Mitochondrial Haplogroups

Ischemic Cardiovascular Disease, Other Diseases, Mortality, and Longevity in the General Population

Marianne Benn, MD, PhD; Marianne Schwartz, MSc, PhD;
Børge G. Nordestgaard, MD, DMSc; Anne Tybjærg-Hansen, MD, DMSc

Background—Rare mutations in the mitochondrial genome may cause disease. Mitochondrial haplogroups defined by common polymorphisms have been associated with risk of disease and longevity. We tested the hypothesis that common haplogroups predict risk of ischemic cardiovascular disease, morbidity from other causes, mortality, and longevity in a general population of European descent.

Methods and Results—We followed 9254 individuals from the Danish general population, in the Copenhagen City Heart Study, prospectively for risk of ischemic cardiovascular disease, morbidity from other causes, and mortality during 25 and 11 years, respectively. Haplogroup frequencies were as follows: H (45.9%), U (15.9%), T (9.9%), J (9.1), K (6.2%), V (4.5%), W/I (3.8%), and Z (3.5%). Hazard ratios for hospitalization due to all cardiovascular disorders (haplogroup U: 1.0 [95% confidence interval{CI}, 0.9 to 1.1]; T: 0.9 [95% CI, 0.8 to 1.0]; J: 1.0 [95% CI, 0.9 to 1.1]; K: 1.0 [95% CI, 0.9 to 1.2]; V: 1.0 [95% CI, 0.9 to 1.2]; W/I: 0.8 [95% CI, 0.7 to 1.0]; Z: 1.0 [95% CI, 0.8 to 1.2]), ischemic heart disease (U: 0.9 [95% CI, 0.8 to 1.1]; T: 0.9 [95% CI, 0.7 to 1.0]; J: 1.1 [95% CI, 0.9 to 1.2]; K: 1.1 [95% CI, 0.9 to 1.3]; V: 1.1 [95% CI, 0.9 to 1.4]; W/I: 1.1 [95% CI, 0.8 to 1.4]; Z: 1.1 [95% CI, 0.8 to 1.4]), and ischemic cerebrovascular disease (U: 1.1 [95% CI, 0.9 to 1.4]; T: 0.9 [95% CI, 0.7 to 1.2]; J: 1.1 [95% CI, 0.9 to 1.4]; K: 1.0 [95% CI, 0.8 to 1.4]; V: 1.1 [95% CI, 0.8 to 1.5]; W/I: 0.8 [95% CI, 0.5 to 1.3]; Z: 0.9 [95% CI, 0.6 to 1.4]) did not differ from 1.0 for any haplogroup versus the most common haplogroup H. Results were similar for hospitalization due to infectious and parasitic diseases, respiratory infections, respiratory disorders, malignant neoplasms, digestive disorders, musculoskeletal disorders, neuropsychiatric disorders, and miscarriages. Likewise, hazard ratios for death from all causes were not different from 1.0 for any haplogroup versus haplogroup H (U: 1.0 [95% CI, 0.8 to 1.1]; T: 0.9 [95% CI, 0.8 to 1.1]; J: 0.9 [95% CI, 0.8 to 1.1]; K: 1.0 [95% CI, 0.8 to 1.2]; V: 1.1 [95% CI, 0.9 to 1.3]; W/I: 0.8 [95% CI, 0.7 to 1.1]; Z: 0.9 [95% CI, 0.7 to 1.2]). Finally, after stratification by major causes of death, hazard ratios remained insignificant.

Conclusions—Our results do not support an association of mitochondrial haplogroups with risk of ischemic cardiovascular disease, morbidity from other causes, mortality, or longevity in a large general population of European descent. **(*Circulation*. 2008;117:2492-2501.)**

Key Words: cardiovascular diseases ■ cerebrovascular disorders ■ genetics ■ morbidity ■ mortality

Mitochondria originate from aerobic bacteria that lived in exosymbiosis with primordial eukaryotic cells 2 to 3 billion years ago.[1] The primordial eukaryotic cell lacked the ability to use oxygen metabolically, and the aerobic bacteria colonized the eukaryotic cell in an endosymbiosis that became permanent. When the aerobic bacteria were integrated into the eukaryotic cells, they brought their own genome, but through evolution the major part of this has been internalized into the genome of the eukaryotic host cell, and today the mitochondrial genome contains only 37 genes.[2] The mitochondria perform pyruvate oxidation and the citric acid cycle and generate energy as ATP by means of the electron transport chain and oxidative phosphorylation. Of the proteins directly needed for the electron transport chain and oxidative phosphorylation, only 13 are encoded by the mitochondrion itself, and ≈80 proteins are coded by the nucleus and subsequently imported from the cytoplasm into the mitochondria.

Editorial p 2431
Clinical Perspective p 2501

Mitochondrial DNA is maternally inherited (for a unique exception, see Schwartz and Vissing[3]) and therefore under-

Received November 30, 2007; accepted February 22, 2008.

From the Departments of Clinical Biochemistry (M.B., A.T.-H.) and Clinical Genetics (M.S.), Rigshospitalet, Copenhagen University Hospital; Department of Clinical Biochemistry, Herlev University Hospital (B.G.N.); and the Copenhagen City Heart Study, Bispebjerg University Hospital (B.G.N., A.T.-H.); all Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

The online-only Data Supplement, which contains morbidity and mortality data, is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.107.756809/DC1.

Correspondence to Anne Tybjærg-Hansen, MD, DMSc, Department of Clinical Biochemistry KB3011, Section for Molecular Genetics, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. E-mail at-h@rh.regionh.dk

© 2008 American Heart Association, Inc.

Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.107.756809

Downloaded from http://ahajournals.org by on March 4, 2022

goes negligible intermolecular recombination. Mutations acquired throughout human history have subdivided the human population into a number of discrete mitochondrial clades or haplogroups.[4] The haplogroups exhibit region-specific sequence variation in indigenous populations, a phenomenon that has been attributed to genetic drift and/or possibly climatic selection.[5–7]

More than 75 human diseases have been associated with rare mutations in the mitochondrial genome, mutations that either impair mitochondrial protein synthesis or impair proteins encoded by the mitochondrial genome.[8] Recently, numerous reports have suggested a role for mitochondrial haplogroups, defined by the presence or absence of common polymorphisms, in the pathogenesis of ischemic cardiovascular disease and other diseases, as well as in longevity.[9,10] However, other reports could not confirm such associations,[10–12] emphasizing the need for large, prospective population-based studies to clarify this. Several studies have speculated that mitochondrial haplogroups are associated with differences in the amount of superoxide and other reactive oxygen species produced in the electron transport chain. This could lead to differences in oxidative stress on cells and thereby to differences in morbidity, mortality, and longevity between individuals with different haplogroups.[9,10]

We tested the hypothesis that mitochondrial haplogroups predict risk of ischemic cardiovascular disease, morbidity from other causes, mortality, and longevity in the general population. For this purpose, we genotyped 9254 individuals from the Danish general population, in the Copenhagen City Heart Study, for the 8 most common haplogroups in European populations. Individuals were followed prospectively for morbidity and mortality for 25 and 11 years, respectively.

Downloaded from http://ahajournals.org by on March 4, 2022

Methods

Participants

The Copenhagen City Heart Study is a prospective study of the Danish general population initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994, and 2001–2003, including individuals randomly selected on the basis of the national Danish Civil Registration System to reflect the adult general population. All 4 examinations included a self-administered questionnaire, a physical examination, and blood samples.[13] Participants in this study were those who gave blood for DNA extraction. Individuals were followed from baseline, defined as the first examination in which they participated, until the occurrence of disease, death, or the beginning of 2004, whichever came first. Participants were followed with their unique Central Person Register number, and follow-up was 100% complete. Median follow-up was 25 years for studies on morbidity and 11 years for studies on mortality. More than 99% of participants were of Northern European descent. The Danish Research Ethics Committee for Copenhagen and Frederiksberg approved this study (No. KF version 100.2039/91). All participants gave written informed consent.

