mean mtDNAcn were observed only in individuals under the age of 60 (mean $\pm$ SD: 1.22 ± 0.36 versus 1.30 ± 0.38 in cases and controls, respectively, $P = 0.004$), with marginal differences in never smokers ($P = 0.08$) and individuals without a history of hypertension ($P = 0.08$). We also evaluated the differences in mean mtDNAcn by various patient characteristics in the healthy control subjects only (Table 2). Our results suggest that median mtDNAcn differs by individual characteristics including age ($P = 0.002$), gender ($P = 0.04$), smoking status ($P = 0.02$) and history of hypertension ($P = 0.03$).

Results from the gender- and age-adjusted unconditional logistic regression analyses suggest that individuals with low mtDNAcn exhibit significantly increased risk of ccRCC (OR = 1.26, 95% CI: 1.01–1.58, $P = 0.04$) (Table 3). In multivariate model adjusted for gender, age, smoking status, BMI, history of hypertension, physical activity and total energy intake, low mtDNAcn was marginally associated with increased risk of ccRCC (OR = 1.28, 95% CI: 0.97–1.68, $P = 0.09$).

In the stratified analyses, lower mtDNAcn was associated with increased ccRCC risk in women (OR = 1.66, 95% CI: 1.03–2.73, $P = 0.04$), younger individuals (age <60, OR = 1.68, 95% CI: 1.13–2.49, $P = 0.01$), never smokers (OR = 1.45, 95% CI: 0.99–2.12, $P = 0.06$), individuals without history of hypertension (OR = 1.62, 95% CI: 1.09–2.41, $P = 0.02$) and individuals with low levels of physical activity (OR = 1.55, 95% CI: 1.02–2.37, $P = 0.05$). Results also suggest significant and marginally significant interactions between age and mtDNAcn and between history of hypertension and mtDNAcn in elevating ccRCC risk (P for interaction = 0.04 and 0.07, respectively) (Table 4).

Because of the evidence for interactions between mtDNAcn and younger age and no history of hypertension, we then evaluated the association between mtDNAcn and ccRCC risk in these subgroups separately. For individuals under the age of 60 (Table 5), associations by gender, smoking status and history of hypertension were consistent with findings from the overall

population. Additionally, low mtDNAcn was associated with increased ccRCC risk in those individuals with medium or low physical activity levels (OR = 2.31, 95% CI: 1.18–4.52 and OR = 2.09, 95% CI: 1.17–3.75, respectively). Results also suggest evidence for an interaction between physical activity and mtDNAcn in ccRCC risk (P interaction = 0.04). In the subset of individuals with no history of hypertension (Table 6), low mtDNAcn was also associated with increased ccRCC risk in women ($P = 0.003$), younger individuals ($P = 0.004$), never smokers ($P = 0.01$) and individuals with low physical activity levels ($P = 0.01$); however, no significant interactions were observed.

Discussion

The data from this case-control study suggest that several established risk factors for ccRCC, including advanced age, male gender, smoking and history of hypertension negatively impact mtDNAcn in PBLs in healthy individuals. Lower mtDNAcn in PBLs was associated with significantly increased risk of ccRCC in younger individuals, women, never smokers (marginal significance, $P = 0.06$), individuals with low levels of physical activity (marginal significance, $P = 0.05$) and those without a history of hypertension. We observed significant or marginally significant interactions between age and mtDNAcn and between history of hypertension and mtDNAcn in elevating the risk of ccRCC.

The number of epidemiologic studies investigating the role of mtDNAcn on cancer risk has been increasing in recent years. Several of these studies show significant associations between mtDNAcn and cancer risk; however, these relationships are often cancer specific (1,5,7,8). The association between mtDNAcn and cancer risk may be impacted by distinct tumor biology and carcinogenic pathways involved in cancer initiation and progression (25).

Low mtDNAcn has been associated previously with increased risk of RCC (1,13), esophageal adenocarcinoma (3), soft tissue sarcoma (26), Ewing's sarcoma (27) and hepatocellular carcinoma (28); whereas high mtDNAcn was identified to associate with increased risk of breast cancer (29), pancreatic cancer (11), non-Hodgkin lymphoma (9), lung cancer (10,30), prostate cancer (31) and colorectal cancer (32). The specific biologic mechanisms by which reduced mtDNAcn may be related to renal carcinogenesis are not fully understood. However, decreases in mtDNAcn could lead to deficiency in oxidative phosphorylation and an enhanced generation of adenosine triphosphate by glycolysis (3,4). Decreased adenosine triphosphate generation by oxidative phosphorylation with associated enhanced glycolysis is often associated with cancer (33).

