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 (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 endosymbiosis with primordial eukaryotic cells 2 to 3 billion years ago. 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. 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.
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.

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 Registry16 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 100 to 199, respectively). Ischemic cardiovascular disease was ischemic heart disease (myocardial infarction and angina pectoris; codes 410 to 414 and 120 to 125, 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, mt12308A>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 (0.05/72<0.0003; Table 2), and <0.001 for mortality (0.05/7<0.001; Table 3). ANOVA and Pearson χ² 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

<table>
<thead>
<tr>
<th>Mitochondrial Haplogroup</th>
<th>H</th>
<th>U</th>
<th>T</th>
<th>J</th>
<th>K</th>
<th>V</th>
<th>WI</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals (%)</td>
<td>4244 (45.9)</td>
<td>1469 (15.9)</td>
<td>912 (9.9)</td>
<td>843 (9.1)</td>
<td>571 (6.2)</td>
<td>412 (4.5)</td>
<td>351 (3.8)</td>
<td>324 (3.5)</td>
<td>...</td>
</tr>
<tr>
<td>Women/men</td>
<td>2341/1903</td>
<td>795/674</td>
<td>535/377</td>
<td>457/386</td>
<td>313/258</td>
<td>220/192</td>
<td>203/174</td>
<td>178/146</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>57.9±15.2</td>
<td>57.1±15.0</td>
<td>58.3±15.0</td>
<td>57.5±15.0</td>
<td>56.8±15.1</td>
<td>57.6±16.1</td>
<td>56.3±15.5</td>
<td>56.0±15.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.2±1.3</td>
<td>6.1±1.3</td>
<td>6.2±1.3</td>
<td>6.2±1.3</td>
<td>6.1±1.3</td>
<td>6.1±1.3</td>
<td>6.2±1.5</td>
<td>6.0±1.2</td>
<td>0.68</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.8±1.2</td>
<td>3.7±1.1</td>
<td>3.8±1.2</td>
<td>3.8±1.2</td>
<td>3.7±1.2</td>
<td>3.7±1.1</td>
<td>3.7±1.2</td>
<td>3.7±1.1</td>
<td>0.78</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.6±0.5</td>
<td>1.6±0.5</td>
<td>1.6±0.5</td>
<td>1.6±0.5</td>
<td>1.6±0.5</td>
<td>1.5±0.5</td>
<td>1.5±0.5</td>
<td>1.5±0.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.9±1.3</td>
<td>1.9±2.3</td>
<td>1.9±1.5</td>
<td>1.9±1.6</td>
<td>1.8±1.2</td>
<td>2.0±1.4</td>
<td>2.1±3.2</td>
<td>1.9±1.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>139±22</td>
<td>138±22</td>
<td>139±23</td>
<td>138±23</td>
<td>137±21</td>
<td>137±24</td>
<td>136±21</td>
<td>135±21</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84±12</td>
<td>83±13</td>
<td>84±13</td>
<td>84±12</td>
<td>83±12</td>
<td>83±12</td>
<td>82±11</td>
<td>83±12</td>
<td>0.08</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6±4.3</td>
<td>25.7±4.4</td>
<td>25.7±4.4</td>
<td>25.6±4.3</td>
<td>25.7±4.2</td>
<td>25.3±4.3</td>
<td>25.3±4.1</td>
<td>25.8±4.3</td>
<td>0.61</td>
</tr>
<tr>
<td>Alcohol, U/wk</td>
<td>16.4±21.6</td>
<td>17.3±22.4</td>
<td>15.7±20.3</td>
<td>16.5±19.4</td>
<td>16.9±21.7</td>
<td>15.9±20.2</td>
<td>15.3±20.5</td>
<td>14.2±16.5</td>
<td>0.31</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>2344 (56)</td>
<td>792 (54)</td>
<td>502 (55)</td>
<td>433 (52)</td>
<td>297 (53)</td>
<td>209 (51)</td>
<td>170 (49)</td>
<td>156 (49)</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>161 (3.4)</td>
<td>76 (5.2)</td>
<td>29 (3.2)</td>
<td>52 (6.2)</td>
<td>26 (4.6)</td>
<td>19 (4.6)</td>
<td>11 (3.1)</td>
<td>14 (3.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>2081 (49)</td>
<td>727 (50)</td>
<td>429 (47)</td>
<td>410 (49)</td>
<td>283 (50)</td>
<td>196 (48)</td>
<td>157 (46)</td>
<td>156 (49)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

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 at 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. Multivariable adjustment was for time-dependent covariates from the general population stratified by mitochondrial haplogroups. 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).

