Final Report

Project Title **Pedigree model of human mtDNA heteroplasmy inheritance**

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Abstract

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Abstract:

โดยทั่วไปนั้น ผู้ป่วยด้วยโรคที่มีสาเหตุมาจากการกลายของยีนในจีโนมไมโทคอนเดรียมักมียีนกลายพันธุ์อยู่ในเซลล์ในสภาพ heteroplasmy คือมีทั้งยีนปกติและยีนกลายผสมกัน ยีนกลายพันธุ์ที่อยู่ในลักษณะนี้สามารถสืบทอดจากแม่สู่ลูกได้โดยแม่ที่มียีนกลายในลักษณะนี้จะถ่ายทอดยีนกลายสู่ลูกแต่ละคนในสัดส่วนที่ไม่เท่ากัน ส่งผลให้สัดส่วนของยีนกลายที่ปรากฏในรุ่นลูกแตกต่างจากในรุ่นแม่และแตกต่างกันระหว่างลูกแต่ละคน ความผันแปรของสัดส่วนของยีนกลายดังกล่าวนี้ทำให้เกิดความซับซ้อนในการประเมินความเสี่ยงของการมีสามาชิกในครอบครัวรุ่นถัดไปที่ป่วยด้วยโรคดังกล่าว ดังนั้นการสร้างความเข้าใจเกี่ยวกับรูปแบบและกลไกที่กำหนดรูปแบบของการถ่ายทอดสารพันธุกรรมในไมโทคอนเดรียที่อยู่ในสภาพ hetetoplasmy จึงนับเป็นขั้นตอนสำคัญที่จะนำไปสู่การพัฒนาวิธีการประเมินความเสี่ยงที่มีความแม่นยำในอนาคต ในงานวิจัยนี้ ผู้วิจัยได้รวบรวมข้อมูลพันธุประวัติของครอบครัวที่มีรายงานว่ามียีนก่อโรคที่พบบ่อยในประชากรอยู่ในสภาพ heteroplasmy เมื่อนำข้อมูลดังกล่าวมาวิเคราะห์ทางสถิติทำให้พบว่า รูปแบบการถ่ายทอดยีนก่อโรคในสภาพ heteroplasmy ของ protein-coding mutation อันได้แก่ G11778A, G3460A และ T8993G มีความแตกต่างจากรูปแบบการถ่ายทอดยีนก่อโรคในสภาพ heteroplasmy ของ tRNA gene mutation อันได้แก่ A8344G และ A3243G จากนั้นผู้วิจัยจึงนำค่า bottleneck parameter ที่คำนวณได้จากข้อมูลพันธุประวัติดังกล่าวมาประยุกต์ใช้ในการสร้างแบบจำลองพันธุประวัติของการถ่ายทอดสารพันธุกรรมในไมโทคอนเดรียที่อยู่ในสภาพ heteroplasmy โดยแบบจำลองดังกล่าวสร้างขึ้นตามสมมุติฐานว่า ความหลากหลายของสัดส่วนของยีนกลายเกิดจากปัจจัย random genetic drift เท่านั้น แบบจำลองดังกล่าวนี้ถูกทดสอบโดยเปรียบเทียบค่าสัดส่วนของยีนกลาย (mutation level) ที่จำลองขึ้นกับค่าที่สำรวจได้ ผลการทดสอบพบว่า แบบจำลองดังกล่าวมีข้อจำกัด คือสามารถใช้อธิบายการถ่ายทอดสารพันธุกรรมในไมโทคอนเดรียที่อยู่ในสภาพ heteroplasmy ของการกลายแบบ A3243G ได้ดีแต่สามารถอธิบายการถ่ายทอดสารพันธุกรรมในไมโทคอนเดรียที่อยู่ในสภาพ heteroplasmy ของการกลายแบบ G3460A, T8993G และ A8344G ได้เพียงบางส่วนเท่านั้น เมื่อนำแบบจำลองดังล่าวไปศึกษาพัฒนาการของ mtDNA mutation level ในครอบครัวจำลองและประยุกต์ใช้ในการประเมินความเสี่ยงของการมีลูกที่ป่วยที่มีสาเหตุจากการมีสัดส่วนของยีนกลายสูงกว่า threshold level พบว่า การเปลี่ยนแปลงของ mtDNA mutation level ของการกลายใน

protein-coding gene เกิดขึ้นเร็วกว่าการเปลี่ยนแปลงของ mtDNA mutation level ของการกลายใน tRNA gene ส่งผลให้ระดับความเสี่ยงของการมีลูกที่ป่วยจากแม่ที่มีการกลายใน protein-coding gene สูงกว่าใน tRNA gene อย่างไรก็ดี ภายได้ข้อจำกัดของแบบจำลองนี้ การนำค่าความน่าจะเป็นต่างๆที่คำนวณได้จากครอบครัวจำลองไปใช้ควรกระทำด้วยความรอบคอบ ในขั้นสุดท้าย ผู้วิจัยได้พัฒนา population model จากแบบจำลองพันธุประวัตินี้และได้ทดลองใช้ประเมินสัดส่วนของ mutant carrier ในประชากรสมมุติ จากผลการทดลองดังกล่าวทำให้ทราบว่า นอกจากปัจจัยการกลายและ random genetic drift ปัจจัยอื่นๆเช่น การคัดเลือก และลักษณะโครงสร้างของประชากร น่าจะเป็นปัจจัยสำคัญในการกำหนดสัดส่วนของ mutant carrier ในประชากรด้วย แต่ในปัจจุบันยังไม่มีข้อมูลเพียงพอที่จะใช้ในการระบุค่าปัจจัยอื่นๆ เหล่านี้ แบบจำลองพันธุประวัติและ population model ที่พัฒนาขึ้นจากงานวิจัยนี้นับเป็นก้าวแรกที่สำคัญในสร้างความเข้าใจเกี่ยวกับรูปแบบการถ่ายทอดโรคที่มีสาเหตุมาจากการกลายของยีนในจีโนมไมโทคอนเดรียและพัฒนาวิธีการป้องกันการถ่ายทอดโรคดังกล่าวได้อย่างมีประสิทธิภาพในอนาคต

Typically an affected individual carried the pathogenic mtDNA mutation in a heteroplasmic condition– a mixture of wild type and mutated mtDNA. Human mtDNA is exclusively maternally inherited, presenting a large inter-generational random shift in mutation level. This random shift complicated recurrence risk estimation in the family carrying the pathogenic mtDNA mutation, emphasizing the need to understand mtDNA heteroplasmy transmission. Various statistical analyses carried out on the pedigree data suggested that the transmission pattern of the mtDNA mutation level of the protein-coding mutations: G11778A, G3460A, and T8993G, is different from that of the tRNA gene mutations: A8344G and A3243G. The bottleneck parameters estimated from the clinical pedigree data were applied to build the pedigree model of human mtDNA heteroplasmy inheritance. This model was built based on the assumption that the transmission of mtDNA mutation levels is solely determined by random genetic drift. Besides the A3243G mutation, the pedigree model could only partly explain the transmission pattern of the G3460A, T8993G and A8344G mutations. Under the simple pedigree structure and random genetic drift theory, the pedigree model suggested that the progression of mtDNA mutation level of the protein-coding mutations is faster that the progression of the tRNA gene mutation, thus the recurrence risk and the probability of having a wild type homoplasmy offspring of the protein-coding mutations are greater that those probabilities of the tRNA gene mutations. Due to the limited validity of the pedigree model, these probabilities should be interpreted with caution. The pedigree model was further developed to the population model and this enlarged scale model was applied to estimate the proportion of mutant carriers in the population. The simulated results of the population model suggested that *de novo* mutation and random drift are not sufficient to explain the distribution of mtDNA mutation levels in general population; however, other factors, such as selection coefficient and mutation specific bottleneck parameter values cannot be defined at the present because of inadequate information. These basic pedigree and population models would be considered as the first step toward understanding the progression of the diseases associated with mtDNA mutations.

Keywords : mtDNA heteroplasmy, random genetic drift, Kimura distribution, pedigree model

Final report content

Abstract

Typically an affected individual carried the pathogenic mtDNA mutation in a heteroplasmic condition– a mixture of wild type and mutated mtDNA. Human mtDNA is exclusively maternally inherited, presenting a large inter-generational random shift in mutation level. This random shift complicated recurrence risk estimation in the family carrying the pathogenic mtDNA mutation, emphasizing the need to understand mtDNA heteroplasmy transmission. Various statistical analyses carried out on the pedigree data suggested that the transmission pattern of the mtDNA mutation level of the protein-coding mutations: G11778A, G3460A, and T8993G, is different from that of the tRNA gene mutations: A8344G and A3243G. The bottleneck parameters estimated from the clinical pedigree data were applied to build the pedigree model of human mtDNA heteroplasmy inheritance. This model was built based on the assumption that the transmission of mtDNA mutation levels is solely determined by random genetic drift. Besides the A3243G mutation, the pedigree model could only partly explain the transmission pattern of the G3460A, T8993G and A8344G mutations. Under the simple pedigree structure and random genetic drift theory, the pedigree model suggested that the progression of mtDNA mutation level of the protein-coding mutations is faster that the progression of the tRNA gene mutation, thus the recurrence risk and the probability of having a wild type homoplasmy offspring of the protein-coding mutations are greater that those probabilities of the tRNA gene mutations. Due to the limited validity of the pedigree model, these probabilities should be interpreted with caution. The pedigree model was further developed to the population model and this enlarged scale model was applied to estimate the proportion of mutant carriers in the population. The simulated results of the population model suggested that *de novo* mutation and random drift are not sufficient to explain the distribution of mtDNA mutation levels in general population; however, other factors, such as selection coefficient and mutation specific bottleneck parameter values cannot be defined at the present because of inadequate information. These basic pedigree and population models would be considered as the first step toward understanding the progression of the diseases associated with mtDNA mutations.

Executive summary

Mitochondrial DNA (mtDNA) mutation has been observed to cause various diseases including deafness, blindness (LHON), and late-onset neurodegenerative diseases. Until recently, there is no effective way to treat patients with the disease caused by mtDNA mutations, thus preventing transmission of the pathogenic mtDNA mutations become an important strategy. Typically an affected individual carried the pathogenic mtDNA mutation in a heteroplasmic condition– a mixture of wild type and mutated mtDNA. The mother who also carried heteroplasmic mtDNA mutations generally transmits a random proportion of mutated mtDNA to her children, generating random shift in heteroplasmy level. This random shift complicates recurrent risk estimation in a

family carrying the mtDNA mutation; therefore, understanding mtDNA heteroplasmy transmission is necessary. This project aims to develop a pedigree model of human mtDNA heteroplasmy inheritance using both computational and statistical techniques

The clinical data of human pedigrees carrying one of the five common pathogenic mtDNA mutations: G11778A, G3460A, T8993G, A8344G and A3243G, was mainly collected from published literature. The mother-offspring pair mutation levels were gathered from this pedigree data and analyzed by both parametric and nonparametric methods. The statistical results suggested that the transmission pattern of the protein-coding mutations: G11778A, G3460A, and T8993G, differed from the pattern of the tRNA gene mutations: A8344G and A3243G. As suggested by the statistical analysis carried out on the mother-offspring pair mutation levels, besides random genetic drift, positive selection should play an important role in determining the mutation level of the offspring carrying protein-coding mutations, while negative selection should play a role in regulating the transmission pattern of the tRNA gene mutations; therefore, the difference of the transmission pattern of the mutation level between the protein-coding mutations and the tRNA gene mutations would be the result of different mechanisms regulating the transmission pattern of these mutations.

The bottleneck parameter values estimated from the clinical pedigree data were further applied to generate the pedigree model that was built based on the assumption that the transmission of mtDNA mutation level is solely determined by random genetic drift. The theoretical Kimura distribution was used for generating the mutation level for each simulated individual. This model was verified by testing whether its behavior is consistent with the random genetic drift theory and the results showed that the model behaved as expected. We further validated the model by comparing the simulated mutation level to the observed mutation levels and the results showed that this model could well predicted the offspring A3243G mutation levels and to some extend can explain other mutation, except the G11778A. The inconsistency between the simulated and the observed data could be caused by the effect of selection or the ascertainment bias presented in the observed clinical pedigree data.

The pedigree model was applied to study the progression pattern of the mtDNA mutation. The simulated data showed that the progression of mtDNA mutation level of the protein-coding mutation was faster than the progression of the tRNA gene mutations. Because the bottleneck parameter values of the protein-coding mutations were higher than the values of the tRNA gene mutations, the difference rate of mutation level development suggested that the lower the bottleneck parameter value, the faster the progression of mtDNA mutation level.

The pedigree model was further applied to estimate recurrence risk in the family carrying a pathogenic mtDNA mutation. Under the assumption of this basic pedigree model, the probability of having a child carrying a high mutation level of the protein-coding mutations is greater than the probability of the tRNA gene mutations. The probability of having a child carrying wild type homoplasmy of the protein-coding mutations is also higher than that probability of the tRNA gene mutations. Due to the limited validity of the pedigree model, the probability values calculated from

the simulated data should be interpreted with caution; the recurrence risk should be considered as the minimum chance of having a potential affected offspring.

The pedigree model was further developed to the population model. Given the *de novo* mutation rate of 0.10%, the initial mutation level of 10% and the bottleneck parameter value of 0.64, the proportion of the mutant carriers could reach 0.54%, the proportion of mutant carriers observed in general population (ELLIOTT *et al.* 2008), at generation 34. The distribution pattern of the simulated mutation levels was not consistent with the pattern of the observed mutation levels. This discrepancy should be caused by no lineage loss, no purifying selection and insufficient effect of random genetic drift defined in the simulation. To improve this population model, the probability of lineage loss, selection coefficient and a set of mutation specific bottleneck parameter values should be included in the model; however, at the present, we did not have enough information at hand to define all these parameters.

The basic pedigree model developed in this project provides the first step toward understanding the progression of the diseases associated with mtDNA mutations. Once other functions and parameters, such as the selection coefficient, the mutation specific bottleneck parameter value, and the penetrance function, were defined, the more realistic pedigree model could be developed from this basic model.

Objective

The specific aims of this project are as follows:

(1) To create a pedigree model of human mtDNA heteroplasmy inheritance

(2) To study the progression across generations of human disease caused by pathogenic mtDNA mutation

(3) To develop a method of recurrence risk estimation

(4) To study the segregation of common pathogenic mtDNA mutation in general population

Research methodology

Pedigree data analysis: Human clinical pedigree data collection

Human clinical pedigrees carrying one of the five common pathogenic mtDNA mutations: G11778A (CARELLI *et al.* 1997; CHUENKONGKAEW *et al.* 2005; HARDING *et al.* 1995; HOLT *et al.* 1989; HOWELL *et al.* 1994; JUVONEN *et al.* 1997; LOTT *et al.* 1990; MARTIN-KLEINER *et al.* 2006; MASHIMA *et al.* 2004; PHASUKKIJWATANA *et al.* 2006; SIMON *et al.* 1999; SWEENEY *et al.* 1992; TANAKA *et al.* 1998; TONSKA *et al.* 2008; ZHU *et al.* 1992), G3460A (BLACK *et al.* 1996; CARELLI *et al.* 1997; GHOSH *et al.* 1996; HARDING *et al.* 1995; HOWELL *et al.* 1991; KAPLANOVA *et al.* 2004; LODI *et al.* 2002; SWEENEY *et al.* 1992; TONSKA *et al.* 2008; VOLODKO *et al.* 2006), T8993G (BARTLEY *et al.* 1996; CAIFALONI *et al.* 1993; CARELLI *et al.* 2002; CHAU *et al.* 2010; DE COO *et al.* 1996; DEGOUL *et al.* 1995; DEGOUL *et al.* 1997; ENNS *et al.* 2006; FERLIN *et al.* 1997; FRYER *et al.* 1994; HOLT *et al.* 1990; HOUSTEK *et al.* 1995; JIANG *et al.* 2002; MAK *et al.* 1996; MAKELA-BENGS *et al.* 1995; MKAOUAR-REBAI *et al.* 2009;

PASTORES *et al.* 1994; PORTO *et al.* 2001; SAKUTA *et al.* 1992; SANTORELLI *et al.* 1993; SHOFFNER *et al.* 1992; STEFFANN *et al.* 2007; TATUCH *et al.* 1992; TSAO *et al.* 2001; TULINIUS *et al.* 1995; UZIEL *et al.* 1997; WHITE *et al.* 1999; WONG *et al.* 2002), A8344G (CANTER *et al.* 2005; CHU *et al.* 1994; GAMEZ *et al.* 1998; HAMMANS *et al.* 1993; HOWELL *et al.* 1996; LARSSON *et al.* 1992; MANCUSO *et al.* 2007; MOLNAR *et al.* 2009; MUNOZ-MALAGA *et al.* 2000; ORCESI *et al.* 2006; PICCOLO *et al.* 1993; SEIBEL *et al.* 1991; SIVESTRI *et al.* 1993; TRAFF *et al.* 1995; TSAO *et al.* 2003; VAN DE GLIND *et al.* 2007; WONG *et al.* 2002), and A3243G (BROWN *et al.* 2001; CERVIN *et al.* 2004; CHINNERY *et al.* 1999; CHOU *et al.* 2004; CIAFALONI *et al.* 1992; DUBEAU *et al.* 2000; FABRIZI *et al.* 1996; FUKAO *et al.* 2009; HAMMANS *et al.* 1995; HARRISON *et al.* 1997; HOSSZUFALUSI *et al.* 2009; HUANG *et al.* 1994; HUANG *et al.* 1996; HUANG *et al.* 1999; IWANISHI *et al.* 1995; JANSEN *et al.* 1997; KO *et al.* 2001; LI *et al.* 1996; LIEN *et al.* 2001; LIOU *et al.* 1994; LU *et al.* 2006; MARTINUZZI *et al.* 1992; MOROVVATI *et al.* 2002; OLSSON *et al.* 1998; ONISHI *et al.* 1998; RUSANEN *et al.* 1994; VERNY *et al.* 2008; VILARINHO *et al.* 1997; WILICHOWSKI *et al.* 1998; ZHANG *et al.* 2009), were collected from the literature. In the case of G11778A mutation, Prof. Dr. Patcharee Lertrit kindly provided extra-unpublished data of eight heteroplasmic families. The pedigree position, gender, relationship with the index case, age at sampling, and blood mtDNA heteroplasmy level of each individual were collected. Because of the maternal inheritance of human mtDNA, only the information of the index cases and their maternal relatives were included in the analyzed data. The individual's pedigree position was recorded with regard to the maternal inheritance of human mtDNA. The relationship between the individual and the index case was recorded as generally defined; for example, mother, sister, brother or uncle. The age at sampling of the individuals is the age when their blood was drawn for mtDNA mutation level measurement.

Pedigree data analysis: Managing mother-offspring pair data

Because this study aims to understand common pathogenic mtDNA heteroplasmy transmission, the mother-offspring pairs, whose mtDNA heteroplasmy level had been reported, were included in the analyzed data. All the mother-offspring pairs whose heteroplasmic offspring was born to the wild type homoplasmic mothers were excluded from the statistical analyses to adjust for the effect of seemingly *de novo* mutation. These *de novo* mutation cases may be the result of the limitation of mtDNA heteroplasmy measurement method. The mother-offspring pairs whose one of them is an index case were also excluded from the statistical analyses to adjust for the effect of ascertainment bias of clinical data. A number of longitudinal studies reported a reduction of blood mutation level toward age (PYLE *et al.* 2007; RAHMAN *et al.* 2001; RAJASIMHA *et al.* 2008; THART *et al.* 1996), which could deceptively generate an inter-generational increase of the mtDNA heteroplasmy level. Hence, the application of the age correction for this reduction is required to reduce the transmission bias due to this longitudinal change. In 2007 Rajasimha et al. propose that this reduction is the result of the selection against high heteroplasmic hematopoietic stem cell. Besides the mechanism, they provided the mathematical formula to correct for this reduction (RAJASIMHA *et al.* 2008). The formula is shown in equation 1.

$$p_{age-corrected} = p_{observed} e^{0.02t} \quad (1)$$

The $p_{observed}$ is the individual's heteroplasmy level and the $p_{age\text{-}corrected}$ is the age-corrected individual's heteroplasmy level. The *t* variable is the individual's age at sampling. After applying this age-correction formula, the families harboring individuals carrying age-corrected A3243G mutation level exceeds 110% were excluded from the analyses because these families may carry a secondary mtDNA mutation that could modify the mtDNA heteroplasmy segregation (CAMPOS *et al.* 1995; MOROVVATI *et al.* 2002).

Calculating statistics based on the Kimura distribution

Kimura distribution is the probability distribution of allele frequencies segregated under random genetic drift process. It was adapted from the work of Motoo Kimura in 1955 (KIMURA 1955), for the full mtDNA heteroplasmy level distribution (WONNAPINIJ *et al.* 2008). It consists of three equations: a probability *f(0)* for carrying wild type homoplasmy, a probability *f(1)* for carrying mutant homoplasmy, and a probability distribution function ϕ *(x)* for carrying *x*% mutation level, as shown in Equation 2 to 4, respectively.

$$f(0) = (1 - p_0) + \sum_{i=1}^{\infty} (2i+1) p_0 (1-p_0) (-1)^i F(1-i, i+2, 2, 1-p_0) b^{\frac{i(i+1)}{2}} \quad (2)$$

$$f(0) = p_0 + \sum_{i=1}^{\infty} (2i+1) p_0 (1-p_0) (-1)^i F(1-i, i+2, 2, p_0) b^{\frac{i(i+1)}{2}} \quad (3)$$

$$\phi(x) = \sum_{i=1}^{\infty} i(i+1)(2i+1) p_0 (1-p_0) F(1-i, i+2, 2, p_0) F(1-i, i+2, 2, x) b^{\frac{i(i-1)}{2}} \quad (4)$$

The 95% confident interval of each mutation was calculated from the Kimura distribution with the *b* parameter value estimated from the offspring heteroplasmy levels. There were two values of the *b* parameter that were calculated in this study: (1) the *b* parameter value calculated from the heteroplasmy levels of the offspring of the mothers carrying an intermediate heteroplasmy level, 40-60% heteroplasmy level and (2) the *b* parameter value calculated from all offspring carrying each mutation. The intermediate heteroplasmy level was chosen because it has the least effect from the average heteroplasmy level (the *p* parameter value) (WONNAPINIJ *et al.* 2010). How to calculate the *b* parameter value is shown in Equation 5.

$$b = 1 - \frac{V}{p(1-p)} \quad (5)$$

The *V* variable is the offspring heteroplasmy level variance and the *p* variable is the offspring heteroplasmy level mean.

Assumed that the distribution of mtDNA mutation levels follows the Kimura distribution, the mtDNA heteroplasmy level variance was normalized by dividing it by the factor p(1-p) (WONNAPINIJ *et al.* 2010). The 95% confidence interval of the normalized offspring mutation level variance and the bottleneck (*b*) parameter values were calculated from 10,000 simulated data (WONNAPINIJ *et al.* 2010). The p_0 and the *b* parameter used for calculating the Kimura distribution were the average offspring mutation level and the b parameters calculated from the pedigree data using Equation 5. The normalization method, the 95% confidence interval of the normalized mutation level variance

and the 95% confidence interval of the *b* parameter value calculation were applied for the comparison of mtDNA heteroplasmy level variance between different mtDNA mutations.

Pedigree data analysis: Data visualization

The 2D scatter diagram was applied to visualize the relationship in heteroplasmy level between offspring and their corresponding mothers. The 95% confident interval was added to the scatter diagram to examine the consistency between the observed mother-offspring pair data and the Kimura distribution. The scatter-diagram smoothing method was applied to define the relationship in heteroplasmy level between offspring and their corresponding mothers. This method uses a locally weighted polynomial regression method (the "lowess" function in R) to analyze the relationship between the response variable and the predictor variable. This method makes only a few initial assumptions about the model; therefore, it can be considered as a non-parametric regression analysis (CLEVELAND 1979). All these plots were created using OriginPro8 (OriginLab)

Pedigree data analysis: Hypothesis testing

Every statistical analysis was done using function in R programming (R foundation for statistical computing). Both parametric and non-parametric approaches: student's t test and Wilcoxon test, were applied to test the hypothesis. The result is considered to be significant different when the p-value is less than the significant level at 0.05.

The one sample t-test was applied to examine whether the average O-M values is significant different from zero. The one-sample Wilcoxon test, which examines the median statistic, was chosen as an alternative non-parametric approach of this t-test. The two-sample t-test with the Welch approximation (the variation of the student's t test proposed to compare two samples possibly having unequal variances) was applied to compare the average O-M values of the female offspring to the statistic of the male offspring. The Mann-Whitney test (the two-sample Wilcoxon test), which is an alternative non-parametric approach of the two-sample t test, was applied to examine the median statistic of this comparison. ANOVA and the Kruskal-Wallis test were applied to examine whether the average O-M values were significance among different mtDNA mutations. Their post hoc test, Tukey test and Wilcoxon test, were applied to examine which pair of mtDNA mutations was significant difference.

The mother-offspring pair data was separated into two groups based on the mother's heteroplasmy level: the group of low heteroplasmic mothers and the group of high heteroplasmic mothers. The group of low heteroplasmic mothers contained the mother-offspring pair data whose mothers carried heteroplasmy level less than 50%. On the contrary, the group of high heteroplasmic mothers contained the mother-offspring pair data whose the mother carried heteroplasmy level greater than of equal to 50%. After separating the mothermother-offspring pair data, the two sample t-test with the Welch approximation and the Mann-Whitney test were applied to compare the average O-M values of the low heteroplasmic mothers to the statistic of the high heteroplasmic mothers.

Pedigree model construction

The pedigree model was designed based on random genetic drift theory using Kimura distribution (shown in Equation 2-4) to calculate the probability of carrying a certain proportion of mutant mtDNA (WONNAPINIJ *et al.* 2008). The proportions of mutant mtDNA were ranged from 0 to 100% (wild type homoplasmy to mutant homoplasmy). The p_0 and b parameter values used in this theoretical distribution were the maternal mutation level and the b parameter estimated from the human clinical pedigree data, respectively. The probabilities of these mutation levels were integrated to generate a cumulative probability distribution for a certain p_0 and b parameter values. To generate the mutation level of each simulated individual, a probability value was randomly chosen from a uniform distribution, then this probability value was used to select a mutation level from the cumulative probability distribution that was previously generated.

