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Mitochondrial Haplogroup X is Associated with Successful Aging in the Amish

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Abstract

Avoiding disease, maintaining physical and cognitive function, and continued social engagement in long-lived individuals describe successful aging (SA). Mitochondrial lineages described by patterns of common genetic variants (“haplogroups”) have been associated with increased longevity in different populations. We investigated the influence of mitochondrial haplogroups on SA in an Amish community sample. Cognitively intact volunteers aged 80 (n=261) were enrolled in a door-to-door survey of Amish communities in Indiana and Ohio. Individuals scoring in the top third for lower extremity function, needing little assistance with self-care tasks, having no depression symptoms, and expressing high life satisfaction were considered SA (n=74). The remainder (n=187) were retained as controls. These individuals descend from 51 matriline in a single 13 generation pedigree. Mitochondrial haplogroups were assigned using the 10 mitochondrial single nucleotide polymorphisms (mtSNPs) defining the nine most common European haplogroups. An additional 17 mtSNPs from a genome-wide association panel were also investigated. Associations between haplogroups, mtSNPs, and SA were determined by logistic regression models accounting for sex, age, body mass index, and matriline via generalized estimating equations. SA cases were more likely to carry Haplogroup X (OR=7.56, p=0.0015), and less likely to carry Haplogroup J (OR=0.40, p=0.0003). Our results represent a novel association of Haplogroup X with SA and suggest that variants in the mitochondrial genome may promote maintenance of both physical and cognitive function in older adults.

Keywords

Genetic association; genetic epidemiology; successful aging; Anabaptist; longevity

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

INTRODUCTION

Aging in humans is a multifactorial process marked by declines in the multiple domains of cognitive ability, physical function, and social engagement (Rowe and Kahn 1987). Avoiding disease and disability, maintaining physical and cognitive function, and continuing social engagement in the context of long life define “successful aging” (SA) (Rowe and Kahn 1997). Although environmental factors and lifestyle choices have a substantial effect on quality of life and rate of decline, the ability to stay healthy and active among the oldest old has multiple heritable components including longevity. Much effort has focused on identifying genetic and environmental factors associated with survival to old age (“longevity”) by comparing long-lived individuals to younger individuals. However, understanding factors underlying the other domains of SA requires that factors associated with maintained cognitive and physical function be studied *among a sample of long lived individuals*. Identifying the genetic determinants of SA will help shape public health responses to an aging population.

One source of potential genes that could influence SA is the mitochondrial genome, given its central role in the Free Radical Theory of Aging (Harman 1956; Harman 1972). This theory attributes the age-related decline in mitochondrial function to the accumulation of deleterious free radicals that are byproducts of oxidative phosphorylation. It also theorizes that cellular free radical damage leads to the common aging phenotypes. Mitochondria contain their own 16 kilobase-pair circular DNA (mtDNA) that is maternally inherited and separate from the nuclear genome. As individuals age, mutations can accumulate in the mtDNA at a rate 5–10 times faster than the nuclear genome (Brown 1979). Since mtDNA is both maternally inherited and non-recombining, mitochondrial lineages can easily be evolutionarily traced and described by patterns of common genetic variants called “haplogroups”. There are nine mitochondrial haplogroups that occur more frequently in individuals of European descent: H, I, J, K, T, U, V, W, and X (Torroni et al. 1996; Torroni et al. 1994). These haplogroups and their subtypes have been associated with aging and various age related diseases including Parkinson disease (van der Walt et al. 2003), age-related macular degeneration (Canter et al. 2008; Udar et al. 2009), Alzheimer disease (Carrieri et al. 2001), and type 2 diabetes (Fuku et al. 2007). While these haplogroups are used as markers of ancestry and mitochondrial genome variation, the functional differences they represent are incompletely described.