End Points

We collected information on morbidity and mortality on all participants (n=9254) from 4 population registries, as follows: (1) Information on morbidity was obtained from the national Danish Patient Registry and was subdivided according to the Global Burden of Disease classification[14]; (2) information on diagnosis of invasive cancer was from the national Danish Cancer Registry[15]; (3) information on cause-specific mortality was from the national Danish Causes of Death Registry[16] and was subdivided according to the Global Burden of Disease classification[14]; and (4) information on any death was from the national Danish Civil Registration System. Diagnoses according to the World Health Organization *International Classification of Diseases, Eighth Revision* and *International Statistical Classification of Diseases, 10th Revision* codes are given in the online-only Data Supplement.

Cardiovascular disorders included chronic rheumatic heart disease, hypertension, ischemic cardiovascular disease, pericarditis, myocarditis, cardiomyopathy, and pulmonary heart disease (World Health Organization *International Classification of Diseases, Eighth Revision* and *International Statistical Classification of Diseases, 10th Revision* codes 390 to 458 and I00 to I99, respectively). Ischemic cardiovascular disease was ischemic heart disease (myocardial infarction and angina pectoris; codes 410 to 414 and I20 to I25, respectively) and ischemic cerebrovascular disease (ischemic stroke, transient ischemic attack, and amaurosis fugax; codes 430 to 437, I63, I65 to I66, and G45). Information on diagnosis of ischemic heart disease was verified by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry and all causes of death entered in the national Danish Causes of Death Registry and medical records from hospitals and general practitioners. Ischemic heart disease was myocardial infarction or characteristic symptoms of angina pectoris based on location, character, and duration of pain and the relation of pain to exercise.[17] A diagnosis of myocardial infarction required the presence of at least 2 of the following criteria: characteristic chest pain, elevated cardiac enzymes, and ECG changes indicative of myocardial infarction.

Experienced neurologists reviewed all potential cases of ischemic cerebrovascular disease, ie, amaurosis fugax (transient blindness in 1 eye only), transient ischemic attack (focal neurological symptoms lasting <24 hours), or ischemic stroke (acute disturbance of focal or global cerebral function with symptoms lasting ≥24 hours or leading to death with presumably no other reason than of vascular origin). Computed tomography or magnetic resonance imaging scan, autopsy, spinal fluid examination, or surgical description distinguished between infarction, intracerebral hemorrhage, and subarachnoid hemorrhage.

Baseline Examination and Laboratory Measures

Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medication. Body mass index was expressed as weight in kilograms divided by height in meters squared. Smokers were defined as current smokers. Diabetes mellitus was defined as a registered diagnosis in the national Danish Patient Registry, self-reported disease, use of antidiabetic drugs, or a nonfasting plasma glucose >11 mmol/L. Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides. Low-density lipoprotein cholesterol was calculated as total cholesterol−high-density lipoprotein cholesterol−(triglycerides×0.45) (all in mmol/L).

Genotype Determination

Genotyping (mtDNA reference sequence, NCBIgi:113200490: mt7028t>c, mt10398a>g, mt11719g>a, nt12308a>g, mt12612a>g, and mt15607a>g) was determined by TaqMan assays (Applied Biosystems Inc, Foster City, Calif). Primers, TaqMan probes, and polymerase chain reaction conditions are available from the authors on request.

Statistical Analysis

Data were analyzed with Stata 9.2. $P<0.05$ on a 2-sided test was considered significant. Probability values were corrected for multiple comparisons by the Bonferroni method (required significance level=0.05/number of comparisons). This changed the required significance levels from <0.05 to <0.004 for risk factors for ischemic cardiovascular disease (0.05/12=0.004; Table 1), <0.0003 for morbidity (0.05/7×22; Table 2), and <0.001 for mortality (0.05/7×7; Table 3). ANOVA and Pearson χ^2 test compared continuous variables and categorical variables between mitochondrial haplogroups, respectively.

With the use of left truncation (or delayed entry), Cox proportional hazards regression models with age as time scale and adjusted for

Table 1. Risk Factors for Ischemic Cardiovascular Disease in Subjects From the General Population by Mitochondrial Haplogroups

	Mitochondrial Haplogroup								
	H	U	T	J	K	V	W/I	Z	*P*
No. of individuals (%)	4244 (45.9)	1469 (15.9)	912 (9.9)	843 (9.1)	571 (6.2)	412 (4.5)	350 (3.8)	324 (3.5)	...
Women/men	2341/1903	795/674	535/377	457/386	313/258	220/192	203/147	178/146	...
Age, y	57.9±15.2	57.1±15.0	58.3±15.0	57.5±15.0	56.8±15.1	57.6±16.1	56.3±15.5	56.0±15.8	0.07
Total cholesterol, mmol/L	6.2±1.3	6.1±1.3	6.2±1.3	6.2±1.3	6.1±1.3	6.1±1.3	6.2±1.5	6.0±1.2	0.68
LDL cholesterol, mmol/L	3.8±1.2	3.7±1.1	3.8±1.2	3.8±1.2	3.7±1.2	3.7±1.1	3.7±1.2	3.7±1.1	0.78
HDL cholesterol, mmol/L	1.6±0.5	1.6±0.5	1.6±0.5	1.6±0.5	1.6±0.5	1.5±0.5	1.6±0.5	1.5±0.5	0.46
Triglycerides, mmol/L	1.9±1.3	1.9±2.3	1.9±1.5	1.9±1.6	1.8±1.2	2.0±1.4	2.1±3.2	1.9±1.5	0.22
Systolic blood pressure, mm Hg	139±22	138±22	139±23	138±23	137±21	137±24	136±21	135±21	0.07
Diastolic blood pressure, mm Hg	84±12	83±13	84±13	84±12	83±12	83±13	82±11	83±12	0.08
Body mass index, kg/m^2	25.6±4.3	25.7±4.4	25.7±4.4	25.6±4.3	25.7±4.2	25.3±4.3	25.3±4.1	25.8±4.3	0.61
Alcohol, U/wk	16.4±21.6	17.3±22.4	15.7±20.3	16.5±19.4	16.9±21.7	15.9±20.2	15.3±20.5	14.2±16.5	0.31
Hypertension, %	2344 (56)	792 (54)	502 (55)	433 (52)	297 (53)	209 (51)	170 (49)	156 (49)	0.04
Diabetes mellitus, %	161 (3.4)	76 (5.2)	29 (3.2)	52 (6.2)	26 (4.6)	19 (4.6)	11 (3.1)	14 (4.3)	0.02
Smokers, %	2081 (49)	727 (50)	429 (47)	410 (49)	283 (50)	196 (48)	157 (46)	156 (49)	0.84

Values are number of individuals (%) or mean±SD. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein. One unit of alcohol=12 g. Bonferroni corrected *P*: <0.05/12=<0.004.

gender (or multivariably) estimated hazard ratios for morbidity and mortality as a function of haplogroups. Age as time scale implies that age is automatically adjusted for. Multivariable adjustment was for gender, age, total cholesterol, body mass index, alcohol consumption, hypertension, diabetes mellitus, smoking, and, for women, menopausal status and use of hormone replacement therapy. Hazard ratios for all cardiovascular disorders, ischemic cardiovascular disease, ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease, and ischemic stroke were in addition adjusted for low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. For multivariable adjustments, we used time-dependent covariates from the 1976–1978, 1981–1983, 1991–1994, and 2001–2003 examinations. Proportionality of hazards over time was tested to ensure that this assumption of the Cox proportional hazards regression was fulfilled on the basis of Schoenfeld residuals. For each analysis, individuals with end points developed before study entry was excluded. Survival curves and log-rank tests compared cumulative incidences of death as a function of age and haplogroups.

