Heliyon 10 (2024) e32512

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

UCP2 polymorphisms, daily step count, and number of teeth associated with all-cause mortality risk in Sado City: A hospital-based cohort study

Han Lyu [a], Noriko Sugita [a,*], Shigeki Komatsu [b], Minako Wakasugi [c], Akio Yokoseki [c], Akihiro Yoshihara [d], Tetsuo Kobayashi [a,e], Kenji Sato [b], Hiroyuki Kawashima [f], Osamu Onodera [g], Ichiei Narita [h], Koichi Tabeta [a]

[a] Division of Periodontology, Department of Oral Biological Science, Faculty of Dentistry & Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan
[b] Sado General Hospital, Niigata, Japan
[c] Department of Inter-Organ Communication Research, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[d] Division of Oral Science and Health Promotion, Faculty of Dentistry & Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan
[e] General Dentistry and Clinical Education Unit, Faculty of Dentistry & Medical and Dental Hospital, Niigata University, Niigata, Japan
[f] Division of Orthopedic Surgery, Niigata University Graduate School of Medical and Dental Science, Niigata, Japan
[g] Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan
[h] Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

ARTICLE INFO

Keywords:
Mitochondria
Physical activity
Oral health
Genetic
Epidemiology

ABSTRACT

Objective: Uncoupling protein 2 (UCP2) is an ion/anion transporter in the mitochondrial inner membrane that plays a crucial role in immune response, regulation of oxidative stress, and cellular metabolism. UCP2 polymorphisms are linked to chronic inflammation, obesity, diabetes, heart disease, exercise efficiency, and longevity. Daily step count and number of teeth are modifiable factors that reduce mortality risk, although the role of UCP2 in this mechanism is unknown. This study aimed to assess the possible effects of UCP2 polymorphisms on the association between daily step count and number of teeth with all-cause mortality.

Methods: This study was conducted as a cohort project involving adult Japanese outpatients at Sado General Hospital (PROST). The final number of participants was 875 (mean age: 69 y). All-cause mortality during thirteen years (from June 2008 to August 2021) was recorded. The functional UCP2 genotypes rs659366 and rs660339 were identified using the Japonica Array®. Survival analyses were performed using multivariate Cox proportional hazard models.

Results: There were 161 deaths (mean observation period: 113 months). Age, sex, daily step count, and the number of teeth were significantly associated with mortality. In females, UCP2 polymorphisms were associated with mortality independent of other factors (rs659366 GA compared to GG + AA; HR = 2.033, p = 0.019, rs660339 C T compared to CC + TT; HR = 1.911, p = 0.029).

Abbreviations: UCP2, uncoupling protein 2; SNP, single nucleotide polymorphism; PROST, The Project in Sado for Total Health; Steps, daily step count; Teeth, number of remaining teeth; BMI, body mass index; Alcohol, alcohol consumption; SD, standard deviation; KM analysis, Kaplan-Meier survival analysis; HR, hazard ratio.

* Corresponding author. Division of Periodontology, Department of Oral Biological Science, Faculty of Dentistry & Graduate School of Medical and Dental Sciences, Niigata University, 2-5274, Gakko-cho, Chuo-ku, Niigata, 951-8514, Japan.
E-mail address: psugita@dent.niigata-u.ac.jp (N. Sugita).

https://doi.org/10.1016/j.heliyon.2024.e32512
Received 8 December 2023; Received in revised form 13 May 2024; Accepted 5 June 2024
Available online 8 June 2024

Multivariate models, with and without UCP2 genotypes, yielded similar results. The interaction terms between UCP2 genotype and daily step count or number of teeth were not significantly associated with mortality.
Conclusion: The effects of UCP2 polymorphisms on the association between daily step count or the number of teeth and all-cause mortality were not statistically significant. In females, UCP2 polymorphisms were significantly associated with all-cause mortality. Our findings confirmed the importance of physical activity and oral health and suggested a role of UCP2 in mortality risk independently with those factors.

1. Introduction

Mortality is a multifaceted outcome influenced by various factors including genetic variations, physical activity levels, and oral health [1,2]. The protective health benefits of physical activity are rooted in the complex interplay between mechanisms that enhance cardiovascular well-being, metabolic equilibrium, and immune competence [3,4]. Daily step count is a dynamic and modifiable lifestyle determinant that quantifies physical activity and is strongly associated with mortality [5–8].

Oral health is a modifiable factor that can improve systemic health. Previous studies have demonstrated that poor oral health, characterized by tooth loss, is associated with an elevated risk of chronic diseases and mortality [9,10]. Periodontitis is a chronic inflammation caused by oral bacteria and is a major cause of tooth loss in adults [11]. Two major pathways may underlie the relationship between tooth loss and mortality: the impact of masticatory dysfunction on dietary behavior, nutrition, and systemic diseases [12–14] and the inflammatory consequences of chronic periodontal infection on the circulatory system [15–17]. Furthermore, a growing body of literature indicates that oral bacterial flora disorders influence immunity, inflammation, and metabolism [18].

Uncoupling protein 2 (UCP2), located in the mitochondrial inner membrane and is a member of the mitochondrial transporter family [19], modulates mitochondrial proton leakage and induces cellular energy expenditure [20]. UCP2 is widely expressed in numerous organs and tissues, including the white blood cells, gingiva, skeletal muscles, skin, brain, liver, and kidneys [21–26]. *In vivo* and *in vitro* studies have suggested that UCP2 plays a significant role in the inhibitory regulation of periodontitis by attenuating macrophage proliferation, migration, proinflammatory cytokine secretion, and ROS production [22].

UCP2 is located on chromosome 11q13 [27]. UCP2 polymorphisms have been associated with a spectrum of health conditions such as hypertension, obesity, diabetes, and heart disease [28–31]. The A allele of the upstream transcript variant rs659366 is associated with higher UCP2 expression levels than the G allele [32,33]. In contrast, rs660339 is a missense variant in exon 4 of UCP2, resulting in the substitution of the amino acid Ala55Val (*C*-to-T transition of nucleotide). In a previous study of healthy subjects, Val/Val homozygotes showed lower energy expenditure, higher exercise efficiency, and lower fat oxidation than Ala/Val and Ala/Ala groups [34].

As mentioned above, daily step count, reflecting physical activity levels, and the number of teeth, representing oral health, have been demonstrated to be associated with mortality risk. We hypothesized that UCP2 influences the impact of these modifiable factors on mortality. Thus, in this study, we explored whether UCP2 polymorphisms induce differences in the association between daily step count or number of teeth and all-cause mortality.

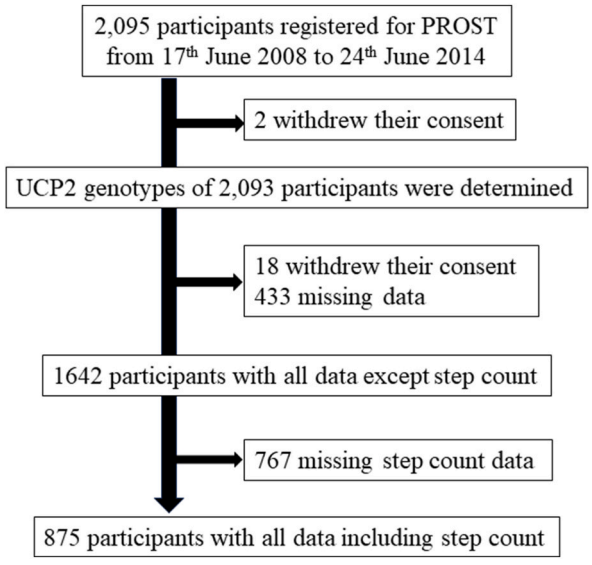

Fig. 1. Study design and flow chart. PROST, The Project in Sado for Total Health; UCP2, uncoupling protein.

2. Methods

2.1. Study participants

This study was part of a hospital-based cohort study called the Project in Sado for Total Health (PROST) conducted at Sado General Hospital, located in Sado City, Niigata, Japan. This hospital is the only major hospital in Sado Island with over 20 departments. PROST began in June 2008 targeting out-patients aged 20 years or older. The rate of individuals over 65 years old is 43.5 % in Sado City [35], which can serve as a model for future super-aging society. On the registration for PROST, all participants underwent the interview, blood sampling, and counting of their remaining teeth. Other data were obtained from medical records according to each patients' medical needs.