The findings from the present study are consistent with two similar previously published retrospective studies (1,13). Both studies suggest that low mtDNAcn is associated with risk of RCC. However, neither of these previous case-control analyses identified significant interactions with known or suspected RCC risk factors in modifying the association between mtDNAcn and cancer risk. Furthermore, this is the first study to focus specifically on the clear cell subtype of RCC. Although our results are consistent with previous findings from case-control analyses (12), in a recently published prospective study, Hofmann et al. (8) determined a positive, instead of inverse, association between mtDNAcn and RCC risk. The heterogeneity of these associations between altered mtDNAcn and RCC risk may due to relatively small to moderate sample sizes, varying study populations, different methods of measuring mtDNAcn or differences in study design (retrospective versus prospective).

Table 1. Characteristics for cases and controls^a

	Case, N (%)	Control, N (%)	χ^2
	(N = 608)	(N = 629)	P value ^b
Age (mean: SD)	58.46 (10.71)	58.57 (10.17)	0.85
Gender			
Female	208 (34.21)	211 (33.55)	0.81
Male	400 (65.79)	418 (66.45)	
Smoking status			
Never smoker	311 (51.15)	342 (54.37)	0.13
Ever smoker	297 (48.85)	287 (45.63)	
High blood pressure/hypertension			
Yes	336 (59.57)	237 (39.63)	<0.001
No	228 (40.43)	361 (60.37)	
BMI (kg/m ²)			
Normal/overweight (<25 kg/m ²)	118 (19.77)	155 (25.04)	0.01
Overweight (25–29 kg/m ²)	234 (39.20)	257 (41.52)	
Obese (≥30 kg/m ²)	245 (41.04)	207 (33.44)	
Physical activity			
Intensive (48+ METs/week)	63 (13.40)	201 (34.54)	<0.001
Medium (30–48 METs/week)	138 (29.36)	217 (37.29)	
Low (<30 METs/week)	269 (57.23)	164 (28.18)	
Total energy intake (kcal)			
Low	96 (16.72)	192 (31.17)	<0.001
Medium	133 (23.17)	193 (31.33)	
High	345 (60.10)	231 (37.50)	

^aIndividuals with missing physical activity (N = 196), BMI (N = 24), total energy (n = 47) intake, history of hypertension (N = 75) and smoking status (N = 11) not included.

^bP value for continuous variables represents P value from Student's t-test.

Table 2. mtDNACn mean and standard deviation by host characteristics of cases and controls

	Case (N = 608)		Control (n = 629)			
	N	Mean (SD)	N	Mean (SD)	P value ^a	P value in controls ^b
Overall	608	1.22 (0.37)	629	1.26 (0.37)	0.06	
Gender						
Female	208	1.28 (0.40)	211	1.30 (0.34)	0.170	0.04
Male	400	1.20 (0.35)	418	1.23 (0.38)	0.20	
Age						
<60	317	1.22 (0.36)	335	1.30 (0.38)	0.002	0.002
60+	291	1.24 (0.38)	294	1.21 (0.34)	0.34	
Smoking status						
Never	311	1.25 (0.37)	342	1.29 (0.39)	0.08	0.02
Ever	297	1.21 (0.38)	287	1.22 (0.33)	0.46	
BMI						
Normal (<25 kg/m ²)	118	1.22 (0.37)	155	1.23 (0.35)	0.56	0.63
Overweight (25–30 kg/m ²)	234	1.24 (0.38)	257	1.28 (0.40)	0.14	
Obese (≥30 kg/m ²)	245	1.23 (0.36)	207	1.26 (0.32)	0.22	
High blood pressure/hypertension						
Yes	336	1.23 (0.38)	237	1.23 (0.33)	0.59	0.03
No	228	1.24 (0.36)	361	1.29 (0.39)	0.08	
Physical activity						
High	63	1.30 (0.36)	201	1.28 (0.38)	0.76	0.27
Medium	138	1.23 (0.40)	217	1.25 (0.36)	0.27	
Low	269	1.22 (0.36)	164	1.24 (0.34)	0.23	
Total energy intake (kcal)						
Low	96	1.20 (0.37)	192	1.26 (0.34)	0.09	0.75
Medium	133	1.21 (0.36)	193	1.27 (0.41)	0.11	
High	345	1.24 (0.37)	231	1.25 (0.35)	0.75	

Individuals with missing physical activity (N = 196), BMI (N = 24), total energy (n = 47) intake, history of hypertension (N = 75) not included.

^aWilcoxon rank-sum test.

^bP value in controls estimated using analysis of covariance.

Table 3. Risk of RCC as estimated by mtDNAcn^a

mtDNAcn	Cases, N (%)	Controls, N (%)	Age and gender adjusted		Multivariate adjusted	
			OR (95% CI)	P	OR (95% CI)	P
≥1.22	192 (44.14)	296 (52.02)	1 (REF)		1 (REF)	
<1.22	243 (55.86)	271 (47.98)	1.26 (1.01–1.58)	0.04	1.28 (0.97–1.68)	0.09

Multivariate model adjusted for gender, age, smoking status, BMI, history of hypertension, physical activity and total energy intake.