Power was calculated with the NCSS-PASS programs and estimated with the use of $\alpha=0.05$ ($P=0.05$), the size of the sample population (ie, 9254 excluding nonincident events), the frequency of the event (morbidity or mortality) in the sample population, and the frequencies of the haplogroups. For comparison with other studies, power was estimated for a given hazard ratio, or hazard ratio was estimated for a given power. Power calculations were not adjusted for multiple testing (corrected type I error rate), and the adjusted power would therefore be lower and the adjusted hazard ratios higher than those given in the Discussion.

All authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Frequencies of Polymorphisms and Haplogroups

Polymorphisms defining haplogroups represented in the Danish population had minor allele frequencies ranging from 0.10 to 0.49 (Figure 1A, left, and 1B). All 6 polymorphisms are located in coding regions; however, 12308a>g is not transcribed and 7028t>c(A341A), 11719a>g(G320G), 12612a>g(V92V), and 15607a>g(K287K) do not result in amino acid substitutions. Only 10398a>g(A114T) changes an amino acid but at a position that is not conserved between orthologous genes (Figure 1A, right). Haplogroup frequencies were 45.9% for haplogroup H, 15.9% for U, 9.9% for T, 9.1% for J, 6.2% for K, 4.5% for V, 3.8% for W/I, and 3.5% for Z. Individuals with combinations of polymorphisms not included in the known European haplogroups (n=126) were excluded from further analyses; among these were 7 individuals who were heteroplasmic verified by sequencing of 2 independent blood samples. With the use of minor allele frequencies of the polymorphisms as a measure of time since they were introduced into the population, a phylogenetic tree was constructed (Figure 1B).

Risk Factors for Ischemic Cardiovascular Disease

Risk factors for ischemic cardiovascular disease of subjects from the general population stratified by mitochondrial haplogroups are shown in Table 1. No convincing associations were found between mitochondrial haplogroups or polymorphisms and major risk factors for ischemic cardiovascular disease, and none remained significant after Bonferroni correction for multiple comparisons (Table 1 and data not shown).

Morbidity From Ischemic Cardiovascular Disease and Other Diseases

Multivariably adjusted hazard ratios for hospitalization due to ischemic cardiovascular disease, ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease, and ischemic stroke did not differ from 1.0 for any of the haplogroups versus the most common haplogroup H (Figure 2 and Table 2). Similar results were seen for hospitalization due to infectious and parasitic diseases, respiratory infections, respiratory disorders, all cardiovascular disorders, malignant neoplasms, digestive disorders, musculoskeletal disorders, neuropsychiatric disorders, and miscarriages (Table 2). However, we observed discrete and nonsystematic deviations for

Downloaded from http://ahajournals.org by on March 4, 2022

Table 2. Morbidity Leading to Hospitalization by Mitochondrial Haplogroups During 25-Year Follow-Up of 9254 Individuals From the General Population

	Mitochondrial Haplogroup							
	H (45.9%)	U (15.9%)	T (9.9%)	J (9.1%)	K (6.2%)	V (4.5%)	W/I (3.8%)	Z (3.5%)
Cardiovascular disorders, all (No. of events=3459)	1.0	1.0 (0.9–1.1)	0.9 (0.8–1.0)	1.0 (0.9–1.1)	1.0 (0.9–1.2)	1.0 (0.9–1.2)	0.8 (0.7–1.0)[a]	1.0 (0.8–1.2)
	n=1654	n=536	n=343	n=319	n=218	n=159	n=112	n=118
Ischemic cardiovascular disease (No. of events=1791)	1.0	0.9 (0.8–1.1)	0.9 (0.7–1.0)	1.1 (0.9–1.2)	1.1 (0.9–1.3)	1.1 (0.9–1.4)	1.1 (0.8–1.4)	1.1 (0.8–1.4)
	n=841	n=273	n=166	n=175	n=119	n=93	n=59	n=65
Ischemic heart disease (No. of events=1451)	1.0	1.0 (0.8–1.1)	0.9 (0.7–1.1)	1.0 (0.8–1.2)	1.1 (0.9–1.4)	1.1 (0.9–1.4)	0.9 (0.7–1.2)	1.2 (0.9–1.6)
	n=678	n=220	n=136	n=143	n=94	n=76	n=48	n=57
Myocardial infarction (No. of events=722)	1.0	1.1 (0.8–1.3)	0.9 (0.7–1.2)	1.1 (0.8–1.4)	1.1 (0.8–1.5)	1.0 (0.7–1.5)	0.8 (0.5–1.3)	0.9 (0.6–1.5)
	n=337	n=118	n=67	n=78	n=43	n=37	n=20	n=22
Ischemic cerebrovascular disease (No. of events=548)	1.0	1.1 (0.9–1.4)	0.9 (0.7–1.2)	1.1 (0.9–1.4)	1.0 (0.8–1.4)	1.1 (0.8–1.5)	0.8 (0.5–1.3)	0.9 (0.6–1.4)
	n=264	n=81	n=48	n=49	n=39	n=38	n=16	n=13
Ischemic stroke (No. of events=439)	1.0	0.9 (0.7–1.2)	0.9 (0.6–1.2)	1.0 (0.7–1.4)	1.2 (0.8–1.7)	1.4 (0.9–2.1)	0.8 (0.4–1.6)	0.7 (0.4–1.4)
	n=210	n=63	n=38	n=42	n=31	n=32	n=12	n=11
Infectious and parasitic diseases (No. of events=1057)	1.0	0.9 (0.8–1.1)	1.0 (0.9–1.3)	1.1 (0.9–1.4)	1.0 (0.8–1.3)	1.2 (0.9–1.6)	1.1 (0.8–1.5)	0.9 (0.8–1.1)
	n=487	n=151	n=112	n=107	n=66	n=56	n=43	n=35
Respiratory infections (No. of events=1274)	1.0	0.9 (0.8–1.1)	1.1 (1.0–1.4)	1.2 (1.0–1.5)*	0.8 (0.6–1.1)	1.1 (0.8–1.4)	1.0 (0.8–1.4)	1.1 (0.8–1.5)
	n=587	n=181	n=144	n=140	n=64	n=64	n=48	n=46
Respiratory disorders (No. of events=1450)	1.0	1.0 (0.8–1.1)	0.9 (0.8–1.1)	0.9 (0.8–1.1)	1.0 (0.8–1.2)	1.0 (0.7–1.2)	1.2 (1.0–1.6)	0.9 (0.7–1.3)
	n=692	n=226	n=140	n=129	n=88	n=64	n=66	n=45
Malignant neoplasms, all (No. of events=1728)	1.0	0.9 (0.8–1.0)	0.9 (0.8–1.1)	1.1 (0.9–1.2)	0.9 (0.7–1.1)	1.0 (0.8–1.2)	1.0 (0.8–1.3)	0.9 (0.7–1.2)
	n=840	n=263	n=164	n=170	n=92	n=83	n=65	n=51
Gastrointestinal cancer (No. of events=328)	1.0	1.2 (0.9–1.7)	1.0 (0.7–1.5)	0.9 (0.6–1.4)	0.6 (0.3–1.1)	1.3 (0.8–2.1)	0.9 (0.5–1.7)	1.0 (0.5–1.8)
	n=152	n=62	n=34	n=27	n=12	n=20	n=11	n=10
Hematologic cancer (No. of events=90)	1.0	0.4 (0.2–0.8)[a]	1.1 (0.6–2.1)	0.6 (0.3–1.4)	0.5 (0.1–1.5)	...	0.7 (0.2–2.4)	0.8 (0.3–2.7)
	n=53	n=6	n=13	n=6	n=3	n=2	n=3	n=4
Respiratory cancer (No. of events=222)	1.0	0.8 (0.5–1.2)	1.0 (0.7–1.6)	1.0 (0.6–1.6)	1.3 (0.8–2.2)	1.1 (0.6–2.0)	1.3 (0.7–2.7)	0.4 (0.1–1.2)
	n=107	n=29	n=23	n=21	n=18	n=12	n=9	n=3
Urologic cancer (No. of events=160)	1.0	1.1 (0.7–1.7)	0.7 (0.4–1.4)	0.9 (0.5–1.6)	1.1 (0.6–2.1)	2.0 (1.1–3.6)*	1.2 (0.5–2.7)	1.7 (0.9–3.5)
	n=71	n=25	n=11	n=12	n=10	n=15	n=7	n=9
Female cancer (No. of events=373)	1.0	0.8 (0.6–1.1)	0.7 (0.5–1.0)	1.3 (0.9–1.7)	0.7 (0.5–1.2)	0.8 (0.5–1.5)	1.0 (0.6–1.6)	1.0 (0.6–1.7)
	n=184	n=53	n=30	n=46	n=18	n=14	n=15	n=13
Male cancer (No. of events=107)	1.0	1.6 (1.0–2.5)	0.8 (0.4–1.6)	1.2 (0.6–2.2)	0.8 (0.3–1.9)	0.5 (0.2–1.6)	...	0.3 (0.0–2.0)
	n=52	n=26	n=8	n=12	n=5	n=3	n=0	n=1
Melanomas and skin cancers (No. of events=563)	1.0	0.8 (0.6–1.0)	1.0 (0.7–1.3)	1.2 (0.9–1.5)	1.0 (0.7–1.4)	0.9 (0.6–1.4)	1.2 (0.8–1.9)	1.1 (0.7–1.7)
	n=267	n=74	n=56	n=60	n=34	n=24	n=28	n=20
Digestive disorders (No. of events=1696)	1.0	1.0 (0.9–1.1)	1.0 (0.9–1.1)	1.0 (0.9–1.2)	0.9 (0.8–1.1)	1.0 (0.8–1.2)	1.1 (0.9–1.3)	1.0 (0.8–1.3)
	n=823	n=267	n=153	n=148	n=110	n=76	n=62	n=57
Diabetes mellitus, types I and II (No. of events=681)	1.0	1.2 (0.9–1.4)	0.8 (0.6–1.1)	1.3 (1.0–1.7)*	1.2 (0.9–1.7)	1.4 (1.0–1.9)	1.0 (0.6–1.6)	1.5 (1.0–2.2)*
	n=293	n=119	n=53	n=80	n=43	n=38	n=22	n=33
Musculoskeletal disorders (No. of events=2663)	1.0	1.0 (0.9–1.1)	1.1 (1.0–1.2)	1.0 (0.9–1.1)	1.1 (1.0–1.3)	1.0 (0.9–1.2)	1.0 (0.8–1.2)	1.0 (0.8–1.2)
	n=1240	n=425	n=276	n=254	n=156	n=121	n=101	n=90
Neuropsychiatric disorders (No. of events=3430)	1.0	1.0 (0.8–1.1)	0.8 (0.7–1.0)*	0.9 (0.8–1.1)	1.0 (0.8–1.3)	0.9 (0.7–1.2)	1.0 (0.8–1.3)	1.0 (0.8–1.3)
	n=1597	n=520	n=370	n=319	n=228	n=156	n=127	n=113
Miscarriage (No. of events=96)	1.0	1.0 (0.3–1.8)	1.0 (0.5–1.9)	1.0 (0.5–2.1)	0.7 (0.2–1.8)	1.3 (0.5–3.0)	0.7 (0.2–2.3)	0.9 (0.3–2.8)
	n=47	n=14	n=10	n=9	n=4	n=6	n=3	n=3