The number of offspring per female and the offspring's gender ratio were used for determining the structure of the simulated pedigree. To simplify the pedigree model, these parameters were set to 1 and 1:0, respectively. The pedigree simulation was written in C/C++ programming language.

Pedigree model verification and validation

The simulation was verified by examining the basic statistical properties of the simulated pedigrees: the mutation level mean and variance, whether they followed random genetic drift theory (HALLIBURTON 2004). 10,000 simulated pedigrees were generated. Each one initialized by a founder female carrying 5% mutation level. Kimura distribution was applied to calculate the probability of carrying x% mutation level. The b parameter values used for generating the simulated data was the value calculated from the mutation levels of the offspring born to the mothers carrying an intermediate mutation level (40-60%). The simulated offspring mutation levels were randomly generated from The number of offspring per female in each generation was fixed to one and the ratio of daughter to son was 1:0, thus no simulated family lost its lineage before the defined maximum number of generations (50 generations). The mean and variance statistics of each generation mutation levels were calculated and plotted against the number of generations.

The confidence interval approach was applied to validate the pedigree model (KELTON and LAW 2000). 200 sets of simulated offspring mutation levels were generated based on the observed maternal mutation levels. The sample size of each set of simulated mutation level was equal to the number of maternal mutation levels of each mtDNA mutation. The b parameter values used for validating the model was calculated from the mean and variance statistics presented in the observed offspring mutation levels. The difference between the simulated and offspring mutation levels was calculated. These differences were averaged to represent the average difference for a set of simulated data, then these average differences were sorted and the 95% confidence interval were calculated based on 200 average difference. If the 95% confidence interval included zero, the simulated offspring mutation levels were considered as being consistent with the observed mutation levels and the model was valid. This validation method was applied to both all offspring and the offspring of the intermediate heteroplasmic mothers of each mtDNA mutation.

Application of the pedigree model to study the progression of mtDNA mutation level

Based on random genetic drift theory, the average mutation level would not change across the number of generations; however, the proportion of individuals carrying wild type homoplasmy, heteroplasmy, and mutant homoplasmy could change across generations. To investigate the progression of mtDNA mutation level toward generations, 10,000 simulated pedigrees were generated. The initial condition of these simulated pedigrees were the same as previously generated for pedigree model verification; the female founder mutation level, the number of offspring per female, the ration of daughter to son, and the number of generations were set at 0.05, 1, 1:0, and 50, respectively. The average probability of carrying wild type homoplasmy, heteroplasmy, and mutant homoplasmy were calculated for each generation and plotted against the number of generations.

Application of the pedigree model to estimate recurrence risk

The mtDNA mutation level has long been considered as one factor determining the expression various mitochondrial diseases caused by mtDNA mutation (DIMAURO and DAVIDZON 2005; GREAVES and TAYLOR 2006); an individual needs to carry a proportion of mutant mtDNA higher than the threshold level of a particular mutation (DIMAURO and DAVIDZON 2005; GREAVES and TAYLOR 2006). In general, the threshold level of mitochondrial disease caused by pathogenic mtDNA mutation is 60%. Based on this concept, recurrence risk in the family carrying pathogenic mtDNA mutations could be in proportion to the probability of carrying mtDNA mutation level greater than the threshold level. The probabilities of harboring the offspring carrying mtDNA mutation level greater than the threshold level were calculated from 10,000 simulated pedigrees. These pedigrees were generated based on the initial conditions that (1) the maternal mutation levels were in the range of 1 to 20%, (2) the number of offspring per mother was set to one, and (3) the ratio of daughter to son was set to 1:0.

Population model development

The population model was developed from the pedigree model. In this model, the founder population contained only wild type homoplasmic females. The *de novo* mutation rate and the initial mutation level determined the mutation levels of first generation population. The *de novo* mutation rate is the proportion of heteroplasmic offspring born to the wild type homoplasmic mother observed in live-birth population of North Cumbria in England (ELLIOTT *et al.* 2008) The limitation of the mutation level measurement used in the North Cumbria population study was used as the initial mutation level. Therefore, the de novo mutation and the initial mutation level used in the population model were 0.10% and 10%, respectively.

After the first generation, the mutation levels of later generation population were determined not only by the *de novo* mutation rate and the initial mutation level, but also by the Kimura distribution (WONNAPINIJ *et al.* 2008). The b parameter value (0.64) used in this model was calculated from the offspring of the intermediate heteroplasmic mothers from all mtDNA mutation included in this study. The wild type homoplasmic mothers could only have either wild type

homoplasmic offspring or heteroplasmic offspring carrying mutation level equal to the initial mutation level, while the heteroplasmic mothers could have offspring carrying mutation levels as generated by the Kimura distribution. In order to simplify the statistical properties of the simulated data, the structure of each pedigree in the population was based on the assumption that each female would only have one daughter, thus no effect of lineage loss was observed. Each simulated pedigree was last for 50 generations.

The proportion of the mutant carriers for each mtDNA mutation was the proportion of simulated individuals who carried mutation level greater than 0%. The proportion of mutant carries was calculated at each generation. The mutation levels of all simulated heteroplasmic individuals were saved for investing the distribution of mutation lelve in the simulated population.

Result

Pedigree data analysis

The data of human clinical pedigrees carrying common pathogenic mtDNA mutations: G11778A, G340A, T8993G, A8344G, and A3243G, was mainly collected from published literature. The mtDNA mutation levels of eight unpublished G11778A families were measured in Mitochondrial genetic lab, Siriraj hospital. The mutation levels of mother-offspring pairs were systematically gathered for statistical analyses aiming to deduce the transmission pattern of each mtDNA mutation. Summary statistics of the mother-offspring pair data were presented in Table 1. The number of transmissions is the number of mother-offspring pairs included in the statistical analyses. The average maternal mutation level is generally lower than the average offspring mutation level, except for the A8344G mutation. The offspring mutation level variances and their normalized value of the protein-coding mutations: G11778A, G3460A, and T8993G, were higher than those statistics of the tRNA gene mutations: A8344G and A3243G; however, these differences may not be statistically significant because the 95% confident interval of the normalized offspring mutation level variance of the protein-coding mutations (0.3671-0.7357) overlapped the confident interval of this statistics of the tRNA gene mutations (0.2580-0.4791).

Theoretical, the bottleneck (*b*) parameter values determine how high the offspring mutation level variance is (WONNAPINIJ *et al.* 2008). As shown in Table 1, this parameter was calculated both from all offspring and from the offspring of the mothers carrying an intermediate mutation level (40-60%). Based on random genetic drift theory, the offspring mutation level variance is least affected by the mutation level mean at the intermediate level, thus the *b* parameter calculated from the offspring of the mother carrying an intermediate mutation levels could be justified as a normalized *b* parameter value. Considered the *b* parameter calculated from all offspring, the values of the protein-coding mutations: G11778A, G3460A, and T8993G, were lower than those of the tRNA gene mutations: A8344G and A3243G; however, the 95% confident intervals of the b parameter values of the protein-coding mutations (0.249-0.633) overlapped the confident interval of this statistics of the tRNA gene mutations (0.521-0.742). The same trend was observed in the b parameter values

calculated from the offspring of the mothers carrying an intermediate mutation level; however, the range of the overlap between the 95% confident interval of the b parameter values of the protein-coding mutation (0.092-0.740) and that of the tRNA gene mutation was smaller (0.696-0.929).

The relationship of mutation levels between offspring and their corresponding mothers was revealed by plotting the offspring mutation levels against the mutation level of their corresponding mothers, as shown in Figure 1a-e. Each figure presents relationship of different pathogenic mtDNA mutations. In each figure, both index cases and their maternal relatives were included. No apparent distinction of mutation levels between index cases and their maternal relatives; however, both Welch's t test and Man-Whitney test indicated that for all mutation, except G3460A, the average mutation level of index cases was significant different from that of their relatives (Table 2). The 95% confident interval of the offspring mutation levels presented in each of these figures was calculated from the Kimura distribution with the maternal mutation level ranged from 0 to 100% and the b parameter estimated from the mutation levels of the offspring of the mother carrying an intermediate mutation level. Regardless of the type of mtDNA mutation, the confident interval covered most offspring mutation levels. Notice that most mutation levels that were not in the range of 95% confident interval were those of the offspring of the mothers carrying an extreme mutation level, either very low or very high mutation level.

To test whether there is any gender bias in the transmission of mtDNA heteroplasmy level, the mother-offspring pair data was separated into two groups based on offspring's gender. For each mtDNA mutation, the average O-M value of the male offspring was statistically compared against that of the female offspring. The results showed that no significant difference of the average O-M value between male and female offspring was observed in all mtDNA mutation included in this study (Table 2).

The pattern of relationship between offspring mutation levels and the maternal mutation levels were deduced by the locally weighted regression analysis. This nonparametric method uses multiple regression models to fit subsets of the data, then these locally fit models are combined to provide the function that can describe the overall data (CLEVELAND 1979). The advantage of this method is that no function needs to be specified prior to analyzing the data. The predicted lines generated based on this method of different mtDNA mutations were compared against each other, as shown in Figure 1f. Besides the predicted lines, the black diagonal line was added in this figure to present the average offspring mutation level expected based on random genetic drift theory. It could be noticed that all predicted lines of protein-coding mutations: G11778A, G3460A, and T8993G, were located above the diagonal line, while all predicted lines of tRNA gene mutations: A8344G and A3243G, were located below the diagonal line. This distinction suggested that the transmission pattern of the protein-coding mutations was different from that of the tRNA gene mutations.

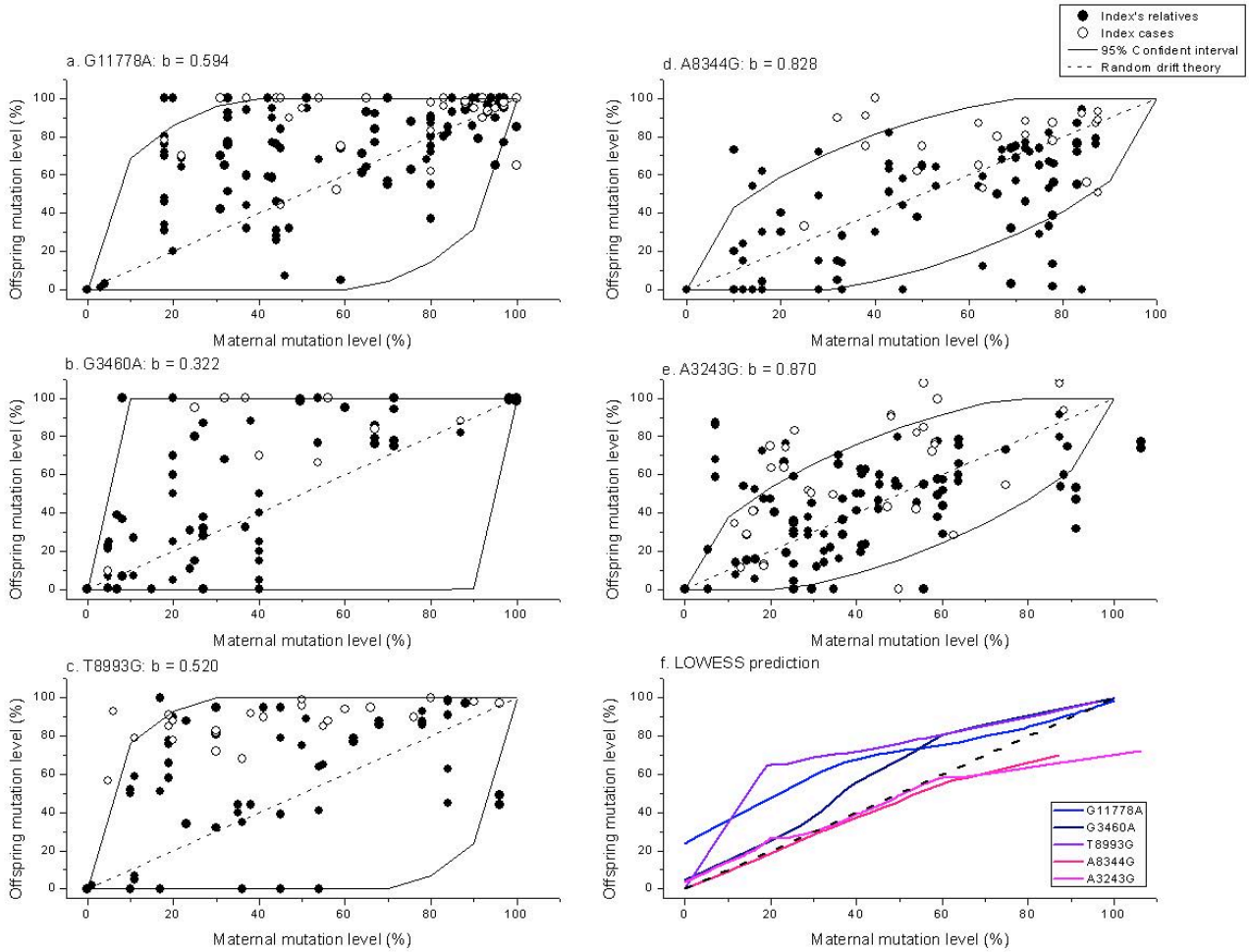

Figure 1: The relationship of the mutation levels between the offspring and their corresponding mothers. The scatter plots present the distribution of the mutation levels of the mother-offspring pairs carrying one of the five mtDNA mutations: (a) G11778A, (b) G3460A, (c) T8993G, (d) A8344G, and (e) A3243G. The 95% confident interval of the offspring mutation level was calculated based on the Kimura distribution with the bottleneck (*b*) parameter values estimated from the mutation levels of the offspring of the mothers carrying 40-60% mutation level. The *b* parameter values were reported in Table 1. The inter-generational relationship of the mutation level was deduced by the locally weighted regression analysis (LOWESS function in R)(f).

Table 1: Summary statistics of the mother-offspring pairs collected from clinical pedigree data

mtDNA mutation	Number of Transmissions [a]	Average maternal mutation level (%)	Average offspring mutation level (%)	Offspring mutation level variance (x 10^{-4})	Normalized offspring mutation level variance [b] (95% confidence interval [c])	Bottleneck parameter [d] (95% confidence interval [c])	
						All offspring	Offspring of the mother carrying 40-60% mutation level [e]
G11778A	165	59.65	74.03	0.0825	0.4292 (0.3671 – 0.4938)	0.571 (0.506 – 0.633)	0.594 (0.452 – 0.717)
G3460A	87	47.57	58.70	0.1607	0.6628 (0.5784 – 0.7509)	0.337 (0.249 – 0.422)	0.322 (0.092 – 0.524)
T8993G	93	28.03	38.97	0.1542	0.6483 (0.5655 – 0.7357)	0.352 (0.264 – 0.435)	0.520 (0.268 – 0.740)
A8344G	89	44.76	37.73	0.0935	0.3978 (0.2580 – 0.3794)	0.602 (0.521 – 0.676)	0.828 (0.696 – 0.926)
A3243G [f]	112	36.28	36.98	0.0739	0.3172 (0.3242 – 0.4791)	0.683 (0.621 – 0.742)	0.870 (0.797 – 0.929)

a: This number represented the number of mother-offspring pairs whose offspring was not the index case. No mother-offspring pair whose heteroplasmic offspring born to the wild type heteroplasmic mother was included in the analysis.

b: Normalized offspring mutation level variance was calculated by dividing the offspring mutation level variance by $p(1-p)$, where p was the offspring mutation level mean.

c: The 95% confidence interval was calculated from the simulated data generated by the Kimura distribution (WONNAPINIJ *et al.* 2010) with the bottleneck (b) parameter value estimated from the mother-offspring pair data.

d: The bottleneck parameter value was calculated from the offspring mutation level mean and variance using the Sewall-Wright variance formula. The detail regarding how to calculate this parameter value was presented in materials and method section.

e: These offspring were chosen because, at this range of maternal mutation levels, the offspring mutation level variance get the least effect from the mutation level mean.

f: The mtDNA mutation level was corrected for a reduction of blood mutation level toward age by applying the age-corrected formula provided by Rajasimha *et al* in 2008 (RAJASIMHA *et al.* 2008)

Table 2: The statistical analyses carried out on the mutation levels and the mutation level differences between offspring and their corresponding mothers (O-M). The average index cases mutation levels were compared against their relatives' mutation levels and the average O-M of females were compared to that of males. One star

mtDNA mutation	Average mutation level				Mutation level differences between offspring and their corresponding mothers (O-M)			
	Index cases (%)	Relatives (%)	p-value of the two-sample Student t-test[a]	p-value of the Mann-Whitney test [b]	Females (%)	Males (%)	p-value of the two-sample Student t-test[a]	p-value of the Mann-Whitney test [b]
G11778A	90.70	74.03	< 0.001***	0.001**	12.00	16.97	0.227	0.171
G3460A	79.21	58.70	0.0779	0.2496	13.43	8.68	0.383	0.959
T8993G	86.98	38.97	< 0.001***	< 0.001***	8.78	15.44	0.261	0.069
A8344G	76.79	37.73	< 0.001***	< 0.001***	-7.17	-6.82	0.947	0.471
A3243G[f]	61.63	36.98	< 0.001***	< 0.001***	2.44	-1.31	0.426	0.609

a: This parametric statistical test with the assumption of unequal variance was applied to examine whether the average values of the two datasets were significant difference.

b: This nonparametric statistical test with the assumption of unequal variance was applied to examine whether the median of the two datasets were significant difference.

*: p-value is less than 0.05 but greater than 0.01.

** : p-value is less than 0.01 but greater than 0.001.

***: p-value is less than 0.001.

The statistical analyses: the one-sample student t-test and the Wilcoxon test, carried out on the mutation level differences between offspring and their corresponding mothers (O-M) were shown in Table 3 and the box and whisker plot of the O-M values was presented in Figure 2a. The results showed that the average O-M values of the protein-coding mutations (G11778A, G3460A and T8993G) were statistically significant and greater than zero. On the other hand, the average O-M values of A8344G and A3243G, the tRNA gene mutations, were not statistically significant different from zero, and statistically significant but lower than zero, respectively. The positive O-M values of the protein-coding mutations and the negative O-M values of the tRNA gene mutations supported the pattern of the inter-generational relationship of the mutation levels predicted by the locally weight regression analysis shown in Figure 1f. The statistically significant difference of the average O-M values compared to zero suggested that, besides random genetic drift, selection may also play a role in determining the offspring mutation levels. In addition, the difference of the average O-M values between protein-coding mutations and tRNA gene mutations suggested that the mechanisms regulating the mtDNA heteroplasmy transmission of these two groups of mutations would be different.

Table 3: The results of the statistical analyses carried out on the mean of the difference between offspring mutation level and maternal mutation level (O-M).

mtDNA mutation	Number of transmission	Mean (%)	Standard error of the mean (%)	p-value of the one-sample Student t-test	p-value of the one-sample Wilcoxon test
G11778A	165	14.38	2.05	< 0.001***	< 0.001***
G3460A	87	11.14	2.74	< 0.001***	< 0.001***
T8993G	93	10.94	2.82	< 0.001***	< 0.001***
A8344G	89	-7.02	2.64	0.009**	0.008**
A3243G[f]	112	0.70	2.32	0.764	0.855

a: This parametric statistical test was applied to examine whether the mean of O-M is equal to zero.
b: This nonparametric statistical test was applied to examine whether the median of O-M is equal to zero.
*: p-value is less than 0.05 but greater than 0.01.
** : p-value is less than 0.01 but greater than 0.001.
***: p-value is less than 0.001.

The average O-M values of the protein-coding mutations were compared against those of the tRNA gene mutations and the results were presented in Table 4. Both ANOVA and Kuskal-Wallis chi-squared test suggested that average O-M values were significant difference among five mtDNA mutations. Tukey multiple comparisons and Wilcoxon rank sum test were further applied to identify which pair of mtDNA mutations was different. The pairwise comparison showed that the average O-

M values of the protein-coding mutations were significant different from those of the tRNA gene mutations; no significant difference of the average O-M values was observed either between different protein-coding mutations or between different tRNA gene mutations.

As shown in Figure 1f, the predicted lines based on the models generated by the locally weight regression analysis suggested that the offspring mutation levels were not simply linearly related to the maternal mutation levels. In fact, every predicted line proposed that the transmission pattern of the low heteroplasmic mothers was different from that of the high heteroplasmic mothers; therefore, we separated the mother-offspring pair data into two groups based on the maternal mutation level: the low and the high heteroplasmic mothers. The low and the high heteroplasmic mothers carried mtDNA mutation level less than 50%, and greater than or equal to 50%, respectively. To examine whether the transmission pattern of the low heteroplasmic mothers was significant different from that of the high heteroplasmic mothers, the average O-M value of the low heteroplasmic mothers was compared against that of the high heteroplasmic mothers. As shown in Figure 2b, among three protein-coding mutations, only the average O-M values of the low G11778A heteroplasmic mothers was significant different from that of the high heteroplasmic mothers. On the other hand, both the average O-M values of the low A8344G and A3243G heteroplasmic mothers were significant different from those of the high heteroplasmic mothers, as shown in Figure 2c. Thus, these results suggested that the transmission patterns of the low heteroplasmic mothers would be different from those of the high heteroplasmic mothers in the case of G11778A and tRNA gene mutations.

Table 4: The results of the statistical analyses applied to compare the average of the difference between offspring mutation level and maternal mutation level (O-M) between different mtDNA mutations. The p-values presented below the diagonal line are the p-values of the Wilcoxon test and the p-values presented above the diagonal line are the p-value of the Tukey multiple comparison test. The Wilcoxon test was used as the post hoc test for the Kruskal-Wallis test and the Tukey test was used as the post hoc test for the ANOVA. Both Kruskal-Wallis and ANOVA showed that the average O-M values were difference among different mtDNA mutations. The p-values of both Kruskal-Wallis and ANOVA were less than 0.001.

	G11778A	G3460A	T8993G	A8344G	A3243G
G11778A		0.877	0.842	< 0.001***	< 0.001***
G3460A	1.000		1.000	< 0.001***	0.038*
T8993G	1.000	1.000		< 0.001***	0.039*
A8344G	< 0.001***	< 0.001***			0.218
A3243G[f]	< 0.001***	0.066	0.028*	0.870	

*: p-value is less than 0.05 but greater than 0.01.

** : p-value is less than 0.01 but greater than 0.001.

***: p-value is less than 0.001.

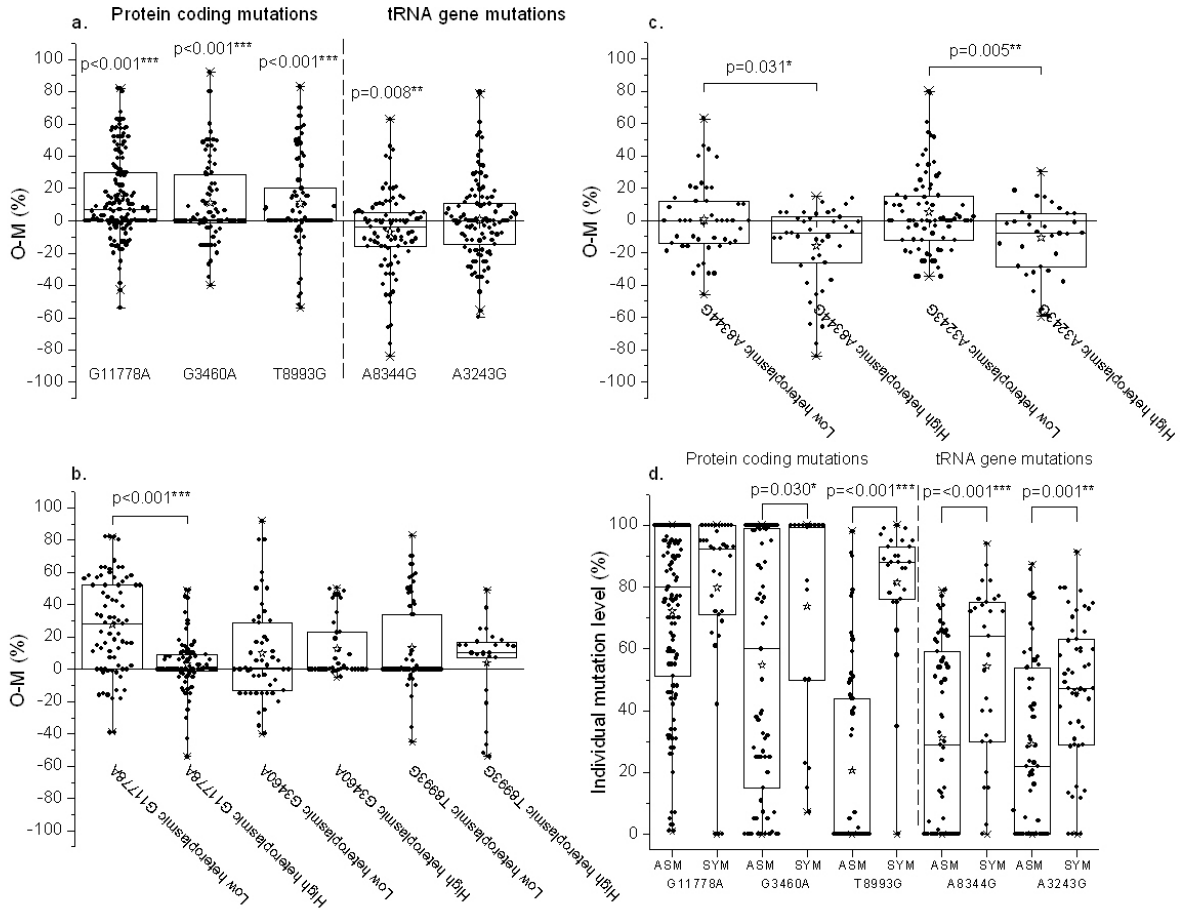

Figure 2: Comparison of the mean of the differences between offspring and maternal mutation levels (O-M) to the expected mean of 0 (a), the mean of the O-M values calculated from the low heteroplasmy mothers to the values calculated from the high heteroplasmy mothers carrying protein coding mutations: G11778A, G3460A and T8993G mutation (b), the mean of the O-M values calculated from the low heteroplasmy mothers to the values calculated from the high heteroplasmy mothers carrying tRNA gene mutations: A8344G and A3243G mutation (c), and the mean of the unaffected individual mutation level to the affected individual mutation level (d). The low heteroplasmy mothers are the mothers carrying mtDNA mutation level less than 50% and the high heteroplasmy mothers are the mothers carrying mtDNA mutation level greater than or equal to 50%. The blasck circle represented each O-M value. The white star represents the mean of the O-M values. The line inside the box represents the median of the O-M values. The height of the box is equal to two times the standard error of the mean. The length of the whisker ranged from 5 to 95 percentile of the O-M values. The p-value is presented only when it is lower than 0.05. The p-value presented in figure (a) is the p-value of the one-sample Wilcoxon test, while the p-values presented in figure (b), (c), and (d) are the p-value of the Mann-Whitney test. ASM and SYM stand for asymptomatic and symptomatic individual, respectively. One (*), two (**) and three (***) stars presented that the p-value is less than 0.05 but greater than 0.01, less than 0.01 but greater than 0.001, and less than 0.001, respectively.