Multiple studies have associated specific mitochondrial haplogroups and polymorphisms with exceptional longevity (survival to age 90) in different populations. Positive haplogroup associations with longevity were found in northern Italians (Haplogroup J; De Benedictis et al. 1999), Irish (a subgroup of Haplogroup J; Ross et al. 2001), Finnish (Haplogroup U and J; Niemi et al. 2003), and Japanese (Haplogroup D; Alexe et al. 2007). Also individual mitochondrial SNPs (mtSNPs) at positions mt9055, mt5178, mt8414, and mt3010 were more frequent in French (Ivanova et al. 1998) and Japanese centenarians (Tanaka et al. 1998). Nevertheless, there is still considerable inconsistency with replication of these associations between or within populations. Divergent populations contain different haplogroup frequencies based on human migration patterns, so even studies of other European-descended populations have produced singular results. For example, Haplogroup

J's positive association with longevity has failed to replicate in Ashkenazi Jews (Shlush et al. 2008) and southern Italians (De Benedictis et al. 1999; Dato et al. 2004). These inconsistencies could reflect population-specific effects, or could suggest that the relationship between mitochondrial haplogroups and aging is more complicated than a simple association with longevity. The health status of the older adults (not considered in many studies, but considered in defining SA) could influence the relationships between mitochondrial haplogroups and aging phenotypes.

The objective of this study is to identify genetic determinants of SA in the Midwestern United States Amish population. The Amish are an isolated religious sect that migrated from Europe to the United States in the 19th century. Their large family sizes, extensive genealogical records, lifestyle, and high standards of living and medical care make them a suitable population for genetic studies of complex traits (Mitchell et al. 2001). In large part, they have strictly maintained their religious and cultural customs and refrained from intermarriage with the general population. As a result of this uniformity, lifestyle differences between individuals are relatively small, minimizing the effect of differing environmental influences on the aging process within the population. Also, their genetic isolation has produced communities with higher frequencies of rare alleles, making it relatively easier to find rare genetic variants than in the general European-descended white population (van der Walt et al. 2005). Previous studies in the Amish have found offspring longevity to be correlated with that of their parents in an additive fashion, with an estimated heritability of lifespan at 0.25 and a mean age of death at 70.7 (Mitchell et al. 2001). These characteristics suggested that genetic studies of aging in the Amish might identify novel genes or variants positively influencing successful aging.

METHODS

Sample Ascertainment

The present study examined individual mtSNPs and haplogroups for associations with SA in this Amish population. The study sample was a subset of the Collaborative Aging and Memory Project (CAMP) that has enrolled Amish age 65 and older since 2002. This subset consisted of cognitively intact participants aged 80 (n=263) that were enrolled in a population-based door-to-door survey of Amish communities in Indiana and Ohio. The overall recruitment and ascertainment methods were described previously (Ashley-Koch et al. 2005; van der Walt et al. 2005; McCauley et al. 2006). Participants were linked in a single 13 generation pedigree constructed from the Anabaptist Genealogy Database (AGDB) (Agarwala et al. 2003). This study was conducted using protocol approved by the Institutional Review Boards at both the University of Miami and Vanderbilt University.

Initial interviews with participants collected basic demographic information, educational, employment, and medical histories, and blood samples. Several tests were then used to determine the physical and cognitive condition of all volunteers. Cognitive impairment was screened with the Modified Mini-Mental State exam (3MS) (Teng and Chui 1987). Individuals who scored <87 after accounting for years of formal education were considered cognitively impaired and referred for follow up neuropsychological evaluation (Khachaturian et al. 2000). If consensus diagnosis after conducting these additional

measures was dementia or cognitive impairment, the individual was removed from the current study. Depression was assessed with the Geriatric Depression Scale (GDS) (Yesavage 1988) and life satisfaction was assessed through a direct question from the population-based survey: “Overall how satisfying is your life: very satisfying, satisfying, or not so satisfying?”

Self-reported assessments of physical function were analyzed in four different components. The ability to handle basic self-care tasks and advanced daily living functions with no or only partial assistance was evaluated with modified versions of the Katz Activities of Daily Living (ADL) (Katz 1983) and instrumental ADL (IADL) scales (Lawton and Brody 1969). Next, functional status and musculoskeletal function were evaluated using the Rosow-Breslau (Rosow and Breslau 1966) and Nagi scales (Nagi 1976) modified for the Established Populations for Epidemiologic Studies of the Elderly (EPESE) (Seeman et al. 1994). In addition, an objective test of lower extremity physical function (the EPESE short physical performance battery) was administered (Seeman et al. 1994).

Determining Successful Aging

Maintaining physical and cognitive function, and continued social engagement are the three determinants of SA. However there is a strong correlation between cognitive and physical declines in individuals with dementia. To better control confounding of these results by cognitive impairment, the analysis was limited to individuals with sustained cognitive function. Thus genetic factors associated with SA could be interpreted as those promoting maintained physical function and social engagement in the context of maintained cognitive function.