The research protocol adhered to the principles outlined in the Declaration of Helsinki of 2013, and was approved by the Ethics Committee of Niigata University (No. G2021-0019). All participants provided written informed consent prior to participation.

As shown in Fig. 1, 2095 patients were initially enrolled in the PROST. They were all Japanese adults, characterized by their independence and non-institutionalized status. The UCP2 genotypes of 2093 participants were determined. Among them, 18 participants withdrew from PROST and 433 had some missing data. Therefore, 1642 participants had all available data except step count. Finally, 875 participants with required data, including daily step counts, were actively engaged in this study. The distribution of participants' ages is shown in Fig. 2.

2.2. Determination of UCP2 genotypes

Genomic DNA was extracted from peripheral blood using a DNA isolation kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction. Genotyping of UCP2 (rs659366/rs660339) polymorphisms was performed using the Japonica SNP array (Tohoku Medical Megabank Organization, Japan) [36]. Genotype data were securely controlled by the Materials and Information Distribution Review Committee of the Tohoku Medical Megabank Project, and data sharing with the researchers was discussed with the review committee. All genotyped samples passed the recommended sample quality control metric for AXIOM arrays (dish QC40.82). The quality value was set at $>$ 99 %. Haploview version 4.2 was used for the linkage disequilibrium analyses.

2.3. Mortality ascertainment

The outcome of this study was all-cause mortality. Participants' deaths were ascertained from medical records, the obituary column of the local newspaper, and information provided by relatives and friends, as described in a previous report by PROST [37].

2.4. Data estimation

Daily step counts were garnered through the utilization of pedometers, coupled with a histogram analysis of the accumulated data. The step count distribution was bifurcated into the lowest quartile (24–2415) and other quartiles (2416–18395), thereby enabling a comprehensive exploration of the step count patterns [38].

Trained technicians counted the number of remaining teeth of all participants upon their registration in PROST, as described in a previous report [39]. Wisdom teeth and residual roots with caps were included in the study. Participants were classified into two groups according to whether the number of remaining teeth was 0–19 or $\geq$20 [40].

Height and weight were measured for all participants, and the body mass index (BMI) was calculated using the following formula: weight (kg) divided by the square of height (m). Blood pressure was recorded as part of the PROST registration procedure. Blood pressure was measured twice; average systolic BP $\geq$ 140 mmHg, average diastolic BP $\geq$ 90 mmHg, or use of antihypertensive medication was defined as hypertension.

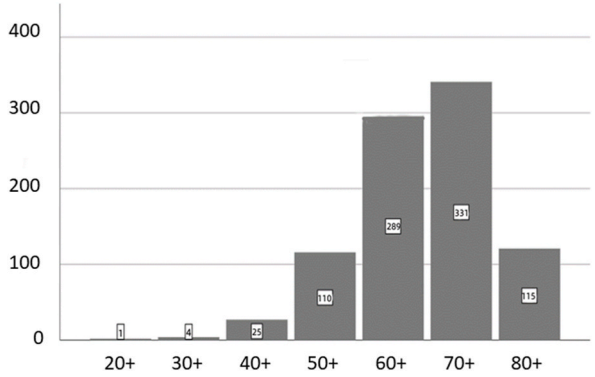

Fig. 2. Histogram of age.

Relevant information was obtained through comprehensive interviews with the participants when registering for PROST using a questionnaire encompassing covariates, such as smoking status, alcohol consumption, and disease history (Supplement 1). As describe in a previous report from PROST [41], the responses to the questionnaire regarding smoking habit were as follows: (1) "I have a smoking habit," (2) "I used to smoke but stopped," and (3) "I do not smoke at all." Past and present smokers were categorized into the "Have smoking habit" group. The responses for alcohol consumption were as follows: (1) "I drink more than 1 day a week," (2) "I was a drinker before," (3) "I'm a social drinker" and (4) "I do not drink alcohol at all." Social drinkers and past drinkers were categorized into the "No drinking habits" and "Drinking habits" groups, respectively.

2.5. Statistical analyses

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 28.0, IBM, Chicago, IL, USA). Initially, Kaplan-Meier survival analysis (KM analysis) was conducted as a univariate assessment of independent variables (i.e., age, sex, daily step count, number of teeth, BMI, hypertension, diabetes, hyperlipidemia, smoking, and alcohol consumption), where several confounding variables exhibited statistically significant outcomes. Subsequently, a multivariable Cox proportional hazard model was used to evaluate the impact of the daily step count, number of teeth, UCP2 polymorphisms, and their interaction terms on all-cause mortality. The outcomes of the Cox analyses were adjusted for covariates that showed significant effects on the KM analyses. Statistical significance was set at a threshold of $p < 0.05$.

The final number of participants in this study was limited (n = 875), because only a part of PROST participants performed step counting, though 1642 participants had all the data except step count. Therefore, we added two types of analyses with the 1642 participants, namely, the association study between UCP2 genotypes and diseases, and the Cox regression analyses of mortality without step count as a predictor.

3. Results

The genotype distribution (rs659366; rs660339) in this study was consistent with the Hardy-Weinberg equilibrium ($\chi^2 = 0.38$ and 0.58, respectively). The D' value was 0.96 estimated for all participants.

An overview of participant characteristics and genotype distributions is presented in Tables 1A and 1B. Of the 875 participants, 161 mortalities were recorded, with an average observation period of 113 months. A chi-square test revealed significant differences in smoking, alcohol consumption, and the distribution of UCP2 genotypes between the male and female groups, whereas no significant differences in other characteristics were found between the genotypes.

Subsequently, KM analysis was performed (Table 2). In each univariate analysis, significant differences in survival were observed in terms of sex, daily step count, number of teeth, smoking status, and alcohol consumption. Survival curves are shown in Fig. 3a-c.

Table 3 presents the results of the Cox proportional hazards models for UCP2 genotypes. Only in the over-dominant models for females did UCP2 polymorphisms show independent associations with mortality (GA compared to GG + AA; HR = 2.033, p = 0.019, CT compared to CC + TT; HR = 1.911, p = 0.029).

We assessed the effects of UCP2 polymorphisms on the associations between daily step count, number of teeth, and mortality in the multivariable Cox proportional hazards model for all participants, males, and females respectively (Tables 4–6). Based on the results shown in Table 3, over-dominant models of UCP2 genotypes were included in the analyses. Two methods were used to assess the effects of UCP polymorphisms. One was a comparison between the results of COX analyses with and without genotypes. The other was COX analysis with the interaction terms.

As shown in Table 4, the analyses for all participants revealed that participants with lower daily step counts experienced a 1.5-fold increased risk of mortality compared to those with higher daily step counts. The effect of the daily step count remained consistent with

Table 1 (A)
Characteristics of the study participants.

Characteristics	All (n = 875)	Males (n = 440)	Females (n = 435)
Number of deaths	161	111	50
Age	68.8 ± 9.9	68.8 ± 10.1	68.8 ± 9.7
Steps	4729 ± 3081	4919 ± 3283	4535 ± 2853
Teeth	17.1 ± 10.4	17.4 ± 10.4	16.8 ± 10.3
BMI	24.2 ± 4.0	24.2 ± 3.35	24.2 ± 4.53
Hypertension	488 (56%)	265 (60%)	223 (51%)
Diabetes	190 (22%)	114 (26%)	76 (17%)
Dyslipidemia	264 (30%)	108 (24%)	156 (36%)
Smoking	351 (40%)	316 (72%)	35 (8%)[a]
Alcohol	343 (39%)	278 (63%)	65 (15%)[a]

UCP2, Uncoupling protein 2; Steps, daily step count; Teeth, the number of teeth; BMI, body mass index. Smoking, smoking habit; Alcohol, alcohol consumption.
Values represent mean ± SD or the number of subjects.
Chi-squared test and Kruskal-Wallis test were performed.
p value $< \alpha$, $\alpha = 0.05$.
[a] There were significant differences of smoking and alcohol consumption status between male and female participants.

Table 1 (B)
Characteristics of the UCP2 genotypes and alleles.

