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Constitutive mitochondrial DNA copy number in peripheral blood of melanoma families with and without *CDKN2A* mutations

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Abstract

Quantitative changes in mitochondrial DNA (mtDNA) have been associated with the risk of a number of human cancers; however, the relationship between constitutive mtDNA copy number in blood and the risk of familial cutaneous malignant melanoma (CMM) has not been reported. We measured mtDNA copy number using quantitative PCR in blood-derived DNA from 136 CMM cases and 302 controls in 53 melanoma-prone families (23 segregating *CDKN2A* germline mutations). MtDNA copy number did not vary by age, sex, pigmentation characteristics, or CMM status. However, germline *CDKN2A* mutation carriers had significantly higher mean mtDNA copy number compared to non-carriers, particularly among CMM cases (geometric mean mtDNA copy number of 144 and 111 for carrier versus non-carrier, respectively; $P=0.02$). When adjusting for age, sex, and familial correlation, having increasing mtDNA copy number was significantly associated with *CDKN2A* mutation status among CMM cases ($OR=1.47$, $P_{trend}=0.024$). In particular, individuals with specific *CDKN2A* mutations with the potential to inactivate or reduce the level of the p16-INK4 reactive oxygen species (ROS) protective function had significantly increased mtDNA copy number levels ($P=0.035$). Future research in prospective studies is required to validate these findings and to further investigate mtDNA copy number in both blood and melanoma tissues in relation to CMM risk and *CDKN2A* mutation status.

Keywords

Familial melanoma; *CDKN2A*; mtDNA copy number; peripheral blood

Introduction

Cutaneous malignant melanoma (CMM) is a potentially fatal form of skin cancer with a heterogeneous etiology [1]. Established risk factors include presence and number of benign

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Conflict of Interest

The authors state no conflict of interest.

and dysplastic nevi (DN), pigmentation phenotype, and sun exposure [2]. Approximately 10% of CMM cases occur in a familial setting [1, 2]. The cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene is one of the two major high-risk melanoma susceptibility genes identified to date. Germline mutations of the *CDKN2A* gene have been described in approximately 20-40% of familial melanoma kindreds [3]. Although germline *CDKN2A* mutations are associated with a high risk of CMM, the penetrance of this gene is incomplete and varies by age and geographic location [4]. Further, phenotypic manifestations such as age at diagnosis, DN, number of melanomas, and co-segregation of pancreatic cancer vary significantly among mutation carriers even within a single family [1, 3]. These findings suggest that other factors may modify the effect of *CDKN2A*.

Mitochondria play an essential role in numerous biological processes such as adenosine triphosphate (ATP) production via oxidative phosphorylation, iron and calcium homeostasis, and apoptosis signaling. Mitochondrial DNA (mtDNA) is an essential multicopy genome consisting of 16.6-kb circular double-stranded DNA molecules that lacks introns, but is compacted into protein-DNA clusters called nucleoids. Due to its lack of histone protection, limited repair capacity, and close proximity to the electron transport chain, which constantly generates superoxide radicals, mtDNA is susceptible to oxidative DNA damage and exhibits high mutation rates [5, 6]. MtDNA copy number (i.e. the total number of mtDNA genomes) per cell may increase to compensate for mtDNA damage/dysfunction and may be a marker of exposure to reactive oxygen species (ROS) and /or oxidative stress [7-9]. Alternatively, a decrease in mtDNA copy number levels could lead to decreased ATP generation by oxidative phosphorylation and concomitant enhanced glycolysis [10, 11], well-known characteristics of cancer cells.

In addition, mtDNA copy number may reflect gene–environment interactions between hereditary factors and exposures such as UV, which can affect the levels of oxidative stress [12, 13].

Recently, a number of epidemiologic studies have reported that mtDNA copy number alterations in peripheral blood are associated with the risk of several cancers [5, 12, 14-23]. MtDNA copy number in blood may be relevant to familial melanoma because impaired DNA repair [24] and UV-mediated oxidative stress play a significant role in melanoma etiology [25]. Also, increased mtDNA copy number has been observed in sun-exposed skin samples compared to less exposed samples, suggesting mtDNA copy number may be a potential biomarker of UV exposure [13]. Expression of p16-INK4 from *CDKN2A* is also induced by exogenous UV-irradiation and endogenous H₂O₂-oxidative stress in murine cells, and p16-INK4 can suppress ROS *in vitro* via a mechanism independent of its cell cycle function [25]. Loss of the p16-INK4 protective ROS function *in vitro* has also been associated with particular familial melanoma-associated point mutations in *CDKN2A* [26].