Median mtDNAcn derived using the distribution in the control population.

^aIndividuals with missing physical activity (N = 196), BMI (N = 24), total energy (n = 47) intake, history of hypertension (N = 75) not included (multivariate N = 1004).

Table 4. Risk of RCC as estimated by mtDNAcn stratified by host characteristics^a

Host characteristics	Case/control	Adjusted OR (95% CI)		P	P interaction
		High	Low		
Gender					
Male	290/373	1 (REF)	1.11 (0.79–1.56)	0.55	
Female	145/196	1 (REF)	1.66 (1.03–2.73)	0.04	0.19
Age					
<60	219/303	1 (REF)	1.68 (1.13–2.49)	0.01	
60+	216/266	1 (REF)	0.95 (0.64–1.43)	0.84	0.04
BMI					
Normal/overweight	260/385	1 (REF)	1.28 (0.90–1.82)	0.18	
Obese	175/184	1 (REF)	1.20 (0.76–1.89)	0.44	0.92
Smoking status					
Never	235/212	1 (REF)	1.45 (0.99–2.12)	0.06	
Ever	200/257	1 (REF)	1.10 (0.73–1.66)	0.63	0.36
History of hypertension					
Yes	256/225	1 (REF)	1.00 (0.74–1.36)	0.98	
No	179/344	1 (REF)	1.62 (1.09–2.41)	0.02	0.07
Physical activity					
High	58/199	1 (REF)	0.87 (0.46–1.67)	0.69	
Medium	128/211	1 (REF)	1.26 (0.79–2.00)	0.39	
Low	249/159	1 (REF)	1.55 (1.02–2.37)	0.05	0.13
Total energy intake					
Low	89/190	1 (REF)	1.32 (0.76–2.32)	0.33	
Medium	122/189	1 (REF)	1.35 (0.82–2.45)	0.24	
High	224/190	1 (REF)	1.22 (0.80–1.86)	0.20	0.77

Multivariate adjusted for gender, age, smoking status, BMI, hypertension, physical activity and total energy intake. Median mtDNAcn derived using the distribution in the control population.

^aIndividuals with missing physical activity (N = 196), BMI (N = 24), total energy (n = 47) intake, history of hypertension (N = 75) not included (multivariate N = 1004).

Our data focused on the clear cell subtype of RCC in a Caucasian population only, which intended to provide a more thorough investigation of the impact of mtDNAcn on a specific histologic subtype of the disease, excluding potential variation due to ethnicity and histological subtypes. We found that several risk factors for ccRCC, including advanced age, male gender, smoking and history of hypertension negatively impacted mtDNAcn in PBLs in healthy individuals. Age-related decline in mtDNAcn was previously found in skeletal muscle and also in human pancreatic islets, both from healthy individuals (34,35). The present study is however, the first to suggest an age effect on mtDNAcn in PBLs in healthy population.

Results from the overall analysis also suggest that the impact of mtDNAcn in increasing ccRCC risk may be limited to women, younger individuals and those with no history of hypertension. Although the exact mechanisms for these differential associations is not clear, our results suggest that potentially, the role of mtDNAcn in contributing to cancer risk is more profound for individuals without traditional ccRCC risk factors. Previous studies have suggested that reduced mtDNAcn may potentially

lead to differential cancer etiology and clinical manifestations between men and women (25) or that possibly, the impact of alterations in mtDNAcn on cancer risk in younger individuals may be indicative of the role of mtDNAcn in early-onset or familial cancers (6).

To our knowledge, this is the first study to indicate differential association of mtDNAcn with ccRCC risk by levels of physical activity. Mitochondria are responsible for synthesizing the molecules essential for the body metabolism and metabolic homeostasis (4,25). Metabolic dysregulation has been determined as a hallmark of cancer (2,7). Endurance activity has been shown to induce metabolic adaptation and various studies have suggested that endurance exercise is associated with the maintenance of homeostatic energy metabolism via mitochondrial biogenesis (36,37). Although mitochondrial decay has been shown to occur with age, it has been suggested that this can be counteracted via increased physical activity (37). Although the exact biological mechanism is unknown, it is possible that low levels of physical activity in younger individuals lead to reduced mtDNAcn and adenosine triphosphate production, resulting in

Table 5. Risk of RCC as estimated by mtDNAcn stratified by host characteristics, age <60 only (N = 522^a)