	UCP2(rs659366)							UCP2(rs660339)						
Characteristics	GG (n = 257)	GA (n = 426)	AA (n = 192)	*p*	G allele (n = 940)	A allele (n = 810)	*p*	CC (n = 257)	CT (n = 424)	TT (n = 194)	*p*	C allele (n = 938)	T allele (n = 812)	*p*
Minor allele frequency						0.463							0.464	
Number of deaths	44 (17 %)	83 (19 %)	34 (18 %)	0.714	171 (18 %)	151 (19 %)	0.808	42 (16 %)	83 (20 %)	36 (19 %)	0.572	167 (18 %)	155 (19 %)	0.489
Age	68.4 ± 10.0	68.9 ± 9.8	69.2 ± 10.1	0.773				68.4 ± 9.9	68.9 ± 9.8	69.2 ± 10.1	0.779			
Number of males	126 (49 %)	200 (47 %)	114 (59 %)	0.015	452 (51 %)	428 (49 %)	0.047	124 (48 %)	201 (47 %)	115 (59 %)	0.017	449 (51 %)	431 (49 %)	0.030
Steps	4661 ± 2998	4835 ± 3060	4583 ± 3239	0.421				4649 ± 2980	4878 ± 3064	4508 ± 3245	0.192			
Teeth	17.5 ± 10.6	17.0 ± 10.2	16.9 ± 10.4	0.588				17.1 ± 10.9	17.5 ± 10.1	16.5 ± 10.6	0.761			
BMI	24.0 ± 3.7	24.2 ± 4.0	24.2 ± 4.3	0.997				23.9 ± 3.5	24.3 ± 4.1	24.3 ± 4.3	0.889			
Hypertension	145 (56%)	237 (56%)	106 (55%)	0.965	527 (56%)	449 (55%)	0.791	146 (57%)	235 (55%)	107 (55%)	0.922	527 (56%)	449 (55%)	0.709
Diabetes	50 (19%)	98 (23%)	42 (22%)	0.551	198 (21%)	182 (22%)	0.477	51 (20%)	94 (22%)	45 (23%)	0.660	196 (21%)	184 (23%)	0.372
Dyslipidemia	80 (31%)	135 (32%)	49 (26%)	0.280	295 (31%)	233 (29%)	0.234	80 (31%)	134 (32%)	50 (26%)	0.316	294 (31%)	234 (29%)	0.251
Smoking	103 (40%)	162 (38%)	86 (45%)	0.284	368 (39%)	334 (41%)	0.375	103 (40%)	161 (38%)	87 (45%)	0.270	367 (39%)	335 (41%)	0.365
Alcohol	106 (41%)	154 (36%)	83 (43%)	0.181	366 (39%)	320 (39%)	0.808	108 (42%)	150 (35%)	85 (44%)	0.075	320 (39%)	320 (39%)	0.868

UCP2, Uncoupling protein 2; Steps, daily step count; Teeth, the number of teeth; BMI, body mass index. Smoking, smoking habit; Alcohol, alcohol consumption.
n = 875. Values represent mean ± SD or the number of subjects.
Chi-squared test and Kruskal-Wallis test were performed.
p value < α, α = 0.05 for genotypes. *p* value < α, α = 0.025 for alleles.

Table 2
Kaplan-Meier survival analyses in different groups.

Variables	All (n = 875)	Males (n = 440)	Females (n = 435)
Sex	<0.001	NA	NA
Steps	<0.001	<0.001	<0.001
Teeth	<0.001	<0.001	0.001
BMI	0.160	0.070	0.893
Hypertension	0.004	0.055	0.104
Diabetes	0.100	0.316	0.769
Dyslipidemia	0.762	0.599	0.932
Smoking	<0.001	0.108	0.155
Alcohol	<0.001	0.586	0.765
rs659366	0.731	0.865	0.142
rs660339	0.560	0.998	0.105

Teeth, the number of teeth; Steps, daily step count; BMI, body mass index.
Smoking, smoking habit; Alcohol, alcohol consumption.
Values represent *p*-values from KM analyses.
We selected variables with *p*-value <0.05 to include in Cox regression analyses.

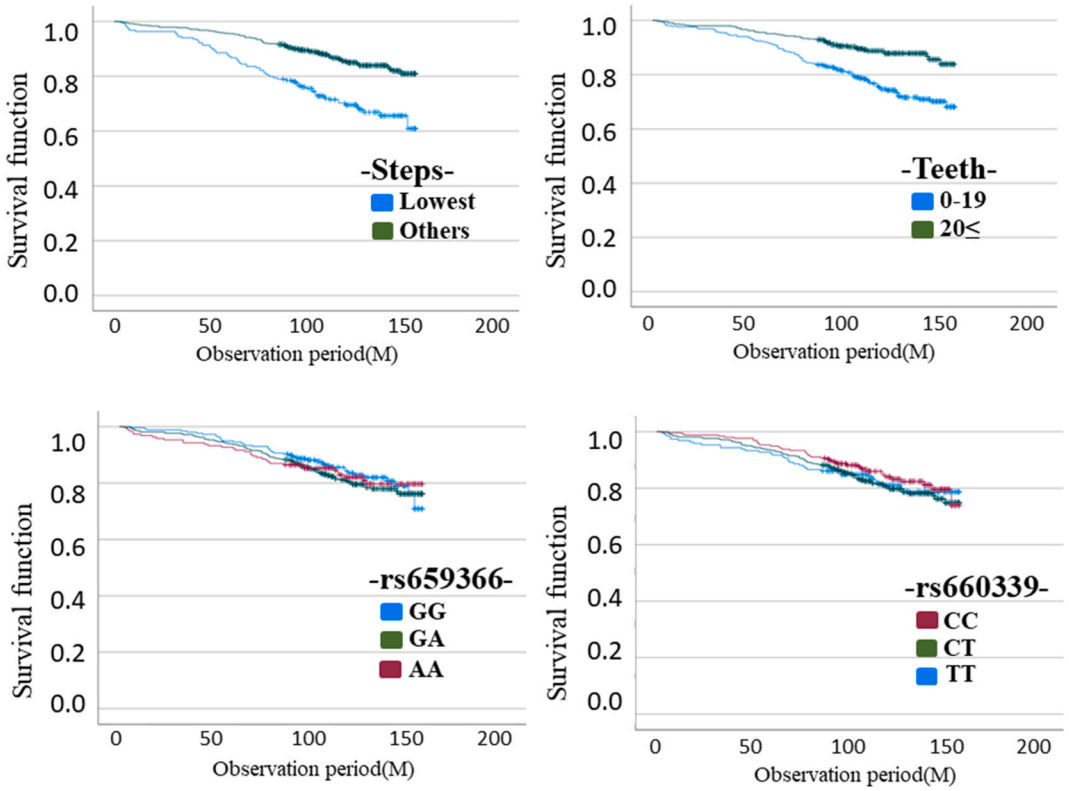

Fig. 3A. Kaplan-Meier curves of all-cause mortality for all participants. Steps, daily step count; Teeth, number of teeth; M, months.

or without adjustment for UCP2 polymorphisms. Table 5 shows that males with a lower number of teeth experienced a 1.5-fold elevated risk of mortality compared to those with a higher number of teeth. The effects of the number of teeth also remained consistent, with or without adjustment for UCP2 polymorphisms. In Table 6, separate analysis for females, showed independent associations between UCP2 polymorphisms and mortality. However, an association between the daily step count and the number of teeth with mortality was not found in females. In all models shown in Tables 4–6, the interactions between UCP2 genotypes and daily step count or number of teeth were not statistically significant. Additionally, none of the other genetic models (co-dominant, dominant, and recessive models) changed the relationships between daily step count or number of teeth and mortality (data not shown).

Table 7 represents the results from the association study between UCP2 genotypes and hypertension, diabetes, dyslipidemia and obesity in 1642 participants using multiple logistic regression analyses. Dyslipidemia was associated with UCP2 rs660339 TT genotype. No other association reached statistical significance between UCP2 genotypes and diseases.