Therefore, studying mtDNA copy number in whole blood and its relationship with melanoma risk and *CDKN2A* status may improve our understanding of melanoma etiology in these families. To our knowledge, no study has investigated the relationship between mtDNA copy number and the risk of familial CMM nor the influence of *CDKN2A* germline mutations on constitutive mtDNA copy number in peripheral blood. In this study, we

determined the relative mtDNA copy number from whole blood from 53 melanoma-prone families (30 *CDKN2A*-mutation negative [*CDKN2A*⁻] and 23 *CDKN2A*-mutation positive [*CDKN2A*⁺]) and evaluated whether constitutive mtDNA copy number varied by CMM and germline *CDKN2A* mutation status.

Materials and Methods

Study population

The details of this family study have previously been described [27, 28]. In brief, U.S. families with at least two living first degree relatives with a history of invasive melanoma were ascertained through health care professionals or self-referrals. All participants in the study underwent a full-body skin examination to characterize phenotypes and completed risk factor questionnaires for sun-related exposures such as tanning ability. All diagnoses of melanoma were confirmed by histologic review of pathologic material and pathology reports. CMM cases and controls with and without *CDKN2A* mutations were selected from families based on the availability of blood DNA. All study participants were Caucasians, and the study population was comprised of 139 CMM cases (61 *CDKN2A*⁺ and 78 *CDKN2A*⁻) and 310 controls (45 *CDKN2A*⁺ and 264 *CDKN2A*⁻). The study was approved by the National Cancer Institute Clinical Center Institutional Review Board and conducted according to the Declaration of Helsinki. Informed consent was obtained from all participants.

Quantitation of relative mtDNA copy number

For this investigation we used DNA samples previously extracted from whole blood using a standardized protocol for total genomic DNA. All extracted DNA samples were quantitated using a NanoDrop (Thermo Scientific, Wilmington, DE, USA). We used a quantitative real-time PCR assay, as described previously in detail [29], to measure relative mtDNA copy number. All laboratory personnel performing the assays were blinded to the case-control status of all DNA samples. Briefly, a fluorescence-based quantitative PCR assay was used to determine the threshold cycle number of a mitochondrial gene (*ND1*) and a nuclear gene (β -globin gene, *HBB*). The ratio of *ND1* and *HBB* threshold cycle numbers, estimated using a linear regression model, is proportional to the mtDNA copy number in each cell. All samples were run in triplicate and for quality control purposes, 38 blinded duplicate samples were interspersed among the test samples. The overall coefficient of variation (CV) for mtDNA copy number for duplicate replicas was 7% and the intra-class correlation coefficient was 0.99. The median overall CV for all test samples was 3%. We were unable to assay samples from three cases and eight controls, therefore analyses were carried out on 302 controls and 136 cases.

Categorization of *CDKN2A* germline mutations

p16-INK4 can suppress ROSs *in vitro* via a mechanism independent of its cell cycle function [25]. Loss of this ROS protective function has also been associated with particular familial melanoma-associated point mutations in *CDKN2A in vitro* [26]. Thus specific *CDKN2A* mutations with the potential to inactivate or reduce the level of the p16-INK4 ROS protective function may result in increased constitutive mtDNA copy number levels in

the blood of *CDKN2A*⁺ mutation carriers compared to non-carriers. Jenkins et al. [26] reported that R87W (and/or mutation at amino acid R87) impairs both the cell cycle and ROS protective functions of p16-INK4 *in vitro*. In contrast, V126D alters only cell cycle function. In addition, independent data suggests that the cell cycle function of p16-INK4 is dependent on amino acids 84-103 within the third ankyrin motif of the protein [30], and melanoma-associated mutations that selectively impair oxidative regulation may be located outside of this region (23). To evaluate if potential ROS-related *CDKN2A* mutations might influence mtDNA copy number in blood, we compared the relative mean mtDNA copy number between *CDKN2A*-carrier controls (n=30) from 13 *CDKN2A*⁺ families that carried mutations located at amino acid R87 and outside the 84-103 amino acid sequence to all non-carrier controls in our study (n=262). Individuals with mutations (n=10) that affected p14ARF primary structure only, located within amino acids 84-103 (G101W), or were previously suggested to alter only cell cycle function and not ROS protection (V126D) were not included in this group. We also compared the relative mean mtDNA copy number between *CDKN2A*-carrier cases (n=36) from 14 *CDKN2A*⁺ families that carried mutations located at amino acid R87 and outside the 84-103 amino acid sequence to all non-carrier cases in our study (n=77) (Table 1).