Table 5: The results of the statistical analyses carried out on the mean and the distribution of the differences between offspring mutation level and mother's mutation level (O-M). The O-M data was separated into two groups based on maternal mutation level: the low heteroplasmy mothers carrying mutation level less than 50% and the high heteroplasmy mothers carrying mutation level greater than or equal to 50%.

mtDNA mutation	Low/High	Number of transmission	Mean (%)	Standard error of the mean (%)	p-value of the one-sample Student t-test[a]	p-value of the one-sample Wilcoxon test[b]	p-value of the two-sample Student t test[c]	p-value of the Mann-Whitney test[d]
G11778A	Low	76	27.54	3.41	< 0.001***	< 0.001***	< 0.001***	< 0.001***
	High	89	3.14	1.72	0.071	0.036*		
G3460A	Low	49	9.90	4.26	0.024*	0.074	0.592	0.242
	High	38	12.72	3.06	< 0.001***	< 0.001***		
T8993G	Low	68	13.44	3.29x	< 0.001***	< 0.001***	0.146	0.445
	High	25	4.12	5.37	0.451	0.226		
A8344G	Low	48	0.38	3.23	0.908	0.879	0.002**	0.031*
	High	41	-15.69	3.94	< 0.001***	< 0.001***		
A3243G	Low	80	5.28	2.72	0.056	0.178	0.001**	0.005**
	High	32	-10.75	3.80	0.008**	0.016*		

a: This parametric statistical test was applied to examine whether the mean of the O-M values is equal to zero.

b: This nonparametric statistical test was applied to examine whether the median of the O-M values is equal to zero.

c: This parametric statistical test was applied to examine whether the mean of the O-M values of the low heteroplasmy mothers is equal to the average value of the high heteroplasmy mothers.

d: This nonparametric statistical test was applied to examine whether the median of the O-M values of the low heteroplasmy mothers is equal to the median value of the high heteroplasmy mothers.

e: This nonparametric statistical test was applied to examine whether the O-M values are normally distributed.

*: p-value is less than 0.05 but greater than 0.01. ** : p-value is less than 0.01 but greater than 0.001. ***: p-value is less than 0.001.

For each mtDNA mutation, the average O-M values of the low and the high heteroplasmic mothers were further statistically compared against zero to examine whether random genetic drift could solely determine the transmission pattern of these mothers' groups. The statistical analyses carried out on these sub-data were presented in Table 5. In the case of low heteroplasmic mothers, the average O-M values of every mtDNA mutations were positive. All tRNA gene mutations and G3460A mutation were not significant different from zero. On the contrary, the average O-M values of the high heteroplasmic mothers were both positive and negative; the average values of the protein-coding mutations were positive, while the average values of the tRNA gene mutations were negative. The statistical analyses showed that every mtDNA mutation, except the T8993G, was significant different from zero. The statistical results carried out on the low heteroplasmic mothers suggested the role of positive selection in determining the transmission pattern of all protein-coding mutations, except the G3460A. On the contrary, the results of the high hetereoplasmic mothers suggested that positive and negative selection may play a role in determining the transmission pattern of the protein-coding mutations, except the T8993G, and the tRNA gene mutation, respectively.

For each mtDNA mutation, the average mutation level of the affected individuals was compared against that of the unaffected individuals, as shown in Figure 2d. Even though, the range of mutation levels of unaffected individuals overlapped that of the affected individuals in all mutations, all mtDNA mutations, except the G11778A, showed significant difference of the average mutation levels between the unaffected and affected individuals. These results suggested that mtDNA mutation level played a role in determining the presence of disease phenotype.

Pedigree model verification and validation

In this study, the pedigree model of mtDNA heteroplasmy inheritance was constructed based on the assumption that only random genetic drift determines the descendent mutation level. Based on this assumption, the mutation level of each simulated offspring was randomly generated based on the Kimura distribution (WONNAPINIJ *et al.* 2008). The p_0 and b parameter values used in this theoretical distribution were the maternal mutation level and the bottleneck parameter estimated from the human clinical pedigree data, respectively. To simplify the statistical properties of the pedigree model, the number of offspring per mother was set to 1 and the ratio of daughter to son was defined as 1:0; therefore, the no lineage loss was observed in the simulated data.

To verify whether the behavior of the model follows random genetic drift theory, 10,000 simulated pedigrees were generated. The general properties of simulated pedigrees were as described in the previous paragraph. The first generation female of each simulated pedigree carries 5% mtDNA mutation level. Five sets of simulated pedigrees were generated based on five b parameter values estimated for five different mtDNA mutations. The average number of individuals per generation was 1 and this average was stable throughout the simulation as shown in Figure 3a. The characteristics of the simulated pedigree were represented by the average statistics of these 10,000 simulated pedigrees. The mutation level mean and variance of these 10,000 simulated

pedigrees were plotted against the number of generations, as shown in Figure 3b-c. The results showed that the average descendant mutation levels of five different mutations were in the range of 4.75% and 5.15%, approximately equal to the founder female mutation level (5%), as shown in Figure 3b, and the mutation level variances increased towards generations, as shown in Figure 3c. These patterns of mutation level mean of variances indicated that the behavior of the model follows random genetic drift theory because, based on random genetic drift theory, the descendant mutation levels should be, on average, equal to the founder female mutation level and the mutation level variance should increase toward generations (HALLIBURTON 2004).

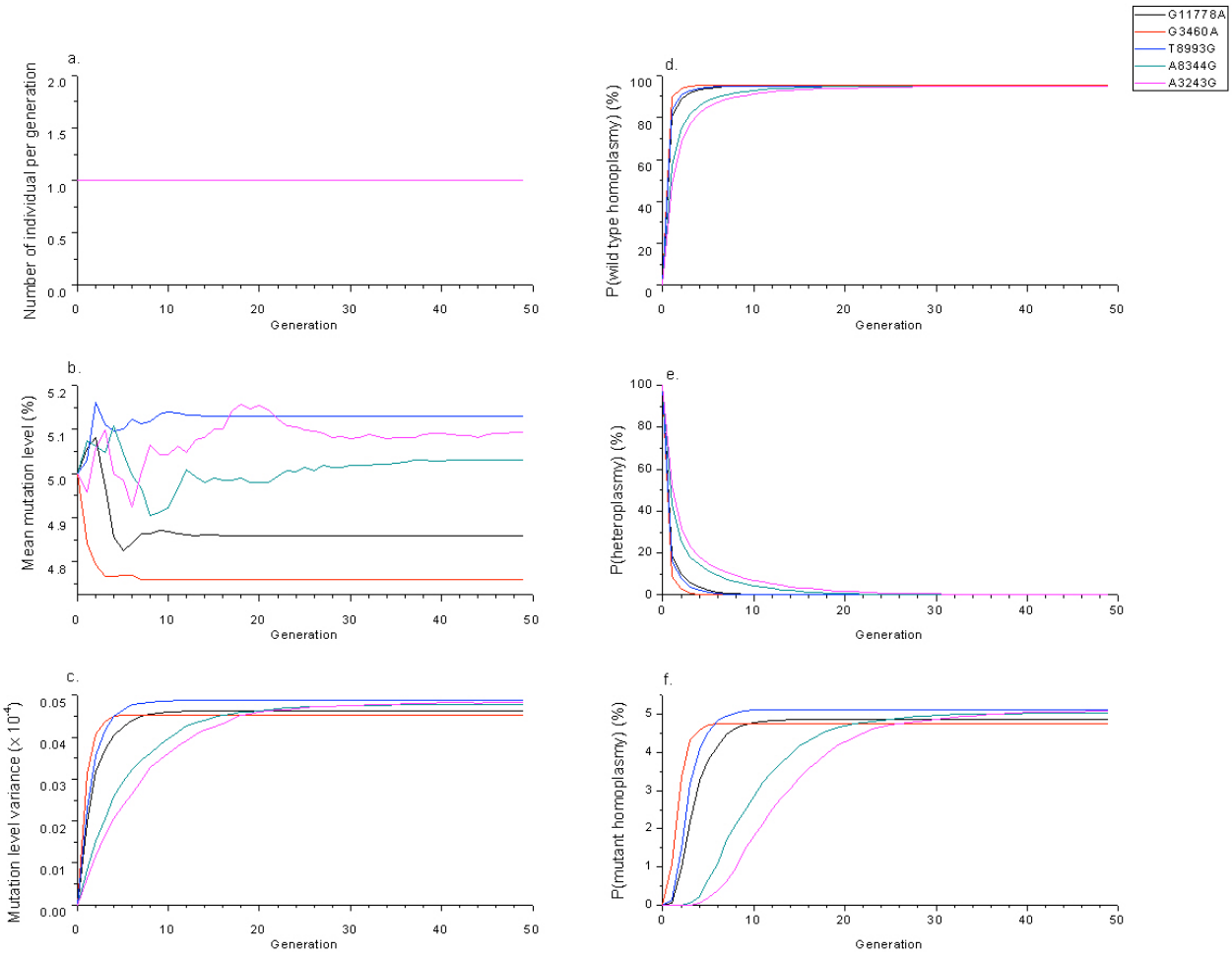

Figure 3: The progression of the number of individuals (a) and the mtDNA mutation levels across generations (b-f). The progression of the mtDNA mutation levels were presented as the progression of the mutation level on average (b), the mutation level variance (c), the probability of carrying wild type homoplasmy (d), the probability of carrying mtDNA heteroplasmy (e), and the probability of carrying mutant homoplasmy (f). These statistics were calculated from 10,000 simulated pedigrees. A female ancestor carrying 5% mutation level initiated each pedigree. The bottleneck parameter values were estimated from the pedigree data, as shown in Table 1.

To validate the pedigree model, 200 sets of simulated pedigrees were generated and compared against the observed pedigrees carrying an mtDNA mutation. For each simulated pedigrees, the differences of offspring mutation levels between simulated and observed data were calculated, then these different values were averaged. Therefore, for each mtDNA mutation, 200 values of average mutation level differences were generated. The 95% confidence interval of the offspring mutation level differences for each mtDNA mutation was derived from these 200 mutation level differences. If the 95% confidence interval included 0, the simulated offspring mutation levels were not statistically significantly different from the observed offspring mutation levels. The upper and lower bounds of the offspring mutation level differences of each mtDNA mutation were presented in Table 6. In the case that all offspring were taken into the analysis, all mtDNA mutations, except the A3243G, showed significant differences between simulated and observed offspring mutation levels. These results were consistent with the Welch and Man-Whitney tests applied on the comparison of the average O-M against 0 shown in Table 3. However, in the case that only the offspring of the intermediate heteroplasmic mothers were included, all mtDNA mutations, except the G11778A, showed significant differences between simulated and observed offspring mutation levels. These results suggested that random genetic drift theory could explain the heteroplasmy transmission pattern of the intermediate heteroplasmic mothers carrying every mutation included in this study, except the G11778A.

Table 6: The 95% confidence interval of the average differences between the simulated and the observed mutation levels. These 95% confidence intervals were calculated from 200 sets of offspring mutation levels. The simulated data was considered to be consistent with the observed data if 0 was in the range of the 95% confidence interval.

mtDNA mutation	All offspring			Offspring of the intermediate heteroplasmic mothers		
	Lower bound (%)	Upper bound (%)	Significant difference? (Y/N)	Lower bound (%)	Upper bound (%)	Significant difference? (Y/N)
G11778A	-18.89	-11.15	N	-24.82	-4.05	N
G3460A	-16.84	-5.50	N	-38.01	2.72	Y
T8993G	-15.42	-6.67	N	-35.87	0.84	Y
A8344G	3.60	10.39	N	-17.68	5.71	Y
A3243G[f]	-3.21	1.97	Y	-8.40	6.37	Y

Progression of mtDNA mutation level across generations

The pedigree model of mtDNA heteroplasmy inheritance developed in this study could be applied to study the progression of mtDNA mutation level across generations. By allowing the

simulation to generate 10,000 pedigrees, each last for 50 generations, we could predict the development of mtDNA mutation level toward 50 generations by observing the changes of mutation level mean, mutation level variance and the probability values toward these generations, as shown in Figure 3b-f. The probabilities values calculated in this study were the probability of carrying wild type homoplasmy, the probability of carrying mtDNA heteroplasmy, the probability of carrying mutant homoplasmy. Different color in each figure represented different types of mtDNA mutations that hold different b parameter values (shown in Table 1). As expected by random genetic drift theory, the progression toward generations of mutation level mean were approximately stable and the mutation level variance increased (Figure 3b-c). In addition, the probabilitiy of carrying homoplasmy, either wild type or mutant, increased toward generations, whiles the probability of carrying mtDNA heteroplasmy decreased toward generations. These trends of probability changes could also be expected from random genetic drift theory (HALLIBURTON 2004).

Even though the progression of mutation level mean, variance, and the probability values followed random genetic drift theory, there are some interesting details that need to be mentioned. As shown in Figure 3b, the greatest changes in average mutation levels were generally presented in the first ten generations. After that, the average mutation levels were stable in the case of protein-coding mutations: G11778A, G3460A, and T8993G, while the average mutation levels of the tRNA mutations: A8344G and A3243G, still randomly changed almost till the last generation of the simulated pedigrees. These changes in mutation level mean were consistent with the changes in mutation level variance presented in Figure 3c that the increase rates of mutation level variance of protein coding mutations were faster than those of tRNA gene mutations. The faster rate of mutation level variance rise of protein coding mutations could be explained by the higher rate of homoplasmy fixation, as shown Figure 3d and f that the probability of carrying wild type and mutant homoplasmy of protein coding mutations increased faster than those probabilities of tRNA gene mutations. In addition, the probability of carrying mtDNA heteroplasmy of protein coding mutations decreased faster that that of tRNA gene mutations as shown in Figure 3e. All these statistics showed that in the long term, the number of generations that the mutant mtDNA could still be observed in the pedigree depended on the b parameter values that were difference among different mutations. On the contrary, in the short term, the differences in the probability of carrying a certain proportion of mutant mtDNA were the result of different b parameter values presented in different mtDNA mutations.

Recurrence risk estimation

We also applied the pedigree model of mtDNA heteroplasmy inheritance to estimate a risk of having an affected child and a probability of having a normal child carrying wild type homoplasmy. Based on the assumption that the affected individual needed to carry a proportion of mutant mtDNA greater than the threshold level (DIMAURO and DAVIDZON 2005; DUBEAU *et al.* 2000; MOSLEMI *et al.* 1998), the phenotype of simulated individual was determined by the individual's mutation level. In

this study, the threshold level was set to 60%, thus the affected individual in the simulation carried mutation level greater than 60%.

As shown in Table 1 that different mtDNA mutations had different b parameter values, we applied those five b parameter values to generate simulated pedigrees. 10,000 simulated pedigrees were generated for each set of parameters: the mother's mutation level and b parameter value. Generally, the low heteroplasmic females should not develop a disease; however, this does not guarantee that the offspring of these females would not carry a high mutation level and more likely to develop the disease. To estimate recurrence risk in these low heteroplasmic females, the maternal mutation levels in the simulation were set to be ranging from 0 to 20%. As expected, the probability of carrying mtDNA mutation level exceeding the threshold level was increase toward the maternal mutation level and the probability of carrying wild type homoplasmy was decrease against the maternal mutation level, as shown in Figure 4 a and b, respectively. Even though the relationship pattern between the probabilities and the maternal mutation level was the same in all mtDNA mutations, the probability values were differences due to different b parameter values.

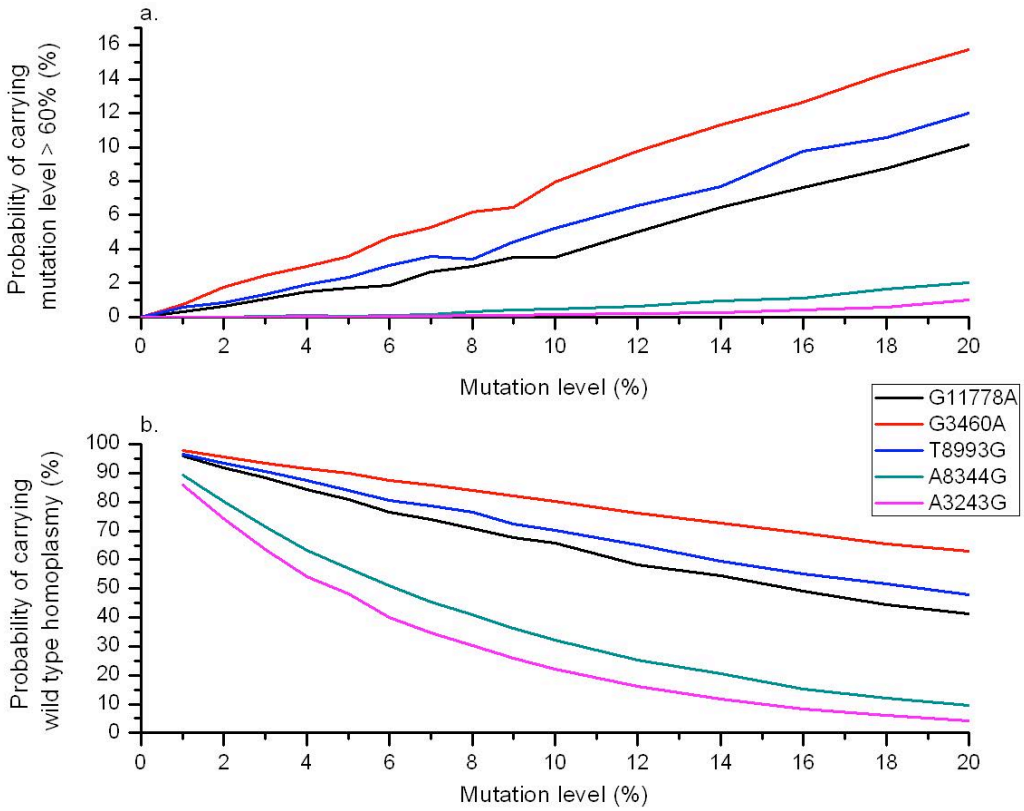

Figure 4: The probability of carrying mutation level higher than the threshold level (60% mutation level) and the probability of carrying wild type homoplasmy. These probability values were calculated from 10,000 simulated pedigrees. Five different bottleneck parameter values represented five different mutations were applied to generate these simulated data. The bottleneck parameter values were shown in Table 1.

Based on the pattern presented in Figure 4a, the probabilities of carrying mtDNA mutation level exceeding the threshold level at 60% of the protein coding mutations (G11778A, G3460A, and T8993G) were all greater than those probabilities of tRNA gene mutations (A8344G and A3243G). In the case of G11778A, G3460A, and T8993G mutations, the mother should carry mutation level no greater than 13%, 10% and 7%, respectively, in order to have approximately 5% probability of having an offspring carrying mtDNA mutation level greater than 60%. On the contrary, the mother who carried tRNA gene mutations could bear mtDNA mutation level greater than 20% and still had less than 5% probability of having an offspring carrying mtDNA mutation level greater than 60%.

On the other hand, the probabilities of carrying wild type homoplasmy of the protein coding mutations were all higher than those of the tRNA gene mutations, as shown in Figure 4b. In the case of protein-coding mutations: G11778A, G3460A, and T8993G, the mother should carry mutation level no greater than 4% in order to have approximately 90% probability of having an offspring carrying wild type homoplasmy. On the contrary, the mother who carried tRNA gene mutations should carrying mutant mtDNA less than 1% in order to have approximately 80% chance of having a wild type homoplasmic offspring.

Proportion of carrier estimation

The proportion of carriers in the population could be estimated by using the population model. The population model was developed from the pedigree model. The founder population contained only wild type homoplasmic females, and then de novo mutation rate and the initial mutation level determined the mutation levels of first generation population. After the first generation, the mutation levels of later generation population were determined not only by the de novo mutation rate and the initial mutation level, but also by the Kimura distribution (WONNAPINIJ *et al.* 2008). The wild type homoplasmic mothers could only have either wild type homoplasmic offspring or heteroplasmic offspring carrying mutation level equal to the initial mutation level, while the heteroplasmic mothers could have offspring carrying mutation levels as generated by the Kimura distribution. In order to simplify the statistical properties of the simulated data, the structure of each pedigree in the population was based on the assumption that each female would only have one daughter, thus no effect of lineage loss was observed.

Three important parameters needed for generating simulation population were the *de novo* mutation rate, the initial mutation level, and the b parameter value. The *de novo* mutation rate of ten common pathogenic mtDNA mutation in general population had been estimated from live births population in England (ELLIOTT *et al.* 2008). The overall *de novo* mutation rate was approximately 0.10%. The initial mutation level, the mutation level of the individual whose mtDNA was mutated by a new mutation, was set as 10% mutation level, the limitation of the detection method used in observing the proportion of carriers in general population (ELLIOTT *et al.* 2008). The b parameter value for all mutations was calculated from the offspring of all mutations who were born to the intermediate heteroplasmic mothers (40-60%). This b parameter value was equal to 0.64.

The proportion of carriers at each generation was calculated from 30,000 simulated pedigrees and the results were plotted against the number of generations, as shown in Figure 5a. This figure showed that if the population size had been constant and no pedigree lost its lineage prior to the end of simulation, the proportion of mtDNA mutant carriers in the population would increase toward generations. The proportion of mutant carriers was approximately equal to 0.54% after developing for 34 generations. Linear regression analysis was applied to examine the relationship between the proportion of carriers and the number of generations and the result showed that these two variables were linearly related with r^2 of 0.97. Based on the linear regression analysis, the rate of increasing the proportion of mutant carriers was 0.01, corresponded well with the *de novo* mutation rate. Therefore, under the constant population size with no lineage loss, radom genetic drift and *de novo* mutation would cause increase of the proportion of mutant carriers would toward generations.

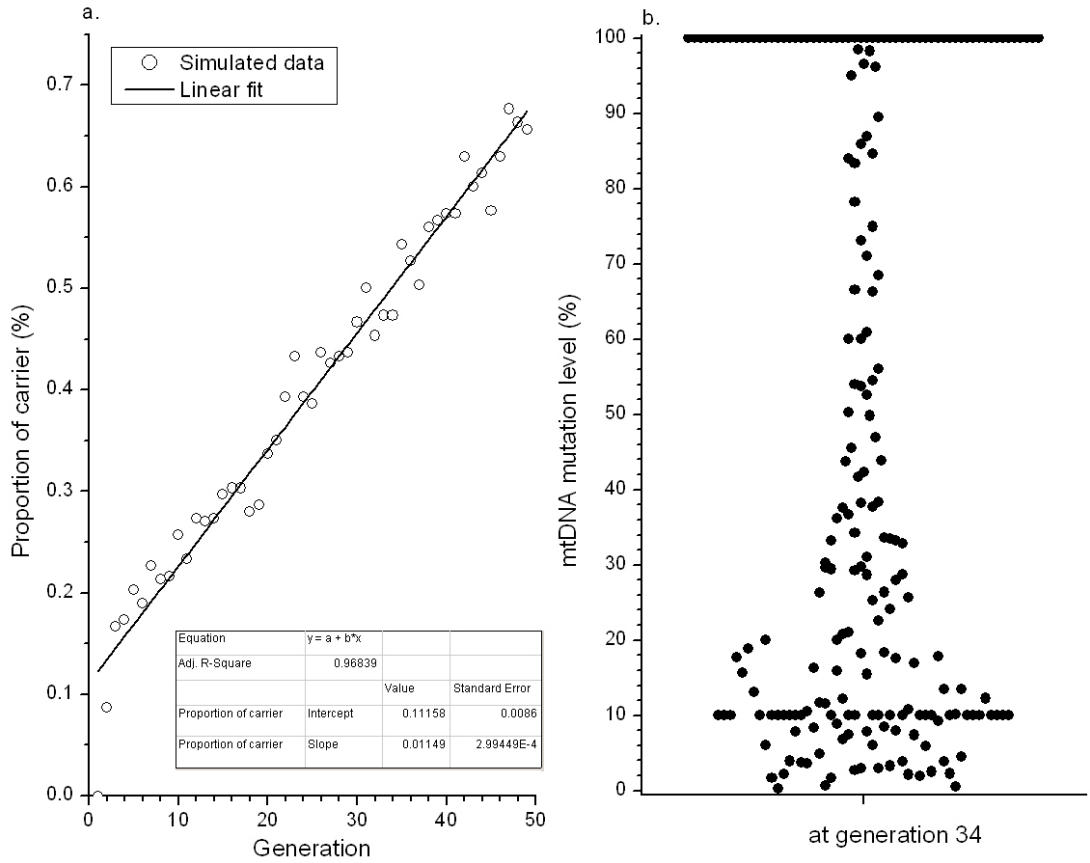

Figure 5: The proportion of mutant carriers and the distribution of the mutation levels calculated from the simulated population. Linear regression analysis was applied to examine whether the increase of the proportion of mutant carriers in the simulated population could be explained by a linear function. The distribution of the mutation levels at generation 34 was presented here because the proportion of mutant carriers at this generation was approximately equal to observed proportion in general population (ELLIOTT *et al.* 2008).