Values are hazard ratio (95% CI); n=number of events in haplogroup. All hazard ratios were adjusted for gender, age, total cholesterol, body mass index, alcohol consumption, hypertension, diabetes mellitus, smoking, and, for women, menopausal status and use of hormone replacement therapy. Hazard ratios for cardiovascular disorders and subgroups thereof were in addition adjusted for low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. For diagnoses, see the online-only Data Supplement.

*$0.01<P<0.05$. Bonferroni-corrected P: $<0.05/22\times7=<0.0003$.

Downloaded from http://ahajournals.org by on March 4, 2022

Table 3. Mortality According to Mitochondrial Haplogroups During 11-Year Follow-Up of 9254 Individuals From the General Population

	Mitochondrial Haplogroup							
	H (45.9%)	U (15.9%)	T (9.9%)	J (9.1%)	K (6.2%)	V (4.5%)	W/I (3.8%)	Z (3.5%)
Death from any cause	1.0	1.0 (0.8–1.1)	0.9 (0.8–1.1)	0.9 (0.8–1.1)	1.0 (0.8–1.2)	1.1 (0.9–1.3)	0.8 (0.7–1.1)	0.9 (0.7–1.2)
(No. of events=2246)	n=1076	n=344	n=222	n=201	n=139	n=121	n=69	n=74
Cardiovascular disorders	1.0	1.0 (0.8–1.3)	0.8 (0.6–1.1)	1.0 (0.8–1.4)	0.9 (0.7–1.3)	0.8 (0.6–1.2)	0.7 (0.4–1.3)	1.2 (0.7–1.9)
(No. of events=608)	n=293	n=102	n=55	n=53	n=39	n=30	n=16	n=20
Infectious and parasitic diseases	1.0	0.8 (0.3–2.7)	2.2 (0.7–7.3)	1.3 (0.4–4.5)	1.3 (0.1–11.3)	2.1 (0.4–11.9)	...	1.3 (0.3–5.6)
(No. of events=55)	n=27	n=7	n=7	n=7	n=2	n=2	n=0	n=3
Respiratory disorders	1.0	0.7 (0.4–1.3)	0.7 (0.4–1.4)	1.1 (0.6–2.1)	0.8 (0.2–2.8)	1.2 (0.6–2.4)	0.9 (0.3–2.3)	0.5 (0.2–1.4)
(No. of events=137)	n=62	n=21	n=13	n=14	n=4	n=12	n=5	n=6
Malignant neoplasm	1.0	0.9 (0.7–1.1)	1.1 (0.8–1.4)	1.0 (0.7–1.3)	0.8 (0.6–1.1)	0.9 (0.6–1.3)	1.2 (0.7–2.0)	0.9 (0.6–1.5)
(No. of events=591)	n=292	n=89	n=55	n=49	n=41	n=27	n=20	n=18
Digestive disorders	1.0	0.8 (0.3–2.1)	0.5 (0.2–1.4)	1.1 (0.3–3.5)	1.9 (0.3–10.8)	1.2 (0.4–3.2)	0.9 (0.2–5.0)	1.0 (0.1–10.1)
(No. of events=67)	n=34	n=9	n=7	n=6	n=2	n=6	n=2	n=1
Other causes of death than above	1.0	1.0 (0.8–1.3)	1.0 (0.8–1.3)	0.9 (0.7–1.2)	0.9 (0.7–1.2)	0.8 (0.6–1.2)	1.0 (0.7–1.5)	0.9 (0.6–1.4)
(No. of events=788)	n=368	n=116	n=85	n=72	n=51	n=44	n=26	n=26

Values are hazard ratio (95% CI); n=number of events in haplogroup. All hazard ratios were adjusted for gender, age, total cholesterol, body mass index, alcohol consumption, hypertension, diabetes mellitus, smoking, and, for women, menopausal status and use of hormone replacement therapy. Hazard ratios for cardiovascular disorders were in addition adjusted for low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. Results were not significant at $P<0.05$. For diagnoses, see the online-only Data Supplement.

cardiovascular disorders, respiratory infections, hematologic cancer, urologic cancer, diabetes mellitus, and neuropsychiatric disorders ($0.01<P<0.05$), which all became nonsignificant when Bonferroni corrected for multiple comparisons (Table 2). Unadjusted hazard ratios and gender-specific hazard ratios were similar (data not shown). Analyzing data for each of the 6 polymorphisms separately (Figure 1A, left column) also did not identify any associations with morbidity (data not shown).