Statistical analyses

Characteristics of cases and controls were compared using logistic regression models. The relative mean mtDNA copy number levels were natural log-transformed to more closely follow a normal distribution; results of analyses of mtDNA copy number as a continuous variable are reported as geometric means. In addition, we also used box-cox power transformed data ($\lambda=-0.75$) to further test our results in terms of significant findings. Linear regression was used to assess whether the distribution of relative mean mtDNA copy number differed significantly between spouse controls and unaffected family members and between cases and controls. We further examined mtDNA copy number as a continuous variable among controls using a multivariable linear regression to assess how age at blood draw, *CDKN2A* mutation status and other CMM risk factors influenced mtDNA copy number levels. Normality of residuals was assessed using the Cramer von Mises test statistic. To assess our main hypothesis, we estimated odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between mtDNA copy number and CMM status using unconditional logistic regression adjusting for age at blood draw (< 35, 35-45, 45-50 and >50 years), sex and *CDKN2A* mutation status. Subjects were categorized into quartiles based on the distribution of mtDNA copy number among controls. For all models, we accounted for familial correlations among family members in the variance computation and *P*-values using a generalized estimating equation (GEE) with the independence working correlation matrix [31]. Tests for trend were computed assuming linear trend across different quartiles. Associations were also evaluated separately for individuals with (+) and without (-) *CDKN2A* germline mutations. To assess how specific germline *CDKN2A* mutations might influence constitutive mtDNA copy number, we collated individuals with specific *CDKN2A* mutations into a single group (Table 1) and compared mtDNA copy number among carrier controls to all non-carrier controls, and among carrier cases to non-carrier cases from *CDKN2A*⁺ families using the GEE regression model adjusting for age at blood

draw, sex, and familial correlation in the variance. All tests were two-sided. Statistical analyses were carried out using SAS software version 9.1 (SAS Institute, Cary, NC).

Results

In total, we analyzed 136 CMM cases (59 *CDKN2A*⁺ and 77 *CDKN2A*⁻) and 302 controls or unaffected individuals (40 *CDKN2A*⁺ and 262 *CDKN2A*⁻) from 53 melanoma-prone families. As expected, *CDKN2A* carrier status, DN, number of moles, number of freckles, solar injury, skin type and *MC1R* genotype were all significantly associated with the risk of CMM in these families (Table 2).

Overall, the mean mtDNA copy number did not differ significantly between non-carrier spouse controls (n= 104) and related family controls that are *CDKN2A*⁻ (n=158) (P=0.72), and we therefore combined these two groups as non-carrier controls. After the adjustment for familial correlation, mtDNA copy number did not show significant associations with age at blood draw, sex, or any CMM risk factor in non-carrier controls (Supplementary Table 1).

In general, mtDNA copy number levels were lower among cases compared to controls across most categories including age at blood draw and sex, but the difference was not significant when comparing all CMM cases to all controls (P=0.094) (Supplementary Table 2). Although the highest quartile of mtDNA copy number levels (Q4, >164) showed a suggestive association with reduced risk of CMM in the multivariate model (OR=0.57, 95% CI=0.31-1.07, P=0.084), the association did not follow the trend ($P_{\text{trend}}=0.169$, Supplementary Table 3). In contrast, we observed that mean mtDNA copy number levels were significantly higher in all cases with *CDKN2A* germline mutations (n=59, mean copy number=144) compared to mutation-negative cases (n=77, mean copy number=111) (P=0.01, Table 3). Increasing mtDNA copy number levels was associated with a significant higher risk of being a *CDKN2A*⁺ carrier case (OR=1.48, $P_{\text{trend}}=0.024$, Table 4). A similar pattern was observed when we compared mean mtDNA copy number in CMM cases carrying potential ROS-related mutations (n=36) to non-carrier cases (n=77) (P=0.035) (Table 3). Similarly, CMM-unaffected individuals carrying potential ROS-related mutations (n=30) also had higher mtDNA copy number compared to non-carrier controls (n=262), although the difference was not statistically significant after adjustment for age at blood draw, sex and familial correlation (P= 0.16, Table 3).