SA status was assigned to individuals (n = 74) meeting the following criteria: education-adjusted 3MS of >86 (or consensus diagnosis of ‘cognitively normal’ after more extensive neuropsychological testing if 3MS <87), GDS of <6, ADL of 1, IADL of 1, Nagi of 1, Rosow-Breslau of 3–4, scoring in the top third of the sample on the EPESE short physical performance battery (>8 out of 12), and indicating a high satisfaction with life, answering either “satisfied” or “very satisfied” on the survey. The remainder of cognitively normal individuals (as assessed by the 3MS) who did not meet at least one of the additional criteria for SA were retained as controls (n=187) (Table 1).

Genotyping

DNA was extracted from lymphocytes using PUREGENE methods (Gentra Systems, Minneapolis, MN) and stored at either the Hussman Institute of Human Genomics (HIHG) at the University of Miami or the Center for Human Genetics Research (CHGR) at Vanderbilt University. To test the association of individual mitochondrial polymorphisms with SA, 27 mtSNPs were genotyped. The first 10 mtSNPs define the nine most common European haplogroups and were used to classify participants into haplogroups for analysis (Torroni et al. 1996). The next 17 mtSNPs were used to test any mitochondrial associations not due to haplogroup. These markers were selected from an ongoing genome-wide association study (GWAS) of 830 CAMP participants over 65 (A.C.C., unpublished data)

using the Affymetrix 6.0 GeneChip® Human Mapping 1 million SNP array set (Affymetrix®, Inc Santa Clara, CA) because they were polymorphic in the Amish sample.

In both cases, DNA samples were arrayed on 384-well plates randomized by phenotype status. DNA samples from the Centre d'Etude du Polymorphisme Humain (CEPH) reference families were replicated on each plate for quality control (QC). QC concordance for each sample was >99%. DNA samples and mtSNPs with less than 98% efficiency were eliminated from the analysis.

Combinatorial Methods

A series of Perl scripts were written to both trace maternal ancestry in the 13 generation Amish pedigree obtained from AGDB and group participants into matrilineal lines descending from female founders. The 263 participants in this study were grouped into 51 matrilineal lines, containing an average of 13 sampled individuals (range: 1 – 93 individuals). There were two individuals whose mtSNP genotypes did not match the rest of their matriline, suggesting either laboratory sample or genealogical record error. These subjects were removed from the analysis, leaving a final sample size of 261 (74 cases and 187 controls). To determine the possible nature of these errors, markers from the ongoing GWAS were used to calculate kinship coefficients and genome-wide identity-by-descent (IBD) estimates for the two subjects. The first individual was an only child with no sampled first degree relatives and could not be analyzed. The second individual shared kinship coefficients of 0.255 with both of its reported full siblings and a proportion of IBD of 0.30 indicating a possible half-sibling relationship from a shared father.

Statistical Analysis

The statistical analysis was performed with SAS software (SAS Institute, Cary, NC). Odds Ratios (OR), 95% confidence intervals (95% CI), and tests of association between mtSNPs and SA were determined by logistic regression via generalized estimating equations (GEE). Age associated covariates age, sex, and body mass index (BMI) were chosen to build the logistic regression model to evaluate the observed data. Number of children of each participant and the inbreeding coefficient have been associated with aging longevity in inbred populations, so they were also included in the model. GEE does not use maximum likelihood estimation so standard assessments of model fit (likelihood ratio test, Akaike's Information Criterion (AIC)) cannot be used. However QIC statistics (analogous to AIC) can be used as goodness-of-fit indicators for GEE regression models. To accurately assess the inclusion of each covariate and the best model fit, a backwards elimination approach was used on the full model with both main effects and interaction terms. The highest model fit was generated from the GEE model containing terms for age, sex, BMI, and number of children (data not shown). Individuals were clustered in the GEE model by matriline, and the independence matrix was used for the initial GEE correlation structure.