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

<table>
<thead>
<tr>
<th>Mitochondrial Haplogroup</th>
<th>Cardiovascular disorders, all (No. of events=3453)</th>
<th>Ischemic cardiovascular disease (No. of events=1791)</th>
<th>Ischemic heart disease (No. of events=1451)</th>
<th>Myocardial infarction (No. of events=722)</th>
<th>Ischemic cerebrovascular disease (No. of events=548)</th>
<th>Ischemic stroke (No. of events=439)</th>
<th>Infectious and parasitic diseases (No. of events=1057)</th>
<th>Respiratory infections (No. of events=1274)</th>
<th>Respiratory disorders (No. of events=1450)</th>
<th>Malignant neoplasms, all (No. of events=1728)</th>
<th>Gastrointestinal cancer (No. of events=328)</th>
<th>Hematologic cancer (No. of events=90)</th>
<th>Respiratory cancer (No. of events=222)</th>
<th>Urologic cancer (No. of events=160)</th>
<th>Female cancer (No. of events=373)</th>
<th>Male cancer (No. of events=107)</th>
<th>Melanomas and skin cancers (No. of events=563)</th>
<th>Digestive disorders (No. of events=1696)</th>
<th>Diabetes mellitus, types I and II (No. of events=681)</th>
<th>Musculoskeletal disorders (No. of events=2663)</th>
<th>Neuropsychiatric disorders (No. of events=3430)</th>
<th>Miscarriage (No. of events=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (45.9%)</td>
<td>1.0</td>
<td>1.0 (0.9–1.1)</td>
<td>0.9 (0.8–1.0)</td>
<td>1.0 (0.9–1.1)</td>
<td>1.0 (0.9–1.2)</td>
<td>1.0 (0.9–1.2)</td>
<td>0.8 (0.7–1.0)*</td>
<td>1.0 (0.8–1.2)</td>
<td>n=1654</td>
<td>n=536</td>
<td>n=343</td>
<td>n=319</td>
<td>n=218</td>
<td>n=159</td>
<td>n=112</td>
<td>n=118</td>
<td>n=841</td>
<td>n=273</td>
<td>n=166</td>
<td>n=175</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U (15.9%)</td>
<td>0.9</td>
<td>0.9 (0.8–1.1)</td>
<td>0.9 (0.7–1.0)</td>
<td>1.1 (0.9–1.2)</td>
<td>1.1 (0.9–1.3)</td>
<td>1.1 (0.9–1.4)</td>
<td>1.1 (0.8–1.4)</td>
<td>1.1 (0.8–1.4)</td>
<td>n=678</td>
<td>n=220</td>
<td>n=136</td>
<td>n=143</td>
<td>n=94</td>
<td>n=76</td>
<td>n=48</td>
<td>n=57</td>
<td>n=337</td>
<td>n=118</td>
<td>n=67</td>
<td>n=78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (9.9%)</td>
<td>1.0</td>
<td>0.9 (0.8–1.1)</td>
<td>0.9 (0.7–1.0)</td>
<td>1.0 (0.8–1.2)</td>
<td>1.1 (0.9–1.4)</td>
<td>0.9 (0.7–1.2)</td>
<td>1.2 (0.9–1.6)</td>
<td>1.0 (0.8–1.4)</td>
<td>n=264</td>
<td>n=81</td>
<td>n=48</td>
<td>n=49</td>
<td>n=39</td>
<td>n=38</td>
<td>n=20</td>
<td>n=22</td>
<td>n=65</td>
<td>n=76</td>
<td>n=43</td>
<td>n=37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J (9.1%)</td>
<td>1.0</td>
<td>1.1 (0.8–1.3)</td>
<td>0.9 (0.7–1.2)</td>
<td>1.1 (0.8–1.4)</td>
<td>1.0 (0.7–1.5)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.9 (0.6–1.5)</td>
<td>0.8 (0.4–1.6)</td>
<td>n=210</td>
<td>n=63</td>
<td>n=38</td>
<td>n=42</td>
<td>n=31</td>
<td>n=32</td>
<td>n=12</td>
<td>n=11</td>
<td>n=105</td>
<td>n=151</td>
<td>n=110</td>
<td>n=127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K (6.2%)</td>
<td>1.0</td>
<td>0.9 (0.7–1.2)</td>
<td>0.9 (0.6–1.2)</td>
<td>1.2 (0.8–1.7)</td>
<td>1.4 (0.9–2.1)</td>
<td>0.8 (0.4–1.6)</td>
<td>0.7 (0.4–1.4)</td>
<td>0.8 (0.4–1.6)</td>
<td>n=487</td>
<td>n=151</td>
<td>n=112</td>
<td>n=107</td>
<td>n=66</td>
<td>n=56</td>
<td>n=43</td>
<td>n=35</td>
<td>n=840</td>
<td>n=263</td>
<td>n=164</td>
<td>n=170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V (4.5%)</td>
<td>1.0</td>
<td>1.1 (0.9–1.4)</td>
<td>0.9 (0.7–1.2)</td>
<td>1.1 (0.9–1.4)</td>
<td>1.1 (0.8–1.5)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.9 (0.6–1.4)</td>
<td>0.8 (0.4–1.6)</td>
<td>n=692</td>
<td>n=226</td>
<td>n=140</td>
<td>n=129</td>
<td>n=88</td>
<td>n=64</td>
<td>n=46</td>
<td>n=45</td>
<td>n=152</td>
<td>n=62</td>
<td>n=34</td>
<td>n=27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/I (3.8%)</td>
<td>1.0</td>
<td>0.9 (0.7–1.2)</td>
<td>0.9 (0.6–1.2)</td>
<td>1.0 (0.7–1.4)</td>
<td>1.0 (0.7–1.2)</td>
<td>1.2 (1.0–1.6)</td>
<td>0.9 (0.7–1.3)</td>
<td>0.9 (0.7–1.3)</td>
<td>n=53</td>
<td>n=56</td>
<td>n=66</td>
<td>n=64</td>
<td>n=64</td>
<td>n=64</td>
<td>n=64</td>
<td>n=64</td>
<td>n=10</td>
<td>n=10</td>
<td>n=12</td>
<td>n=20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z (3.5%)</td>
<td>1.0</td>
<td>0.8 (0.7–1.2)</td>
<td>0.7 (0.6–1.2)</td>
<td>1.0 (0.7–1.4)</td>
<td>1.1 (0.8–1.4)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.9 (0.6–1.4)</td>
<td>1.0 (0.8–1.4)</td>
<td>n=107</td>
<td>n=29</td>
<td>n=23</td>
<td>n=21</td>
<td>n=18</td>
<td>n=12</td>
<td>n=9</td>
<td>n=3</td>
<td>n=7</td>
<td>n=11</td>
<td>n=12</td>
<td>n=20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 <0.05/22×7=0.0003.
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 I A, 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.  