Discussion

In this study, we evaluated whether mtDNA copy number in blood-derived DNA varied by CMM and germline *CDKN2A* mutation status in melanoma-prone families. Our results suggest that melanoma cases carrying *CDKN2A* mutations, including those with ROS-related mutations, had significantly higher mtDNA copy number levels compared to non-carrier cases. To our knowledge, this is the first study evaluating mtDNA copy number levels in blood in relation to CMM risk and germline *CDKN2A* mutations.

Overall, we did not see a significant difference in mtDNA copy number between cases and controls, although the highest quartile of mtDNA copy number levels (Q4, >164) showed a suggestive association with reduced risk of CMM in our melanoma families. Both increasing

and decreasing mtDNA copy number in blood have been previously associated with cancer risk [5, 12, 14-23]; this has been suggested to reflect the variation in sample size, study population, study design, or tumor-specificity. In our study, blood was collected after melanoma diagnosis for the vast majority of melanoma cases (n=110), which prohibited us from evaluating mtDNA copy number in pre-diagnostic samples. Nevertheless, we did observe higher mtDNA copy number levels in blood samples from CMM cases obtained before diagnosis (n=26; mean mtDNA copy number=153) compared to post-diagnosis (n=110; mean mtDNA copy number=118), suggesting that the observed reduced mtDNA copy number may be a melanoma-related effect rather than a marker of risk, and/or may be attributable to a general immune response to melanoma in our cases. As surgery remains the mainstay of melanoma therapy, this result is unlikely to reflect a response to treatment in cases [32].

Oxidative stress has been shown to increase mtDNA content *in vitro* [8]. Expression of p16-INK4 from *CDKN2A* is induced by exogenous UV-irradiation and endogenous oxidative stress, and p16-INK4 can suppress the generation of ROS *in vitro* [25]. Different murine skin cells including melanocytes have been shown to exhibit increased levels of intracellular ROS in the context of deficiency of p16-INK4, which can be restored upon re-expression. The regulation of oxidative stress by p16-INK4 was shown to occur independently of Rb or its cell cycle control function in skin cells [25]. Germline *CDKN2A* mutations are associated with a high risk of familial CMM and the loss of the p16-INK4 protective ROS function has been associated with specific *CDKN2A* familial melanoma-associated point mutations [26]. Thus, we hypothesized that individuals with specific germline *CDKN2A* mutations conferring a potential reduction or elimination of p16-INK4 ROS protective function would characterize higher constitutive mtDNA copy number levels in blood DNA compared to individuals with no germline *CDKN2A* mutations. Consistent with our hypothesis, we observed a significantly higher mtDNA copy number in *CDKN2A*⁺ carrier cases compared to *CDKN2A*-mutation negative cases. The association was similar when we analyzed pre-diagnostic and post-diagnostic cases separately. Similarly, *CDKN2A* mutation carrier controls also had a slight increase in mtDNA copy number compared to non-carrier controls. Although these findings are biologically plausible, the clinical relevance of increased mtDNA copy number in peripheral blood of *CDKN2A*⁺ melanoma cases remains unclear. It is possible that an already high constitutive mtDNA copy number level occurring in *CDKN2A*⁺ melanoma cases may be further increased in tumor tissues to compensate for more mtDNA damage by oxidative stress and mitochondrial dysfunction. In line with this hypothesis, increased mtDNA copy number in serum has been correlated with TNM stage of lung cancer [33].