Several individuals (n=21) had missing genotypes for one or more haplogroup-defining SNPs. However, since matriline is a valid predictor of haplogroup in the absence of mutation, 18 individuals with missing data were placed into the appropriate haplogroup by imputing the missing values from their respective matrilineal lines. The remaining three

individuals were the only representatives of their matriline and could not be used in the analysis. In addition, there were 18 subjects from three distinct matriline that shared the same genotypes but did not belong to one of the nine European haplogroups being studied. They were grouped together and analyzed as Haplogroup “Other”. There were no representatives for Haplogroups I, K, or W although they were previously reported to exist in our Amish population at low frequencies (van der Walt et al. 2005). The observed haplogroups were evaluated simultaneously in one logistic regression model via GEE using the most common haplogroup, HV, as the reference. Statistical significance was assessed at $\alpha = 0.05$:

$$\text{Logit}(\text{SA}) = \beta_0 + \beta_1 \text{AGE} + \beta_2 \text{SEX} + \beta_3 \text{BMI} + \beta_4 \text{Parity} + \beta_5 \text{J} + \beta_6 \text{T} + \beta_7 \text{U} + \beta_8 \text{V} + \beta_9 \text{X}$$

Next the additional mtSNPs were analyzed with the minor allele as the risk allele. Statistical significance was assessed at $p = 0.002$ after Bonferroni correction for multiple testing:

$$\text{Logit}(\text{SA}) = \beta_0 + \beta_1 \text{AGE} + \beta_2 \text{SEX} + \beta_3 \text{BMI} + \beta_4 \text{Parity} + \beta_5 \text{mtSNP}$$

RESULTS

For the haplogroup analysis, most individuals (and matriline) belonged to Haplogroup H (N=104, 40.31%) or T (N=67, 25.97%). Haplogroup V's frequency was too low in our population (N=2, 0.0078%) to analyze separately, but since Haplogroup V is most closely related to Haplogroup H, its individuals were grouped in the referent category, Haplogroup HV (N= 106, 41.09%). Significant associations with SA were found with two haplogroups. A positive association was found with Haplogroup X (odds ratio [OR]=7.56, $p = 0.0015$), while a negative association was found with Haplogroup J (OR=0.40, $p = 0.0003$) (Table 2). Also despite increased power due to the relative homogeneity of our study sample, the small sample size prevented more in-depth analysis of SA associations within sub-haplogroups and between matriline.

Next, positive associations with SA were found with two of the additional mtSNPs typed in the GWAS: rs2854122 (OR=10.59, $p = 0.0068$) and rs3135030 (OR=9.13, $p = 0.0003$) (Supplemental Table 2). However, these mtSNPs were strongly correlated with the haplogroup-defining mtSNPs for Haplogroups J and X ($R^2 > 0.70$) and did not extend the haplogroup findings. Furthermore despite a few strongly correlated mtSNP pairs, four of the ten haplogroup-defining mtSNPs could not be imputed from the 17 mtSNPs available on the Affymetrix 6.0 chipset (data not shown). Therefore, the GWAS mtSNPs could not determine Haplogroups I, K, V or W and should not be substituted for the “classical” haplogroup-defining SNPs as described by Torroni et al. to categorize the common European haplogroups.

DISCUSSION

In this study we tested specific mitochondrial SNPs and haplogroups for association with SA in an Amish sample. We found associations with two evolutionarily distinct haplogroups, a

novel positive association of SA with Haplogroup X and a negative association with Haplogroup J that is opposite of previous studies of mitochondrial haplogroups and longevity.

The association of Haplogroup X with SA is novel. No significant associations of Haplogroup X to age-related disease, aging, or significantly altered mitochondrial function have been previously reported. Unlike most “European” haplogroups, Haplogroup X is also found in West Eurasians and Native Americans (Reidla et al. 2003). Haplogroup X occurs in <5% of European populations and can be further divided into two sub-haplogroups, X1 and X2, with most Europeans, including our sample subset, falling into X2 (Reidla et al. 2003). Haplogroup X2 accounts for 7% of individuals in our Amish sample, and this frequency is higher than other European populations (Melton et al. 2010). This enrichment could have originated from a founder effect of small numbers of Swiss and German Amish immigrants (van der Walt et al. 2005) and has allowed detection of an effect that studies on other European populations with lower frequencies have missed. The excess of Haplogroup X in Amish SA cases (15% vs. 3% in controls) suggests the presence of alleles on this mitochondrial lineage associated with achieving a long healthy life.