The distribution of mtDNA mutation levels of all carriers at generation 34 was presented in Figure 5b. At this generation, the proportion of mutant carriers is approximately equal to the observed proportion of carriers in generation population (ELLIOTT *et al.* 2008). The mutation levels were in the range of 0 to 100% with the average mutation level of 48%. The distribution of these mtDNA mutation levels was not symmetric around the mutation level mean; the number of low heteroplasmic individuals, carrying mutation level < 48%, (n_{low} = 120) was greater than the number of high heteroplasmic individuals, carrying mutation level >= 48% (n_{high} = 88).

Conclusion and Discussion

Transmission pattern of mtDNA heteroplasmy levels deduced from clinical pedigree data

The aims of analyzing the pedigree data were to obtain the bottleneck (b) parameter values that were later used in the pedigree model and to understand the transmission pattern of mtDNA heteroplasmy level. Various statistical analyses were carried out on the pedigree data of five common pathogenic mtDNA mutaions: G11778A, G3460A, T8993G, A8344G and A3243G. The results mainly showed that the transmission patterns of the protein-coding mutations: G11778A, G3460A, and T8993G, heteroplasmy levels were different from the patterns of the tRNA gene mutations: A8344G and A3243G. This difference suggested that the mechanisms regulating the transmission pattern of protein-coding mutations would be different from those regulating the transmission pattern of tRNA gene mutations.

As shown in Figure 1a-e, the mother-offspring pair mutation levels were randomly distributed above and below the line represented the expected offspring mutation levels. In addition, the 95% confidence intervals calculated from the theoretical Kimura distribution for each mutation could explain most offspring mutation levels. Based on both the distribution of the mother-offspring pair mutation levels and the coverage of the theoretical 95% confidence intervals, we may concluded that random genetic drift would play a major role in determining the transmission of mtDNA heteroplasmy levels.

However, the average maternal mutation level of every mutation was different from the average offspring mutation level (Table 1). This difference suggested that selection may play a role in regulating the transmission pattern of mtDNA mutation level. In order to examine whether the average mutation level of the offspring was statistical significant different from that of their corresponding mothers, the average mutation level differences between offspring and their corresponding mothers (O-M) of each mutation was compared against zero (Table 3 and Figure 2A). The results showed that the average O-M of all mutations, except the A3243G mutation, were statistically significant different from zero. These results were consistent with the previous study, except for the significant different of the average O-M value of the G3460A mutation (CHINNERY *et al.* 2000). We would argue that this inconsistency would be the result of a larger sample size in our G3460A pedigree data that could increase the power of the hypothesis testing because the average O-M value calculated in this study was in the same range as the average O-M value calculated in

the previous study. Therefore, the significant difference of the average O-M value presented in this study suggested that selection would also play a role in determining the offspring mutation level of every mtDNA mutation, except the A3243G mutation.

The role of selection in determining the transmission pattern of all mtDNA mutation level, except the A3243G, was supported by the pattern of the inter-generational relationship of the mtDNA mutation deduced by the locally weight regression analysis (CLEVELAND 1979). The advantage of this nonparametric method is that no prior assumption about the relationship pattern needs to be defined. The lines presented the transmission pattern of the mtDNA mutations predicted by this nonparametric method were compared against the straight line represented the average offspring mutation level expected by random genetic drift theory. The location of the predicted lines presenting the transmission pattern of protein coding mutations were all located above the expected line, suggesting the role of positive selection on determining the offspring mutation levels. On the other hand, the predicted line presenting the transmission pattern of the A8344G was located below the expected line, indicating that purifying selection may play a role in determining the transmission pattern of A8344G mutation, while the predicted line of the A3243G was located around the expected line, supporting the role of random drift in determining the offspring mutation level. Therefore these patterns of predicted line not only supported the role of selection and random drift in regulating the transmission pattern of mtDNA mutation, but also suggested that different mutations have different pattern of mtDNA heteroplasmy transmission. Based on the location of the predicted lines, we would also suggest that the transmission pattern of the protein coding mutations was different from that of the tRNA gene mutation.

Both parametric and nonparametric hypothesis tests were applied to examine whether the transmission pattern of the protein-coding mutation was significant different from that of the tRNA gene mutations. As shown in Table 4, only the average O-M of the protein-coding mutations were significant different from the average O-M of the tRNA gene mutations. These statistical results supported that the transmission pattern of the protein-coding mutations would be different from that of the tRNA gene mutations.

The pattern of the predicted lines presented in Figure 1f further suggested that the transmission pattern of the low heteroplasmic mothers would be different from that of the high heteroplasmic mothers, regardless of the type of mtDNA mutations. This difference may be the result of different mechanisms regulating the inheritance of mtDNA mutation level. In order to test this hypothesis, the mother-offspring pairs carrying each mtDNA mutation were separated into two groups based on the maternal mutation level. The group of low heteroplasmic mothers contained mother-offsping pairs whose maternal mutation levels were lower than 50% and the group of high heteroplasmic mothers contained mother-offsping pairs whose maternal mutation levels were greater than or equal to 50%. The statistical analyses were applied to compare the average O-M of the group of low heteroplasmic mothers to that of the high heteroplasmic mothers. The comparison of the average O-M values between the group of low and high heteroplasmic mothers showed that the

significant difference between these groups were observed in all tRNA gene mutations (A8344G and A3243G) and only one protein-coding mutation (G11778A). To some extend, these results did not only support that the transmission pattern of the low heteroplasmi mothers is different from that of the high heteroplasmic mothers, but also suggested the difference in transmission pattern between protein-coding mutation and tRNA gene mutations.

The statistical analyses were also applied to compare the average O-M of each group against zero. In the case of the protein-coding mutations, the statistical results suggested that positive selection should play an important role in determining the offspring mutation level both of the low and the high heteroplasmic mothers. In the case of LHON mutations: G11778A and G3460A, the positively significant difference of the average O-M value could be the result of the incomplete penetrance of these mutations (HUDSON *et al.* 2007; HUDSON *et al.* 2005; TONG *et al.* 2007) and the effect of ascertainment bias (CHINNERY *et al.* 2001), while, in the case of T8993G mutation, the positively significant difference of the average O-M value could involve a high mutation rate of the *mt-ATP6* gene (STEWART *et al.* 2008). Interestingly, the average O-M of the high heteroplasmic T8993G mothers was not significant different from zero, suggesting that the effect of positive selection might be balanced by the negative selection. On the other hand, the statistical result carried out on the average O-M of the low and the high heteroplasmic tRNA gene mutation suggested that random drift and purifying selection should play important role in determining the offspring mutation level of the low and the high heteroplasmic mothers, respectively. The observation of the purifying selection could be expected because of the pathogenicity of these tRNA gene mutations. Some females carrying the A3243G mutation experienced spontaneous abortions (CALLAGHAN *et al.* 2009; MURPHY *et al.* 2008; NAN *et al.* 2002; OHKUBO *et al.* 2001; YANAGISAWA *et al.* 1995), which could explain the purifying selection of this mutation observed in this study. These results supported that, for each mutation, different mechanisms regulating the transmission of the mutation level from the of the low and the high heteroplasmic mothers, and different set of forces could driven the transmission of mutation level of the protein-coding mutations and the tRNA gene mutations.

The properties of the pedigree model

In order to gain insight into the transmission pattern of mtDNA heteroplamy inheritance without concerning about the effect of ascertainment bias, the pedigree model was constructed. In 2008, Kimura distribution, the distribution of allele frequencies in the population subjected to a pure random genetic drift, was proposed as the theoretical distribution that can describe the distribution of mtDNA mutatation levels in various organism (WONNAPINIJ *et al.* 2008). In this study, the theoretical Kimura distribution was applied to generate mutation level for the simulated individual. The bottleneck (b) parameter used in the model was the value estimated from clinical pedigree data (Table 1). The b parameter values calculated from the mutation levels of the offspring born to the intermediate heteroplasmic mothers were used in the pedigree model to generated simulated pedigrees because these values were least subjected to the effect of mutation level mean. The

simplest form of pedigree structure, only one daughter for each mother, was applied to generate the simulated pedigree to simplify the statistical properties of the simulated pedigree. Therefore, this pedigree model was built based on the assumption that the individual mutation level was solely determined by random genetic drift and the transmission of mtDNA mutation level was continued until the end of simulation.

The pedigree model was verified by comparing the simulated data to the expected value based on random genetic drift theory. The comparison results showed that the progression of mtDNA mutation level mean of every mutation included in this study was approximately constant (Figure 3b) as expected by random genetic drift theory that the average descendant allele frequency would be equal to the ancestor allele frequency (HALLIBURTON 2004). As suggested by Sewall-Wright variance formula that the mutation level variance should increase toward generations (HALLIBURTON 2004), the progression of mutation level variance of every mutation in the simulation followed this suggestion (Figure 3c). Based on the progression of mutation level mean and variance, the behavior of the pedigree model developed in this project corresponded well with the concept of random genetic drift theory.

To examine the validity of the pedigree model in explaining the transmission pattern of common pathogenic mtDNA mutations, the simulated pedigrees were generated and compared against the observed clinical pedigrees. Based on the 95% confidence interval of the differences between simulated and observed mutation levels shown in Table 6, only the simulated offspring A3243G mutation levels were not statistically significant different from the observed mutation level, indicating that the pedigree model could well explain the transmission pattern of A3243G mutation level. This comparison result was consistent with the Welch's t-test and the Wilcoxon test carried out on the average of the difference of mutation levels between offspring and their corresponding mothers (O-M) shown in Table 3. Therefore, the inconsistency between simulated offspring mutation levels and the observed offspring mutation levels of every mutation, except the A3243G, would be the result of other mechanism, such as selection, that could also played a role in determining offspring mutation levels.

Interestingly, the 95% confidence interval of the differences between simulated and observed mutation levels of the offspring born to the intermediate heteroplasmic mothers (Table 6) showed that simulated offspring mutation levels of every mutation, except the G11778A, were not statistically significant different from the observed mutation levels. The discrepancy between the comparison results of all offspring and those of offspring born to intermediate heteroplasmic mothers could be caused by the effect of selection on the mutation level of the offspring born to the low and high heteroplasmic mothers. Based on the statistical analysis carried out on the average O-M values of the low and the high heteroplasmic mothers (Table 4), positive and/or negative selection could play a role in determining the mutation level of the offspring of these mothers; therefore, the random genetic drift theory could not be sufficient to predict the offspring mutation levels of these extreme heteroplasmic mothers, leading to the inconsistency between simulated and the observed mutation

levels when all offspring were taken into the analysis. Another possible reason for this discrepancy would be the effect of ascertainment bias on the extreme heteropalsmic mothers, leading to observing deceived high heteroplasmic offspring. Therefore, we may concluded that, to some extend, the pedigree model could explain the heteroplasmy transmission of all mtDNA mutations included in this study, except the G11778A.

The application of pedigree simulation

The pedigree model was further applied to study the progression of mtDNA mutation levels across generations, as shown in Figure 3b-f. Regardless of the type of mtDNA mutation, the mutation level mean was not change, while the mutation level variance increase toward generation (Figure 3b-c). These results indicated that on each generation, the mutation levels were randomly varied which, on average, the mutation level of the later generation would be approximately equal to that of the previous generation. The variation of mutation levels in each generation could be revealed by the probability of carrying wild type homoplasmy, mtDNA hteroplasmy, and mutant homoplasmy. Because the simulated pedigree was initialized by a founder female carrying 5% mutation level, the probability of carrying wild type homoplasmy was the greatest on each generation. As shown in Figure 3d-e, the probability of carrying homoplasmy, either wild type or mutant, kept rising toward generation, while the probability of carrying mtDNA heteroplasmy decrease toward generation. These changes of probability values toward generation indicated that, without a new mutation, the mtDNA heteroplasmy could not be maintained in the pedigree forever; however, the mutant mtDNA could be kept in the family in homoplasmic condition if there is no purifying selection to eliminate mutant mtDNA.

As shown in Table 1 that different mutations carried different values of the b parameter, this difference affected the progression of mtDNA mutation across generations. At a certain generation, the probability of carrying wild type and the probability of carrying mutant homoplasmy of the protein coding mutations: G11778A, G3460A, and T8993G, were greater than those probabilities of tRNA gene mutations: A8344G and A3243G (Figure 3d-e). On the contrary, the probability of carrying heteroplasmic mutations of the protein coding mutations was lower than the probability of the tRNA gene mutations (Figure 3f). Therefore, the progression to the equilibrium state of the protein coding mutation level was faster than the progression of the tRNA gene mutations, corresponded to the lower b parameter value of the protein coding mutations.

We also applied the pedigree model to estimate recurrence risk in the low heteroplasmic females whose mutation level is in the range of 0 to 20%. As expected, the probability of carrying mtDNA mutation level greater than the threshold level increase toward the maternal mutation level; on the other hand, the probability of carrying wild type homoplasmy decrease toward the maternal mutation level, as shown in Figure 4 a and b. Therefore, the higher the mutation level in the mother, the greater the chance that the mother will have a high heteroplasmic offspring who is likely to develop the mitochondrial disease.

The estimate recurrence risks presented in this report were different from the estimate risks previously reported in the literature (SAMUELS *et al.* 2013). In the present study, five different b parameter values were applied to calculate recurrence risks for five different mutations. The b parameter values used in this study were in the range between 0.322 to 0.870, thus the b parameter value used in the previous study was approximately the average of all b parameter values used in this study. Therefore, the probability values presented here could be considered as the lower and upper bound values of the previous study. Based on the pattern presented in Figure 4a and b, the probability of having an affected offspring of the protein coding mutations was greater that that of the tRNA gene mutations and the probability of having a healthy child, carrying wild type homoplasmy, of the protein coding mutations was lower than that of the tRNA gene mutations. Therefore, these results suggested that if two females harboring different mtDNA mutations carry the same mutation level and the threshold levels of these different mutations are equal, the mother carrying protein-coding mutation have a greater risk of bearing an affected child.

Due to the limited validity of the pedigree model, the recurrence risk and the probability of having a healthy offspring presented in Figure 4a and b should be interpreted with caution. As shown in Table 6, the pedigree model could well explain only the transmission pattern of A3243G mutation, somewhat describe the transmission pattern of G3460A, T8993G and A8344G, but not sufficiently depict transmission pattern of G11778A. Therefore, except the A3243G, the recurrence risk estimated from the pedigree model should be considered as the minimum value and the probability of having a healthy child should be considered as the maximum value.

Population model and its application

The population model was developed from the pedigree model thus, the transmission of mtDNA mutation level in this model was determined purely by random genetic drift.. The first generation population of the simulation carried wild type homoplasmy. The mutation level of later generation individual whose mother carried wild type homoplasmy depended on the probability of getting a new mutation that was determined by the mutation rate. If the simulated offspring born to the heteroplasmic mother, the mutation level of this individual was randomly generated from the Kimura distribution. Therefore, the chance that a simulated individual will carry mutant mtDNA depended on the probability of getting a new mutation determined by the *de novo* mutation rate and the Kimura distribution with the width determined by the b parameter value.

This model was applied to estimate the proportion of mutant carriers in the population. Given the *de novo* mutation rate at 0.10%, the initial mutation level of 10% and the b parameter values of 0.64, the results (Figure 5) showed that under the constant population size with no lineage loss and only random drift determining the transmission of mtDNA mutation level, the proportion of mutant carriers kept rising toward generations with the increase rate of 0.01%, corresponded well with the *de novo* mutation rate. The variation of the proportion of mutant carriers could be the result of random genetic drift. If the effect random genetic drift is strong enough to counteract the effect of mutation, the proportion of mutant carriers should not increase toward generation (HALLIBURTON

2004). Therefore, the increase of the proportion of mutant carriers suggested that under the constant population size with no lineage loss and the moderate b parameter value, the proportion of mutant carriers depended on the *de novo* mutation rate.

The distribution of simulated mutation levels at generation 34 was shown in Figure 5b. The mutation levels at this generation were chosen because the proportion of mutant carrier was approximately equal to the proportion observed in general population (ELLIOTT *et al.* 2008). The pattern presented in this plot showed that the mutation levels were not equally distributed around the mutation level mean at 48%; their distribution was rather skewed to the left presenting a greater number of low heteroplasmic individuals. This distribution pattern was not consistent with the distribution of the carrier mutation levels presented in the previous report because the previous report on the proportion of mutant carrier presented that the distribution of mutation levels were symmetric around the mutation level mean (ELLIOTT *et al.* 2008). The distinction of the mutation level distribution between the simulated and the observed data would be caused by no lineage loss, no purifying selection and insufficient effect of random genetic drift defined in the simulation. The unlimited time of lineage existence would help maintaining a new mutation that was arisen in the past until random drift or purifying selection eliminate it from the population. In out population model, the purifying selection was not included in the model due to insufficient data to define selection coefficient and the effect of random genetic drift defined in the model was insufficient to counteract the effect of mutation. Therefore, at this generation, some low heteroplasmic individuals would be newly generated by the de novo mutation and some would be the descendants of the heteroplasmic ancestor. Because the observed proportion of carriers was based on many mtDNA mutations (ELLIOTT *et al.* 2008), the insufficiency of the effect of random drift in the model may be caused by using only one b parameter value to represent the transmission of every mutation. In order to construct a more realistic population model, zthe probability of lineage loss, purifying selection and multiple b parameter values should be included in the model; however, at the present, we did not have enough information at hand to define all these parameters.

In summary, the statistical analysis carried out on the pedigree data suggested that the transmission pattern of the mtDNA mutation level of the protein-coding mutations: G11778A, G3460A, and T8993G, is different from that of the tRNA gene mutations: A8344G and A3243G. This difference would be the result of different mechanism regulating the transmission patterns of these mtDNA mutations. The bottleneck parameters estimated from the clinical pedigree data were applied to build the pedigree model of human mtDNA heteroplasmy inheritance. This model was built based on the assumption that the transmission of mtDNA mutation levels is solely determined by random genetic drift. Besides the A3243G mutation, the pedigree model could only partly explain the transmission pattern of other mutations: G3460A, T8993G and A8344G. The pedigree model was applied to study the progression pattern of the mtDNA mutation and estimating recurrence risk in the family carrying a pathogenic mtDNA mutation. Because of the limited validity of the pedigree model, the probability values calculated from the simulated data should be interpreted with caution;

the recurrence risk should be considered as the minimum chance of having a potential affected offspring. The pedigree model was further developed to the population model. This enlarge scale of the pedigree model was applied to estimate the proportion of mutant carriers in the population. Under the simplest scenario used in the population simulation, the proportion of carriers depended largely on the *de novo* mutation rate.

The result of this project only provides the first step toward understanding the progression of the diseases associated with mtDNA mutations. Regarding the transmission of mtDNA mutation level, the bottleneck parameter value of other pathogenic mtDNA mutation and the selection coefficient of the mutant mtDNA should be defined. In order to further understand the progression of these complex diseases, many functions and parameters, such as the penetrance function, the nuclear-mitochondrial interaction function and the threshold level for a specific disease phenotype, have to be defined (SCHORK and GUO 1993). Once these parameters and functions were identified, they could be added to the basic model developed in this study and develop the better pedigree model that will be more efficient in recurrence risk estimation.

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Appendix

The attached manuscript needs to be revised prior to submission because new unpublished pedigrees were added into the pedigree data.

Difference in mtDNA heteroplasmy inheritance between protein coding and tRNA gene mutations

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Abstract

The G11778A, G3460A, T8993G, A8344G and A3243G variations are common pathogenic mitochondrial DNA (mtDNA) mutations observed in association with various diseases. Affected individuals typically carry these mutations in a heteroplasmic condition, a mixture of mutated and wild type mtDNA. The heteroplasmy mothers generally transmit random proportions of mutated mtDNA to their offspring, generating a large random shift in mutation level. This study aims to understand the mtDNA heteroplasmy transmission of these common mutations by statistically analyzing human clinical pedigrees collected from published literature. In the case of G11778A, G3460A and T8993G variations (protein-coding mutations), the mothers carrying low levels of the pathogenic mutation tend to have an offspring carrying mutation level higher than them, while the mothers carrying high levels of the pathogenic mutation tend to have offspring carrying mutation either higher than or approximately equal to them. On the contrary, in the cased of A8344G and A3243G (tRNA gene mutations), the low heteroplasmy mothers generally have offspring carrying mutation level approximately equal to them, while the high heteroplasmy mothers tend to have offspring carrying mutation level lower than them. These results suggested that, besides random drift, positive selection would also play a role in regulating the heteroplasmy transmission of the protein-coding mutations. On the other hand, both random drift and negative selection would play a role in determining the heteroplasmy transmission of the tRNA gene mutations.

Introduction

The G11778A, G3460A, T8993G, A8344G and A3243G variations are common pathogenic mtDNA mutations that have long been observed in association with various diseases, especially neurodegenerative disorders. The G11778A and G3460A variations are point mutations in the *mt-ND4* and *mt-ND1* genes, respectively. These protein-coding genes encode subunits of complex I functioning in the oxidative phosphorylation process. Both the G11778A and G3460A variations have been observed to cause Leber's hereditary optic neuropathy (LHON)[1]. The T8993G variation is located in the *mt-ATP6* gene, coding for ATPase6 that is a subunit of complex V also functioning in the OXPHOS process. This point mutation has been has been observed to cause Leigh's syndrome and NARP (neurogenic muscle weakness, ataxia and retinitis pigmentosa) disease[2,3]. The A8344G variation is located in the *mt-tRNA*^{Lys} gene. It has long been observed to cause MERRF (myoclonic epilepsy and ragged-red fiber) disease[4]. Finally, the A3243G variation is located in the *mt-tRNA*$^{Leu(UUR)}$ gene. This variation has been observed in association with several diseases, including diabetes, deafness, and MELAS (mitochondrial encephalomyopathy lactic acidosis and stroke-like episode)[5-7].

Typically, an affected individual carries the pathogenic mtDNA mutation in a heteroplasmic condition, as a mixture of wild type and mutated mtDNA. The mtDNA mutation level is the primary factor determining an expression of mtDNA-mutation causing diseases. The proportion of mutate mtDNA is required to exceed the threshold level before the expression of disease phenotype[8]. The threshold level varies between different pathogenic mtDNA mutations and among different tissues[8]. No threshold level has been reported for the G11778A and G3460A mutation probably because of the incomplete penetrance of these mutations[9-11]. In the case of T8993G variation, the affected individual needs to carry at least 80% as measure in the blood sample[12]. The threshold level of the A8344G and A3243G variations were reported as 73-98% and 60-90%, respectively, depending on the associated diseases[8]. Even though exceeding the threshold level is necessary, it may not be sufficient to determine the expression of many disease phenotypes. Several modifying factors have been identified, including the secondary mtDNA mutations[10], nuclear DNA variations[9], and mtDNA haplotype[13].

A study of the prevalence of ten common pathogenic mtDNA mutations, including all variations in the study, in the general population of the North Cumbria in England showed that approximately 1 in 200 of the population carrying one of these ten mutations[14]. The A3243G mutation was reported as the most common pathogenic mtDNA mutation in this population (approximately 1 in 700 of the population), while the A8344G and T8993G variations were not observed in this population[14]. A number of epidemiological studies showed that different pathogenic mtDNA mutations have difference prevalence in the population and the prevalence of a

particular pathogenic mtDNA mutation generally varies between different populations[14-21].

Human mtDNA is exclusively maternally inherited[22]. The mother carrying mutated mtDNA in heteroplasmic condition generally transmits a random proportion of mutated mtDNA to her offspring, generating a large inter-generational random shift in mutation level[23-26]. This random shift in mutation level complicates a prediction of the recurrences risk in a family carrying a pathogenic mtDNA mutation, leading to an uncertainty in genetic counseling for the family carrying the mtDNA mutation[27-28].

Mitochondrial genetic bottleneck has been hypothesized as a mechanism generating the random shift in mutation level between generations. This process generated a variation of mutation levels by randomly sampling a certain number of mtDNA molecules to be inherited to the next generation. The mitochondrial genetic bottleneck hypothesis was supported by the experimental results in the mouse model carrying a neutral NZB/BALB mtDNA[29]. The results showed that the average mtDNA mutation level of the offspring was approximately equal to their corresponding mothers' mutation level, agreeing to the random drift theory[30]. The comparison of mtDNA mutation level variance across different stages of female germ line development, from the primordial germ cell to the offspring, indicated that the variation of mtDNA mutation level was largely generated during the proliferation of the primordial germ cell[29]. Later, several studies attempted to identify what mitochondrial segregating unit is and when the largest mutation level variance was generated; however, these issues are still under debated[31-35].

On the basis of random genetic drift theory and standard haploid population genetics, the mutation level mean of the offspring would be equal to the maternal mutation level and the variance of the offspring mutation level would have the following form (cite):

$$V_t = p_0(1-p_0)\left(1-e^{-t/N_{eff}}\right)$$

The mutation level variance (V_t) of a group of offspring of a single mother can be calculated from the maternal mutation level (p_0), the number of generations (t), and the effective population size (N_{eff}). This equation is generally referred as the Sewall-Wright variance formula (cite). It was introduced to the field of mitochondrial genetics by applying it to describe the variation of mtDNA mutation levels measured from *Drosophila* (cite). The variance of mtDNA mutation level is useful but very limited measurement. It is lack of a capability to describe the distribution pattern of mtDNA mutation level, thus Wonnapinij *et al.* applied a set of probability distribution functions developed by Motoo Kimura in 1995 (cite) to estimate the distribution of mtDNA heteroplasmy levels. This probability model consists of three equations: a probability $f(0,t)$ for losing an allele, a probability $f(1,t)$ for fixing an allele, and a probability distribution function $\phi(x,t)$ that the allele is present at frequency x in the population.

$$f(0,t) = (1-p_0) + \sum_{i=1}^{\infty}(2i+1)p_0(1-p_0)(-1)^i F(1-i,i+2,2,1-p_0)e^{-\left(\frac{i(i+1)}{2N_{eff}}\right)t}$$

$$\phi(x,t) = \sum_{i=1}^{\infty} i(i+1)(2i+1)p_0(1-p_0)F(1-i,i+2,2,p_0)F(1-i,i+2,2,x)e^{-\left(\frac{i(i+1)}{2N_{eff}}\right)t}$$

$$f(1,t) = p_0 + \sum_{i=1}^{\infty}(2i+1)p_0(1-p_0)(-1)^i F(1-i,i+2,2,p_0)e^{-\left(\frac{i(i+1)}{2N_{eff}}\right)t}$$

The definition of each variable in these equations is the same as for the Sewall-Wright variance formula. In terms of mtDNA mutation level, the $f(0,t)$ and the $f(1,t)$ are the probabilities of having offspring carrying wild type and mutant homoplasmy, respectively, and the $\phi(x,t)$ is the probability of having offspring carrying x×100% mutation level. These equations have been referred as the Kimura distribution. This theoretical Kimura distribution was compared against human, mouse and *Drosophila* data set and the results showed that the fit Kimura distribution is consistence with the observed mtDNA heteroplasmy level distribution (cite).