Cox regression analyses of mortality in 1642 participants are shown in Table 8. Sex, number of teeth, BMI, and hypertension were

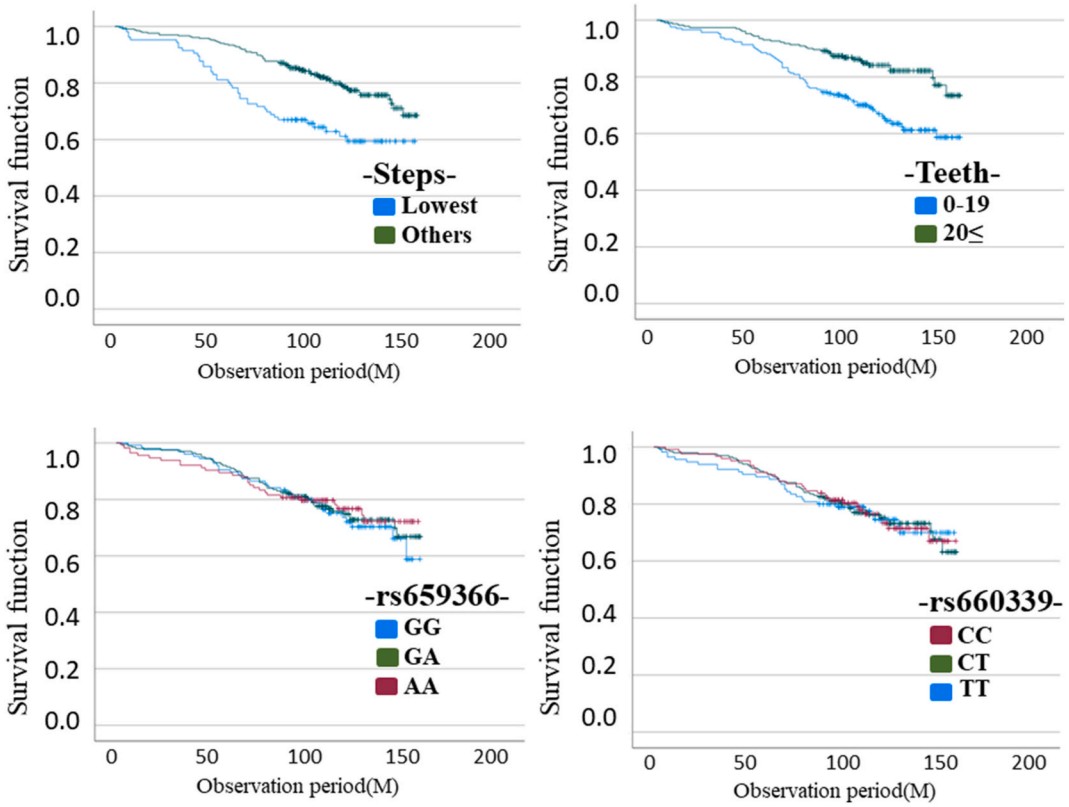

Fig. 3B. Kaplan-Meier curves of all-cause mortality for males. Steps, daily step count; Teeth, number of teeth; M, months.

associated with mortality. In the male group, only the number of teeth was a risk for mortality. In the female group, UCP2 polymorphisms, number of teeth, BMI were associated with mortality. Step count was not included in the analyses (Table 8).

4. Discussion

The results of the present study did not demonstrate significant effects of UCP2 polymorphisms on the association between daily step count or the number of teeth and all-cause mortality, despite previous reports indicating crucial roles of UCP2 in biological processes and the association of its genetic polymorphisms with various health conditions [28,42,43]. This inconsistency with our initial hypothesis might be explained by the limited influence of UCP2 polymorphisms. Additionally, the dual nature of UCP2 function may attenuate the aggregated effects. Elevated UCP2 expression can offer benefits such as mitigating oxidative stress, metabolic disorders, and inflammation [44–46]. However, it may also compromise cellular energy efficiency, rendering cells less proficient at handling stressors and metabolic demands [47]. Moreover, decreased production of reactive oxygen species can attenuate immune responses and potentially lead to energy wastage, thereby increasing susceptibility to chronic diseases and infections [21,48,49]. Another point that may have influenced the results of this study is that the quantity of UCP2 protein is not solely dictated by alleles; however, is subjected to intricate regulation. Precise research to distinguish between UCP2 mRNA and protein expression levels, including tissue-specific regulation, will provide clearer insights.

This study provides further evidence of the independent associations between daily step count and the number of teeth with all-cause mortality, even after adjusting for various covariates, including UCP2 genotypes. Lower number of teeth was an obvious risk of mortality in this study, especially in Table 8, consistent in all participants, male group, and female group. The hazard ratio for number of teeth was higher than both BMI and hypertension. Our findings underscore the importance of maintaining an active lifestyle and good oral health as distinct yet potent contributors to reducing mortality risk [18]. Although there are many unresearched factors in survival analyses, the present study clearly indicates that oral health is an essential factor in the investigations of mortality risk.

It is worth noting that in female participants, UCP2 heterozygotes of both rs659366 and rs660339 polymorphisms had a higher risk of all-cause mortality than homozygotes. Rose et al. assessed the associations between longevity and eight polymorphisms in UCP genes in Italian participants and reported as a part of results that each dominant model for UCP2 rs660339 and UCP3 rs1800849 was associated with longevity phenotype [50]. It remains unclear why heterozygotes are associated with a higher risk of mortality, although the dual nature of UCP2 functions described above might be involved in this mechanism. Moreover, a previous study suggested that UCP2 polymorphisms influence human leukocyte telomere length, potentially affecting cellular aging and longevity [51]. Although presumed based on these previous studies, this is the first study to demonstrate an independent association between UCP2

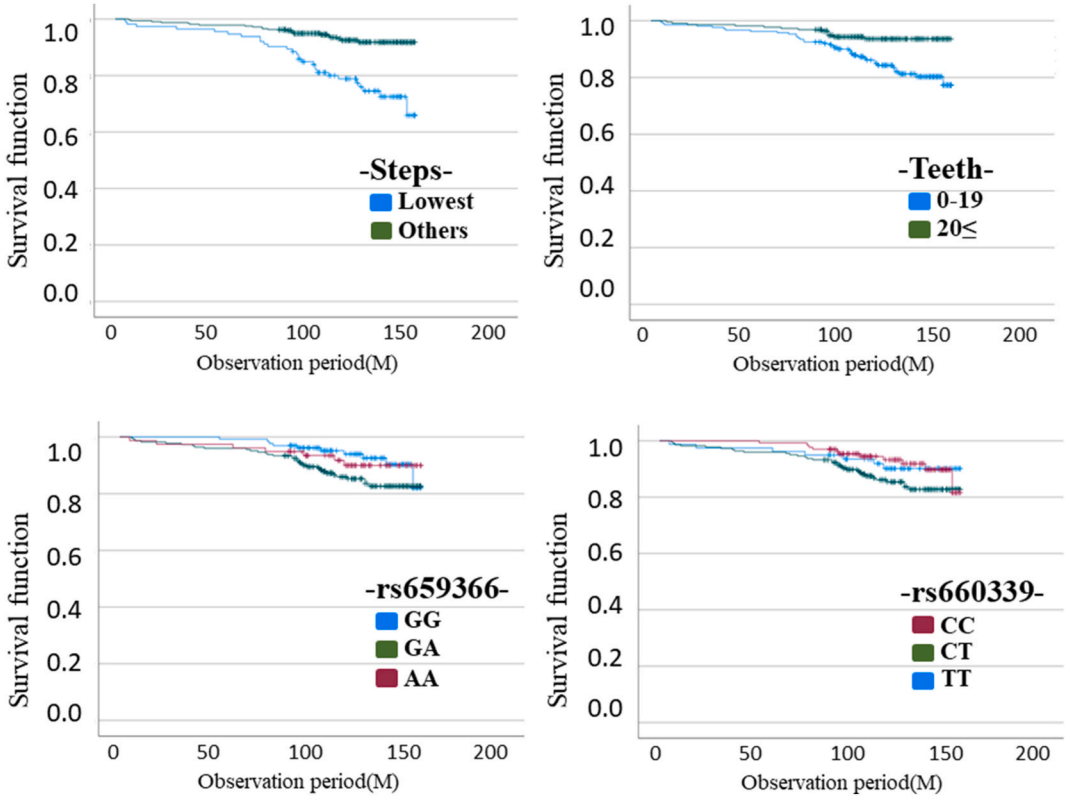

Fig. 3C. Kaplan-Meier curves of all-cause mortality for females. Steps, daily step count; Teeth, number of teeth; M, month.