Consistent with previous findings, we found no significant association between mtDNA copy number and age at blood draw [19, 21, 34] among unaffected individuals. Taking advantage of the detailed pigmentation phenotypes we have collected for our family members, we examined the influence of established CMM risk factors on mtDNA copy number among unaffected individuals. Melanin and UV irradiation have been shown to alter mtDNA copy number in human melanoma cell lines [35] and mtDNA copy number may be a potential biomarker of UV exposure, a major risk factor for CMM [13]. Increasing

mtDNA copy number has been observed in sun exposed skin samples [13] and in normal skin from CMM patients with high-risk pigmentation phenotypes [36]. However, in the present study, we found no significant association for mtDNA copy number in blood with any of the CMM risk factors examined including solar injury, tanning ability and/or pigmentation phenotypes (skin type, eye color, hair color, moles, etc.), which might suggest that the ability of these factors to alter mtDNA copy number may be more important for cells directly exposed to UV irradiation. A marginally non-significant association for mtDNA copy number in blood with solar injury ($P=0.09$) was observed in *CDKN2A*⁺ carrier controls. However, while this result is based on a small number of *CDKN2A*⁺ carrier controls ($n=40$) in our study, it suggests that the association of solar injury with mtDNA copy number in blood may also vary by *CDKN2A* mutations status and the potential of p16-INK4 to protect against UV-induced oxidative stress. Notably, melanoma in melanoma-prone families with *CDKN2A* mutations is associated with chronic exposure to UV radiation [4], which may further exacerbate an already altered constitutive mtDNA copy via generation of ROS in skin.

While not a primary aim of our study, recent evidence suggests that telomere length is associated with mitochondrial biogenesis and function, as well as increased ROS levels [37] and that mtDNA copy number positively correlates with telomere length in blood from elderly women [38]. Also, TERT reverse transcriptase localizes to the mitochondria (mt) and mtTERT improves mitochondrial function and stress resistance, independently of its telomeric function [39]. Taking advantage of previously published telomere length data from our melanoma-prone families [40], we assessed the correlation between telomere length and mtDNA copy number in blood from the same set of controls ($n=158$), however, no significant correlation was found. Similarly, we did not observe any significant association between common genetic variants in TERT ($n=12$ tagging SNPs) [40] and mtDNA copy number (control= 158 and cases= 77) (data not shown).

This study is the first to examine mtDNA copy number in blood with risk of melanoma in melanoma-prone families with and without *CDKN2A* mutations as well as the influence of specific germline *CDKN2A* mutations on mtDNA copy number. Our findings further highlight that until we have a thorough understanding of the genetic and non-genetic factors that may influence mtDNA copy number levels, we need to be cautious when viewing mtDNA copy number in blood as a biomarker of cancer risk.

Our families had well annotated sun exposure and pigmentation data, were strongly influenced by genetic factors, and were of Caucasian ethnicity, minimizing the influence of race and genetic background on mtDNA copy number. However, our study is limited by its small sample size. In addition, our families were ascertained primarily through self- or physician-referral, which might affect the generalizability of the results.

In conclusion, our data did not support a significant difference in mtDNA copy number in blood between CMM cases and controls. However, CMM cases (and to a lesser extent unaffected individuals) with germline *CDKN2A* mutations had significantly higher mtDNA copy number suggesting that specific *CDKN2A* mutations can alter constitutive mtDNA copy number, possibly as a result of increased susceptibility to oxidative stress. Future

research in prospective studies is required to validate our results and to investigate the functional significance of altered mtDNA copy number in both blood and melanoma tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Germline *CDKN2A* mutations in melanoma-prone families. CMM unaffected and affected individuals with germline *CDKN2A* mutations from mutation-positive melanoma families were collated into a single group (shaded gray) with potential to influence ROS protection and cell cycle function. Mutations were grouped based on location outside the cell-cycle dependent amino acid sequence 84-103 and previous evidence suggesting a dual impact on ROS protection and cell cycle functions in vitro (23, 28). Families with individuals with *CDKN2A* mutations which only affected p14ARF primary structure, located within the amino acid sequence 84-103 (G101W) or were previously suggested to alter only cell cycle function and not ROS protection (V126D) were not included in this group and/or analyzed separately due to insufficient numbers.