Different pathogenic mtDNA mutations presented different sizes of the inter-generational random shift in the mutation level. A large random shift has commonly observed in the families carrying T8993G variation[36-37], while the A8344G families tended to have a small random shift[38]. Different rate of mtDNA heteroplasmy segregation could be caused either by different size of mitochondrial bottleneck or an existence of other forces interacting to the random drift. An experiment in the mutator mice showed that non-synonymous mutations in protein-coding genes were subjected to purifying selection, while the synonymous mutations in these genes were randomly segregated[39]. The authors also showed that the mutation rates of some protein-coding genes were higher than others, such as the *mt-Cyb*, *mt-ATP6* and *mt-ATP8* genes. In the case of the tRNA and rRNA genes, the mutation rates of genes in these categories were reported to be high which is inconsistent with the mutation rates observed in natural mouse strain and humans. The authors thus concluded that purifying selection would also play a role in determining the transmission pattern of the non-synonymous mutations of the protein-coding genes.

In the previous study by Chinnery *et al*, the authors studied single-generation transmissions of the six common pathogenic mtDNA mutations: G11778A, G3460A, T8993G, T8993C, A8344G and A3243G[23]. They examined whether random drift or selection plays a role in determining the transmission pattern of these mtDNA mutations by comparing the distribution of the difference of the mutation level between offspring and their corresponding mothers (O-M) to the normal distribution. Their results showed that the transmissions of the G11778A, T8993G and A3243G mutations were in favor of inheriting mutated mtDNA, whereas the transmission of the A8344G was in favor of inheriting wild type mtDNA. These results suggested that positive selection might

play a role in determining the transmission pattern of the G11778A, T8993G and A3243G mutations, while purifying selection might play a role in regulating the transmission pattern of the A8344G mutation. Even though this study showed that different mtDNA mutation would have different pattern of mtDNA heteroplasmy transmission, the authors did not directly examine this difference by comparing the statistical properties of one mutation against other mutations. They also did not examine the effect of maternal mutation level on the transmission pattern of the mtDNA mutation. In addition, the blood A3243G mutation level used in this previous study was not corrected for the reduction of blood mutation level toward age. This correction is necessary because the A3243G mutation level as measured in blood sample has been observed to decrease with increasing age and this reduction of the blood mutation level would deceive the transmission pattern of the A3243G mutation.

In this study, we statistically analyzed human clinical pedigrees data carrying one of the five common pathogenic mtDNA mutations: G11778A, G3460A, T8993G, A8344G, and A3243G mutation. This study aims to understand the transmission pattern of the pathogenic mtDNA mutation level by comparing the statistical properties of these mutations to the expected statistics based on random drift theory. We also compared the statistics of one mutation to another mutation to examine whether different mutations has different pattern of mtDNA heteroplasmy transmission. This comparison was done especially to examine our hypothesis that the mtDNA heteroplasmy transmission patterns of the protein coding mutations (G11778A, G3460A and T8993G) are different from the patterns of the tRNA gene mutations (A8344G and A3243G). The effect of maternal mutation level on the transmission pattern of the mtDNA mutation level was also examined by comparing the statistical properties of the low heteroplasmy mothers to those of the high heteroplasmy mothers. The solution for correcting the reduction of blood A3243G mutation[40] was applied to the blood A3243G measurements in our study to help adjusting the confounding effect caused by this reduction of blood mutation level.

Materials and Methods

Collecting human clinical pedigree data

Human clinical pedigrees of the families carrying one of the five common pathogenic mtDNA mutations: G11778A [41-54], G3460A [11; 46; 47; 55-59], T8993G [2; 3; 12; 26; 36; 60-83], A8344G [24; 26; 84-103] and A3243G [5-7; 26; 104-142], were collected from published literatures. The pedigree position, gender, relationship with the index case, age at sampling, and blood mtDNA heteroplasmy level of each individual were collected. Because of the maternal inheritance of human mtDNA, only the information of the index cases and their maternal relatives were included in the analyzed data. The individual's pedigree position was recorded with regard to the maternal inheritance of human mtDNA. The relationship between the individual and the index case was recorded as generally defined; for example, mother, sister, brother or uncle. The age at sampling of the individuals is the age when their blood was drawn for mtDNA mutation level measurement. The pedigree data used for this study is shown in Supplementary Table 1

Managing mother-offspring pair data

Because this study aims to understand common pathogenic mtDNA heteroplasmy transmission, the mother-offspring pairs, whose mtDNA heteroplasmy level had been reported, were included in the analyzed data. All the mother-offspring pairs whose heteroplasmic offspring was born to the wild type homoplasmic mothers were excluded from the statistical analyses to adjust for the effect of seemingly *de novo* mutation. These *de novo* mutation cases may be the result of the limitation of mtDNA heteroplasmy measurement method. The mother-offspring pairs whose one of them is an index case were also excluded from the statistical analyses to adjust for the effect of ascertainment bias of clinical data.

A number of longitudinal studies reported a reduction of blood mutation level toward age [40; 143-145], which could deceptively generate an inter-generational increase of the mtDNA heteroplasmy level. Hence, the application of the age correction for this reduction is required to reduce the transmission bias due to this longitudinal change. In 2007 Rajasimha *et al.* propose that this reduction is the result of the selection against high heteroplasmic hematopoietic stem cell. Besides the mechanism, they provided the mathematical formula to correct for this reduction [40]. The formula is shown in equation 1.

$$p_{age-corrected} = p_{observed} e^{(0.02 \times t)} \qquad \text{Equation 1}$$

The $p_{observed}$ is the individual's heteroplasmy level and the $p_{age\text{-}corrected}$ is the age-corrected individual's heteroplasmy level. The t variable is the individual's age at sampling. After applying this age-correction formula, the families harboring individuals carrying age-corrected A3243G mutation level exceeds 110% were excluded from the analyses because these families may carry a secondary mtDNA mutation that could modify the mtDNA heteroplasmy segregation [108; 129].

Calculating statistics based on the Kimura distribution

The 95% confident interval of each mutation was calculated from the Kimura distribution with the *b* parameter value estimated from the offspring heteroplasmy levels. There were two values of the *b* parameter that were calculated in this study: (1) the *b* parameter value calculated from the heteroplasmy levels of the offspring of the mothers carrying an intermediate heteroplasmy level, 40-60% heteroplasmy level and (2) the *b* parameter value calculated from all offspring carrying each mutation. The intermediate heteroplasmy level was chosen because it has the least effect from the average heteroplasmy level (the *p* parameter value) [146]. How to calculate the *b* parameter value is shown in Equation 2 [147].

$$b = 1 - \frac{V}{p(1-p)} \qquad \text{Equation 2}$$

The *V* variable is the offspring heteroplasmy level variance and the *p* variable is the offspring heteroplasmy level mean.

Assuming that the distribution of mtDNA heteroplasmy level follows the Kimura distribution, the mtDNA heteroplasmy level variance was normalized by dividing it by the factor *p(1-p)* [146]. The error bar of the mtDNA heteroplasmy level variance of each mtDNA mutation was also calculated based on the Kimura distribution [146]. Both the normalization method and the variance error bar calculation were applied for the comparison of mtDNA heteroplasmy level variance between different mtDNA mutations.

Performing statistical analyses

Two categories of statistical analyses were used in this study: data visualization and hypothesis testing.

Data visualization

Histogram was applied to explore the distribution of the differences between offspring heteroplasmy level and mother's heteroplasmy level (O-M values). The 2D scatter diagram was applied to visualize the relationship in heteroplasmy level between offspring and their corresponding mothers. The 95% confident interval was added to the scatter diagram to examine the consistency between the observed mother-offspring pair data and the Kimura distribution. The scatter-diagram smoothing method was applied to define the relationship in heteroplasmy level between offspring and their corresponding mothers. This method uses a locally weighted polynomial regression method (the "lowess" function in R) to analyze the relationship between the response variable and the predictor variable. This method makes only a few initial assumptions about the model; therefore, it can be considered as a non-parametric regression analysis [148]. All these plots were created using OriginPro8 (OriginLab)

Hypothesis testing

Every statistical analysis was done using function in R programming (R foundation for statistical computing). Both parametric and non-parametric approaches: student's t test and Wilcoxon test, were applied to test the hypothesis. The result is considered to be significant different when the p-value is less than the significant level at 0.05.

The Kolmogorov-Smirnov (KS) test was applied to examine whether the observed O-M values is normally distributed. The one sample *t*-test was applied to examine whether the average O-M values is significant different from zero. The one-sample Wilcoxon test, which examines the median statistic, was chosen as an alternative non-parametric approach of this t-test. The two-sample *t*-test with the Welch approximation (the variation of the student's t test proposed to compare two samples possibly having unequal variances) was applied to compare the average O-M values of the female offspring to the statistic of the male offspring. The Mann-Whitney test (the two-sample Wilcoxon test), which is an alternative non-parametric approach of the two-sample t-test, was applied to examine the median statistic of this comparison.

The mother-offspring pair data was separated into two groups based on the mother's heteroplasmy level: the group of low heteroplasmic mothers and the group of high heteroplasmic mothers. The group of low heteroplasmic mothers contained the mother-offspring pair data whose mothers carried heteroplasmy level less than 50%. On the contrary, the group of high heteroplasmic mothers contained the mother-offspring pair data whose the mother carried heteroplasmy level greater than of equal to 50%. After separating the mother-offspring pair data, the two sample *t*-test with the Welch approximation and the Mann-Whitney test were applied to compare the average O-M values of the low heteroplasmic mothers to the statistic of the high heteroplasmic mothers.

Results

In this study, the data of human clinical pedigrees carrying pathogenic mtDNA mutations: G11778A, G3460A, T8993G, A8344G, and A3243G, was collected from the publish literature. The mutation levels of mother-offspring pairs were systematically gathered for statistical analyses aiming to deduce the transmission pattern of each mtDNA mutation. Summary statistics of the mother-offspring pair data were shown in Table 1. The number of mother-offspring pairs carrying each mtDNA mutation used for the statistical analyses is the number of transmission reported in this Table. The highest offspring mutation level variance was observed in the offspring carrying G3460A mutation, while the lowest value was observed in the offspring carrying A3243G mutation. In general, the offspring mutation level variances of the protein-coding mutations: G11778A, A8344G and T8993G, were greater than the variances of the tRNA gene mutations: A8344G and A3243G. Since the mutation level variance is influenced by the mutation level mean, the variance of each mtDNA mutation was normalized by dividing it by $p(1-p)$ where p is the offspring mutation level mean. The normalized offspring mutation level variance of each mtDNA mutation was reported in Table 1. The greatest normalized offspring mutation level variance was observed from the offspring carrying G3460A mutation, while the lowest normalized variance was obtained from the offspring carrying A3243G mutation. As the results of offspring mutation level variance, the normalized mutation level variance of the protein coding mutations: G11778A, G3460A and T8993G mutation, was generally greater than the normalized variance of the tRNA gene mutations: A8344G and A3243G mutation.

The bottleneck (b) parameter value determining the width of the offspring mutation level distribution was calculated both from all offspring and from the offspring of the mother carrying an intermediate mutation level (40-60%), as shown in Table 1. This intermediate maternal mutation level was chosen because, based on random genetic drift theory, the offspring mutation level variance is least affected by the mutation level mean. The detail regarding the bottleneck parameter calculation was shown in the material and method section. Because the bottleneck parameter value is inversely related to the normalized mutation level variance, the highest and the lowest bottleneck parameter values were observed in the case of A3243G and G3460A mutation, respectively. The bottleneck parameter values of the protein coding mutations were typically lower than the values of the tRNA gene mutations both when this value was calculated from all offspring and from the offspring of the mother carrying an intermediate mutation level.

For every mtDNA mutation included in this study (except the G11778A), at least one heteroplasmy offspring born to the wild type homoplasmy mother was observed, as shown in Table 2. This observation indicated that *de novo* mutation may existed to maintain mutated mtDNA in human population; however, it is also possible that the limitation of detecting a small proportion of

mutated DNA from the blood sample generated this transmission pattern. Assumed that the lowest mutation level that the measurement method can detect the mutated mtDNA is 6%, we calculated the probability of obtaining heteroplasmy offspring from the Kimura distribution given the maternal mutation level of 5% and the bottleneck parameter values calculated from the offspring of the intermediate heteroplasmy mothers. The expected number of heteroplasmy offspring was calculated from this probability value. The Fisher's exact test was applied to compare the observed to the expected number of heteroplasmy offspring born to the apparent wild type homoplasmy mothers. Due to a very small sample size in the case of G3460A mutation, the comparison was not done for this mtDNA mutation. The comparison results showed that only the observed number of T8993G heteroplasmy offspring was statistically significant different from the expected value (p-value < 0.001).

Figure 1a-e present the comparison of the observed O-M values to the normal distribution with the mean and standard deviation estimated from the observed data. The peak of the observed O-M distribution of every mutation, except the A8344G mutation, was consistent with the peak of the fitted normal distribution. As reported in Table 3, the skewness statistics of the O-M values of the protein-coding mutations: G11778A, G3460A, and T8993G, and the A3243G mutation were positive, indicating that the distribution were skewed to the right, while this statistics of the A8344G mutation was negative, indicating that its distribution was skewed to the left. The p-value of the Kolmogorov-Smirnov (KS) test showed that the O-M distributions of the protein-coding mutations were significantly deviated from the normal distribution. On the contrary, the O-M distributions of the tRNA gene mutations: A8344G and A3243G were consistent with the normal distribution.

The relationship of mutation level between offspring and their corresponding mothers was initially examined by plotting the offspring mutation levels against the maternal mutation levels, as shown in Figure 1f-j. The index cases and *de novo* mutation cases, the heteroplasmy offspring born to the wild type homoplasmy mothers, were also included in the plots. Based on these scatter plots, the index cases carrying protein-coding mutations generally carried a high proportion of mutated mtDNA, while the index cases carrying tRNA gene mutations carried a random proportion of the mutated mtDNA. Summary statistics and the mutation level distribution of these index cases were presented in Figure 2. The difference of the index case mutation level between protein-coding mutations (G11778A, G3460A, and T8993G) and tRNA gene mutations (A8344G and A3243G) was supported by the p-values of the Welch two-sample t test and the Mann-Whitney test.

Figure 1f-j also presented the 95% confident interval for the offspring mutation level of each mtDNA mutation. This 95% confident interval was calculated from the Kimura distribution with the maternal mutation level ranged from 0 to 100% and the bottleneck parameter value estimated

from the mutation levels of the offspring born to the mother carrying an intermediate mutation level (40-60%). Regardless of the type of mtDNA mutation, the 95% confident interval covered approximately 95% of the offspring mutation levels. Notice that most of the offspring carrying mutation level lower of greater than the 95% confident level was born to the mothers who carried an extreme mutation level, either very low or very high mutation level.

The statistical analyses carried out on the mean and the distribution of the differences of the mutation level between offspring and their corresponding mothers (O-M) were shown in Table 3. The box and whisker plot of the O-M value was presented in Figure 3a. The one-sample student t-test and the Wilcoxon test were applied to examine whether the average O-M value of each mutation significantly differed from zero. If the average O-M value was significant difference from zero, selection might also play a role in determining the offspring mutation level. The results showed that the average O-M value of G11778A, G3460A and T8993G mutation (the protein-coding mutations) were positive and statistically significant difference from zero, while, in the case of tRNA gene mutations, only the average O-M value of the A3243G mutation was positive but not significant difference from zero. The average O-M value of the A8344G mutation (another tRNA gene mutation) was negative and statistically significant difference from zero. The difference of the sign of the average O-M value between the protein-coding mutations and the tRNA gene mutation (the A8344G) suggested that the offspring mutation level of these mtDNA mutations would be determined by different mechanisms.

The difference of the average O-M value between the protein-coding mutations and the tRNA gene mutations was confirmed by the comparison of the average O-M values between different mtDNA mutations. As shown in Table 4, the comparison results provided by the Welch two-sample t test and the Mann-Whitney test showed that no significant difference was observed either when the average O-M values of the G11778A, G3460A and T8993G mutation were compared against each other or when the average O-M value of the A8344G mutation was compared against this statistics of the A3243G mutation. Interestingly, the significant difference of the average O-M value was observed only when the average value of the G11778A, G3460A or T8993G was compared against the average value of the A8344G or A3243G. These statistically significant differences of the average O-M value between the protein-coding mutations and the tRNA gene mutations suggested that the mechanisms regulating the mtDNA heteroplasmy transmission of these two groups of mtDNA mutations are different.

The locally weighted regression analysis was applied to describe the relationship of mutation level between offspring and their corresponding mother. This method is a nonparametric method that used multiple regression models to locally fit subsets of the data. The function that can describe the data is built upon these locally fit models. The advantage of this method is that neither

linear nor nonlinear function needs to be specified prior to analyze the data. The scatter-diagram smoothing line of each mutation obtained from this method was compared against each other, as shown in Figure 3b. The null hypothesis based on a random genetic drift theory was that the mutation level of the offspring would be approximately equal to the mutation level of their corresponding mothers. This null hypothesis was shown in the figure as the straight black line. The smoothing lines presenting the inter-generational relationship of mutation level of the G11778A, A8344G and T8993G (the protein-coding mutations) were located above the null hypothesis line. On the contrary, the smoothing lines presenting the inter-generational relationship of the A8344G and A3243G mutation level were located below the null hypothesis line. The location of these smoothing lines compared to the null hypothesis line supported the results of the previous analysis on the average O-M values that the mtDNA heteroplasmy transmission pattern of the protein-coding mutations are different from the pattern of the tRNA gene mutations.

No scatter smoothing line, presented in Figure 3b, showed a straight-line relationship, thus a simple linear model could not explain the inter-generational relationship of the mutation level of any mtDNA mutation included in this study. In fact, every smoothing line presented two different patterns of the inter-generational relationship of the mutation level: one for the low heteroplasmy mother and another for the high heteroplasmy mother. Therefore, we further analyzed the O-M values of the low and the high heteroplasmy mothers. The low heteroplasmy mothers carried mtDNA mutation level less than 50% and the high heteroplasmy mothers carried mtDNA mutation level greater than or equal to 50%. To test whether the mtDNA heteroplasmy transmission pattern of the low heteroplasmy mother differs from the pattern of the high heteroplasmy mother, the average O-M values of the low heteroplasmy mothers were compared against the average values obtained from the high heteroplasmy mothers. In addition, the average O-M values of the low and the high heteroplasmy mothers were compared against zero to examine whether the random drift process could solely determine the offspring mutation level. The resulted of these statistical analyses were shown in Table 5. The box and whisker plots of the O-M values separated by the maternal mutation level of the protein-coding mutations (G11778A, G3460A, and T8993G) and the tRNA gene mutations (A8344G and A3243G) were presented in Figure 3c and d, respectively.

For the protein-coding mutations, the average O-M values of the low and the high heteroplasmy mothers were positive, as shown in Table 5 and Figure 3c. These findings agreed to the location of the smoothing lines obtained from the locally weighted regression analysis (Figure 3b). The comparison of the average O-M value between the low and the high heteroplamy mothers showed that the average values of the low heteroplasmy mothers were not statistically significant difference from the average values of the high heteroplasmy mothers, except for the G11778A mutation. The non-significant difference of the average O-M values between the low and the high

heteroplasmy mothers did not contradicted the pattern of the scatter smoothing lines representing the inter-generational relationship of the mutation level because the average O-M values provided less detail than the scatter smoothing lines. These non-significant differences of the average O-M values suggested that the same mechanisms regulated mtDNA heteroplasmy transmission of the low and the high heteroplasmy mothers carrying these protein-coding mutations.

We further compared the average O-M values of the low and the high heteroplasmy mothers against the null hypothesis based on random drift theory. As shown in Table 5 and Figure 3c, the results presented that only the average O-M values of the high heteroplasmy mothers of the G11778A and T8993G mutation were not statistically significant difference from zero. The non-significant difference from the null hypothesis of the average O-M value of the high heteroplasmy G11778A mothers should be interpreted with caution because only the p-value of the one-sample Student t-test showed non-significant difference. Since the one-sample Student t-test is a parametric statistical analysis with prior assumption that the data is normally distributed, this test may not be sensitive enough to detect the significant deviation of the average O-M of the high heteroplasmy G11778A mother due to non-normal distribution of the O-M value (p-value of the KS test = 0.017). The significant difference from zero of the average O-M values of the low and the high heteroplasmy mothers carrying protein-coding mutations indicated that other mechanisms, besides random drift, would also play a role in determining the offspring mutation level.

For the tRNA gene mutations, the average O-M values of the low heteroplasmy mother were positive, while the average values of the high heteroplasmy mothers were negative (Table 5 and Figure 3d). The comparison of the average O-M values between the low and the high heteroplasmy mothers showed that these average O-M values were statistically significant difference, corresponding to the pattern of the scatter smoothing lines representing the inter-generational relationship of the mutation level. The comparison of the average O-M values of the low and the high heteroplasmy mothers against the null hypothesis showed that only the average values of the high heteroplasmy mothers were significant difference from zero. Even though the p-value of the one sample Student t-test that used for comparing the average O-M value of the low heteroplasmy A3243G mothers indicated that this O-M mean significantly differed from zero, this conclusion was not supported by the p-value of the one sample Wilcoxon test, the alternative non-parametric approach of the Student t-test. These statistical analysis results of the tRNA gene mutations suggested that the mechanisms regulating the mtDNA heteroplasmy transmission of the low heteroplasmy mothers would differ from the ones regulating the transmission of the high heteroplasmy mothers.

Discussion

This study aims to understand transmission pattern of mtDNA heteroplasmy level and the mechanisms determining the pattern. To achieve this goal, we had statistically analyzed human clinical pedigrees carrying one of the five common pathogenic mtDNA mutations: G11778A, G3460A, T8993G, A8344G and A3243G mutation. The results mainly showed that the heteroplasmy transmission pattern of the protein coding mutations: G11778A, G3460A and T8993G, differed from that pattern of the tRNA gene mutations: A8344G and A3243G. This difference indicated that the mechanisms determining the heteroplasmy transmission of these two groups of mtDNA mutations should be different. We proposed that random drift and *de novo* mutation determined the heteroplasmy transmission of the protein coding mutations, while the random drift and purifying selection determined the heteroplasmy transmission of the tRNA gene mutations.

The statement that random genetic drift plays a major role in determining the transmission of mtDNA heteroplasmy level is supported by the highly percentage of offspring heteroplasmy levels that were in the range of the estimated 95% confident interval calculated based on the Kimura distribution, more than 95% of the offspring carrying protein coding mutations and approximately 90% of the offspring carrying tRNA gene mutations, as shown in Figure 1f-j and Supplementary Figure 1. Some offspring heteroplasmy levels were out of the range of the estimated 95% confident interval. We can notice that these offspring were born to the mothers carrying either too low or relatively high heteroplasmy level. These deviations suggested that other mechanisms might also play a role in determining an offspring heteroplasmy level.

The role of *de novo* mutation on determining the heteroplasmy transmission of the protein coding mutations was suggested by the positively high values of the average of the differences of the mtDNA heteroplasmy level between the offspring and their corresponding mothers (O-M values), as shown in Table 2 and the position of the smoothing lines above the intended line, as shown in Figure 2b.

As one would expected the distribution of the O-M values to be normal with the mean of zero if random genetic drift is solely responsible for the mtDNA heteroplasmy transmission [23], the significant positive difference from zero of the average statistics and the significant deviation from the normal distribution of these values suggested that the *de novo* mutation may play a role in determining the transmission pattern of these mutations heteroplasmy level. Our findings seemed to be contradicted to the previous observation by Chinnery P.F *et al.* [23] in which they observed no significant deviation of the O-M values from the normal distribution and no significant difference from zero of the O-M values of the G3460A mutation. However, considering the average O-M values of these mutations, the average values observed in this study were in the same range as the

values observed in the previous study; therefore, the statistical significance observed in this study would be caused by a larger sample size which could increase the power of the hypothesis testing.

The intended line was generated based on the random genetic drift theory that the offspring heteroplasmy level would be approximately equal to their corresponding mothers' heteroplasmy level[23]. The smoothing lines, shown in Figure 2b, presented the relationship in heteroplasmy level between offspring and their corresponding mothers without making any prior assumptions about the model that can explain this relationship. The position of these smoothing lines above the intended line suggested that the offspring tends to carry a larger proportion of mutant mtDNA than their corresponding mothers, thus indicating the role of *de novo* mutation on determining the offspring heteroplasmy level.

The pattern of the smoothing lines, as shown in Figure 2b, further suggested the non-linear relationship in heteroplamy level between offspring and their corresponding mothers carrying these protein-coding mutations. The pattern showed the possibility that the mechanisms regulating the mtDNA heteroplasmy transmission of the low heteroplasmic mothers, carrying heteroplasmy level less than 50%, differs from those regulating the mtDNA heteroplasmy transmission of the high heteroplasmic mothers, carrying heteroplasmy level greater than or equal to 50%.

The O-M values of the low heteroplasmic mothers were skewed but not significantly deviated from the normal distribution except for the T8993G mutation; however, the O-M values of the high heteroplasmic mothers were skewed and significant deviated from the normal distribution. The average O-M values of the low heteroplasmic mothers and the high heteoplasmic mothers were all positive and significant difference from zero, except for the average O-M of the high heteroplasmic mothers carrying T8993G mutation. Both the skewed distributions and the significant differences of the average O-M values of the G11778A and G3460A mutation suggested the role of *de novo* mutation on determining the heteroplasmy transmission of these mutations. The right skewed distribution of the O-M values and the positively significant difference from zero of the average O-M values of the low heteroplasmic T8893G mutation also supported the role of *de novo* mutation on regulating the heteroplasmy transmission of the mothers carrying protein-coding mutations. However, the left-skewed distribution of the O-M values and approximately zero of the average O-M values suggested other mechanisms. The left-skewed distribution suggested a role of purifying selection, while the approximately zero of the average O-M values seemed to be generated by random genetic drift. We proposed that this pattern should be generated by the interaction between purifying selection and random drift under the effect of ascertainment bias.

