Objective: To determine whether variants of the mitochondrial genome influence the risk of developing age-related hearing loss (ARHL).

Design: Cross-sectional study.

Setting: Eligible participants were noninstitutionalized permanent residents 49 years or older identified in a door-to-door census of 2 suburban postcode areas, west of Sydney, Australia.

Participants: The Blue Mountains Hearing Study (BMHS) was a population-based survey of hearing loss, conducted during 1997 to 1999, among the participants of the Blue Mountains Eye Study cohort.

Main Outcome Measures: We defined hearing impairment as the pure-tone average of audiometric hearing thresholds at 500, 1000, 2000, and 4000 Hz (>25- but ≤40-dB hearing level [HL] [mild hearing loss], >40- but ≤60-dB HL [moderate hearing loss], or >60-dB HL [severe hearing loss]) in the better of the 2 ears.

Results: Of the 2765 BMHS participants, 912 (33%) were found to have ARHL. After adjusting for other hearing loss risk factors, mitochondrial DNA (mtDNA) haplogroups U and K were independently associated with a higher prevalence of ARHL compared with subjects with other haplogroups. Haplogroup U was significantly associated with moderate to severe ARHL (multivariable-adjusted odds ratio, 1.63; 95% confidence interval, 1.10-2.41). Haplogroup K was associated with severity types of ARHL in persons aged 50 to 59 years (odds ratio, 3.02; 95% confidence interval, 1.30-6.99). There was also a joint effect between mtDNA haplogroups U and K and other known hearing loss risk factors such as diabetes and past noise exposure.

Conclusion: Findings from this older Australian population demonstrate an association between certain mtDNA haplogroups and ARHL, as well as a link to the susceptibility of other known risk factors for ARHL.

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Mitochondrial DNA (mtDNA) “polymorphisms” are maternally transmitted and typically reflect different ethnic backgrounds. Specific mtDNA polymorphisms have now been classified into a number of specific mitochondrial haplogroups.10 There are 10 recognized mtDNA haplogroups within the European community, 4 in the Asian community, and 1 in the African community. Mitochondrial DNA haplogroups have recently been identified to be associated with a number of neurodegenerative conditions.11-20 Rather than merely representing the presence of “neutral” polymorphisms reflecting different ethnic backgrounds, different mtDNA haplogroups may cause mild deleterious bioenergetic abnormalities. Impairment in mitochondrial function due to mutations in the mitochondrial genome (mtDNA) is associated with an insidious decline in physiologic and biochemical performance that contributes to the aging process and to the ultimate death of the organ.21 There is a growing body of evidence suggesting that mtDNA haplogroups may play a role in the etiology of ARHL.

Hearing loss is the most frequent sensory disorder worldwide.1 The most common form of hearing impairment in humans is age-related hearing loss (ARHL, also termed presbycusis),2 with prevalence estimates in Western societies ranging between 29% and 46%.3,4 Age-related hearing loss is characterized by bilateral high-frequency hearing loss resulting from degeneration of cochlear structures within the inner ear.5 Both genetic and environmental factors contribute causally to ARHL.2,5 Although age is the strongest predictor for developing ARHL,6 other known risk factors for ARHL include male sex,5,6 family history of hearing loss, occupational and recreational noise exposure,7 type 2 diabetes mellitus,8 and smoking.9

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Author Affiliations: Department of Neurogenetics, Kolling Institute (Drs Manwaring, Jones, and Sue and Mr Howard), and Department of Ophthalmology, Centre for Vision Research, Westmead Millennium Institute (Drs Wang and Mitchell and Ms Rochtchina), University of Sydney, and Department of Linguistics, Macquarie University (Mr Newall), Australia.

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function have been reported in human models of this, mutations in mtDNA and reduced mitochondrial function in patients with mitochondrial disease.28

We hypothesized that certain mtDNA haplogroups may improve the risk of ARHL in some individuals. We aimed to determine whether mtDNA haplogroups are genetic markers for ARHL in a large representative sample of older Australians living in a geographically defined area, west of Sydney, Australia.