Mortality

Multivariably adjusted hazard ratios for death from all causes were not different from 1.0 for any of the haplogroups (Table 3). Unadjusted hazard ratios and gender-specific hazard ratios were similar (data not shown). After stratification by major causes of death, hazard ratios remained nonsignificant (Table 3). On survival plots, longevity as a function of age did not differ when we compared haplogroups with the most common haplogroup H (median survival [95% CI], 79 years [78 to 80]; overall log rank, $P=0.42$). Apparent differences in longevity were found between the V haplogroup with the shortest median survival (78 years [75 to 80]) and the W/I and T haplogroups with the longest median survival (84 years [80 to 85] and 81 years [79 to 83], respectively, by log-rank tests) ($P=0.01$ and 0.03, respectively) (Figure 3). After correction for multiple comparisons by the Bonferroni method, these differences were no longer significant.

Discussion

In this large prospective study of a general population of Northern European descent, no evidence was found for consistent and robust associations between mitochondrial haplogroups and risk of ischemic cardiovascular disease, morbidity from other causes, or mortality. In contrast, several smaller case-control studies have shown associations between mitochondrial haplogroups and myocardial infarction, cerebral infarction, cancer, diabetes mellitus, and neurodegenerative diseases.[18–36]

The Asian N9b haplogroup (defined by polymorphisms at positions mt5147 and mt16519) has been reported to protect against myocardial infarction in Japanese men (odds ratio [95% CI], 0.2 [0.1 to 0.5]; 920 cases/522 controls) but not in Japanese women (695 cases/434 controls).[19] It has been speculated that this reduction in risk of myocardial infarction could be due to a reduction in the production of superoxide and other reactive oxygen species associated with this particular haplogroup[19] that thus might confer resistance to myocardial infarction.[19,37] In another Japanese study, haplogroup A (defined by polymorphism at position mt663) has been suggested to be associated with increased risk of atherothrombotic cerebral infarction in women (odds ratio [95% CI], 2.1 [1.0 to 4.2]; 112 cases/328 controls) but not in men (234 cases/407 controls).[18] In the present study, we did not find any associations between the 8 common haplogroups examined or any of the 6 polymorphisms defining these haplogroups and risk of ischemic cardiovascular disease, even when we further subdivided the diagnostic group into ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease, and ischemic stroke. In the present study, we have 80% power to exclude a hazard ratio of ≥ 1.1 or ≤ 0.8 (ie, protective) for risk of ischemic heart disease and 1.3 and 0.7 for ischemic cerebrovascular disease for the 4 most common haplogroups H, U, T, and J (80% of the population), equivalent to the odds ratios found in previous studies.[18,19]

Several haplogroups or polymorphisms have been associated with risk of cancer in case-control studies. The mt10398a>g polymorphism present in European haplogroups J, K, and Z has been associated with increased risk of invasive breast cancer in black women (48 cases/54 controls and validated in 654 cases/605 controls) but not in white women (879 cases/760 controls).[20] The mt16189t>c polymorphism (defining a subgroup of haplogroup T) has been associated with risk of endometrial cancer in

Downloaded from http://ahajournals.org by on March 4, 2022

A

Mt	Rare allele frequency (%)	Mitochondrial haplogroup								Gene	Residue	*Homo sapiens*	*Pan troglodytes*	*Macaca mulatta*	*Canis familiaris*	*Rattus norvegicus*	*Mus musculus*	*Monodelphis domestica*	*Gastrosteus aculeatus*	*Gallus gallus*	*Caenorhabditis elegans*
		H	HV	W/I	U	K	Z	J	T												
mt7028 t>c	0.47	c	t	t	t	t	t	t	t	*COX1*	A341A	A	A	A	A	A	A	A	A	A	A
mt10398 a>g	0.19	a	a	a	a	g	g	g	a	*ND3*	A114T	A	T	A	T	T	T	A	A	A	V
mt11719 g>a	0.49	g	g	a	a	a	a	a	a	*ND4*	G320G	G	G	G	G	G	G	G	G	G	G
mt12308 a>g	0.22	a	a	a	g	g	a	a	a	*TRNL2*		a	a	a	a	a	a	-	-	-	-
mt12612 a>g	0.09	a	a	a	a	a	a	g	a	*ND5*	V92V	V	V	I	V	V	V	I	V	I	I
mt15607 a>g	0.10	a	a	a	a	a	a	a	g	*CYTB*	K287K	K	K	K	K	K	K	K	K	K	K

B

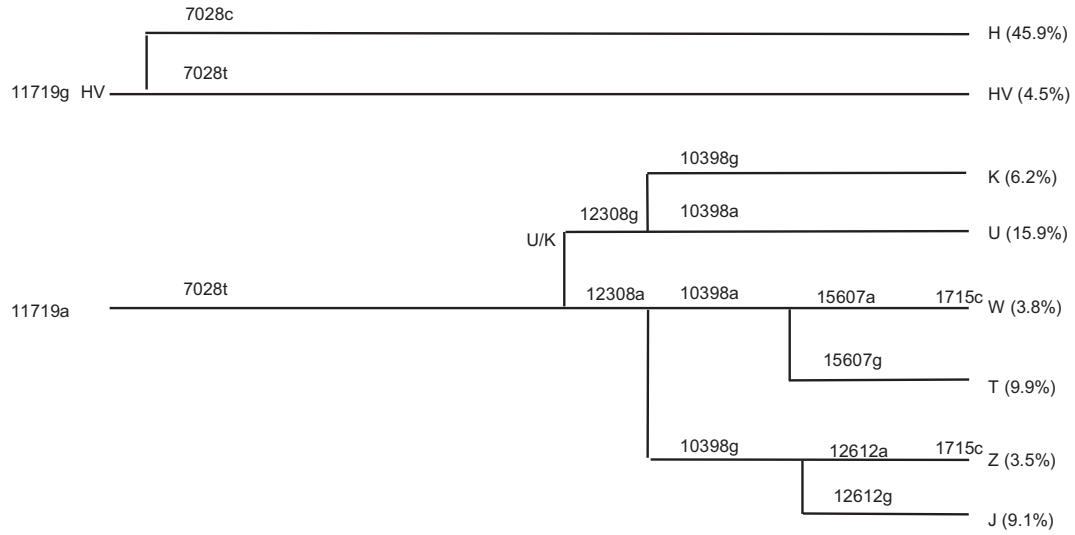

Figure 1. Mitochondrial haplogroups in 9254 individuals from the Danish general population. A, Frequencies and characterization of the European haplogroups, frequencies of the polymorphisms defining these haplogroups, the affected mitochondrial gene and amino acid residue, and the evolutionary sequence conservation for each residue. In the left panel, the shaded boxes mark the presence of the rare alleles of the corresponding polymorphisms that together define the haplogroups. In the right panel, shaded boxes mark the amino acids conserved between humans and the aligned species (right panel). Lower-case letters represent nucleotides, and capital letters represent amino acids. Mt indicates mitochondrial; -, no sequence available. B, Phylogenetic tree depicting the origin and evolution of mitochondrial haplogroups in a Northern European population. The assumption is that the most frequent polymorphisms are also the oldest.