A large number of heteroplasmic offspring born to the wild type homoplasmic mothers has been widely observed in the families carrying T8993G mutation, indicating a high rate of *de novo* mutation [26; 36; 63; 64; 68-70; 75; 81]. An excess of the mtDNA mutations in the *mt-ATP6* gene in the

mutator mice could also supported the high mutation rate of this mtDNA mutation [39]. Thus, these evidences supported that *de novo* mutation plays a role in regulating the T8993G heteroplasmy transmission.

However, neither the large number of heteroplasmic offspring offspring born to the wild type homoplasmic mothers nor the high mutation rate of the G11778A and G3460A had been observed; therefore, the statistically significant results of these two mutations may be caused by (1) the longitudinal change of blood heteroplasmy level, (2) the less-intense purifying selection of these mutations, or (3) the ascertainment bias of human clinical data. The reduction of the blood heteroplasmy level toward age, approximately 3% in 2-4 years, has been observed in the individuals carrying G3460A mutation [11]; therefore the observed inter-generational increase of blood G3460A mutation may be the result of this reduction. Besides that, both the G3460A and G11778A mutation have been observed in association with LHON disease and generally found to have incomplete penetrance [9; 10; 13]. Some mutant homoplasmic individuals have been observed to be asymptomatic [41; 42; 45-48; 52-54]. Several secondary mtDNA mutations have been observed to play a role as a modifying factor determining an expression of LHON disease [149-153]. Therefore, the less-intense purifying selection of these LHON mutations may generate the statistically significant results. In addition, the lack of low heteroplasmic offspring born to the high heteroplasmic mother found in the G3460A pedigree data, as shown in Figure 1g, pointed to the existence of the ascertainment bias of the human clinical data. Thus, even though all the index cases were removed from the analyzed data to reduce the effect of the ascertainment bias, this process cannot completely eliminate the effect of this bias on the statistical analyses [154].

In this study, we had not observed purifying selection regulating the heteroplasmy transmission of the protein coding mutation. This finding seemed to be contradicted to the purifying selection in protein coding genes observed in the mutator mice [39]; however, this contradiction may be caused either by the effect of ascertainment bias or the less-intense purifying selection of the mutations chosen to study. The ascertainment bias tends to generate an inter-generational increase of the mtDNA heteroplasmy level, suggesting the role of *de novo* mutation, because the families with multiple affected offspring carrying high heteroplasmy level tends to be collected for the clinical study [154]. This bias may confound the effect of purifying selection. The confounding effect of the ascertainment bias may explain why we detected that the T8993G heteroplasmy transmission of the high heteroplasmic mothers is consistent with random genetic drift theory.

The role of purifying selection on determining the heteroplasmy transmission of the tRNA gene mutations was suggested by the negative values of the average O-M values of the high heteroplasmic mothers, as shown in Figure 2d, and the position of the partial smoothing lines below

the intended line, as shown in Figure 2b. The part of smoothing lines that are located below the intended line is the part that presents the relationship in heteroplasmy level between the offspring and their corresponding high heteroplasmic mothers, thus agreeing with the negative average O-M statistic. The left-skewed distribution of the O-M values of the high heteroplasmic mothers also suggested the role of purifying selection on the mtDNA heteroplasmy transmission of these tRNA gene mutations. The observation of the purifying selection could be expected because of the pathogenicity of these mutations. Some females carrying the A3243G mutation experienced spontaneous abortions [128; 155-159], which could explain the purifying selection of this mutation observed in this study. The purifying selection of the tRNA gene mutations observed in this study seemed to be contradicted to the lack of purifying selection of the mtDNA mutation in tRNA genes observed in the mutator mice [39]. We would argue that they might not be able to detect the effect of purifying selection because their mice may carry inadequate proportions of the tRNA gene mutations.

The slightly left-skewed distribution of the O-M values and the negatively significant difference of the average O-M values from zero of the A8344G mutation indicated that purifying selection plays a role in determining the mtDNA heteroplasmy transmission of this mutation. These results were consistent with the previous study by Chinnery *et al.* [154]. The distribution of the O-M values of the low heteroplasmic A8344G mother was slightly right-skewed but not significantly deviated from the normal distribution. The average O-M of these mothers was statistically non-significant positive value. These statistical results agreed with the pattern of the smoothing line shown in Figure 2b. Only the beginning part of the line presenting the relationship in heteroplasmy level between the offspring and their corresponding low heteroplasmic mothers was consistent with the intended line. Both the statistical results carried out on the O-M values and the smoothing line suggested that random drift plays a role in determining the mtDNA heteroplasmy transmission of these low heteroplasmic mothers. Thus, the distribution and the average statistic of the overall O-M values of this mutation should be driven by the O-M values of the high heteroplasmic mothers.

On the other hand, the slightly right-skewed distribution of the O-M values and the positively average O-M values of the A3243G mutation suggested the role of *de novo* mutation; however, neither the distribution nor the average of the O-M values reach significant difference. These results partly disagreed with the previous study by Chinnery *et al.* [154] in which their results showed the statistically significant difference from zero of the average O-M values of the A3243G mutation. This difference would be the result of the application of the age-correction for a reduction of blood heteroplasmy level toward age to the blood A3243G heteroplasmy measurements in this study. This application should decrease the difference in heteroplasmy level between offspring and their corresponding mothers, leading to non-significant difference between

the offspring heteroplasmy level and the mother's heteroplasmy level. Regarding the O-M values of the low heteroplasmic A3243G mother, the approximate normal distribution with the average around zero and the consistence of the beginning part of the smoothing line shown in Figure 2b, with the intended line suggested the role of random genetic drift on the heteroplasmy transmission. This conclusion agreed with the study by Brown *et al.* [160]. They observed that the segregation of the human primary oocytes A3243G heteroplasmy levels agreed with the random genetic drift theory.

No gender bias of the mtDNA heteroplasmy transmission was observed both in the protein coding and tRNA gene mutations, except for the T8993G mutation when the Mann-Whitney test was applied. The mean ($mean_{feamle}$= 8.34, $mean_{male}$ = 16.38) and median ($median_{female}$=0, $median_{male}$=10.5) statistics of the O-M values of this mutation showed that the mothers carrying this mutation transmitted a higher proportions of this mutant mtDNA to their sons than to their daughters. This result corresponded to the study by Wong *et al.* [26]. They also observed that the intergenerational increase of the proportions of this mutant mtDNA in male offspring was larger than in female offspring: however, this difference did not reach the statistical significance. More families had been added to the pedigree data used in this analysis. The larger the samples size, the higher the power of the hypothesis testing, thus generating the significant difference.

Conclusion

The results of this study suggested that random genetic drift plays a major role in determining the heteroplasmy transmission of the five common pathogenic mtDNA mutations: G11778A, G3460A, T8993G, A8344G, and A3243G. The heteroplasmy transmission pattern of the protein coding mutations: G11778A, G3460A, and T8993G, differs from the pattern of the tRNA gene mutations: A8344G and A3243G. These differences are caused by different mechanisms interacting with the random drift, which the *de novo* mutation increases the heteroplasmy level of the offspring carrying protein-coding mutation and the purifying selection decreases the heteroplasmy level of the offspring carrying tRNA gene mutation. Our findings would be useful for developing an effective method for recurrence risk estimation and for preventing transmission of these pathogenic mtDNA mutations with the help of assisted reproductive technologies [161].

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Table 1: Summary statistics of the mother-offspring pairs collected from the published human clinical pedigrees

mtDNA mutation	Number of transmission[a]	Average maternal mutation level (%)	Average offspring mutation level (%)	Offspring mutation level variance (x 10^{-4})	Normalized offspring mutation level variance[b] (standard error[c])	Bottleneck parameter[d]	
						All offspring	Offspring of the mother carrying 40-60% mutation level[e]
G11778A	123	63.49	76.12	0.0806	0.4435 (0.0564)	0.5565	0.5396
G3460A	83	49.12	60.62	0.1558	0.6526 (0.0545)	0.3474	0.3855
T8993G	96	27.53	38.46	0.1534	0.6480 (0.0520)	0.3520	0.5198
A8344G	86	43.48	36.18	0.0943	0.4085 (0.0458)	0.5915	0.7971
A3243G[f]	110	37.35	38.37	0.0772	0.3265 (0.0334)	0.6735	0.8587

a: The number of transmission was the number of mother-offspring pairs whose neither the mother nor the offspring is an index case. The number of heteroplasmy offspring born to the wild type homoplasmy mother was not included in this number.
b: Normalized offspring mutation level variance was equal to the offspring mutation level variance divided by $p(1-p)$ where p is the average offspring mutation level.
c: Standard error of the normalized variance was calculated by dividing Kimura distribution model based standard error of variance by $p(1-p)$ where p is the average offspring mutation level (cite).
d: The bottleneck parameter value was calculated from the offspring mutation level mean and variance using the Sewall-Wright variance formula. The detail regarding how to calculate this parameter value was presented in materials and method section.
e: These offspring were chosen because, at this range of maternal mutation levels, the offspring mutation level variance should have the least effect from the mutation level mean.
f: The mtDNA mutation level was corrected for a reduction of blood mutation level toward age by applying the age-corrected formula provided by Rajasimha *et al* in 2008 (cite)

Table 2: The statistical analysis results carried out on the *de novo* mutation cases.

mtDNA mutation	Observed number of wild type homoplasmy mothers	Observed number of offspring		Expected number of offspring		p-value of Fisher's exact test
		Wild type homoplasmy offspring	Heteroplasmy offspring	Wild type homoplasmy offspring[a]	Heteroplasmy offspring[b]	
G11778A	0	0	0	ND	ND	ND
G3460A	1	0	1	ND	ND	ND
T8993G	17	7	18	21	4	< 0.001***
A8344G	5	9	5	9	5	1.00
A3243G	9	11	13	12	12	1.00

a: The expected number of wild type homoplasmy offspring was equal to the probability of obtaining wild type homoplasmy offspring given the mother carrying 5% mutation level. This probability value was calculated based on the Kimura distribution using the bottleneck parameter values reported in Table 1.
b: The expected number of heteroplasmy offspring was equal to the probability of obtaining heteroplasmy offspring given the mother carrying 5% mutation level. This probability value was calculated based on the Kimura distribution using the bottleneck parameter values reported in Table 1.
*: p-value is less than 0.05 but greater than 0.01.
** : p-value is less than 0.01 but greater than 0.001.
***: p-value is less than 0.001.
ND: Not determined

Table 3: The results of the statistical analyses carried out on the mean and the distribution of the difference between offspring mutation level and maternal mutation level (O-M).

mtDNA mutation	Number of transmission	Mean (standard error[a]) (%)	p-value of the one-sample Student t-test[b]	p-value of the one-sample Wilcoxon test[c]	Skewness	p-value of the Kolmogorov-Smirnov test[d]
G11778A	123	12.63 (2.31)	< 0.001***	<0.001***	0.6445	0.0038**
G3460A	83	11.49 (2.85)	< 0.001***	<0.001***	0.8782	0.0053**
T8993G	96	10.93 (2.75)	< 0.001***	<0.001***	0.3716	0.0021**
A8344G	86	-7.30 (2.72)	0.0088**	0.0068**	-0.3919	0.1609
A3243G	110	1.02 (2.38)	0.6678	0.8700	0.6816	0.0654

a: Standard error of the mean
b: This parametric statistical test was applied to examine whether the mean of O-M is equal to zero.
c: This nonparametric statistical test was applied to examine whether the median of O-M is equal to zero.
d: This nonparametric statistical test was applied to examine whether the O-M values are normally distributed.
*: p-value is less than 0.05 but greater than 0.01.
** : p-value is less than 0.01 but greater than 0.001.
***: p-value is less than 0.001.

Table 4: The results of the statistical analyses applied to compare the average of the difference between offspring mutation level and maternal mutation level (O-M) between different mtDNA mutations. The p-values presented below the diagonal line are the p-values of the Welch two-sample t test and the p-values presented above the diagonal line are the p-value of the Mann-Whitney test. Bonferroni correction was applied, thus the p-value was considered as significant difference only when it is lower than 0.0125. The star (*) symbol represented the significant p-value.

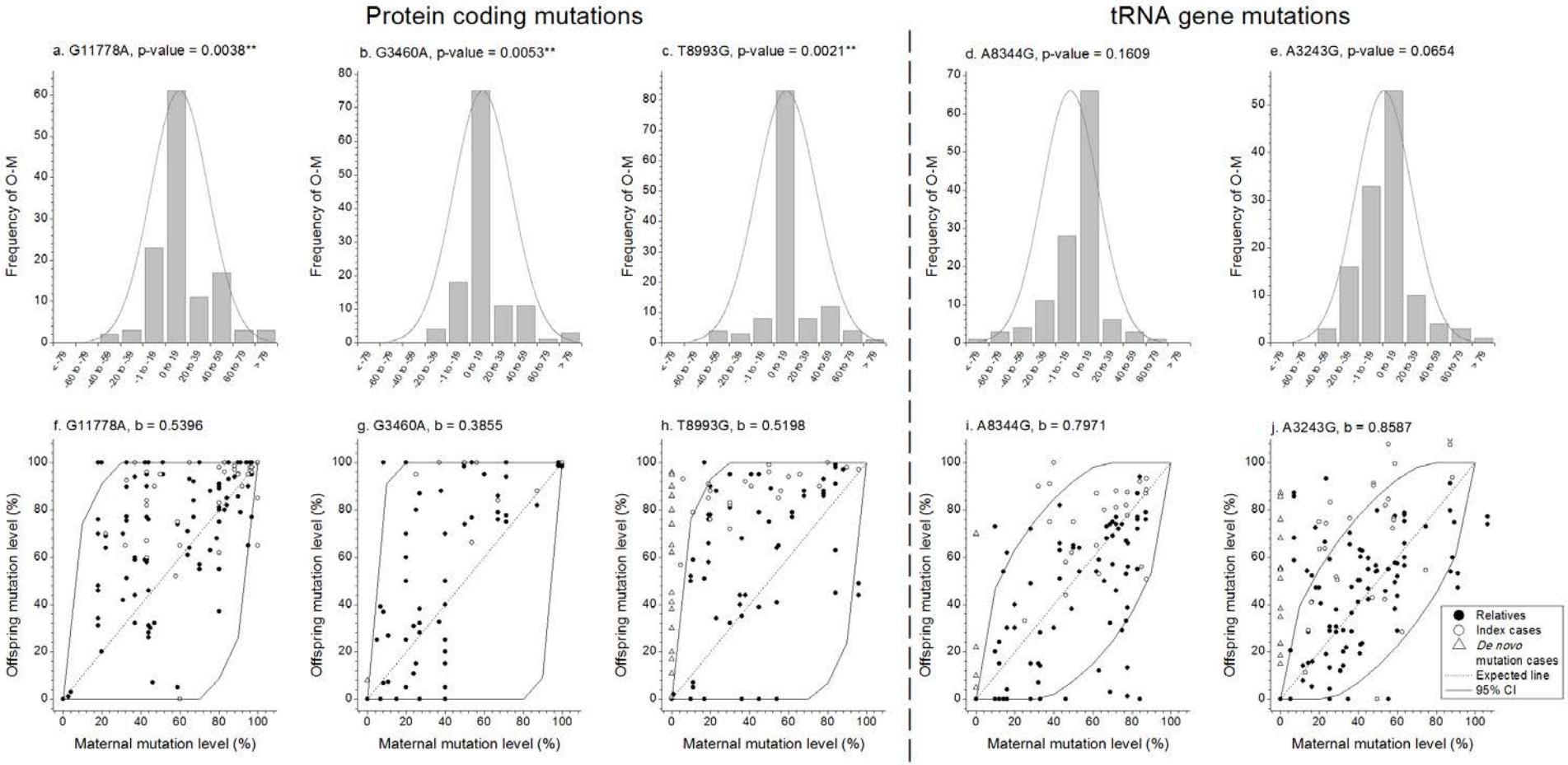
	G11778A	G3460A	T8993G	A8344G	A3243G
G11778A		0.5598	0.7698	<0.001*	<0.001*
G3460A	0.7573		0.7931	<0.001*	0.0061*
T8993G	0.6360	0.8866		<0.001*	0.0019*
A8344G	<0.001*	<0.001*	<0.001*		0.0721
A3243G	<0.001*	0.0054*	0.0070*	0.0223	

Table 5: The results of the statistical analyses carried out on the mean and the distribution of the differences between offspring mutation level and mother's mutation level (O-M). The O-M data was separated into two groups based on maternal mutation level: the low heteroplasmy mothers carrying mutation level less than 50% and the high heteroplasmy mothers carrying mutation level greater than or equal to 50%. The one-star symbol (*) represents the p-value is less than 0.05 but greater than 0.01.

mtDNA mutation	Low/ High	Number of transmission	Mean (standard error of the mean)	p-value of the one-sample Student t-test[a]	p-value of the one-sample Wilcoxon test[b]	p-value of the Kolmogorov-Smirnov test[e]	p-value of the two-sample Student t test[c]	p-value of the Mann-Whitney test[d]
G11778A	Low	48	27.12 (4.33)	< 0.001***	< 0.001***	0.8272		
	High	75	3.35 (1.96)	0.0917	0.0427*	0.0171*	< 0.001***	< 0.001***
G3460A	Low	48	12.40 (4.54)	0.0089**	0.0315*	0.1964		
	High	35	10.25 (2.72)	< 0.001***	< 0.001***	0.0036**	0.6869	0.6666
T8993G	Low	71	13.32 (3.17)	< 0.001***	< 0.001***	< 0.001***		
	High	25	4.12 (5.37)	0.4506	0.2256	0.0205*	0.1475	0.4477
A8344G	Low	47	0.21 (3.28)	0.9486	0.8504	0.1628		
	High	39	-16.36 (4.10)	< 0.001***	< 0.001***	0.1088	0.0023**	0.0296*
A3243G	Low	79	6.33 (2.77)	0.0251*	0.0823	0.0629		
	High	31	-12.50 (3.66)	0.0018**	0.0038**	0.3361	< 0.001***	< 0.001***

a: This parametric statistical test was applied to examine whether the mean of the O-M values is equal to zero.
b: This nonparametric statistical test was applied to examine whether the median of the O-M values is equal to zero.
c: This parametric statistical test was applied to examine whether the mean of the O-M values of the low heteroplasmy mothers is equal to the average value of the high heteroplasmy mothers.
d: This nonparametric statistical test was applied to examine whether the median of the O-M values of the low heteroplasmy mothers is equal to the median value of the high heteroplasmy mothers.
e: This nonparametric statistical test was applied to examine whether the O-M values are normally distributed.
*: p-value is less than 0.05 but greater than 0.01.
** : p-value is less than 0.01 but greater than 0.001.
***: p-value is less than 0.001.

Figure 1: Histogram and scatter plots of the mother-offspring pairs are presented. The histograms present the distribution of the differences between offspring and maternal mutation level (O-M) of the five common pathogenic mtDNA mutations: (a) G11778A, (b) G3460A, (c) T8993G, (d) A8344G, and (e) A3243G. The p-value presented within each histogram is the p-value of the Kolmogorov-Smirnov test that was applied to examine whether the distribution of the observed O-M is normally distributed. The scatter plots present the distribution of the mutation levels of the mother-offspring pairs carrying one of the five mtDNA mutations: (f) G11778A, (g) G3460A, (h) T8993G, (i) A8344G, and (j) A3243G. The 95% confident interval of the offspring mutation level was calculated based on the Kimura distribution with the bottleneck (b) parameter values estimated from the mutation levels of the offspring of the mothers carrying 40-60% mutation level. The b parameter values were reported in Table 1.



*: p-value is less than 0.05 but greater than 0.01. ,** : p-value is less than 0.01 but greater than 0.001., ***: p-value is less than 0.001.

Figure 2: Comparison of the index cases mutation levels between different mtDNA mutations were shown in box and whisker plots. The black triangle represents the mean and the line inside the box represents the median. The height of the box is equal to two times the standard error of the mean. The length of the whisker ranged from 5 to 95 percentile of data. The p-values are the p-values of the Welch two-sample t-test and the Mann-Whitney test, respectively. Bonferroni correction was applied, thus the p-value was considered as significant difference only when it is lower than 0.0125. Only the significant p-values were presented in this figure.

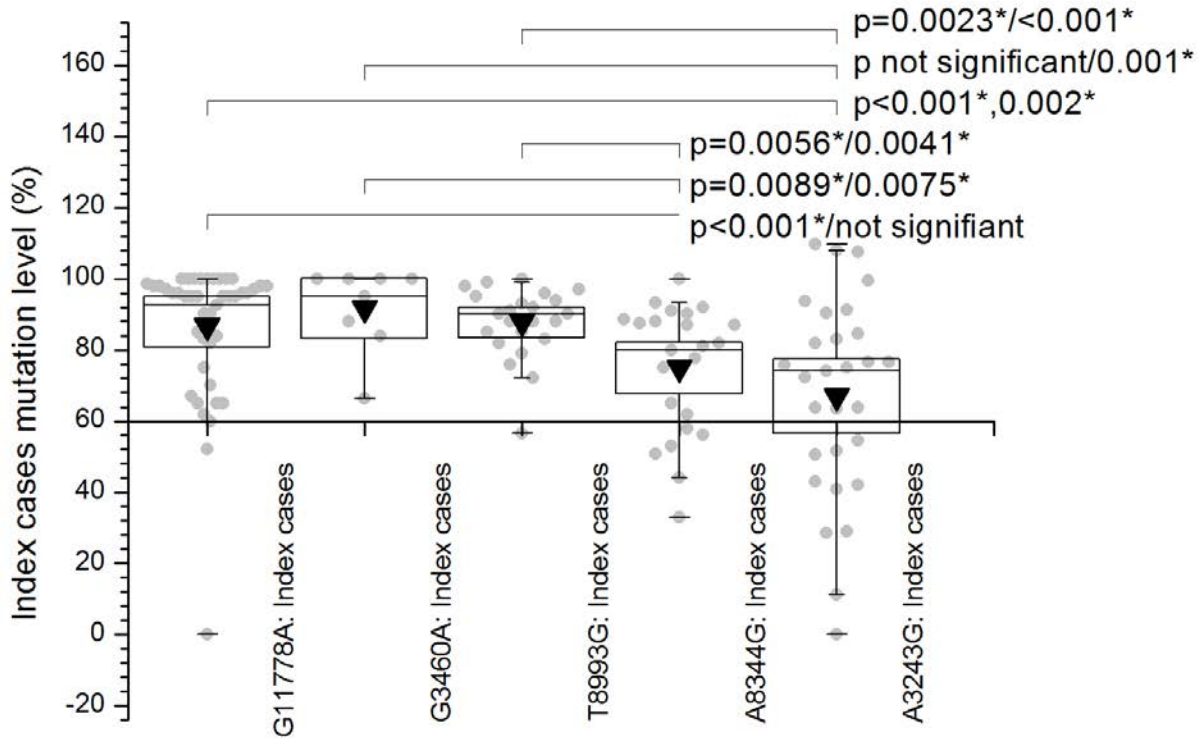
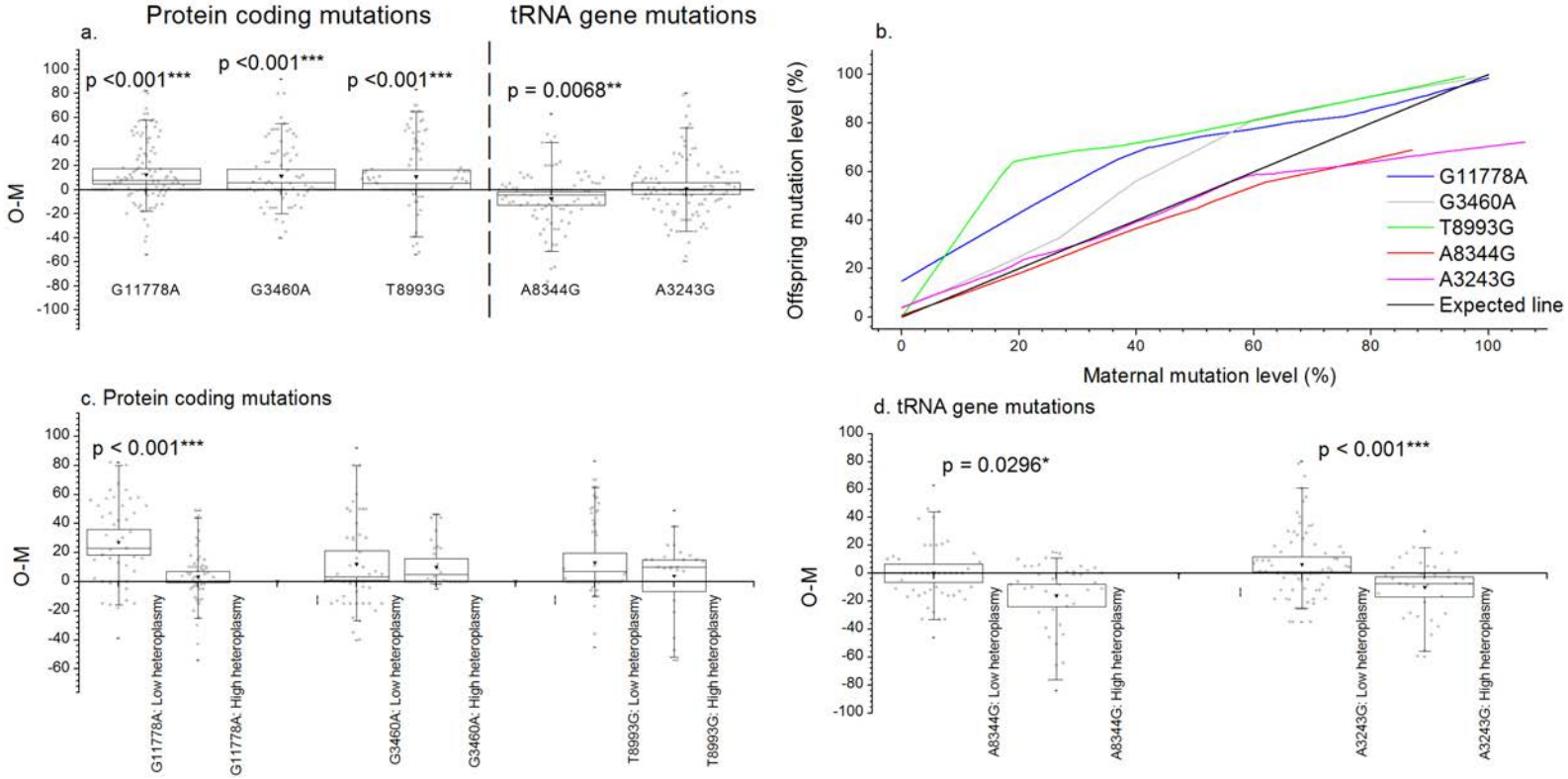


Figure 3: Comparison of the mean of the differences between offspring and maternal mutation levels (O-M) to the expected mean of 0 (a), the relationship pattern derived by the locally weighted regression analysis (b), the mean of the O-M values calculated from the low heteroplasmy mothers to the values calculated from the high heteroplasmy mothers carrying protein coding mutations: G11778A, G3460A and T8993G mutation (c), and the mean of the O-M values calculated from the low heteroplasmy mothers to the values calculated from the high heteroplasmy mothers carrying tRNA gene mutations: A8344G and A3243G mutation (d). The low heteroplasmy mothers are the mothers carrying mtDNA mutation level less than 50% and the high heteroplasmy mothers are the mothers carrying mtDNA mutation level greater than or equal to 50%. The grey circle represented each O-M value. The black triangle represents the mean of the O-M values. The line inside the box represents the median of the O-M values. The height of the box is equal to two times the standard error of the mean. The length of the whisker ranged from 5 to 95 percentile of the O-M values. The p-value is presented only when it is lower than 0.05. The p-value presented in figure (b) is the p-value of the one-sample Wilcoxon test, while the p-value presented in figure (c) and (d) is the p-value of the Mann-Whitney test.