METHODS

We studied 2765 individuals from the Blue Mountains Hearing Study (BMHS) cohort. The BMHS was a population-based survey of hearing loss conducted during the years 1997 to 1999 among participants of the Blue Mountains Eye Study (BMES) cohort.29 In 1991, we identified 4443 eligible noninstitutionalized permanent residents 49 years or older in a door-to-door census of 2 urban postcode areas, west of Sydney. Of this target population, 3654 persons (82.4%) participated in the BMES baseline survey (1992-1994, BMES I). During the years 1997 to 1999, 2335 of the 3111 survivors (75.1%) participated in 5-year follow-up examinations (BMES II A). In 1999, a repeated door-to-door census was conducted in the same area; 1174 of the 1378 newly eligible residents who had moved into the study area or entered into the study age group (85.2%) participated in BMES II B during the 1999 to 2000 period. Of these 3509 participants, 2956 (84.2%) also agreed to take part in the BMHS and were examined. All participants gave written informed consent and the institutional local human ethics committees approved the study.

A face-to-face interview was conducted, and a comprehensive medical history that included information on hearing and lifestyle factors was obtained from all participants. An audiologist-administered questionnaire included demographic and socioeconomic characteristics, history of any self-perceived hearing problem, including its severity, onset and duration, whether primary care practitioners or other professionals had been consulted, and if a hearing aid had been provided. The medical history included cardiovascular disease and risk factors, medications used, exercise, smoking, and caffeine or alcohol consumption. Hearing-related questions included family history of hearing loss, past medical or surgical treatment of otologic conditions, diseases associated with hearing loss, and risk factors for ear disease. Other questions addressed exposure to noise at work or during military service or leisure activities and past use of ototoxic drugs. The severity of the noise exposure was subjectively classified in 3 ways: mostly quiet, tolerable level, or unable to hear speech. The duration of the noise exposure was also categorized in years. Blood pressure, height, and weight were measured, and body mass index was calculated. Participants also completed a food frequency questionnaire, which provided dietary zinc and fat intakes.

Pure-tone audiometry was performed by audiologists in sound-treated booths, using standard TDH-39 earphones and Madsen OB822 audiometers (Madsen Electronics, Copenhagen, Denmark), which were calibrated regularly during the study period to Australian standards. Testing was performed by audiologists who also examined the ears for wax occlusion. If present, the subject was asked to return for assessment after treatment. Audiometric thresholds for air-conduction stimuli (in both ears) were established for frequencies at 250, 500, 1000, 2000, 4000, 6000, and 8000 Hz, with 3000 Hz added if a 20-dB difference existed between the 2000- and 4000-Hz thresholds. Bone conduction was evaluated whenever air conduction thresholds were greater than 15-dB hearing level (dB HL) for frequencies of 500, 1000, 2000, and 4000 Hz. Subjects were examined for any evidence of collapsed canals, and if present, air-conduction thresholds at the higher frequencies were reassessed, taking care to reduce the pressure on the external ear. For the purposes of this analysis, we defined hearing impairment as the pure-tone average of audiometric hearing thresholds at 500, 1000, 2000, and 4000 Hz (>25- but ≤40-dB HL [mild hearing loss], >40- but ≤60-dB HL [moderate hearing loss], or >60-dB HL [severe hearing loss]) in the better of the 2 ears.