Haplogroup X2 can be further subdivided into six subclades (X2a–X2f) that are characterized by both synonymous and nonsynonymous mtDNA mutations (Reidla et al. 2003). Haplogroup X2 has 13 nonsynonymous changes outlined by Riedla (2003) that are located in coding regions, and three of these mtSNPs have been previously associated with disease (Penisson-Besnier et al. 2001; Zhang et al. 2008; Andreu et al. 1999; Andreu et al. 2000). Nine variants cause amino acid changes in NADH dehydrogenase subunits 2, 4, 5, and 6 in ETC Complex I while three produce amino acid changes in the cytochrome b subunit of ETC Complex III. Two variants change amino acids in ATP Synthase F0 subunits 8 and 9 (Ingman and Gyllenstein 2006). Since mitochondria are responsible for ATP production and the generation of harmful reactive oxygen species (ROS), these nonsynonymous changes may positively alter the effectiveness of the electron transport chain and ATP production or decrease production of ROS and oxidative stress. Our results indicate that inherited mtDNA polymorphisms associated with Haplogroup X2 may help to protect against these harmful processes and positively influence aging.

Unlike Haplogroup X, Haplogroup J has been widely reported as having a positive, though population specific, association with longevity (Dato et al. 2004). Studies of mitochondrial function have found that along with the evolutionarily closely related Haplogroup T, Haplogroup J is characterized by less efficient oxidative phosphorylation. This deficiency requires individuals to burn more calories to create the needed amount of ATP. It was theorized that this higher caloric need would lead to fewer available reducing agents and electrons to make the deleterious ROS that contribute to aging (Coskun et al. 2003). This functional advantage has not been validated, however, and past sequencing of Haplogroup J produced no functional polymorphisms or haplotypes associated with longevity (Rose et al. 2001). On the other hand, Haplogroup J and its oxidative phosphorylation deficiency have been implicated in and positively associated with several diseases, including LHON (Torroni et al. 1997), multiple sclerosis (Reynier et al. 1999), and AIDS progression (Hendrickson et al. 2008).

Our findings suggest an inverse association of Haplogroup J with successful aging, and Ren et al. (2008) found a similar effect after sequencing the mitochondrial genomes of healthy nonagenarians and healthy young controls in the Chinese Uygur population. It is not clear why our SA results differ from previously reported positive associations with longevity, but it may reflect the differences in the phenotypes and individuals studied. For example, an age at death analysis for ancestors of participants in this study produced no significant haplogroup associations (data not shown). Therefore, Haplogroup J might not be associated with longevity in this Amish population. Also, Haplogroup J was present at a lower frequency in our sample relative to other populations in Western Europe, where past studies originated (De Benedictis et al. 1999; Ross et al. 2001; Niemi et al. 2003). If the inconsistent associations between longevity and Haplogroup J are due to mutations in specific sub-haplogroups of J, then the lower prevalence of Haplogroup J in our study might have reduced the chances of sampling Amish individuals in those sub-haplogroups conferring longevity. Nevertheless, our methods are also different than those previously reporting positive associations for Haplogroup J (De Benedictis et al. 1999; Ross et al. 2001; Niemi et al. 2003). For example, we used the three-part definition of SA rather than just chronological age to choose our cases. Past aging studies focused only on longevity, but we believe that finding factors promoting long life without age-related disability has more public health relevance. Also, this is one of the first studies to test mitochondrial associations with indicators of SA in age-matched cases and controls. Past aging studies usually compared genotypes from long-lived cases to younger controls from the general population and inferred that the mtSNPs enriched in the older population might have an effect that allowed for increased longevity. Our method allowed us to look at the effects that genotype differences between normally aged and SA subjects might produce on long-lived individuals' function and levels of physical decline. This had not been done in the previous studies identifying Haplogroup J, and we found that most Amish carrying Haplogroup J did not meet our criteria for SA. Therefore, while J may be more common in longer-lived people, it may not be associated with preserved physical and cognitive function.