Table 3
Cox regression analyses of mortality in different models.

UCP2 genotypes	All (n = 875)		Males (n = 440)		Females (n = 435)	
	HR (95 % CI)	p value	HR (95 % CI)	p value	HR (95 % CI)	p value
rs659366						
GG	Reference		Reference		Reference	
GA	1.184 (0.820–1.709)	0.368	0.929 (0.600–1.438)	0.741	1.864 (0.917–3.792)	0.086
AA	0.881 (0.562–1.382)	0.581	0.838 (0.504–1.392)	0.494	0.816 (0.306–2.178)	0.685
Dominant model (GG vs GA/AA)	0.929 (0.656–1.316)	0.679	1.117 (0.745–1.675)	0.591	0.649 (0.323–1.303)	0.224
Recessive model (AA vs GG/GA)	0.793 (0.541–1.161)	0.233	0.876 (0.567–1.354)	0.551	0.525 (0.232–1.185)	0.121
Over-dominant model (GA vs GG/AA)	1.253 (0.917–1.711)	0.157	1.009 (0.693–1.467)	0.965	2.033 (1.126–3.668)	0.019
rs660339						
CC	Reference		Reference		Reference	
CT	1.279 (0.880–1.858)	0.197	1.044 (0.667–1.634)	0.851	1.711 (0.858–3.413)	0.127
TT	0.954 (0.609–1.495)	0.838	0.949 (0.570–1.581)	0.840	0.766 (0.292–2.005)	0.587
Dominant model (CC vs CT/TT)	0.863 (0.606–1.228)	0.412	0.992 (0.655–1.504)	0.971	0.707 (0.360–1.390)	0.315
Recessive model (TT vs CC/CT)	0.819 (0.562–1.194)	0.299	0.924 (0.602–1.418)	0.718	0.527 (0.234–1.189)	0.123
Over-dominant model (CT vs CC/TT)	1.306 (0.954–1.788)	0.095	1.071 (0.736–1.559)	0.722	1.911 (1.067–3.425)	0.029

HR, hazard ratio.
Analyses for all participants were adjusted for age, sex, daily step count, the number of teeth, hypertension, smoking and alcohol consumption.
Analyses for males or females were adjusted for age, daily step count and the number of teeth.
The reference genotypes of rs659366: GA/AA in the dominant model, GG/GA in the recessive model, and GG/AA in the over-dominant model.
The reference genotypes of rs660339: CT/TT in the dominant model, CC/CT in the recessive model, and CC/TT in the over-dominant model.
p value $< \alpha$, $\alpha = 0.05$.

polymorphisms and all-cause mortality.

Previous reports have demonstrated associations between hypertension and rs659366, and between obesity and rs660339 both in Japanese [52,53]. In this study, significant association was observed between rs660339 and dyslipidemia (Multiple logistic regression analyses, Table 7, n = 1642). However, dyslipidemia was not significant risk in the Kaplin-Meier survival analysis (Table 2) nor in Cox regression analyses of mortality (Table 8).

Table 4
Cox regression analysis of mortality with and without genotypes and interaction terms for all participants (n = 875).

Variables	With genotypes				Without genotypes	
	rs659366		rs660339		HR (95 % CI)	*p* value
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value		
Steps	1.597 (1.131–2.254)	0.008	1.622 (1.147–2.293)	0.006	1.559 (1.107–2.198)	0.011
Teeth	1.387 (0.978–1.966)	0.067	1.391 (0.981–1.972)	0.064	1.395 (0.984–1.977)	0.062
Genotypes	1.253 (0.917–1.711)	0.157	1.306 (0.954–1.788)	0.095		

Variables and interactions	rs659366				rs660339			
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Steps	1.595 (1.130–2.251)	0.008	1.448 (0.906–2.315)	0.122	1.621 (1.146–2.293)	0.006	1.346 (0.841–2.155)	0.216
Teeth	1.309 (0.811–2.115)	0.271	1.382 (0.974–1.960)	0.070	1.498 (0.914–2.457)	0.109	1.389 (0.980–1.968)	0.065
Genotypes	1.159 (0.671–2.002)	0.597	1.155 (0.768–1.736)	0.490	1.438 (0.831–2.489)	0.194	1.118 (0.743–1.682)	0.593
Genotypes* Steps			1.213 (0.648–2.273)	0.545			1.449 (0.773–2.715)	0.247
Genotypes* Teeth	1.122 (0.578–2.179)	0.734			0.867 (0.445–1.688)	0.674		

Steps, daily step count; Teeth, the number of teeth; HR, hazard ratio.
HR for the lowest quartile of steps (reference: the other quartiles), and for 0–19 teeth (reference: ≥ 20 teeth) are represented.
Over-dominant models (GA vs GG/AA for rs659366 and CT vs CC/TT for rs660339) were adopted.
The reference genotypes were GG/AA and CC/TT, respectively. HR for the GA or CT genotypes are represented.
Adjusted for age, sex, hypertension, smoking and alcohol consumption.
p value $< \alpha$, $\alpha = 0.05$.

Table 5
Cox regression analysis of mortality with and without genotypes and interaction terms for males (n = 440).

Variables	With genotypes				Without genotypes	
	rs659366		rs660339		HR (95 % CI)	*p* value
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value		
Steps	1.414 (0.937–2.134)	0.099	1.425 (0.943–2.154)	0.093	1.414 (0.937–2.132)	0.099
Teeth	1.517 (1.013–2.272)	0.043	1.151 (1.011–2.269)	0.044	1.517 (1.013–2.271)	0.043
Genotypes	1.009 (0.693–1.467)	0.965	1.071 (0.735–1.559)	0.722		

Variables and interactions	rs659366				rs660339			
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Steps	1.415 (0.937–2.135)	0.099	1.203 (0.697–2.077)	0.506	1.421 (0.939–2.150)	0.096	1.103 (0.637–1.911)	0.727
Teeth	1.522 (0.896–2.586)	0.120	1.503 (1.003–2.252)	0.048	1.804 (1.039–3.135)	0.036	1.510 (1.009–2.261)	0.045
Genotypes	1.014 (0.537–1.912)	0.966	0.886 (0.554–1.416)	0.613	1.365 (0.727–2.563)	0.333	0.874 (0.548–1.396)	0.573
Genotypes* Steps			1.434 (0.658–3.126)	0.364			1.768 (0.813–2.004)	3.848
Genotypes* Teeth	0.992 (0.452–2.178)	0.984			0.687 (0.314–1.502)	0.347		

Steps, daily step count; Teeth, the number of teeth; HR, hazard ratio.
HR for the lowest quartile of steps (reference: the other quartiles), and for 0–19 teeth (reference: ≥ 20 teeth) are represented.
Over-dominant models (GA vs GG/AA for rs659366 and CT vs CC/TT for rs660339) were adopted.
The reference genotypes were GG/AA and CC/TT, respectively. HR for the GA or CT genotypes are represented.
Adjusted for age.
p value $< \alpha$, $\alpha = 0.05$.

The divergent impact of UCP2 polymorphisms on mortality risk in male and female groups can be attributed to sex-specific biological variations in immune response and metabolism, including lipid metabolism and energy balance [54], all of which are intricately linked to UCP2. Moreover, sex hormones, which are pivotal in regulating the immune system, oxidative stress, and cellular metabolism [55,56], may interact with UCP2, thereby contributing to female-specific associations.

Daily step count was significantly associated with mortality in all participants but not in the male and female groups ($p > 0.05$).

Table 6
Cox regression analysis of mortality with and without genotypes and interaction terms for females (n = 435).