DNA was available from hair follicles and/or blood in 2856 of the 2956 BMHS participants (96.6%). Ninety participants, classified as having either childhood onset hearing loss (n = 14), conductive hearing loss (n = 38), or otosclerosis (n = 18), were excluded, as was 1 participant without complete audiological data, resulting in 2756 participants (93.5%) with complete data available for analysis. Total DNA was isolated from each subject's hair follicles and/or blood by standard laboratory techniques.30 Ten European mtDNA haplogroups (H, I, J, K, M, T, U, V, W, and X) and the African superhaplogroup L were categorized by the presence or absence of the well-defined restriction enzyme recognition sites as determined by polymerase chain reaction and restriction fragment length polymorphism analysis.31 A comparison of specific mtDNA haplogroup prevalence in persons with and without hearing loss was performed using χ² statistics. Logistic regression analyses, adjusting for known hearing loss risk factors (age, male sex, family history, noise exposure at work, diabetes, and smoking) were performed to assess specific mtDNA haplogroup associations with hearing loss, using other haplogroups as the reference group. Further analyses stratified by haplogroup explored possible interactions between mtDNA haplogroups and these risk factors. Odds ratios (ORs) and 95% confidence intervals (CIs) are presented.

RESULTS

PREVALENCE OF ARHL

The 2765 BMHS participants in this study comprised 1574 women (56.9%) and 1191 men (43.1%), with a mean age of 67.4 years. Age-related hearing loss was present in 912 (33.0%) and was further classified as mild in 625 (22.6%), moderate in 244 (8.8%), and severe in 43 (1.6%). Severe hearing loss was much more frequent in men (60.5%) than in women (39.5%), while no sex difference was found in the prevalence of mild or moderate hearing loss (Table 1). Age-related hearing loss was present in 116 of 277 participants with diabetes (41.9%), in 392 of 1017 participants who gave a history of noise exposure at work (38.5%), and in 81 of 266 participants who were current smokers (30.5%).

PREVALENCE OF SPECIFIC mtDNA HAPLOGROUPS

Of the 2765 participants, 2647 (95.7%) could be categorized into 1 of the 10 recognized European mtDNA haplogroups (H, I, J, K, T, U, V, W, X, and M) or into the
African’s superhaplogroup L, as shown in Table 1. Haplogroup H was the most frequent (43.1%), followed by haplogroups U (14.4%), J (10.6%), T (9.3%), and K (7.9%). There were 118 participants (4.3%) who could not be classified with any of the polymorphisms associated with these 11 predefined haplogroups and were designated as “unknown” according to standard practice.10

The distribution of mtDNA haplogroups in this study reflected the predominantly northern European heritage of the population, being relatively similar to reported haplogroup prevalence in European populations.31-33

ASSOCIATIONS WITH ARHL

Age-related hearing loss was associated with age (OR per year, 1.16; 95% CI, 1.14-1.17), male sex (OR, 1.49; 95% CI, 1.21-1.82), family history of hearing loss (OR, 1.58; 95% CI, 1.31-1.92), noise exposure at work (OR, 1.71; 95% CI, 1.39-2.10), diabetes (OR, 1.48; 95% CI, 1.11-1.98), and current smoking (OR, 1.55; 95% CI, 1.13-2.13) (Table 2). After controlling for these variables, we found that haplogroup U was associated with moderate to severe ARHL (OR, 1.63; 95% CI, 1.10-2.41) compared with other non-U haplogroups (Table 3). Although the mean age of subjects with hearing loss did not vary significantly between haplogroups (range, 71.0-76.6 years), subgroup analysis showed that haplogroup K was associated with ARHL among persons aged 50 to 59 years (OR, 3.02; 95% CI, 1.30-6.99) compared with non-U and non-K haplogroups. No haplogroup was associated with a reduced likelihood of having ARHL.

The association with ARHL remained when persons with haplogroup K or U were grouped (cluster U/K). Subjects within haplogroup U/K (22% of this population) had a moderately increased risk of developing moderate to severe hearing loss (OR, 1.51; 95% CI, 1.07-2.11) compared with subjects with non-U and non-K haplogroups.