*: p-value is less than 0.05 but greater than 0.01. ,** : p-value is less than 0.01 but greater than 0.001., ***: p-value is less than 0.001.

Supplementary Table 1: Data of human clinical pedigrees carrying common pathogemic mtDNA mutations: G11778A, G3460A, T8993G, A8344G and A3243G. The mutation level is the blood mutation level reported in the published literature. "Relationship" means relationship with the index case. "NA" means not available.

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	1	1	1	Grandmother	F	NA	NA	NA	41
G11778A	1	2	1	Aunt	F	NA	NA	NA	41
G11778A	1	2	2	Uncle	M	NA	NA	NA	41
G11778A	1	2	3	Mother of Index1	F	66	95	NA	41
G11778A	1	2	4	Aunt	F	NA	NA	NA	41
G11778A	1	2	5	Aunt	F	NA	NA	NA	41
G11778A	1	2	6	Mother of Index2	F	60	97	NA	41
G11778A	1	2	7	Aunt	F	56	79	NA	41
G11778A	1	3	1	Cousin	F	NA	NA	NA	41
G11778A	1	3	2	Cousin	F	NA	NA	NA	41
G11778A	1	3	3	Cousin	F	NA	NA	NA	41
G11778A	1	3	4	Cousin	F	NA	NA	NA	41
G11778A	1	3	5	Brother of Index1	M	NA	NA	95	41
G11778A	1	3	6	Index1	M	32	95	95	41
G11778A	1	3	7	Brother of Index1	M	30	95	95	41
G11778A	1	3	8	Brother of Index1	M	28	95	95	41
G11778A	1	3	9	Brother of Index2	M	27	77	97	41
G11778A	1	3	10	Sister of Index2	F	24	95	97	41
G11778A	1	3	11	Index2	M	23	96	97	41
G11778A	1	3	12	Cousin	M	24	NA	79	41
G11778A	1	3	13	Cousin	M	22	68	79	41

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	2	1	1	Mother	F	NA	65	NA	42
G11778A	2	2	1	Index	M	NA	100	65	42
G11778A	2	2	2	Sister	F	NA	93	65	42
G11778A	2	2	3	Brother	M	NA	100	65	42
G11778A	2	2	4	Sister	F	NA	64	65	42
G11778A	3	1	1	Grandmother	F	NA	42	NA	42
G11778A	3	2	1	Mother	F	NA	59	42	42
G11778A	3	3	1	Index	M	NA	75	59	42
G11778A	4	1	1	Greatgrandmother	F	NA	NA	NA	43
G11778A	4	2	1	Grandmother's brother	M	NA	60	NA	43
G11778A	4	2	2	Grandmother	F	NA	80	NA	43
G11778A	4	3	1	Uncle	M	NA	83	80	43
G11778A	4	3	2	Uncle	M	NA	75	80	43
G11778A	4	3	3	Mother	F	NA	90	80	43
G11778A	4	4	1	Index	M	NA	95	90	43
G11778A	4	4	2	Sister	F	NA	95	90	43
G11778A	5	1	1	Mother	F	50	80	NA	44
G11778A	5	2	1	Index	M	26	83	80	44
G11778A	5	2	2	Brother	M	NA	NA	80	44
G11778A	6	1	1	Grandmother	F	64	67	NA	44
G11778A	6	2	1	Aunt	F	47	84	67	44
G11778A	6	2	2	Uncle	M	44	77	67	44
G11778A	6	2	3	Mother	F	43	92	67	44

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	6	3	1	Cousin	F	23	82	84	44
G11778A	6	3	2	Cousin	F	15	85	84	44
G11778A	6	3	3	Index	M	15	90	92	44
G11778A	7	1	1	Mother	F	58	47	NA	44
G11778A	7	2	1	Index	M	26	90	47	44
G11778A	7	2	2	Sister	F	32	32	47	44
G11778A	7	3	1	Niece	F	NA	NA	32	44
G11778A	8	1	1	Greatgrandmother	F	NA	NA	NA	44
G11778A	8	2	1	Grandmother	F	NA	NA	NA	44
G11778A	8	2	2	Grandmother's sister	F	89	32	NA	44
G11778A	8	2	3	Grandmother's sister	F	NA	NA	NA	44
G11778A	8	3	1	Mother	F	66	80	NA	44
G11778A	8	3	2	Aunt	F	NA	NA	32	44
G11778A	8	3	3	Uncle	M	NA	NA	32	44
G11778A	8	3	4	Aunt	F	NA	NA	NA	44
G11778A	8	4	1	Sister	F	36	90	80	44
G11778A	8	4	2	Brother	M	34	37	80	44
G11778A	8	4	3	Sister	F	33	80	80	44
G11778A	8	4	4	Index	M	30	62	80	44
G11778A	8	4	5	Brother	M	29	55	80	44
G11778A	8	4	6	Sister	F	27	89	80	44
G11778A	8	4	7	Sister	F	25	91	80	44
G11778A	8	4	8	Cousin	F	NA	NA	NA	44

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	8	4	9	Cousin	M	NA	NA	NA	44
G11778A	8	4	10	Cousin	M	NA	NA	NA	44
G11778A	8	4	11	Cousin	M	NA	NA	NA	44
G11778A	8	4	12	Cousin	M	NA	NA	NA	44
G11778A	8	4	13	Cousin	M	NA	NA	NA	44
G11778A	9	1	1	Index1	F	68	32.73	NA	45
G11778A	9	2	1	Daughter of Index1	F	NA	89.74	32.73	45
G11778A	9	2	2	Daughter of Index1	F	NA	75.47	32.73	45
G11778A	9	2	3	Daughter of Index1	F	NA	51.11	32.73	45
G11778A	9	2	4	Son of Index1	M	NA	77.27	32.73	45
G11778A	9	2	5	Son of Index1	M	NA	100	32.73	45
G11778A	9	2	6	Index2	M	29	92.5	32.73	45
G11778A	9	3	1	Nephew of Index2	M	NA	95.24	89.74	45
G11778A	9	3	2	Nephew of Index2	M	NA	100	89.74	45
G11778A	9	3	3	Nephew of Index2	M	NA	85.71	89.74	45
G11778A	9	3	4	Niece of Index2	F	NA	100	89.74	45
G11778A	9	3	5	Nephew of Index2	M	NA	87.88	75.47	45
G11778A	9	3	6	Niece of Index2	F	NA	62.86	75.47	45
G11778A	9	3	7	Nephew of Index2	M	NA	95	51.11	45
G11778A	9	3	8	Nephew of Index2	M	NA	100	51.11	45
G11778A	9	3	9	Niece of Index2	F	NA	100	51.11	45
G11778A	10	1	1	Mother	F	NA	95	NA	46
G11778A	10	2	1	Sister	F	NA	65	95	46

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	10	2	2	Sister	F	NA	95	95	46
G11778A	10	2	3	Index	M	NA	95	95	46
G11778A	10	2	4	Brother	M	NA	NA	95	46
G11778A	11	1	1	Index	F	NA	95	NA	46
G11778A	11	2	1	Son	M	NA	90	95	46
G11778A	11	2	2	Son	M	NA	95	95	46
G11778A	12	1	1	Mother	F	NA	50	NA	46
G11778A	12	1	2	Aunt	F	NA	45	NA	46
G11778A	12	2	1	Index1	M	NA	95	50	46
G11778A	12	2	2	Index2	M	NA	95	50	46
G11778A	12	2	3	Brother	M	NA	95	50	46
G11778A	12	2	4	Cousin	F	NA	30	45	46
G11778A	13	1	1	Mother of Index1	F	NA	NA	NA	47
G11778A	13	2	1	Sister of Index1	F	NA	100	NA	47
G11778A	13	2	2	Index1	M	NA	100	NA	47
G11778A	13	2	3	Sister of Index1	F	NA	91	NA	47
G11778A	13	2	4	Brother of Index1	M	NA	NA	NA	47
G11778A	13	2	5	Sister of Index1	F	NA	100	NA	47
G11778A	13	2	6	Brother of Index1	M	NA	NA	NA	47
G11778A	13	2	7	Brother of Index1	M	NA	NA	NA	47
G11778A	13	2	8	Sister of Index1	F	NA	85	NA	47
G11778A	13	3	1	Index2	M	NA	100	100	47
G11778A	13	3	2	Brother of Index2	M	NA	100	100	47

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	13	3	3	Sister of Index2	F	NA	100	100	47
G11778A	13	3	4	Daughter of Index1	F	NA	79	91	47
G11778A	13	3	5	Daughter of Index1	F	NA	100	91	47
G11778A	13	3	6	Index3	M	NA	100	100	47
G11778A	13	3	7	Cousin	M	NA	100	85	47
G11778A	13	3	8	Cousin	F	NA	NA	85	47
G11778A	14	1	1	Greatgrandmother	F	NA	NA	NA	47
G11778A	14	2	1	Mother of the Father	F	NA	NA	NA	47
G11778A	14	2	2	Grandmother	F	NA	NA	NA	47
G11778A	14	3	1	Father's brother	M	NA	NA	NA	47
G11778A	14	3	2	Father's brother	M	NA	NA	NA	47
G11778A	14	3	3	Father's brother	M	NA	100	NA	47
G11778A	14	3	4	Father	M	NA	100	NA	47
G11778A	14	3	5	Mother	F	NA	58	NA	47
G11778A	14	3	6	Aunt	F	NA	NA	NA	47
G11778A	14	4	1	Index	F	NA	52	58	47
G11778A	14	4	2	Cousin	F	NA	NA	NA	47
G11778A	15	1	1	Grandmother of Index1-3	F	NA	NA	NA	47
G11778A	15	2	1	Mother of Index1-3	F	NA	NA	NA	47
G11778A	15	2	2	Aunt of Index1-3	F	NA	NA	NA	47
G11778A	15	3	1	Index1	F	NA	92	NA	47
G11778A	15	3	2	Brother of Index1-3	M	NA	NA	NA	47
G11778A	15	3	3	Index2	F	NA	100	NA	47

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	15	3	4	Brother of Index1-3	M	NA	NA	NA	47
G11778A	15	3	5	Index3	F	NA	85	NA	47
G11778A	15	3	6	Sister of Indec1-3	F	NA	NA	NA	47
G11778A	15	3	7	Index4	M	NA	100	NA	47
G11778A	15	4	1	Index5	M	NA	85	100	47
G11778A	15	4	2	Doughter of Index2	F	NA	NA	100	47
G11778A	15	4	3	Index6	M	NA	65	100	47
G11778A	15	4	4	Cousin of Index5-6	F	NA	NA	85	47
G11778A	15	4	5	Cousin of Index5-6	M	NA	NA	85	47
G11778A	15	4	6	Cousin of Index5-6	M	NA	NA	85	47
G11778A	16	1	1	Grandmother	F	NA	NA	NA	48
G11778A	16	1	2	Grandmother's brother	M	NA	NA	NA	48
G11778A	16	1	3	Grandmother's brother	M	NA	NA	NA	48
G11778A	16	1	4	Grandmother's sister	F	NA	NA	NA	48
G11778A	16	1	5	Grandmother's brother	M	NA	NA	NA	48
G11778A	16	2	1	Mother	F	NA	97	NA	48
G11778A	16	2	2	Uncle	M	NA	30	NA	48
G11778A	16	2	3	Aunt	F	NA	NA	NA	48
G11778A	16	2	4	Aunt	F	NA	0	NA	48
G11778A	16	2	5	Uncle	M	NA	14	NA	48
G11778A	16	2	6	Mother's cousin	M	NA	0	NA	48
G11778A	16	2	7	Mother's cousin	M	NA	0	NA	48
G11778A	16	3	1	Brother	M	NA	100	97	48

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	16	3	2	Index	M	NA	98	97	48
G11778A	16	3	3	Cousin	F	NA	37	NA	48
G11778A	16	4	1	Cousin's son	M	NA	32	37	48
G11778A	16	4	2	Cousin's son	M	NA	60	37	48
G11778A	17	1	1	Mother	F	NA	88	NA	48
G11778A	17	1	2	Aunt	F	NA	0	NA	48
G11778A	17	2	1	Index	M	NA	98.5	88	48
G11778A	17	2	2	Sister	F	NA	94	88	48
G11778A	17	3	1	Niece	F	NA	100	94	48
G11778A	17	3	2	Nephew	M	NA	100	94	48
G11778A	18	1	1	Grandmother	F	84	43	NA	49
G11778A	18	2	1	Uncle	M	63	77	43	49
G11778A	18	2	2	Uncle	M	59	90	43	49
G11778A	18	2	3	Aunt	F	56	59	43	49
G11778A	18	2	4	Uncle	M	54	58	43	49
G11778A	18	2	5	Mother	F	50	95	43	49
G11778A	18	2	6	Uncle	M	48	95	43	49
G11778A	18	3	1	Cousin	M	33	74	59	49
G11778A	18	3	2	Cousin	M	31	5	59	49
G11778A	18	3	3	Sister	F	28	90	95	49
G11778A	18	3	4	Index	M	25	95	95	49
G11778A	19	1	1	Mother of Index1	F	NA	NA	NA	50
G11778A	19	2	1	Brother of Index1	M	NA	NA	NA	50

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	19	2	2	Sister of Index1	F	NA	NA	NA	50
G11778A	19	2	3	Index1	F	NA	83	NA	50
G11778A	19	2	4	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	5	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	6	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	7	Sister of Index1	F	NA	NA	NA	50
G11778A	19	2	8	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	9	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	10	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	11	Brother of Index1	M	NA	NA	NA	50
G11778A	19	2	12	Sister of Index1	F	NA	NA	NA	50
G11778A	19	3	1	Index2	M	NA	100	83	50
G11778A	19	3	2	Index3	M	NA	NA	83	50
G11778A	19	3	3	Sister of Index2, 3 and 4	F	NA	80	83	50
G11778A	19	3	4	Index4	F	NA	96	83	50
G11778A	19	3	5	Cousin of Index 2-4	F	NA	14	NA	50
G11778A	19	3	6	Cousin of Index 2-4	F	NA	28	NA	50
G11778A	19	4	1	Nephew of Index 2-4	M	NA	81	80	50
G11778A	19	4	2	Nephew of Index 2-4	M	NA	98	80	50
G11778A	19	4	3	Nephew of Index 2-4	M	NA	NA	80	50
G11778A	19	4	4	Index5	M	NA	98	96	50
G11778A	19	4	5	Sister of Index5	F	NA	100	96	50
G11778A	19	4	6	Brother of Index5	M	NA	NA	96	50

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	20	1	1	Index	M	NA	93	NA	51
G11778A	20	1	2	Index	F	NA	10	NA	51
G11778A	20	1	3	Index	F	NA	78	NA	51
G11778A	20	2	1	Index	M	NA	100	78	51
G11778A	21	1	1	Index	F	NA	35	NA	51
G11778A	21	1	2	Index	F	NA	62	NA	51
G11778A	21	2	1	Index	M	NA	85	35	51
G11778A	21	2	2	Index	M	NA	95	62	51
G11778A	22	1	1	Index	F	NA	43	NA	51
G11778A	22	2	1	Index	M	NA	77	43	51
G11778A	22	2	2	Index	M	NA	90	43	51
G11778A	22	2	3	Index	F	NA	59	43	51
G11778A	22	2	4	Index	M	NA	58	43	51
G11778A	22	2	5	Index	F	NA	95	43	51
G11778A	22	2	6	Index	M	NA	95	43	51
G11778A	22	3	1	Index	M	NA	74	59	51
G11778A	22	3	2	Index	M	NA	5	59	51
G11778A	22	3	3	Index	F	NA	90	95	51
G11778A	22	3	4	Index	M	NA	95	95	51
G11778A	23	1	1	Grandmother	F	NA	NA	NA	52
G11778A	23	2	1	Aunt	F	NA	NA	NA	52
G11778A	23	2	2	Aunt	F	NA	NA	NA	52
G11778A	23	2	3	Mother	F	71	22	NA	52

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	23	2	4	Aunt	F	67	0	NA	52
G11778A	23	2	5	Aunt	F	NA	NA	NA	52
G11778A	23	3	1	Cousin	M	NA	NA	NA	52
G11778A	23	3	2	Cousin	F	NA	NA	NA	52
G11778A	23	3	3	Cousin	F	NA	NA	NA	52
G11778A	23	3	4	Cousin	M	NA	NA	NA	52
G11778A	23	3	5	Cousin	F	NA	NA	NA	52
G11778A	23	3	6	Cousin	M	NA	NA	NA	52
G11778A	23	3	7	Cousin	F	NA	NA	NA	52
G11778A	23	3	8	Index	F	49	70	22	52
G11778A	23	3	9	Brother	M	41	69	22	52
G11778A	23	3	10	Sister	F	38	64	22	52
G11778A	23	3	11	Sister	F	NA	NA	22	52
G11778A	23	3	12	Sister	F	NA	NA	22	52
G11778A	23	3	13	Cousin	M	NA	NA	0	52
G11778A	23	3	14	Cousin	M	NA	NA	0	52
G11778A	23	3	15	Cousin	F	45	0	0	52
G11778A	23	3	16	Cousin	F	43	0	0	52
G11778A	23	3	17	Cousin	F	NA	NA	NA	52
G11778A	23	3	18	Cousin	M	NA	NA	NA	52
G11778A	23	4	1	Relative	F	NA	NA	NA	52
G11778A	23	4	2	Relative	M	NA	NA	NA	52
G11778A	23	4	3	Relative	M	NA	NA	NA	52

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	23	4	4	Relative	F	NA	NA	NA	52
G11778A	23	4	5	Nephew	M	18	61	64	52
G11778A	23	4	6	Niece	F	16	71	64	52
G11778A	23	4	7	Nephew	M	18	96	NA	52
G11778A	23	4	8	Niece	F	10	99	NA	52
G11778A	23	4	9	Nephew	M	NA	NA	NA	52
G11778A	23	4	10	Relative	M	NA	NA	0	52
G11778A	23	4	11	Relative	F	19	0	0	52
G11778A	23	4	12	Relative	M	NA	NA	0	52
G11778A	23	4	13	Relative	F	NA	NA	NA	52
G11778A	23	4	14	Relative	M	NA	NA	NA	52
G11778A	24	1	1	Great-grandmother	F	NA	NA	NA	53
G11778A	24	2	1	Grandmother	F	NA	NA	NA	53
G11778A	24	2	2	Grandmother	F	NA	20	20	53
G11778A	24	3	1	Mother	F	50	100	NA	53
G11778A	24	3	2	Mother	F	53	100	20	53
G11778A	24	4	1	Sister	F	NA	NA	100	53
G11778A	24	4	2	Index	M	27	100	100	53
G11778A	24	4	3	Index	M	25	100	100	53
G11778A	24	4	4	Brother	M	NA	NA	100	53
G11778A	24	4	5	Sister	F	NA	NA	100	53
G11778A	25	1	1	Great-grandmother	F	NA	NA	NA	54
G11778A	25	2	1	Grandmother	F	NA	18	NA	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	25	2	2	Grandmother's brother	M	NA	0	NA	54
G11778A	25	2	3	Grandmother's brother	M	NA	NA	NA	54
G11778A	25	3	1	Aunt	F	NA	34	18	54
G11778A	25	3	2	Aunt	F	NA	31	18	54
G11778A	25	3	3	Aunt	F	NA	46	18	54
G11778A	25	3	4	Uncle	M	NA	NA	18	54
G11778A	25	3	5	Mother	F	NA	100	18	54
G11778A	25	3	6	Aunt	F	NA	70	18	54
G11778A	25	3	7	Uncle	M	NA	48	18	54
G11778A	25	3	8	Uncle	M	NA	100	18	54
G11778A	25	3	9	Uncle	M	NA	NA	18	54
G11778A	25	3	10	Uncle	M	NA	76	18	54
G11778A	25	4	1	Cousin	M	NA	NA	31	54
G11778A	25	4	2	Cousin	F	NA	NA	31	54
G11778A	25	4	3	Cousin	M	NA	NA	31	54
G11778A	25	4	4	Cousin	M	NA	70	31	54
G11778A	25	4	5	Cousin	M	NA	NA	46	54
G11778A	25	4	6	Cousin	F	NA	NA	46	54
G11778A	25	4	7	Cousin	F	NA	NA	46	54
G11778A	25	4	8	Index	M	NA	100	100	54
G11778A	25	4	9	Brother	M	NA	100	100	54
G11778A	25	4	10	Cousin	F	NA	57	70	54
G11778A	25	4	11	Cousin	F	NA	55	70	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	25	4	12	Cousin	F	NA	100	70	54
G11778A	25	5	1	Relative	F	NA	NA	NA	54
G11778A	25	5	2	Relative	F	NA	NA	NA	54
G11778A	26	1	1	Grandmother	F	NA	NA	NA	54
G11778A	26	2	1	Aunt	F	NA	4	NA	54
G11778A	26	2	2	Uncle	M	NA	NA	NA	54
G11778A	26	2	3	Aunt	F	NA	NA	NA	54
G11778A	26	2	4	Mother	F	NA	37	NA	54
G11778A	26	2	5	Uncle	M	NA	NA	NA	54
G11778A	26	2	6	Uncle	M	NA	NA	NA	54
G11778A	26	3	1	Cousin	F	NA	NA	4	54
G11778A	26	3	2	Cousin	M	NA	NA	4	54
G11778A	26	3	3	Cousin	F	NA	NA	4	54
G11778A	26	3	4	Cousin	F	NA	3	4	54
G11778A	26	3	5	Cousin	M	NA	NA	4	54
G11778A	26	3	6	Cousin	M	NA	NA	4	54
G11778A	26	3	7	Brother	M	NA	NA	37	54
G11778A	26	3	8	Brother	M	NA	59	37	54
G11778A	26	3	9	Sister	F	NA	44	37	54
G11778A	26	3	10	Sister	F	NA	100	37	54
G11778A	26	3	11	Brother	M	NA	100	37	54
G11778A	26	3	12	Brother	M	NA	94	37	54
G11778A	26	3	13	Index	M	NA	100	37	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	26	4	1	Relative	F	NA	1	3	54
G11778A	26	4	2	Relative	M	NA	NA	3	54
G11778A	26	4	3	Relative	M	NA	NA	3	54
G11778A	26	4	4	Relative	M	NA	NA	3	54
G11778A	26	4	5	Relative	M	NA	76	44	54
G11778A	26	4	6	Relative	F	NA	NA	44	54
G11778A	26	4	7	Relative	F	NA	NA	100	54
G11778A	26	4	8	Relative	M	NA	100	100	54
G11778A	26	5	1	Relative	M	NA	NA	1	54
G11778A	26	5	2	Relative	M	NA	NA	1	54
G11778A	26	5	3	Relative	Fetus	NA	NA	NA	54
G11778A	27	1	1	Relative	F	NA	NA	NA	54
G11778A	27	2	1	Relative	M	NA	NA	NA	54
G11778A	27	2	2	Relative	M	NA	NA	NA	54
G11778A	27	2	3	Relative	F	NA	NA	NA	54
G11778A	27	2	4	Relative	F	NA	NA	NA	54
G11778A	27	2	5	Relative	F	NA	NA	NA	54
G11778A	27	2	6	Relative	M	NA	NA	NA	54
G11778A	27	3	1	Greatgrandmother's cousin	F	NA	NA	NA	54
G11778A	27	3	2	Greatgrandmother's cousin	F	NA	NA	NA	54
G11778A	27	3	3	Greatgrandmother's cousin	M	NA	NA	NA	54
G11778A	27	3	4	Greatgrandmother's	F	NA	NA	NA	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
				cousin					
G11778A	27	3	5	Greatgrandmother's cousin	M	NA	NA	NA	54
G11778A	27	3	6	Greatgrandmother's cousin	M	NA	NA	NA	54
G11778A	27	3	7	Greatgrandmother's cousin	F	NA	44	NA	54
G11778A	27	3	8	Greatgrandmother's cousin	M	NA	NA	NA	54
G11778A	27	3	9	Greatgrandmother's cousin	F	NA	85	NA	54
G11778A	27	3	10	Greatgrandmother	F	NA	100	NA	54
G11778A	27	3	11	Greatgrandmother's brother	M	NA	100	NA	54
G11778A	27	4	1	Grandmother's cousin	M	NA	NA	NA	54
G11778A	27	4	2	Grandmother's cousin	M	NA	NA	NA	54
G11778A	27	4	3	Grandmother's cousin	M	NA	NA	NA	54
G11778A	27	4	4	Grandmother's cousin	M	NA	NA	NA	54
G11778A	27	4	5	Grandmother's cousin	M	NA	NA	NA	54
G11778A	27	4	6	Grandmother's cousin	M	NA	NA	NA	54
G11778A	27	4	7	Grandmother's cousin	F	NA	NA	NA	54
G11778A	27	4	8	Grandmother's cousin	F	NA	NA	NA	54
G11778A	27	4	9	Grandmother's cousin	F	NA	46	44	54
G11778A	27	4	10	Grandmother's cousin	M	NA	100	44	54
G11778A	27	4	11	Grandmother's cousin	F	NA	31	44	54
G11778A	27	4	12	Grandmother's cousin	F	NA	26	44	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	27	4	13	Grandmother's cousin	F	NA	NA	44	54
G11778A	27	4	14	Grandmother's cousin	M	NA	28	44	54
G11778A	27	4	15	Grandmother's cousin	M	NA	NA	44	54
G11778A	27	4	16	Grandmother's cousin	M	NA	93	85	54
G11778A	27	4	17	Grandmother's cousin	M	NA	NA	85	54
G11778A	27	4	18	Grandmother's cousin	M	NA	NA	85	54
G11778A	27	4	19	Grandmother's cousin	M	NA	NA	85	54
G11778A	27	4	20	Grandmother's cousin	F	NA	NA	85	54
G11778A	27	4	21	Grandmother's cousin	F	NA	NA	85	54
G11778A	27	4	22	Grandmother	F	NA	100	100	54
G11778A	27	4	23	Grandmother's sister	F	NA	100	100	54
G11778A	27	4	24	Grandmother's brother	M	NA	NA	100	54
G11778A	27	4	25	Grandmother's brother	M	NA	100	100	54
G11778A	27	4	26	Grandmother's brother	M	NA	100	100	54
G11778A	27	4	27	Grandmother's sister	F	NA	100	100	54
G11778A	27	4	28	Grandmother's sister	F	NA	100	100	54
G11778A	27	5	1	Mother's cousin	M	NA	NA	46	54
G11778A	27	5	2	Mother's cousin	M	NA	NA	46	54
G11778A	27	5	3	Mother's cousin	M	NA	NA	46	54
G11778A	27	5	4	Mother's cousin	F	NA	7	46	54
G11778A	27	5	5	Mother's cousin	F	NA	42	31	54
G11778A	27	5	6	Mother's cousin	F	NA	NA	31	54
G11778A	27	5	7	Mother's cousin	F	NA	NA	31	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	27	5	8	Mother's cousin	M	NA	NA	31	54
G11778A	27	5	9	Mother's cousin	F	NA	NA	31	54
G11778A	27	5	10	Mother's cousin	M	NA	NA	31	54
G11778A	27	5	11	Mother's cousin	M	NA	NA	26	54
G11778A	27	5	12	Mother's cousin	M	NA	NA	26	54
G11778A	27	5	13	Mother's cousin	M	NA	NA	26	54
G11778A	27	5	14	Mother's cousin	M	NA	NA	26	54
G11778A	27	5	15	Mother's cousin	F	NA	NA	NA	54
G11778A	27	5	16	Mother's cousin	M	NA	NA	NA	54
G11778A	27	5	17	Mother's cousin	M	NA	NA	NA	54
G11778A	27	5	18	Mother's cousin	M	NA	NA	NA	54
G11778A	27	5	19	Mother's cousin	F	NA	NA	NA	54
G11778A	27	5	20	Mother's cousin	M	NA	NA	NA	54
G11778A	27	5	21	Mother	F	NA	100	100	54
G11778A	27	5	22	Aunt	F	NA	NA	100	54
G11778A	27	5	23	Uncle	M	NA	NA	100	54
G11778A	27	5	24	Mother's cousin	F	NA	100	100	54
G11778A	27	5	25	Mother's cousin	M	NA	100	100	54
G11778A	27	5	26	Mother's cousin	F	NA	100	100	54
G11778A	27	5	27	Mother's cousin	M	NA	100	100	54
G11778A	27	5	28	Mother's cousin	F	NA	100	100	54
G11778A	27	6	1	Cousin	M	NA	NA	7	54
G11778A	27	6	2	Cousin	M	NA	NA	7	54