Host characteristics	Case/control	Adjusted OR (95% CI)		P	P interaction
		High	Low		
Gender					
Male	155/214	1 (REF)	1.38 (0.87–2.22)	0.17	0.12
Female	64/89	1 (REF)	2.84 (1.33–6.06)	0.01	
BMI					
Normal/overweight	128/208	1 (REF)	1.57 (0.95–2.52)	0.08	0.8
Obese	91/95	1 (REF)	1.68 (0.89–3.21)	0.11	
Smoking status					
Never	132/189	1 (REF)	1.85 (1.12–3.05)	0.02	0.53
Ever	87/114	1 (REF)	1.48 (0.78–2.82)	0.23	
History of hypertension					
Yes	104/78	1 (REF)	1.09 (0.56–2.10)	0.81	0.11
No	115/225	1 (REF)	2.08 (1.26–3.44)	0.004	
Physical activity					
High	21/103	1 (REF)	0.42 (0.14–1.26)	0.12	0.04
Medium	64/107	1 (REF)	2.31 (1.18–4.52)	0.02	
Low	134/93	1 (REF)	2.09 (1.17–3.75)	0.01	
Total energy intake					
Low	32/84	1 (REF)	1.20 (0.49–2.96)	0.68	0.6
Medium	68/110	1 (REF)	1.81 (0.90–3.63)	0.09	
High	119/109	1 (REF)	1.78 (1.01–3.17)	0.05	

Multivariate adjusted for gender, age, smoking status, BMI, history of hypertension, physical activity and total energy intake.

Median mtDNAcn derived using the distribution in the control population.

^aIndividuals with missing physical activity, BMI, total energy intake, history of hypertension not included.

Table 6. Risk of RCC as estimated by mtDNAcn stratified by host characteristics, no history of hypertension only (N = 523^a)

Host characteristics	Case/control	Adjusted OR (95% CI)		P	P interaction
		High	Low		
Gender					
Male	119/226	1 (REF)	1.21 (0.75–1.96)	0.44	0.05
Female	60/118	1 (REF)	3.27 (1.51–7.17)	0.003	
Age					
<60	115/225	1 (REF)	2.08 (1.26–3.44)	0.004	0.07
60+	64/119	1 (REF)	1.01 (0.52–1.98)	0.97	
BMI					
Normal/overweight	134/274	1 (REF)	1.52 (0.96–2.40)	0.07	0.74
Obese	45/70	1 (REF)	1.80 (0.77–4.18)	0.17	
Smoking status					
Never	104/203	1 (REF)	2.04 (1.20–3.46)	0.01	0.22
Ever	75/141	1 (REF)	1.17 (0.63–2.17)	0.61	
Physical activity					
High	23/126	1 (REF)	0.80 (0.31–2.08)	0.65	0.16
Medium	55/123	1 (REF)	1.59 (0.82–3.09)	0.17	
Low	101/95	1 (REF)	2.21 (1.19–4.11)	0.012	
Total energy intake					
Low	38/105	1 (REF)	1.34 (0.60–3.00)	0.47	0.96
Medium	48/125	1 (REF)	2.31 (1.11–4.84)	0.13	
High	93/114	1 (REF)	1.57 (0.86–2.89)	0.15	

Multivariate adjusted for gender, age, smoking status, BMI, history of hypertension, physical activity and total energy intake. Median mtDNAcn derived using the distribution in the control population.

^aIndividuals with missing physical activity, BMI, total energy intake, history of hypertension not included.

an increase in cancer risk (37). Further research in this area is necessary to elucidate these associations.

The strength of the present study includes its large sample size, histologically confirmed cases and its focus on the clear cell subtype of RCC. Additionally, this is the first study to take into account suspected ccRCC factors, such as physical activity, in reference to the association between mtDNAcn and ccRCC

risk. The present study is also the first to suggest differential associations between mtDNAcn and ccRCC risk based on age and history of hypertension.

Despite these strengths, there are also several limitations to the present study. Firstly, mtDNAcn measurement in individual subjects was not repeated and it is possible that a single measurement may not reflect mtDNAcn over a lifetime (12).

However, previous research suggests that mtDNAcn is relatively stable over time. Therefore, this is unlikely to have impacted our results significantly. Although our sample size was large relative to other studies of this type, and adequate for overall analyses, power was more limited for the stratified and interaction analyses. Further analyses with larger sample sizes are required to validate these results. Finally, reverse causation is always a primary concern in studies that utilize a retrospective case-control design. The present study includes only newly diagnosed (within 1 year of diagnosis) cases for which samples were drawn at recruitment. However, we cannot rule out tumor-related effects on mtDNAcn. Therefore, prospective studies of mtDNAcn and ccRCC involving prediagnostic blood specimens are needed to confirm these results.

In conclusion, our results indicate that the association between mtDNAcn in PBLs and ccRCC risk may be modified by various host characteristics. The results of this study provide novel insight into the role of mtDNA in modulating ccRCC susceptibility and suggest mtDNAcn in PBLs as a promising biomarker in kidney cancer risk assessment and prevention.

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