JOINT EFFECTS OF mtDNA HAPLOGROUPS AND ARHL RISK FACTORS

Table 2 also shows the associations of ARHL risk factors in persons with haplogroups U, K, or others. Among persons with haplogroup U, those with diabetes had a nearly 4-fold higher risk of moderate to severe ARHL (OR, 3.82; 95% CI, 1.42-10.26) compared with those with the same haplogroup but without diabetes. This risk from diabetes was higher than the similar risk among subjects with other haplogroups (OR, 1.67; 95% CI, 1.00-2.78) (Table 2). Among persons with haplogroup K, those with a history of noise exposure at work were more likely to have any type of ARHL (OR, 2.60; 95% CI, 1.20-5.68) or moderate to severe ARHL (OR, 5.59; 95% CI, 1.42-21.96) than those without a past work-related noise exposure (Table 2).

Among persons with no history of noise exposure at work, those with haplogroup U were more likely than those with other haplogroups to have any type of ARHL (adjusted OR, 1.47; 95% CI, 1.04-2.07) or moderate to severe ARHL (OR, 2.26; 95% CI, 1.37-3.74). In contrast, among persons with a history of noise exposure at work, haplogroup U was not associated with any ARHL (OR, 0.81; 95% CI, 0.54-1.23) or with moderate to severe ARHL (OR, 1.00; 95% CI, 0.53-1.90).

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We found a significantly higher prevalence of ARHL in subjects with mtDNA haplogroups U and K. Haplogroup U was associated with moderate to severe ARHL and haplogroup K was associated with ARHL in individuals aged 50 to 59 years. These haplogroup associations were independent of other known ARHL risk factors. No haplogroups were associated with reduced ARHL prevalence.

Our findings suggest that haplogroups U and K are genetic markers of ARHL susceptibility. This hypothesis is strengthened by the joint effects found between these haplogroups and known ARHL risk factors: the risk of ARHL was greater in the subgroup of persons with diabetes belonging to haplogroup U and in the subgroup of persons with a history of noise exposure at work belonging to haplogroup K. Our results further support the concept that certain mtDNA haplogroups may cause mild
Table 2. Risk Factors for Age-Related Hearing Loss (ARHL) in the Whole Study Sample and in Stratified Subgroups of Haplogroups U, K, and Others

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Haplogroup, Any ARHL</th>
<th>Haplogroup, Moderate to Severe ARHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year; OR (95% CI)</td>
<td>1.16 (1.14-1.17)</td>
<td>1.19 (1.14-1.23)</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.14 (1.10-1.19)</td>
<td>1.20 (1.17-1.22)</td>
</tr>
<tr>
<td>Subjects, No. (%)</td>
<td>474 (38.6)</td>
<td>44 (42.7)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.49 (1.21-1.82)</td>
<td>1.59 (1.30-2.02)</td>
</tr>
<tr>
<td>Noise exposure at work</td>
<td>64 (35.4)</td>
<td>350 (38.6)</td>
</tr>
<tr>
<td>Subjects, No. (%)</td>
<td>405 (38.8)</td>
<td>302 (38.6)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.71 (1.39-2.01)</td>
<td>1.96 (1.41-2.72)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>44 (24.2)</td>
<td>32 (42.9)</td>
</tr>
<tr>
<td>Subjects, No. (%)</td>
<td>117 (41.9)</td>
<td>90 (41.9)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.15 (1.06-1.27)</td>
<td>1.09 (1.00-1.20)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>160 (13.0)</td>
<td>132 (12.6)</td>
</tr>
<tr>
<td>Subjects, No. (%)</td>
<td>1.65 (1.36-2.00)</td>
<td>1.82 (1.18-2.81)</td>
</tr>
<tr>
<td>Family history of hearing loss</td>
<td>21 (16.5)</td>
<td>18 (15.7)</td>
</tr>
<tr>
<td>Subjects, No. (%)</td>
<td>1.74 (1.28-2.37)</td>
<td>1.98 (1.41-2.77)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

a Each haplogroup was compared with other haplogroups, such as H vs non-H haplogroups.

b All models were simultaneously adjusted for age, sex, noise exposure at work, diabetes, current smoking, and family history of hearing loss.

deliberious bioenergetic abnormalities rather than merely representing the “neutral” polymorphisms reflecting different ethnic backgrounds. It is possible that genetic variants in specific mtDNA haplogroups may impair respiratory chain function within the cochlea to increase the risk of developing ARHL. A number of studies have suggested associations between various mtDNA haplogroups and a variety of medical conditions, including Parkinson disease, Alzheimer disease, occipital stroke, Leber hereditary optic neuropathy, and multiple sclerosis. Of particular interest, haplogroup U has previously been associated with occipital stroke, azoospernia, and Alzheimer disease.