Chinese women (51 cases/107 controls),[21] and the U haplogroup has been associated with increased risk of prostate cancer in white North American men (odds ratio [95% CI], 1.95 [CI not reported]; 221 cases/246 controls and validated in 71 cases/128 controls).[22,23] In the present study, we did not find any associations between the 8 haplogroups examined and risk of cancer or between any of the polymorphisms defining these haplogroups and risk of cancer, even when they were further subdivided into the diagnostic groups of female and male cancer and breast, cervix uteri, corpus uteri, ovarian and vaginal cancers, and testis and prostate cancers, respectively (data not shown). In the present study, we have 90% power to exclude a hazard ratio of ≥1.65 for increased risk of male cancer for haplogroup U and 95% power to exclude a hazard ratio of ≥1.95, equivalent to the odds ratios found in previous studies.[22,23]

A role for mitochondrial haplotypes in diabetes mellitus was first suggested in 1998 by Poulton et al,[24] who, in a study of 251 men from the United Kingdom, found that the mt16189t>c polymorphism, defining a subgroup of haplogroup T, was associated with impaired glucose tolerance (odds ratio [95% CI], 2.4 [1.1 to 5.6]). The authors later verified this finding in another case-control study, now observing an association between the mt16189 polymorphism and a diagnosis of type 2 diabetes mellitus (odds ratio [95% CI], 1.6 [1.0 to 2.7]; 552 cases/552 controls).[25] The lack of association between haplogroup T and diabetes mellitus types I and II in the present study is in accordance with a case-control study of a Finnish population that also failed to find any association between both haplogroup T and the mt16189t>c polymorphism and type 2 diabetes (762 cases/402 controls)[26] and a recent study including 3304 cases

Downloaded from http://ahajournals.org by on March 4, 2022

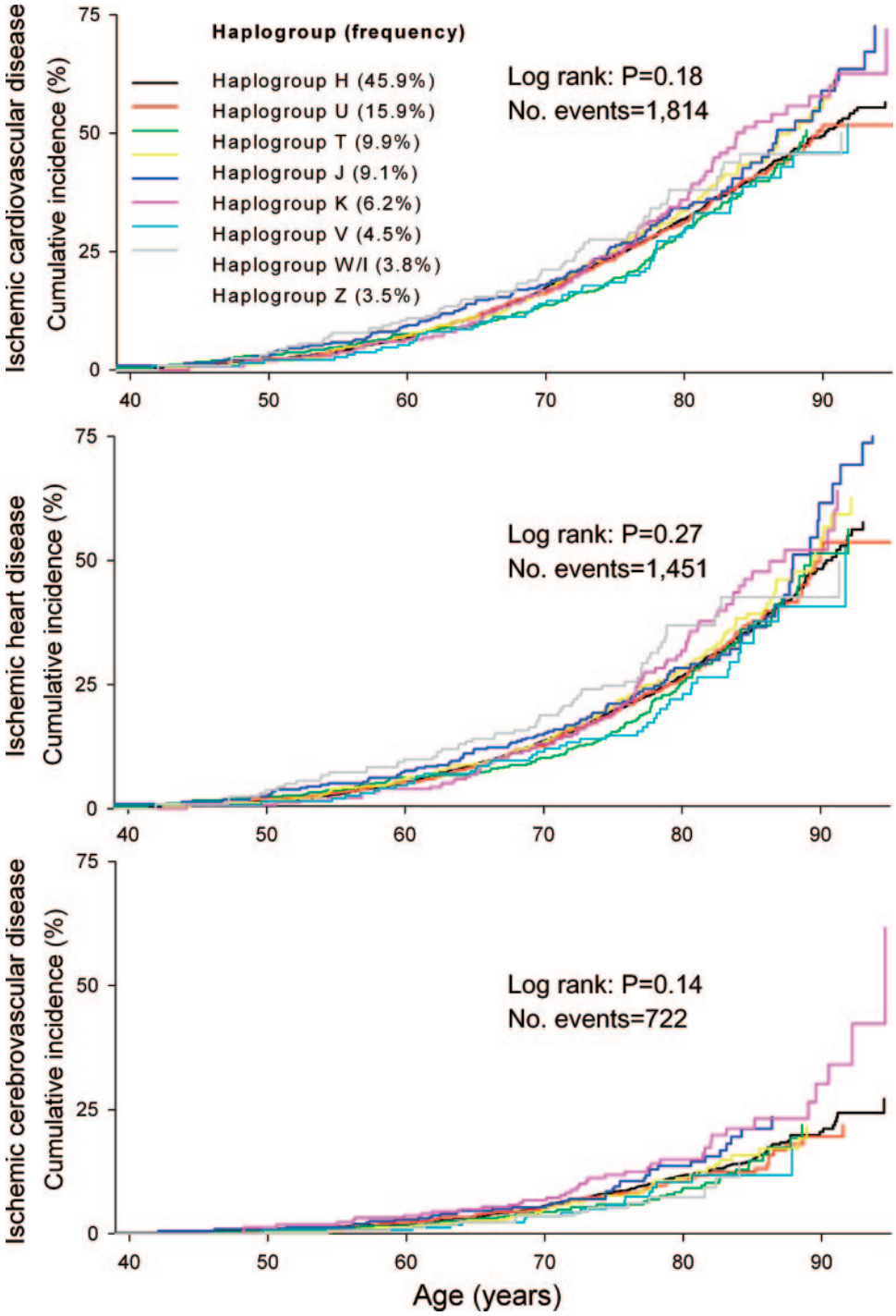

Figure 2. Cumulative incidence of ischemic cardiovascular disease (top), ischemic heart disease (middle), and ischemic cerebrovascular disease (bottom) as a function of age and by mitochondrial haplogroups in 9254 individuals from the Danish general population.

with type 2 diabetes and 3304 matched nondiabetic individuals.[12] The latter study also failed to show association with any "metabolic" quantitative phenotypic trait such as systolic and diastolic blood pressure, lipid levels, and glucose levels, exactly like the present study. We have 90% power to exclude a hazard ratio of 1.35 for diabetes mellitus for haplogroup T and 99% power to exclude a hazard ratio of 1.6, equivalent to the odds ratio found in a previous study.[25]

These conflicting results probably reflect that all the diseases studied are complex disorders, in which each polymorphism either does not play a role or plays only a modest role, and that most studies have been severely underpowered with a high risk of type 1 error, ie, accepting a chance finding as a true difference or association. In the present study, we did not see any robust associations between haplogroups and risk of any of the diseases studied.

Mitochondrial haplogroups have also been associated with longevity, and in both an Italian and an Irish study, haplogroup J was overrepresented in centenarians (109 cases/125 controls and 129 cases/100 controls, respectively)[32–34]; in a Finnish study

Downloaded from http://ahajournals.org by on March 4, 2022

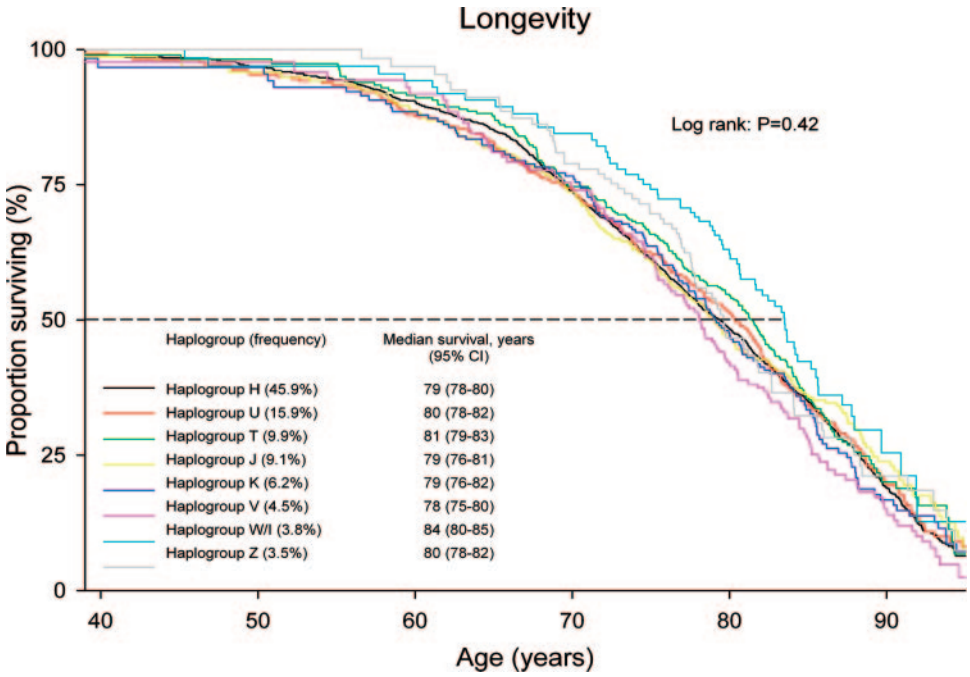

Figure 3. Longevity by mitochondrial haplogroups in 9254 individuals from the Danish general population.