Family	<i>CDKN2A</i> Mutation	Base change	p16 AA change
A	23ins24	g.32-33ins9-32	p.M1-S8dup
A7	M53I	g.159G>C / g.202G>C	p.M53I
AP	IVS2-105A/G	g.458-105A>G	p.156-157del
AY	D153spl	g.384_457del	p.R128fs137X
B	L16R	g.47T>G	p.L16R
C	M53I	g.159G>C / g.202G>C	p.M53I
D	R58X	g.172C>T / g.215C>T	p.R58X
D1	R87W	g.259C>T/g.302C>T	p.R87W
E	N71S	g.212A>G / g.255A>G	p.N71S
F	R87P	g.260G>C / g.303G>C	p.R87P
N	167del31	g.167_197del / g.210_240del	p.G67fsX145
O	225del19	g.225_243del / g.268_286del	p.A76fs139X
P	240del14	g.240_253del / g.283_296del	p.1-80p16:100-133p14
Q	IVS2+1	g.457+1G>T	p.Ex1a-Ex3delEx2 / p.R128fs137X
AH	Intron 1	IVS1b+2T>C	NONE*
AN	Exon 1b del	Exon1b del	NONE*
G, CC, H and I	G101W	g.301G>T / g.344G>T	p.G101W
J, K and L	V126D	g.377T>A	p.V126D

* p16 amino acid (AA) changes designated NONE= Mutation that alters primary structure of p14ARF only. The study did not include any unaffected individuals from the mutation-positive family Q.

Table 2

Distribution of age, gender, *CDKN2A*, pigmentation phenotype, and sun exposure variables in 53 melanoma-prone families by CMM status. P-values were obtained by comparing CMM cases to unaffected individuals using logistic regression with a generalized estimating equation and adjusting for familial correlation in the variance.

	Unaffected Individuals (n=302)		CMM Cases (n=136)		P
	N	%	N	%	
Age at blood draw					
<35	74	24.5	31	22.8	
35-45	89	29.5	33	24.3	
46-50	35	11.6	19	14.0	
50+	104	34.4	53	39.0	0.616
Gender					
Female	172	57.0	73	53.7	
Male	130	43.1	63	46.3	0.537
<i>CDKN2A</i>					
Non-Carrier	262	86.8	77	56.6	
Carrier	40	13.3	59	43.4	<0.0001
Dysplastic nevi					
Unaffected	143	47.4	2	1.5	
Affected	119	39.4	103	97.7	<0.0001
Moles					
0-24	93	30.8	5	3.6	
25-99	105	34.8	27	19.9	
100+	81	28.8	81	59.6	<0.0001
Freckles					
Non/few	92	30.4	17	12.5	
Moderate	51	16.9	19	14.0	
Many	75	24.8	49	36.0	<0.0001
Solar injury					
None/mild	184	60.9	52	38.2	
Moderate	58	19.2	38	27.9	
Severe	38	12.6	23	16.9	0.0002
Tanning ability					
Tan/Little burn	147	48.7	44	14.6	
Burn/Little tan	118	39.1	68	22.5	0.0116
Skin type					
Dark/medium	87	28.8	17	12.5	
Pale/fair	187	61.9	96	70.6	0.0013
Eye color					
Black/brown	69	22.8	19	14.0	
Hazel	62	20.5	25	18.4	

	Unaffected Individuals (n=302)		CMM Cases (n=136)		P
	N	%	N	%	
Green/gray	24	7.9	17	12.5	0.1626
Blue	120	39.7	52	38.2	
Hair color					
Black/brown	124	41.1	35	25.7	0.1598
Blonde brown/light brown	74	24.5	27	19.9	
Blonde	47	15.6	12	8.8	
Red	30	9.9	13	9.6	

Table 4

Association between mtDNA copy number in blood and risk of being a *CDKN2A*⁺ carrier case. Odds ratios and P-values were computed using an unconditional logistic regression model adjusted for age at blood draw, sex, and familial correlation using a generalized estimating equation approach.

mtDNA _{CoN} #	CMM Cases				Model OR (95% CI)	P
	CDKN2A ⁻ Non-Carrier (n=77)	CDKN2A ⁺ Carrier (n=59)	N	%		
Q1 (<83)	22	(28.6)	11	(18.6)	Ref	
Q2 (83-106)	20	(26.0)	17	(28.2)	1.69 (0.66-4.4)	0.273
Q3 (107-164)	26	(33.8)	13	(22.0)	0.98 (0.36-2.65)	0.961
Q4 (>164)	9	(11.7)	18	(30.5)	5.04 (1.5-17.0)	0.009
P_{trend}						0.024

Abbreviations: mtDNA_{CoN} = mitochondrial DNA copy number; OR = odds ratio; CI = confidence interval; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3 and Q4 = quartile 4

= exponentiation of log-transformed mtDNA copy number.