Although initially recognized as a separate haplogroup, haplogroup K is regarded as a subgroup, or haplotype, of U itself by some researchers. A similar mtDNA haplogroup cluster analysis showed a protective association of cluster UKJT with Parkinson disease, and other studies have shown an association between clusters UK and WIX with longevity. These observations suggest that certain haplogroup clusters may modify disease susceptibility.

A strength of this study lies in its large population-based sample of participants sourced from the general community, rather than an exclusively disease-specific group, which could potentially be subject to selection bias. In this older Australian population, prevalence of ARHL (in relation to age, sex, hearing loss severity, and other known risk factors) and of each mtDNA haplogroup was similar to data reported from studies of European and North American populations, suggesting that our results would likely be applicable to other white-based societies.

In conclusion, our findings suggest that mtDNA haplogroups U and K are independent genetic markers for moderate to severe ARHL and may modify susceptibility associated with some known risk factors. Mitochondrial DNA may play a role in the pathogenesis of ARHL. 

Table 3. Associations Between the 5 Most Prevalent Mitochondrial DNA Haplogroups and Age-Related Hearing Loss (ARHL)

<table>
<thead>
<tr>
<th>Haplogroup</th>
<th>Mild ARHL</th>
<th>Moderate to Severe ARHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No. (%)</td>
<td>Age- and Sex-Adjusted OR (95% CI)</td>
<td>Multivariate Adjusted OR (95% CI)</td>
</tr>
<tr>
<td>H</td>
<td>280 (44.8)</td>
<td>1.03 (0.84-1.26)</td>
</tr>
<tr>
<td>J</td>
<td>72 (11.5)</td>
<td>1.18 (0.86-1.62)</td>
</tr>
<tr>
<td>K</td>
<td>58 (9.3)</td>
<td>1.15 (0.80-1.65)</td>
</tr>
<tr>
<td>T</td>
<td>53 (8.5)</td>
<td>0.96 (0.68-1.38)</td>
</tr>
<tr>
<td>U</td>
<td>87 (13.9)</td>
<td>1.09 (0.81-1.46)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

a Each haplogroup was compared with other haplogroups, such as H vs non-H haplogroups.

b Adjusted for age, sex, noise exposure at work, diabetes, current smoking, and family history of hearing loss.
but our findings need to be corroborated by future studies to confirm the prevalence of mtDNA haplogroups in other populations of individuals affected with ARHL. The precise mechanism underlying how mtDNA haplogroups increase genetic risk for ARHL remains to be clarified. If these findings are confirmed, introduction of preventive strategies to minimize environmental causes could be implemented to reduce the overall risk of a genetically susceptible individual of developing ARHL.

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Correspondence: Carolyn M. Sue, MBBS, PhD, Department of Neurogenetics, Clinic 4, Royal North Shore Hospital, Reserve Road, St Leonards, New South Wales, Australia 2065 (csue@med.usyd.edu.au).

Author Contributions: Dr. Sue had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Mitchell and Sue. Acquisition of data: Manwaring, Wang, Howard, Newall, and Sue. Analysis and interpretation of data: Jones, Wang, Roehchina, and Sue. Drafting of the manuscript: Manwaring, Jones, Newell, and Sue. Critical revision of the manuscript for important intellectual content: Jones, Wang, Roehchina, Howard, and Mitchell. Statistical analysis: Roehchina. Obtained funding: Wang, Newall, Mitchell, and Sue. Administrative, technical, and material support: Manwaring and Howard. Study supervision: Wang, Mitchell, and Sue.

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REFERENCES