Another interesting observation in our data set is the unequal distribution of males and females in SA cases and normally aged controls. Although there were more females in our sample population (58%, n=149), only 35% (n=26) of individuals categorized as SA were female. This does not match previous longevity studies, census records (Bonneux et al. 2010), or the pronounced trend often documented in nature, where females usually live longer than males (Austad 2006). However, past research has shown that although there are greater percentages of female centenarians and supercentenarians, it is often their male counterparts that reach long life through healthier trajectories (Franceschi et al. 2000). In a recent study using physical and cognitive measurements similar to our own, Christensen et al. (2007) found that women actually contract age-related disabilities and lose their independence at a faster rate than their long-lived male counterparts. Seeman et al. (1994) also found similar results when males between 70–79 years old in the MacArthur Studies of Successful Aging performed better physically than their female peers. These observations were consistent in our sample population, where higher percentages of female subjects were categorized as controls, after scoring too high on the ADL, IADL, or Nagi tests which measure levels of independence with daily health care tasks. No specific measure appeared

to contribute more to “control” status (data not shown). Franceschi et al. (2000) proposed that women may reach extreme longevity more because of healthier life style choices than specific genetic effects, unlike their male counterparts. Although we did not observe a statistically significant interaction between mtSNP genotypes and sex, our results support this proposal, as females are living just as long as their male peers, but have not aged as successfully.

In summary, this study tested specific mitochondrial SNPs and haplogroups for association with SA in an Amish sample. We found a significant enrichment of long-lived Amish individuals in Haplogroup X who had aged successfully by avoiding disease, maintaining physical and cognitive function, and continuing social engagement. This novel association of Haplogroup X with SA suggests variants on that mitochondrial background promote SA or its sub-domains, and this finding provides strong preliminary evidence for the existence of functionally significant variation on mitochondrial Haplogroup X that promote successful aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic and Phenotypic Characteristics of 74 successfully aged cases and 187 normally aged controls. All participants were cognitively intact, older than age 80, and were recruited through door-to-door surveys of Amish communities in Indiana and Ohio.

Variable	SA Cases	Normal Controls	Total
Total	74	187	261
Age (mean $\pm$ SD)	82 $\pm$ 2.5	84 $\pm$ 3.6	83 $\pm$ 3.4
Gender (% female)	35	66	58
Body Mass Index (mean $\pm$ SD)	26.70 $\pm$ 4.23	28.24 $\pm$ 5.38	27.74 $\pm$ 5.07
3MS (Median[Range])	94[87–103]	92[58–101]	92[58 ^a –103]
GDS (Median[Range])	1[0–4]	2[0–14]	2[0–14]
ADL (Median[Range])	0[0–1]	1[0–9]	0[0–9]
IADL (Median[Range])	0[0–1]	1[0–12]	0[0–12]
Nagi (Median[Range])	0[0–1]	2[0–5]	1[0–5]
Rosow-Breslau (Median[Range])	3[3–4]	4[3–6]	3[3–6]
EPESE (Median[Range])	10[9–12]	7[0–12]	8[0–12]

^aIndividuals with 3MS<86 were found to be cognitively intact upon neuropsychological exam and included in the study

Table 2

Association of the most common European haplogroups with successful aging in 74 successfully aged Amish cases and 187 normally aged Amish controls. Estimates of the odds ratio and 95% confidence interval for each haplogroup (using H/V as a referent) were obtained from a generalized estimating equation model with an independence correlation matrix, clustering individuals by matriline and adjusting for age, sex, body mass index, and inbreeding coefficient.

Observed Haplogroups ^a	Matrilines (N)	Freq Cases (N/Total)	Freq Controls (N/Total)	Odds Ratio	95% Confidence Interval	P Value
H/V ^b	19	0.41 (30/74)	0.41 (76/187)		-----Referent-----	
J	2	0.03 (2/74)	0.06 (11/187)	0.40	0.24 – 0.65	0.0003
T	12	0.24 (18/74)	0.26 (49/187)	0.87	0.34 – 2.22	0.768
U	7	0.12 (9/74)	0.15 (28/187)	0.76	0.28 – 2.05	0.5892
X	6	0.15 (11/74)	0.03 (6/187)	7.56	2.17 – 26.28	0.0015
Other ^c	4	0.04 (3/74)	0.08 (15/187)	0.47	0.15 – 1.54	0.2136
Missing ^d	3	0.01 (1/74)	0.01 (2/187)		-----	

Freq, frequency

^aHaplogroup I, K, and W did not appear in our study population.

^bHaplogroup V was too infrequent to be analyzed independently (N=2, 0.0078%).

^cThis group contains individuals all of whom share the same alleles at typed SNPs and did not belong to one of the nine haplogroups being studied (N = 18, 0.0698)

^dThese individuals could not be grouped or analyzed due to missing mtSNP data