of individuals aged >90 years, haplogroups J and U were enriched (225 cases/657 controls)[35]; and in a Japanese study of centenarians, haplogroup D was enriched (11 cases/43 controls).[36] From these studies, it has been concluded that specific mitochondrial DNA lineages from Europe and Asia are "protective against the ravage of aging."[9] The mechanism for this suggested effect on aging is unknown, but from previous data it has been suggested that haplogroups associated with a longer life span are those associated with a partial uncoupling of oxidative phosphorylation and therefore reduced production of reactive oxygen species.[9] Despite our well-powered prospective study with 90% power to detect a reduction in mortality as small as a change in hazard ratio from 1.0 to 0.8 for haplogroup J (9.1%), we were unable to detect any association of mitochondrial haplogroups with mortality. Thus, our results do not support the hypothesis that common mitochondrial haplogroups play a role in longevity. Although we observed small variations in median survival and apparent differences between haplogroup V with the shortest median survival and haplogroup T and W/I with the longest survivals, these results were no longer significant after correction for multiple comparisons.

The differences in outcome of the present study and most previous studies could be due to the obvious differences in study design, ie, prospective versus case-control studies, number of events/cases included, information on confounders and ability to adjust for these, and differences in the descent of study populations. In previous studies, speculations on the mechanism for association of haplogroups with disease and longevity have been on the role of free radicals produced during electron transfer, apoptosis, and variation in cellular energy production. The present study does not address these questions directly; however, it does not support the notion that mitochondrial haplogroups are of major importance for risk of ischemic cardiovascular disease, morbidity from other causes, or longevity.

Our results should not be taken as addressing the broader hypothesis that mitochondrial mutations contribute to specific diseases because we only examined the role of common polymorphisms defining mitochondrial haplogroups. The study also does not address the effect of age-dependent somatic deletions in the mitochondrial genome or the effect of heteroplasmy because the 7 heteroplasmic individuals identified were excluded from further analysis. Some of the reasons mitochondrial haplogroups may not play a major role in morbidity and mortality are as follows: (1) From an evolutionary point of view, it would be disastrous for the primitive mitochondrion to harm its host, and mitochondria with harmful genetic variants would be eradicated from the population if they were associated with early disease or not compatible with life. In the present study, the polymorphisms defining the haplogroups are common, with rare allele frequencies between 0.1 and 0.5, and must at least have been fairly harmless to have reached such high frequencies. (2) The diseases studied in the present study are complex diseases in which oxidative phosphorylation, as hypothesized in previous studies, may play a role; however, the polymorphisms defining the mitochondrial haplogroups are, for the most part, synonymous. Thus, only a minor part of a total of ≈80 different protein subunits in the mitochondrial electron transport chain and oxidative phosphorylation system would be affected because the vast majority of these proteins are encoded by nuclear genes.

We recognize that the present study has limitations and that further analysis may be required to clarify the contribution of mitochondrial haplogroups to variation in morbidity and mortality. Although our study includes a large number of carefully evaluated individuals with a long follow-up in a prospective setting, the study only includes individuals of Northern European descent, and our results are only applica-

Downloaded from http://ahajournals.org by on March 4, 2022

ble to populations with a similar ethnic background. In regard to events, some of the diagnoses used as end points in this study are obviously less certain than others, and some are more subjectively assessed, but the registers from which the data have been drawn are well validated,[15,16] and minor diagnostic misclassifications must be assumed to be randomly distributed between haplogroups. Finally, power calculations were not adjusted for multiple testing, and the adjusted power would therefore be lower and the hazard ratios higher than those given above.

In conclusion, our results do not support an association of mitochondrial haplogroups with risk of ischemic cardiovascular disease, morbidity from other causes, mortality, or longevity in a large general population of European descent.

Acknowledgments

The authors thank Karin Møller Hansen for expert technical assistance. We are indebted to the staff and participants of the Copenhagen City Heart Study for their important contributions.

Sources of Funding

This work was supported by grants from the Danish Heart Foundation, the Danish Medical Research Council, Ingeborg and Leo Dannin's Grant, and Henry Hansen's and Wife's Grant.

Disclosures

None.