Variables	With genotypes				Without genotypes	
	rs659366		rs660339		HR (95 % CI)	*p* value
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value		
Steps	1.748 (0.941–3.246)	0.077	1.762 (0.948–3.327)	0.073	1.647 (0.886–3.063)	0.115
Teeth	1.193 (0.614–2.318)	0.603	1.225 (0.628–2.387)	0.552	1.185 (0.602–2.333)	0.623
Genotypes	2.033 (1.126–3.668)	0.019	1.911 (1.067–3.425)	0.029		

Variables and interactions	rs659366				rs660339			
	HR (95 % CI)	*p* value	HR (95%CI)	*p* value	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Steps	0.576 (0.311–1.066)	0.079	3.303 (1.030–10.591)	0.044	1.744 (0.941–3.232)	0.077	2.598 (0.881–7.665)	0.084
Teeth	0.833 (0.275–2.525)	0.747	1.180 (0.605–2.300)	0.627	0.848 (0.283–2.546)	0.769	1.226 (0.628–2.394)	0.551
Genotypes	1.384 (0.448–4.280)	0.572	3.581 (1.212–10.584)	0.021	1.280 (0.412–3.973)	0.670	2.707 (0.997–7.345)	0.051
Genotypes* Steps			0.410 (0.110–1.532)	0.185			0.569 (0.163–1.985)	0.377
Genotypes* Teeth	1.689 (0.442–6.447)	0.443			1.716 (0.452–6.514)	0.427		

Steps, daily step count; Teeth, the number of teeth; HR, hazard ratio.
HR for the lowest quartile of steps (reference: the other quartiles), and for 0–19 teeth (reference: ≥ 20 teeth) are represented.
Over-dominant models (GA vs GG/AA for rs659366 and CT vs CC/TT for rs660339) were adopted.
The reference genotypes were GG/AA and CC/TT, respectively. HR for the GA or CT genotypes are represented.
Adjusted for age.
p value < α, α = 0.05.

Table 7
Associations between UCP2 genotypes and diseases (n = 1642).

Genotypes		Outcomes							
		Hypertension		Diabetes		Dyslipidemia		Obesity	
		OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*	OR (95 % CI)	*p*
UCP2 (rs659366)	GG	Reference		Reference		Reference		Reference	
	AG	1.055 (0.823–1.353)	0.673	0.846 (0.617–1.161)	0.301	0.934 (0.706–1.236)	0.633	0.980 (0.762–1.261)	0.877
	AA	0.925 (0.699–1.224	0.586	0.934 (0.654–1.334)	0.707	0.775 (0.570–1.055)	0.106	1.165 (0.880–1.543)	0.287
UCP2 (rs660339)	CC	Reference		Reference		Reference		Reference	
	CT	1.071 (0.845–1.357)	0.572	1.065 (0.696–1.261)	0.667	1.156 (0.895–1.493)	0.268	0.860 (0.678–1.092)	0.215
	TT	1.117 (0.846–1.476)	0.435	0.937 (0.748–1.518)	0.727	1.380 (1.012–1.882)	0.042	0.894 (0.676–1.183)	0.432

Multiple logistic regression analyses were performed.
Adjusted for age, sex, smoking and alcohol consumption.
p value < α, α = 0.05.

This inconsistency might be caused by the relatively small number of participants in the male and female groups compared with all participants. Additionally, we entered a limited number of independent variables (age, daily step count, number of teeth, and genotype) into the analyses of each sex group, whereas sex, hypertension, smoking, and alcohol consumption were included in the analyses of all participants.

Since strong linkage disequilibrium exists between UCP2 rs659366 and rs660339, the association of one polymorphism with mortality may be caused by linkage disequilibrium with the other. Similarly, significant linkage disequilibrium has been reported between UCP2 and UCP3 polymorphisms [50,57]. UCP3 is predominantly expressed in skeletal muscles [58]. Therefore, future analyses that include UCP3 polymorphisms may reveal their significant effects on the association between the daily step count and mortality risk.

Gender-based differences were observed in lifestyle habits and health consciousness. In our study, significant differences in smoking and alcohol consumption were observed between men and women (Tables 1A and 1B). The effects of these differences on the results add complexity and require further research.

This study has several limitations. First, we assessed only two previously reported UCP2 polymorphisms. Broader research, including other genetic polymorphisms, is needed to better understand their impact on mortality and their relationship with step count

Table 8
Cox regression analyses of mortality in 1642 participants without step count as a predictor.

Variables	UCP2 (rs659366)							UCP2 (rs660339)					
	All (n = 1642)		Males (n = 865)		Females (n = 777)			All (n = 1642)		Males (n = 865)		Females (n = 777)	
	HR	p value	HR	p value	HR	p value		HR	p value	HR	p value	HR	p value
Sex	2.008	<0.001	–	–	–	–`		2.061	<0.001	–	–	–	–
Age	1.071	<0.001	1.060	<0.001	1.100	<0.001		1.071	<0.001	1.060	<0.001	1.099	<0.001
GG	Reference		Reference		Reference		CC	Reference		Reference		Reference	
GA	1.092	0.501	0.853	0.317	1.789	0.019	CT	1.193	0.203	1.064	0.712	1.719	0.027
AA	0.871	0.386	0.769	0.156	1.159	0.646	TT	1.106	0.524	1.262	0.208	1.218	0.527
Teeth	1.524	<0.001	1.464	0.010	1.658	0.039		1.529	<0.001	1.460	0.011	1.698	0.031
BMI	0.929	<0.001	0.952	0.084	0.903	<0.001		0.929	<0.001	0.951	0.083	0.902	<0.001
Hypertension	1.287	0.035	1.213	0.188	1.373	0.139		1.280	0.039	1.211	0.191	1.385	0.130
Diabetes	1.168	0.252	1.156	0.358	1.141	0.628		1.174	0.238	1.155	0.360	1.151	0.606
Dyslipidemia	1.008	0.954	1.039	0.812	0.989	0.961		1.006	0.960	1.040	0.807	0.989	0.961
Smoking	1.185	0.256	1.152	0.372	1.890	0.097		1.190	0.244	1.157	0.359	1.901	0.095
Alcohol	1.005	0.969	1.054	0.708	1.319	0.418		1.003	0.985	1.050	0.726	1.324	0.413

Teeth, the number of teeth; HR, hazard ratio. HR for 0–19 teeth (reference: $\geq$ 20 teeth) are represented.
Adjusted for all variables shown in this table.
p value $< \alpha$, $\alpha = 0.05$.

and number of teeth. Second, we could not obtain valuable information, such as the relatedness of the participants, masticatory function, periodontal status, and physical activity indicators other than step count. Third, we could not perform cause-specific mortality analyses due to the limited number of participants. These limitations highlight the need for further large-scale studies to validate and refine our understanding of the relationships among genetic variants, physical activity, oral health, and mortality.

5. Conclusion

In a hospital-based cohort study of Japanese adults, the effects of UCP2 polymorphisms on the association of daily step count and the number of teeth with all-cause mortality were not statistically significant. UCP2 polymorphisms were significantly associated with all-cause mortality only in the female participants. Our findings confirmed the importance of physical activity and oral health and suggested a role of UCP2 in mortality risk independently with those factors.

Ethics statement

The protocol of this study was approved by the Ethics Committee of Niigata University (No. G2021-0019). All participants provided written informed consent prior to participation.

Funding

This work was supported by Grant-in-Aid for the Project in Sado for Total Health (PROST) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid for PROST from Niigata-ken Koseiren, and JSPS KAKENHI Grant Number 22K17055.

Research data for this article

Due to the sensitive nature of the data obtained in this study, survey respondents were assured raw data would remain confidential and would not be shared.

CRediT authorship contribution statement

Han Lyu: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Noriko Sugita:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Shigeki Komatsu:** Writing – review & editing, Investigation. **Minako Wakasugi:** Writing – review & editing, Project administration, Data curation, Conceptualization. **Akio Yokoseki:** Writing – review & editing, Project administration, Investigation, Data curation, Conceptualization. **Akihiro Yoshihara:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Tetsuo Kobayashi:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Kenji Sato:** Writing – review & editing, Investigation, Conceptualization. **Hiroyuki Kawashima:** Writing – review & editing, Supervision. **Osamu Onodera:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Ichiei Narita:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Koichi Tabeta:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank all participants, staff, and investigators of the PROST for their valuable contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32512.