The Asian N9b haplogroup (defined by polymorphisms at positions mt1547 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). 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 that might confer resistance to myocardial infarction. 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). 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.

Several haplogroups or polymorphisms have been associated with risk of cancer in case-control studies. The mt10398a 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). The mt16189c polymorphism (defining a subgroup of haplogroup T) has been associated with risk of endometrial cancer in...
Chinese women (51 cases/107 controls), 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). 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

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.

<table>
<thead>
<tr>
<th>Mt</th>
<th>Rare allele frequency (%)</th>
<th>Mt17028 t&gt;c</th>
<th>H</th>
<th>HV</th>
<th>U/K</th>
<th>Z</th>
<th>T</th>
<th>Gene</th>
<th>Residue</th>
<th>Homo sapiens</th>
<th>Pan troglodytes</th>
<th>Macaca mulatta</th>
<th>Canis lupus familiaris</th>
<th>Rattus norvegicus</th>
<th>Mus musculus</th>
<th>Monodelphis domestica</th>
<th>Gallus gallus</th>
<th>Gasterosteus aculeatus</th>
<th>Carassius auratus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.47</td>
<td>t</td>
<td>l</td>
<td>l</td>
<td>l</td>
<td>l</td>
<td>COX1</td>
<td>A341A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.19</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>g</td>
<td>g</td>
<td>ND3</td>
<td>A1141T</td>
<td>A</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>V</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.49</td>
<td>g</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>ND4</td>
<td>G320G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.22</td>
<td>a</td>
<td>a</td>
<td>g</td>
<td>a</td>
<td>a</td>
<td>TRNL2</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.09</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>g</td>
<td>ND5</td>
<td>V92V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>CYTB</td>
<td>K287K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td>K</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A role for mitochondrial haplotypes in diabetes mellitus was first suggested in 1998 by Poulton et al., 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
with type 2 diabetes and 3304 matched nondiabetic individu-
als.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 poly-
morphism 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 I 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

**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.
of individuals aged >90 years, haplogroups J and U were enriched (225 cases/657 controls); and in a Japanese study of centenarians, haplogroup D was enriched (11 cases/43 controls). From these studies, it has been concluded that specific mitochondrial DNA lineages from Europe and Asia are “protective against the ravage of aging.” 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. 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-
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, 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


**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.
Mitochondrial Haplogroups: Ischemic Cardiovascular Disease, Other Diseases, Mortality, and Longevity in the General Population
Marianne Benn, Marianne Schwartz, Børge G. Nordestgaard and Anne Tybjærg-Hansen

Circulation. 2008;117:2492-2501; originally published online May 5, 2008; doi: 10.1161/CIRCULATIONAHA.107.756809

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/117/19/2492

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2008/05/14/CIRCULATIONAHA.107.756809.DC2
http://circ.ahajournals.org/content/suppl/2008/05/05/CIRCULATIONAHA.107.756809.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/