References

1. Margulis L. *Symbiosis in Cell Evolution*. New York, NY: WH Freeman & Co Ltd; 1981.
2. Gabaldon T, Huynen MA. Lineage-specific gene loss following mitochondrial endosymbiosis and its potential for function prediction in eukaryotes. *Bioinformatics*. 2005;21:ii144–ii150.
3. Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. *N Engl J Med*. 2002;347:576–580.
4. Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. *Nature*. 1987;325:31–36.
5. Johnson MJ, Wallace DC, Ferris SD, Rattazzi MC, Cavalli-Sforza LL. Radiation of human mitochondria DNA types analyzed by restriction endonuclease cleavage patterns. *J Mol Evol*. 1983;19:255–271.
6. Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, Sukernik RI, Olckers A, Wallace DC. Natural selection shaped regional mtDNA variation in humans. *Proc Natl Acad Sci U S A*. 2003;100:171–176.
7. Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC. Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science*. 2004;303:223–226.
8. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med*. 2003;348:2656–2668.
9. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*. 2005;39:359–407.
10. Herrnstadt C, Howell N. An evolutionary perspective on pathogenic mtDNA mutations: haplogroup associations of clinical disorders. *Mitochondrion*. 2004;4:791–798.
11. Elson JL, Herrnstadt C, Preston G, Thal L, Morris CM, Edwardson JA, Beal MF, Turnbull DM, Howell N. Does the mitochondrial genome play a role in the etiology of Alzheimer's disease? *Hum Genet*. 2006;119:241–254.
12. Saxena R, de Bakker PI, Singer K, Mootha V, Burtt N, Hirschhorn JN, Gaudet D, Isomaa B, Daly MJ, Groop L, Ardlie KG, Altshuler D. Comprehensive association testing of common mitochondrial DNA variation in metabolic disease. *Am J Hum Genet*. 2006;79:54–61.
13. Dahl M, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. *J Exp Med*. 2004;199:1391–1399.
14. Mathers CD, Stein C, Fat DM, Rao C, Inoue M, Tomijima N, Bernard C, Lopez AD. *Global Burden of Disease 2000: Version 2, Methods and Results*. Geneva, Switzerland: World Health Organization; 2002:1–108.
15. Storm HH, Michelsen EV, Clemmensen IH, Pihl J. The Danish Cancer Registry: history, content, quality and use. *Dan Med Bull*. 1997;44:535–539.
16. Juel K, Helweg-Larsen K. The Danish registers of causes of death. *Dan Med Bull*. 1999;46:354–357.
17. Julian DG, Bertrand ME, Hjalmarson A, Fox K, Simoons ML, Ceremuzynski L, Maseri A, Meinertz T, Meyer J, Pyorala K, Rehnqvist N, Tavazzi L, Toutouzas P, Treasure T. Management of stable angina pectoris: recommendations of the Task Force of the European Society of Cardiology. *Eur Heart J*. 1997;18:394–413.
18. Nishigaki Y, Yamada Y, Fuku N, Matsuo H, Segawa T, Watanabe S, Kato K, Yokoi K, Yamaguchi S, Nozawa Y, Tanaka M. Mitochondrial haplogroup A is a genetic risk factor for atherothrombotic cerebral infarction in Japanese females. *Mitochondrion*. 2007;7:72–79.
19. Nishigaki Y, Yamada Y, Fuku N, Matsuo H, Segawa T, Watanabe S, Kato K, Yokoi K, Yamaguchi S, Nozawa Y, Tanaka M. Mitochondrial haplogroup N9b is protective against myocardial infarction in Japanese males. *Hum Genet*. 2007;120:827–836.
20. Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. *Cancer Res*. 2005;65:8028–8033.
21. Liu VW, Wang Y, Yang HJ, Tsang PC, Ng TY, Wong LC, Nagley P, Ngan HY. Mitochondrial DNA variant 16189T>C is associated with susceptibility to endometrial cancer. *Hum Mutat*. 2003;22:173–174.
22. Booker LM, Habermacher GM, Jessie BC, Sun QC, Baumann AK, Amin M, Lim SD, Fernandez-Golarz C, Lyles RH, Brown MD, Marshall FF, Petros JA. North American white mitochondrial haplogroups in prostate and renal cancer. *J Urol*. 2006;175:468–472.
23. Canter JA, Kallianpur AR, Fowke JH. Re: North American white mitochondrial haplogroups in prostate and renal cancer. *J Urol*. 2006;176:2308–2309.
24. Poulton J, Brown MS, Cooper A, Marchington DR, Phillips DI. A common mitochondrial DNA variant is associated with insulin resistance in adult life. *Diabetologia*. 1998;41:54–58.
25. Poulton J, Luan J, Macaulay V, Hennings S, Mitchell J, Wareham NJ. Type 2 diabetes is associated with a common mitochondrial variant: evidence from a population-based case-control study. *Hum Mol Genet*. 2002;11:1581–1583.
26. Mohlke KL, Jackson AU, Scott LJ, Peck EC, Suh YD, Chines PS, Watanabe RM, Buchanan TA, Conneely KN, Erdos MR, Narisu N, Enloe S, Valle TT, Tuomilehto J, Bergman RN, Boehnke M, Collins FS. Mitochondrial polymorphisms and susceptibility to type 2 diabetes-related traits in Finns. *Hum Genet*. 2005;118:245–254.
27. Chagnon P, Gee M, Filion M, Robitaille Y, Belouchi M, Gauvreau D. Phylogenetic analysis of the mitochondrial genome indicates significant differences between patients with Alzheimer disease and controls in a French-Canadian founder population. *Am J Med Genet*. 1999;85:20–30.
28. van der Walt JM, Dementieva YA, Martin ER, Scott WK, Nicodemus KK, Kroner CC, Welsh-Bohmer KA, Saunders AM, Roses AD, Small GW, Schmechel DE, Murali DP, Gilbert JR, Haines JL, Vance JM, Pericak-Vance MA. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. *Neurosci Lett*. 2004;365:28–32.
29. Chinnery PF, Taylor GA, Howell N, Andrews RM, Morris CM, Taylor RW, McKeith IG, Perry RH, Edwardson JA, Turnbull DM. Mitochondrial DNA haplogroups and susceptibility to AD and dementia with Lewy bodies. *Neurology*. 2000;55:302–304.
30. van der Walt JM, Nicodemus KK, Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP, Haines JL, Koller WC, Lyons K, Pahwa R, Stern MB, Colcher A, Hiner BC, Jankovic J, Ondo WG, Allen FH Jr, Goetz CG, Small GW, Mastaglia F, Stajich JM, McLaurin AC, Middleton LT, Scott BL, Schmechel DE, Pericak-Vance MA, Vance JM. Mitochondrial polymorphisms significantly reduce the risk of Parkinson disease. *Am J Hum Genet*. 2003;72:804–811.
31. Ross OA, McCormack R, Maxwell LD, Duguid RA, Quinn DJ, Barnett YA, Rea IM, El Agnaf OM, Gibson JM, Wallace A, Middleton D, Curran MD. mt4216C variant in linkage with the mtDNA TJ cluster may confer a susceptibility to mitochondrial dysfunction resulting in an increased risk of Parkinson's disease in the Irish. *Exp Gerontol*. 2003;38:397–405.
32. De Benedictis G, Rose G, Carrieri G, De Luca M, Falcone E, Passarino G, Bonafe M, MontiD, Baggio G, Bertolini S, Mari D, Mattace

Downloaded from http://ahajournals.org by on March 4, 2022

R, Franceschi C. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J*. 1999;13: 1532–1536.
33. Rose G, Passarino G, Carrieri G, Altomare K, Greco V, Bertolini S, Bonafe M, Franceschi C, De Benedictis G. Paradoxes in longevity: sequence analysis of mtDNA haplogroup J in centenarians. *Eur J Hum Genet*. 2001;9:701–707.
34. Ross OA, McCormack R, Curran MD, Duguid RA, Barnett YA, Rea IM, Middleton D. Mitochondrial DNA polymorphism: its role in longevity of the Irish population. *Exp Gerontol*. 2001;36:1161–1178.
35. Niemi AK, Hervonen A, Hurme M, Karhunen PJ, Jylha M, Majamaa K. Mitochondrial DNA polymorphisms associated with longevity in a Finnish population. *Hum Genet*. 2003;112:29–33.
36. Tanaka M, Gong J, Zhang J, Yamada Y, Borgeld HJ, Yagi K. Mitochondrial genotype associated with longevity and its inhibitory effect on mutagenesis. *Mech Ageing Dev*. 2000;116:65–76.
37. Guzik TJ, Sadowski J, Guzik B, Jopek A, Kapelak B, Przybylowski P, Wierzbicki K, Korbut R, Harrison DG, Channon KM. Coronary artery superoxide production and Nox isoform expression in human coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2006;26:333–339.

CLINICAL PERSPECTIVE

More than 75 human diseases have been associated with rare mutations in the mitochondrial genome; these mutations either impair mitochondrial protein synthesis or impair proteins encoded by the mitochondrial genome. Recently, numerous reports have suggested a role for mitochondrial haplogroups, defined by the presence or absence of common polymorphisms, in the pathogenesis of ischemic cardiovascular disease and other diseases, as well as in longevity. However, other reports could not confirm such associations, emphasizing the need for large prospective population-based studies to clarify this. In the present study, we tested the hypothesis that common haplogroups predict risk of ischemic cardiovascular disease, morbidity from other causes, mortality, and longevity in a general population of European descent. A total of 9254 individuals from the Danish general population, in the Copenhagen City Heart Study, were followed prospectively for risk of ischemic cardiovascular disease, morbidity, and mortality during 25 and 11 years, respectively. Hazard ratios for hospitalization due to all cardiovascular disorders and ischemic cerebrovascular disease did not differ from 1.0 for any haplogroup versus the most common haplogroup H. Results were similar for hospitalization due to infectious diseases, respiratory disorders, malignant neoplasms, digestive disorders, musculoskeletal disorders, and miscarriages. Likewise, hazard ratios for death from all causes were not different from 1.0 for any haplogroup. Finally, after stratification by major causes of death, hazard ratios remained insignificant. Our results do not support an association of mitochondrial haplogroups with risk of ischemic cardiovascular disease, morbidity from other causes, mortality, or longevity in a large general population of European descent.

Downloaded from http://ahajournals.org by on March 4, 2022