References

[1] K.S. Hall, E.T. Hyde, D.R. Bassett, et al., Systematic review of the prospective association of daily step counts with risk of mortality, cardiovascular disease, and dysglycemia, Int. J. Behav. Nutr. Phys. Activ. 17 (1) (2020) 78, https://doi.org/10.1186/s12966-020-00978-9.

[2] S. Koka, A. Gupta, Association between missing tooth count and mortality: a systematic review, J Prosthodont Res 62 (2) (2018) 134–151, https://doi.org/10.1016/j.jpor.2017.08.003.

[3] U. Ekelund, J. Steene-Johannessen, W.J. Brown, et al., Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women, Lancet 388 (10051) (2016) 1302–1310, https://doi.org/10.1016/S0140-6736(16)30370-1.

[4] Y. Qiu, B. Fernández-García, H.I. Lehmann, et al., Exercise sustains the hallmarks of health, J Sport Health Sci 12 (1) (2023) 8–35, https://doi.org/10.1016/j.jshs.2022.10.003.

[5] T. Yates, S.M. Haffner, P.J. Schulte, et al., Association between change in daily ambulatory activity and cardiovascular events in people with impaired glucose tolerance (NAVIGATOR trial): a cohort analysis, Lancet 383 (9922) (2014) 1059–1066, https://doi.org/10.1016/S0140-6736(13)62061-9.

[6] F.C. Bull, S.S. Al-Ansari, S. Biddle, et al., World Health Organization 2020 guidelines on physical activity and sedentary behaviour, Br. J. Sports Med. 54 (24) (2020) 1451–1462, https://doi.org/10.1136/bjsports-2020-102955.

[7] A.E. Paluch, S. Bajpai, D.R. Bassett, et al., Daily steps and all-cause mortality: a meta-analysis of 15 international cohorts, Lancet Public Health 7 (3) (2022) e219–e228, https://doi.org/10.1016/S2468-2667(21)00302-9.

[8] B. del Pozo Cruz, M.N. Ahmadi, I.M. Lee, E. Stamatakis, Prospective associations of daily step counts and intensity with cancer and cardiovascular disease incidence and mortality and all-cause mortality, JAMA Intern. Med. 182 (11) (2022) 1139–1148, https://doi.org/10.1001/jamainternmed.2022.4000.

[9] P.K. Friedman, I.B. Lamster, Tooth loss as a predictor of shortened longevity: exploring the hypothesis, Periodontol 72 (1) (2000. 2016) 142–152, https://doi.org/10.1111/prd.12128.

[10] Y.H. Yu, W.S. Cheung, B. Steffensen, D.R. Miller, Number of teeth is associated with all-cause and disease-specific mortality, BMC Oral Health 21 (1) (2021) 568, https://doi.org/10.1186/s12903-021-01934-0.

[11] B.L. Pihlstrom, B.S. Michalowicz, N.W. Johnson, Periodontal diseases, Lancet 366 (9499) (2005) 1809–1820, https://doi.org/10.1016/S0140-6736(05)67728-8.

[12] H.C. Hung, G. Colditz, K.J. Joshipura, The association between tooth loss and the self-reported intake of selected CVD-related nutrients and foods among US women, Community Dent. Oral Epidemiol. 33 (3) (2005) 167–173, https://doi.org/10.1111/j.1600-0528.2005.00200.x.

[13] R.E. Nowjack-Raymer, A. Sheiham, Numbers of natural teeth, diet, and nutritional status in US adults, J. Dent. Res. 86 (12) (2007) 1171–1175, https://doi.org/10.1177/154405910708601206.

[14] P. Moynihan, M. Thomason, A. Walls, et al., Researching the impact of oral health on diet and nutritional status: methodological issues, J. Dent. 37 (4) (2009) 237–249, https://doi.org/10.1016/j.jdent.2008.12.003.

[15] R.A. Seymour, P.M. Preshaw, J.M. Thomason, J.S. Ellis, J.G. Steele, Cardiovascular diseases and periodontology, J. Clin. Periodontol. 30 (4) (2003) 279–292, https://doi.org/10.1034/j.1600-051x.2003.00291.x.

[16] G.R. Persson, R.E. Persson, Cardiovascular disease and periodontitis: an update on the associations and risk, J. Clin. Periodontol. 35 (8 Suppl) (2008) 362–379, https://doi.org/10.1111/j.1600-051X.2008.01281.x.

[17] M. Kebschull, R.T. Demmer, P.N. Papapanou, "Gum bug, leave my heart alone!"–epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis, J. Dent. Res. 89 (9) (2010) 879–902, https://doi.org/10.1177/0022034510375281.

[18] J. Yu, W. Qin, W. Huang, K. Thomas, Oral health and mortality among older adults: a doubly robust survival analysis, Am. J. Prev. Med. 64 (1) (2023) 9–16, https://doi.org/10.1016/j.amepre.2022.08.006.

[19] P.J. Kaisaki, P.Y. Woon, R.H. Wallis, A.P. Monaco, M. Lathrop, D. Gauguier, Localization of tub and uncoupling proteins (Ucp) 2 and 3 to a region of rat chromosome 1 linked to glucose intolerance and adiposity in the Goto-Kakizaki (GK) type 2 diabetic rat, Mamm. Genome 9 (11) (1998) 910–912, https://doi.org/10.1007/s003359900895.

[20] S.R. Krauss, G. de Haan, Epigenetic perturbations in aging stem cells, Mamm. Genome 27 (7–8) (2016) 396–406, https://doi.org/10.1007/s00335-016-9645-8.

[21] C. Pecqueur, M.C. Alves-Guerra, C. Gelly, et al., Uncoupling protein 2, in vivo distribution, induction upon oxidative stress, and evidence for translational regulation, J. Biol. Chem. 276 (12) (2001) 8705–8712, https://doi.org/10.1074/jbc.M006938200.

[22] X. Yan, Z. Yuan, Y. Bian, et al., Uncoupling protein-2 regulates M1 macrophage infiltration of gingiva with periodontitis, Cent. Eur. J. Immunol. 45 (1) (2020) 9–21, https://doi.org/10.5114/ceji.2020.94664.

[23] S. Mori, N. Yoshizuka, M. Takizawa, et al., Expression of uncoupling proteins in human skin and skin-derived cells, J. Invest. Dermatol. 128 (8) (2008) 1894–1900, https://doi.org/10.1038/jid.2008.20.

[24] F. Ji, T. Shen, W. Zou, J. Jiao, UCP2 Regulates embryonic neurogenesis via ROS-mediated Yap alternation in the developing neocortex, Stem Cell. 35 (6) (2017) 1479–1492, https://doi.org/10.1002/stem.2605.

[25] L.F. Iannucci, J. Sun, B.K. Singh, et al., Short chain fatty acids induce UCP2-mediated autophagy in hepatic cells, Biochem. Biophys. Res. Commun. 480 (3) (2016) 461–467, https://doi.org/10.1016/j.bbrc.2016.10.072.

[26] B.M. de Souza, M. Michels, D.A. Sortica, et al., Polymorphisms of the UCP2 gene are associated with glomerular filtration rate in type 2 diabetic patients and with decreased UCP2 gene expression in human kidney, PLoS One 10 (7) (2015) e0132938, https://doi.org/10.1371/journal.pone.0132938.

[27] N. Tu, H. Chen, U. Winnikes, et al., Structural organization and mutational analysis of the human uncoupling protein-2 (hUCP2) gene, Life Sci. 64 (3) (1999) PL41–PL50, https://doi.org/10.1016/s0024-3205(98)00555-4.

[28] X.Y. Tian, S. Ma, G. Tse, W.T. Wong, Y. Huang, Uncoupling protein 2 in cardiovascular health and disease, Front. Physiol. 9 (2018) 1060, https://doi.org/10.3389/fphys.2018.01060.

[29] N. Kaul, Y.P. Singh, A.J. Bhanwer, The influence of ethnicity in the association of WC, WHR, hypertension and PGC-1α (Gly482Ser), UCP2 -866 G/A and SIRT1 -1400 T/C polymorphisms with T2D in the population of Punjab, Gene 563 (2) (2015) 150–154, https://doi.org/10.1016/j.gene.2015.03.019.

[30] M. Zhou, S. He, F. Ping, et al., Uncoupling protein 2 and peroxisome proliferator-activated receptor γ gene polymorphisms in association with diabetes susceptibility in Chinese Han population with variant glucose tolerance, Internet J. Endocrinol. 2018 (2018) 4636783, https://doi.org/10.1155/2018/4636783.

[31] S. Zhang, X. Liu, T. Wang, et al., Association and interaction effect of UCP2 gene polymorphisms and dietary factors with congenital heart diseases in Chinese Han population, Sci. Rep. 11 (1) (2021) 8699, https://doi.org/10.1038/s41598-021-88057-2.

[32] M. Sasahara, M. Nishi, H. Kawashima, et al., Uncoupling protein 2 promoter polymorphism -866G/A affects its expression in beta-cells and modulates clinical profiles of Japanese type 2 diabetic patients, Diabetes 53 (2) (2004) 482–485, https://doi.org/10.2337/diabetes.53.2.482.

[33] S. Vogler, R. Goedde, B. Miterski, et al., Association of a common polymorphism in the promoter of UCP2 with susceptibility to multiple sclerosis, J. Mol. Med. (Berl.) 83 (10) (2005) 806–811, https://doi.org/10.1007/s00109-005-0661-5.

[34] A. Astrup, S. Toubro, L.T. Dalgaard, S.A. Urhammer, T.I. Sorensen, O. Pedersen, Impact of the v/v 55 polymorphism of the uncoupling protein 2 gene on 24-h energy expenditure and substrate oxidation, Int. J. Obes. Relat. Metab. Disord. 23 (10) (1999) 1030–1034, https://doi.org/10.1038/sj.ijo.0801040.

[35] Prefecture Niigata, Curr. Situation of the Elderly, 2022, pp. 7–8, pref.niigata.lg.jp/uploaded/attachment/343537.pdf.

[36] Y. Kawai, T. Mimori, K. Kojima, et al., Japonica array: improved genotype imputation by designing a population-specific SNP array with 1070 Japanese individuals, J. Hum. Genet. 60 (10) (2015) 581–587, https://doi.org/10.1038/jhg.2015.68.

[37] M. Wakasugi, A. Yokoseki, M. Wada, et al., Vegetable and fruit intake frequency and mortality in patients with and without chronic kidney disease: a hospital-based cohort study, J. Ren. Nutr. 33 (4) (2023) 566–574, https://doi.org/10.1053/j.jrn.2023.01.011.

[38] P.F. Saint-Maurice, R.P. Troiano, D.R. Bassett Jr., et al., Association of daily step count and step intensity with mortality among US adults, JAMA 323 (12) (2020) 1151–1160, https://doi.org/10.1001/jama.2020.1382.

[39] A. Kuroki, N. Sugita, S. Komatsu, et al., The number of remaining teeth as a risk indicator of cognitive impairment: a cross-sectional clinical study in Sado Island, Clin. Exp. Dent Res. 4 (6) (2018) 291–296, https://doi.org/10.1002/cre2.147.
[40] S. Ishikawa, T. Konta, S. Susa, et al., Association between presence of 20 or more natural teeth and all-cause, cancer-related, and cardiovascular disease-related mortality: yamagata (Takahata) prospective observational study, BMC Oral Health 20 (1) (2020) 353, https://doi.org/10.1186/s12903-020-01346-6.
[41] K. Takamisawa, N. Sugita, S. Komatsu, et al., Association between serum IgG antibody titers against Porphyromonas gingivalis and liver enzyme levels: a cross-sectional study in Sado Island, Heliyon 6 (11) (2020) e05531, https://doi.org/10.1016/j.heliyon.2020.e05531, 2020.
[42] C. Fleury, M. Neverova, S. Collins, et al., Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia, Nat. Genet. 15 (3) (1997) 269–272, https://doi.org/10.1038/ng0397-269.
[43] M. Hirose, P. Schilf, F. Lange, et al., Uncoupling protein 2 protects mice from aging, Mitochondrion 30 (2016) 42–50, https://doi.org/10.1016/j.mito.2016.06.004.
[44] L. Zhu, H. Xie, Q. Liu, F. Ma, H. Wu, Klotho inhibits H2 O2 -induced oxidative stress and apoptosis in periodontal ligament stem cells by regulating UCP2 expression, Clin. Exp. Pharmacol. Physiol. 48 (10) (2021) 1412–1420, https://doi.org/10.1111/1440-1681.13547.
[45] B. Buemann, B. Schierning, S. Toubro, et al., The association between the val/ala-55 polymorphism of the uncoupling protein 2 gene and exercise efficiency, Int. J. Obes. Relat. Metab. Disord. 25 (4) (2001) 467–471, https://doi.org/10.1038/sj.ijo.0801564.
[46] X. Yu, S. Wieczorek, A. Franke, et al., Association of UCP2 -866 G/A polymorphism with chronic inflammatory diseases, Gene Immun. 10 (6) (2009) 601–605, https://doi.org/10.1038/gene.2009.29.
[47] R.E. Gimeno, M. Dembski, X. Weng, et al., Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis, Diabetes 46 (5) (1997) 900–906, https://doi.org/10.2337/diab.46.5.900.
[48] D. Arsenijevic, H. Onuma, C. Pecqueur, et al., Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production, Nat. Genet. 26 (4) (2000) 435–439, https://doi.org/10.1038/82565.
[49] H. Bo, N. Jiang, G. Ma, et al., Regulation of mitochondrial uncoupling respiration during exercise in rat heart: role of reactive oxygen species (ROS) and uncoupling protein 2, Free Radic. Biol. Med. 44 (7) (2008) 1373–1381, https://doi.org/10.1016/j.freeradbiomed.2007.12.033.
[50] G. Rose, P. Crocco, F. De Rango, A. Montesanto, G. Passarino, Further support to the uncoupling-to-survive theory: the genetic variation of human UCP genes is associated with longevity, PLoS One 6 (12) (2011) e29650, https://doi.org/10.1371/journal.pone.0029650.
[51] Y. Zhou, D. Simmons, B.D. Hambly, C.S. McLachlan, Interactions between UCP2 SNPs and telomere length exist in the absence of diabetes or pre-diabetes, Sci. Rep. 6 (2016) 33147, https://doi.org/10.1038/srep33147.
[52] Q. Ji, H. Ikegami, T. Fujisawa, et al., A common polymorphism of uncoupling protein 2 gene is associated with hypertension, J. Hypertens. 22 (1) (2004) 97–102, https://doi.org/10.1097/00004872-200401000-00018.
[53] K. Kosuge, M. Soma, T. Nakayama, et al., Human uncoupling protein 2 and 3 genes are associated with obesity in Japanese, Endocrine 34 (1–3) (2008) 87–95, https://doi.org/10.1007/s12020-008-9111-9.
[54] M. Oliva, M. Muñoz-Aguirre, S. Kim-Hellmuth, et al., The impact of sex on gene expression across human tissues, Science 369 (6509) (2020) eaba3066, https://doi.org/10.1126/science.aba3066.
[55] Cognuck S. Quirós, W.L. Reis, M. Silva, et al., Sex differences in body composition, metabolism-related hormones, and energy homeostasis during aging in Wistar rats, Phys. Rep. 8 (20) (2020 Oct) e14597, https://doi.org/10.14814/phy2.14597.
[56] D.N. Di Florio, J. Sin, M.J. Coronado, P.S. Atwal, Sex differences in inflammation, redox biology, mitochondria and autoimmunity, Redox Biol. 31 (2020) 101482, https://doi.org/10.1016/j.redox.2020.101482.
[57] S. Kim, L. Myers, E. Ravussin, K.E. Cherry, S.M. Jazwinski, Single nucleotide polymorphisms linked to mitochondrial uncoupling protein genes UCP2 and UCP3 affect mitochondrial metabolism and healthy aging in female nonagenarians, Biogerontology 17 (4) (2016) 725–736, https://doi.org/10.1007/s10522-016-9643-y.
[58] O. Boss, S. Samec, A. Paoloni-Giacobino, et al., Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression, FEBS Lett. 408 (1) (1997) 39–42, https://doi.org/10.1016/s0014-5793(97)00384-0.