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G11778A	27	6	3	Cousin	M	NA	NA	42	54
G11778A	27	6	4	Cousin	F	NA	100	42	54
G11778A	27	6	5	Cousin	M	NA	NA	NA	54
G11778A	27	6	6	Cousin	F	NA	NA	NA	54
G11778A	27	6	7	Cousin	F	NA	70	NA	54
G11778A	27	6	8	Cousin	M	NA	NA	NA	54
G11778A	27	6	9	Index	M	NA	100	100	54
G11778A	27	6	10	Brother	M	NA	100	100	54
G11778A	27	6	11	Brother	M	NA	100	100	54
G11778A	27	6	12	Cousin	F	NA	100	NA	54
G11778A	27	6	13	Cousin	F	NA	NA	NA	54
G3460A	1	1	1	Mother	F	56	56	NA	58
G3460A	1	2	1	Index	M	33	100	56	58
G3460A	1	2	2	Sister	F	25	100	56	58
G3460A	1	2	3	Sister	F	25	100	56	58
G3460A	2	1	1	Mother	F	NA	0	NA	47
G3460A	2	2	1	Index	F	NA	8	0	47
G3460A	3	1	1	Mother	F	NA	50	NA	47
G3460A	3	2	1	Index	M	NA	100	50	47
G3460A	3	2	2	Sister	F	NA	74	50	47
G3460A	4	1	1	Greatgrandmother	F	NA	NA	NA	46
G3460A	4	1	2	Greatgranduncle	M	NA	NA	NA	46
G3460A	4	1	3	Greatgrandaunt	F	NA	NA	NA	46

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	4	1	4	Greatgrandaunt	F	NA	NA	NA	46
G3460A	4	1	5	Greatgrandaunt	F	NA	NA	NA	46
G3460A	4	2	1	Granduncle	M	NA	NA	NA	46
G3460A	4	2	2	Granduncle	M	NA	NA	NA	46
G3460A	4	2	3	Granduncle	M	NA	NA	NA	46
G3460A	4	2	4	Grandmother	F	NA	5	NA	46
G3460A	4	2	5	Grandmother's cousin	M	NA	NA	NA	46
G3460A	4	2	6	Grandmother's cousin	M	NA	NA	NA	46
G3460A	4	2	7	Grandmother's cousin	F	NA	NA	NA	46
G3460A	4	3	1	Mother	F	NA	25	5	46
G3460A	4	4	1	Index	M	NA	95	25	46
G3460A	4	4	2	Brother	M	NA	15	25	46
G3460A	4	4	3	Sister	F	NA	80	25	46
G3460A	5	1	1	Grandmother	F	NA	NA	NA	46
G3460A	5	2	1	Mother	F	NA	67	NA	46
G3460A	5	2	2	Half-uncle	M	NA	NA	NA	46
G3460A	5	2	3	Half-uncle	M	NA	NA	NA	46
G3460A	5	2	4	Half-aunt	F	NA	NA	NA	46
G3460A	5	3	1	Index	M	NA	84	67	46
G3460A	5	3	2	Brother	M	NA	76	67	46
G3460A	5	3	3	Brother	M	NA	86	67	46
G3460A	5	3	4	Brother	M	NA	79	67	46
G3460A	6	1	1	Mother	F	NA	71.43	NA	55

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	6	2	1	Brother	M	NA	77.78	71.43	55
G3460A	6	2	2	Brother	M	NA	75	71.43	55
G3460A	6	2	3	Brother	M	NA	94.12	71.43	55
G3460A	6	2	4	Index	M	NA	100	71.43	55
G3460A	7	1	1	Greatgrandmother	F	NA	NA	NA	57
G3460A	7	2	1	Grandaunt	F	NA	NA	NA	57
G3460A	7	2	2	Grandaunt	F	NA	12.6	NA	57
G3460A	7	2	3	Granduncle	M	NA	11.1	NA	57
G3460A	7	2	4	Grandmother	F	NA	8.2	NA	57
G3460A	7	2	5	Granduncle	M	NA	21.2	NA	57
G3460A	7	2	6	Granduncle	M	NA	3.5	NA	57
G3460A	7	3	1	Mother	F	NA	36.8	8.2	57
G3460A	7	3	2	Aunt	F	NA	6.9	8.2	57
G3460A	7	3	3	Aunt	F	NA	100	8.2	57
G3460A	7	4	1	Index	M	23	100	36.8	57
G3460A	7	4	2	Brother	M	NA	32.5	36.8	57
G3460A	7	4	3	Cousin	M	NA	0	6.9	57
G3460A	7	4	4	Cousin	F	NA	39.1	6.9	57
G3460A	7	4	5	Cousin	F	NA	100	100	57
G3460A	7	4	6	Cousin	M	NA	100	100	57
G3460A	8	1	1	Great-greatgrandmother	F	NA	NA	NA	56
G3460A	8	2	1	Greatgrandmother	F	NA	NA	NA	56
G3460A	8	2	2	Greatgranduncle	M	NA	NA	NA	56

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	8	2	3	Greatgranduncle	M	NA	NA	NA	56
G3460A	8	3	1	Grandmother	F	86	20	NA	56
G3460A	8	3	2	Granduncle	M	NA	NA	NA	56
G3460A	8	3	3	Grandaunt	F	81	40	NA	56
G3460A	8	3	4	Grandaunt	F	73	100	NA	56
G3460A	8	3	5	Granduncle	M	70	20	NA	56
G3460A	8	3	6	Grandaunt	F	67	100	NA	56
G3460A	8	4	1	Uncle	M	59	100	20	56
G3460A	8	4	2	Aunt	F	57	NA	20	56
G3460A	8	4	3	Mother	F	55	100	20	56
G3460A	8	4	4	Aunt	F	NA	NA	20	56
G3460A	8	4	5	Aunt	F	49	60	20	56
G3460A	8	4	6	Uncle	M	47	50	20	56
G3460A	8	4	7	Aunt	F	45	70	20	56
G3460A	8	4	8	Uncle	M	45	50	20	56
G3460A	8	4	9	Mother's cousin	F	50	20	40	56
G3460A	8	4	10	Mother's cousin	F	49	25	40	56
G3460A	8	4	11	Mother's cousin	M	48	50	40	56
G3460A	8	4	12	Mother's cousin	M	41	70	40	56
G3460A	8	4	13	Mother's cousin	F	45	40	40	56
G3460A	8	4	14	Mother's cousin	F	47	15	40	56
G3460A	8	4	15	Mother's cousin	F	39	100	100	56
G3460A	8	4	16	Mother's cousin	M	38	100	100	56

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	8	4	17	Mother's cousin	M	32	100	100	56
G3460A	8	4	18	Mother's cousin	M	28	100	100	56
G3460A	8	5	1	Index	M	30	100	100	56
G3460A	8	5	2	Sister	F	28	100	100	56
G3460A	8	5	3	Brother	M	24	100	100	56
G3460A	8	5	4	Cousin	M	20	NA	NA	56
G3460A	8	5	5	Cousin	M	18	70	NA	56
G3460A	8	5	6	Cousin	F	15	95	60	56
G3460A	8	5	7	Daughter of the mother's cousin	F	28	25	20	56
G3460A	8	5	8	Son of the mother's cousin	M	27	25	20	56
G3460A	8	5	9	Daughter of the mother's cousin	F	25	5	20	56
G3460A	8	5	10	Son of the mother's cousin	M	21	5	20	56
G3460A	8	5	11	Son of the mother's cousin	M	27	25	40	56
G3460A	8	5	12	Daughter of the mother's cousin	F	26	5	40	56
G3460A	8	5	13	Son of the mother's cousin	M	19	0	40	56
G3460A	8	5	14	Son of the mother's cousin	M	22	0	15	56
G3460A	8	5	15	Daughter of the mother's cousin	F	15	0	15	56
G3460A	8	5	16	Daughter of the mother's cousin	F	10	0	15	56

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	9	1	1	Aunt	F	NA	100	NA	11
G3460A	9	1	2	Aunt	F	NA	23.9	NA	11
G3460A	9	1	3	Mother	F	NA	53.7	NA	11
G3460A	9	1	4	Aunt	F	NA	49.6	NA	11
G3460A	9	2	1	Cousin	M	NA	100	100	11
G3460A	9	2	2	Cousin	M	NA	100	100	11
G3460A	9	2	3	Cousin	F	NA	100	100	11
G3460A	9	2	4	Cousin	F	NA	98.2	100	11
G3460A	9	2	5	Cousin	F	NA	10.8	23.9	11
G3460A	9	2	6	Cousin	M	NA	30.9	23.9	11
G3460A	9	2	7	Brother	M	NA	100	53.7	11
G3460A	9	2	8	Index	M	NA	66.3	53.7	11
G3460A	9	2	9	Brother	M	NA	100	53.7	11
G3460A	9	2	10	Brother	M	NA	76.6	53.7	11
G3460A	9	2	11	Sister	F	NA	NA	53.7	11
G3460A	9	2	12	Cousin	F	NA	99.8	49.6	11
G3460A	9	2	13	Cousin	M	NA	99.4	49.6	11
G3460A	9	2	14	Cousin	F	NA	98.2	49.6	11
G3460A	9	3	1	Niece	F	NA	100	100	11
G3460A	9	3	2	Nephew	M	NA	98.8	100	11
G3460A	9	3	3	Niece	F	NA	100	98.2	11
G3460A	9	3	4	Nephew	M	NA	98.6	98.2	11
G3460A	9	3	5	Niece	F	NA	7.2	10.8	11

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	9	3	6	Niece	F	NA	26.8	10.8	11
G3460A	9	3	7	Niece	F	NA	100	99.8	11
G3460A	9	3	8	Nephew	M	NA	99.6	99.8	11
G3460A	9	3	9	Niece	F	NA	100	99.8	11
G3460A	9	3	10	Niece	F	NA	99	98.2	11
G3460A	10	1	1	Great-greatgrandmother	F	NA	NA	NA	59
G3460A	10	2	1	Great-grandaunt	F	NA	NA	NA	59
G3460A	10	2	2	Great-grandaunt	F	NA	NA	NA	59
G3460A	10	2	3	Greatgrandmother	F	NA	NA	NA	59
G3460A	10	2	4	Greatgrandaunt	F	NA	NA	NA	59
G3460A	10	2	5	Greatgrandaunt	F	NA	NA	NA	59
G3460A	10	2	6	Greatgrandaunt	F	NA	NA	NA	59
G3460A	10	2	7	Greatgranduncle	M	NA	NA	NA	59
G3460A	10	2	8	Greatgrandaunt	F	NA	NA	NA	59
G3460A	10	2	9	Greatgrandaunt	F	NA	NA	NA	59
G3460A	10	2	10	Greatgrandaunt	F	NA	NA	NA	59
G3460A	10	3	1	Grandmother's cousin	M	NA	NA	NA	59
G3460A	10	3	2	Grandmother's cousin	M	NA	NA	NA	59
G3460A	10	3	3	Grandmother's cousin	M	NA	NA	NA	59
G3460A	10	3	4	Grandmother's cousin	F	NA	0	NA	59
G3460A	10	3	5	Granuncle	M	NA	NA	NA	59
G3460A	10	3	6	Grandmother	F	NA	27	NA	59
G3460A	10	3	7	Grandmother's cousin	F	NA	NA	NA	59

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	10	3	8	Grandmother's cousin	F	NA	0	NA	59
G3460A	10	3	9	Grandmother's cousin	F	NA	NA	NA	59
G3460A	10	3	10	Grandmother's cousin	M	NA	NA	NA	59
G3460A	10	3	11	Grandmother's cousin	M	NA	NA	NA	59
G3460A	10	3	12	Grandmother's cousin	M	NA	NA	NA	59
G3460A	10	3	13	Grandmother's cousin	F	NA	NA	NA	59
G3460A	10	3	14	Grandmother's cousin	F	NA	NA	NA	59
G3460A	10	4	1	Mother's cousin	F	NA	0	0	59
G3460A	10	4	2	Mother's cousin	F	NA	0	0	59
G3460A	10	4	3	Mother's cousin	F	NA	NA	0	59
G3460A	10	4	4	Mother's cousin	M	NA	NA	0	59
G3460A	10	4	5	Half-sister of the mother	F	NA	0	27	59
G3460A	10	4	6	Uncle	M	NA	NA	27	59
G3460A	10	4	7	Uncle	M	NA	NA	27	59
G3460A	10	4	8	Mother	F	NA	87	27	59
G3460A	10	4	9	Uncle	M	NA	28	27	59
G3460A	10	4	10	Uncle	M	NA	NA	27	59
G3460A	10	4	11	Uncle	M	NA	32	27	59
G3460A	10	4	12	Aunt	F	NA	38	27	59
G3460A	10	4	13	Mother's cousin	F	NA	NA	NA	59
G3460A	10	4	14	Mother's cousin	F	NA	0	NA	59
G3460A	10	4	15	Mother's cousin	M	NA	NA	NA	59
G3460A	10	4	16	Mother's cousin	M	NA	0	NA	59

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	10	4	17	Mother's cousin	F	NA	NA	NA	59
G3460A	10	4	18	Mother's cousin	F	NA	NA	NA	59
G3460A	10	4	19	Mother's cousin	F	NA	NA	NA	59
G3460A	10	4	20	Mother's cousin	M	NA	NA	NA	59
G3460A	10	5	1	Index's cousin	M	NA	0	0	59
G3460A	10	5	2	Index's cousin	F	NA	NA	0	59
G3460A	10	5	3	Index's cousin	M	NA	0	0	59
G3460A	10	5	4	Index's cousin	F	NA	0	NA	59
G3460A	10	5	5	Index's cousin	M	NA	NA	NA	59
G3460A	10	5	6	Index	M	NA	88	87	59
G3460A	10	5	7	Sister	F	NA	82	87	59
G3460A	10	5	8	Index's cousin	M	NA	NA	38	59
G3460A	10	5	9	Index's cousin	F	NA	88	38	59
G3460A	10	5	10	Index's cousin	M	NA	NA	0	59
G3460A	10	5	11	Index's cousin	M	NA	NA	0	59
G3460A	10	5	12	Index's cousin	F	NA	0	NA	59
G3460A	10	5	13	Index's cousin	F	NA	0	NA	59
G3460A	10	5	14	Index's cousin	F	NA	NA	NA	59
G3460A	10	5	15	Index's cousin	M	NA	NA	NA	59
G3460A	10	5	16	Index's cousin	M	NA	0	NA	59
G3460A	10	5	17	Index's cousin	M	NA	NA	NA	59
G3460A	10	5	18	Index's cousin	F	NA	NA	NA	59
G3460A	10	5	19	Index's cousin	M	NA	NA	NA	59

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
G3460A	10	5	20	Index's cousin	F	NA	NA	NA	59
T8993G	1	1	1	Mother	F	NA	NA	NA	60
T8993G	1	1	2	Uncle	M	76	14	NA	60
T8993G	1	1	3	Uncle	M	78	6	NA	60
T8993G	1	1	4	Uncle	M	88	9	NA	60
T8993G	1	2	1	Index	F	47	82	NA	60
T8993G	1	2	2	Sister	F	52	23	NA	60
T8993G	1	3	1	Nephew	M	29	34	23	60
T8993G	1	3	2	Niece	F	29	88	23	60
T8993G	1	4	1	Niece's daughter	F	3	97	88	60
T8993G	2	1	1	Mother	F	NA	4.8	NA	3
T8993G	2	2	1	Index	F	7	56.6	4.8	3
T8993G	3	1	1	Grandmother	F	NA	NA	NA	61
T8993G	3	1	2	Grandmother's brother	M	NA	NA	NA	61
T8993G	3	2	1	Mother	F	NA	68	NA	61
T8993G	3	2	2	Aunt	F	NA	0	NA	61
T8993G	3	2	3	Aunt	F	NA	0	NA	61
T8993G	3	2	4	Uncle	M	NA	NA	NA	61
T8993G	3	3	1	Index1	F	NA	NA	68	61
T8993G	3	3	2	Brother	M	11	86	68	61
T8993G	3	3	3	Brother	M	14	88	68	61
T8993G	3	3	4	Index2	F	NA	NA	68	61
T8993G	4	1	1	Greatgrandmother	F	NA	0	NA	12

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	4	2	1	Grandmother	F	NA	45	0	12
T8993G	4	3	1	Mother	F	NA	39	45	12
T8993G	4	3	2	Aunt	F	NA	95	45	12
T8993G	4	3	3	Aunt	F	NA	NA	45	12
T8993G	4	3	4	Uncle	M	NA	NA	45	12
T8993G	4	3	5	Aunt	F	30	0	45	12
T8993G	4	3	6	Uncle	M	33	79	45	12
T8993G	4	4	1	Index	F	0.58	NA	39	12
T8993G	4	4	2	Cousin	M	NA	0	0	12
T8993G	5	1	1	Mother	F	NA	76	NA	62
T8993G	5	2	1	Index1	F	6.5	90	76	62
T8993G	5	2	2	Inde2	F	2.5	NA	76	62
T8993G	6	1	1	Mother	F	23	53	NA	63
T8993G	6	2	1	Brother	M	NA	NA	53	63
T8993G	6	2	2	Sister	F	NA	NA	53	63
T8993G	6	2	3	Index	M	2	NA	53	63
T8993G	6	2	4	Sister	F	NA	NA	53	63
T8993G	7	1	1	Grandmother	F	NA	0	NA	63
T8993G	7	2	1	Mother	F	NA	41	0	63
T8993G	7	2	2	Aunt	F	NA	NA	0	63
T8993G	7	2	3	Aunt	F	NA	NA	0	63
T8993G	7	2	4	Uncle	M	NA	NA	0	63
T8993G	7	3	1	Sister	F	NA	NA	41	63

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	7	3	2	Brother	M	NA	NA	41	63
T8993G	7	3	3	Index	M	6.5	NA	41	63
T8993G	8	1	1	Mother	F	NA	NA	NA	63
T8993G	8	2	1	Brother	M	5	NA	NA	63
T8993G	8	2	2	Index	M	1.5	NA	NA	63
T8993G	9	1	1	Grandmother	F	NA	NA	NA	63
T8993G	9	2	1	Mother	F	NA	NA	NA	63
T8993G	9	2	2	Uncle	M	NA	NA	NA	63
T8993G	9	2	3	Uncle	M	NA	NA	NA	63
T8993G	9	3	1	Index1	M	2.5	NA	NA	63
T8993G	9	3	2	Index2	M	1.25	NA	NA	63
T8993G	10	1	1	Grandmother	F	NA	NA	NA	63
T8993G	10	2	1	Mother	F	NA	NA	NA	63
T8993G	10	2	2	Aunt	F	NA	0	NA	63
T8993G	10	3	1	Index	M	2	NA	NA	63
T8993G	11	1	1	Mother	F	NA	56	NA	63
T8993G	11	2	1	Index	M	0.75	88	56	63
T8993G	12	1	1	Mother	F	NA	NA	NA	63
T8993G	12	2	1	Brother	M	NA	NA	NA	63
T8993G	12	2	2	Index	M	10	NA	NA	63
T8993G	13	1	1	Grandmother	F	NA	0	NA	63
T8993G	13	2	1	Mother	F	26	62	0	63
T8993G	13	2	2	Aunt	F	NA	0	0	63

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	13	2	3	Aunt	F	NA	0	0	63
T8993G	13	3	1	Brother	M	NA	79	62	63
T8993G	13	3	2	Brother	M	NA	77	62	63
T8993G	13	3	3	Index	F	NA	NA	62	63
T8993G	14	1	1	Mother	F	NA	NA	NA	63
T8993G	14	2	1	Sister	F	NA	NA	NA	63
T8993G	14	2	2	Index	F	0.5	NA	NA	63
T8993G	15	1	1	Grandmother	F	NA	NA	NA	2
T8993G	15	2	1	Mother	F	39	78	NA	2
T8993G	15	2	2	Uncle	M	35	NA	NA	2
T8993G	15	2	3	Uncle	M	NA	NA	NA	2
T8993G	15	2	4	Uncle	M	NA	NA	NA	2
T8993G	15	2	5	Uncle	M	NA	NA	NA	2
T8993G	15	2	6	Uncle	M	NA	NA	NA	2
T8993G	15	2	7	Aunt	F	NA	NA	NA	2
T8993G	15	3	1	Index	M	3.5	NA	78	2
T8993G	15	3	2	Brother	M	14	88	78	2
T8993G	15	3	3	Brother	M	NA	NA	78	2
T8993G	15	3	4	Brother	M	NA	86	78	2
T8993G	15	3	5	Brother	M	11	87	78	2
T8993G	15	3	6	Sister	F	6	93	78	2
T8993G	15	3	7	Sister	F	4.5	93	78	2
T8993G	16	1	1	Grandmother	F	NA	0	NA	64

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	16	2	1	Mother	F	31	38	0	64
T8993G	16	2	2	Aunt	F	NA	NA	0	64
T8993G	16	2	3	Aunt	F	NA	NA	0	64
T8993G	16	2	4	Uncle	M	NA	NA	0	64
T8993G	16	2	5	Uncle	M	NA	NA	0	64
T8993G	16	3	1	Sister	F	NA	NA	38	64
T8993G	16	3	2	Brother	M	NA	NA	38	64
T8993G	16	3	3	Index	M	0.5	NA	38	64
T8993G	17	1	1	Mother	F	NA	84	NA	65
T8993G	17	2	1	Miscarriage sibling	Fetus	NA	NA	84	65
T8993G	17	2	2	Miscarriage sibling	Fetus	NA	NA	84	65
T8993G	17	2	3	Brother	M	NA	NA	84	65
T8993G	17	2	4	Sister	F	NA	99	84	65
T8993G	17	2	5	Sister	F	NA	63	84	65
T8993G	17	2	6	Sister	F	NA	91	84	65
T8993G	17	2	7	Index	F	9	NA	84	65
T8993G	17	2	8	Brother	M	NA	45	84	65
T8993G	17	2	9	Brother	M	NA	NA	84	65
T8993G	17	2	10	Brother	M	NA	98	84	65
T8993G	18	1	1	Mother	F	NA	50	NA	66
T8993G	18	2	1	Index1	M	NA	96	50	66
T8993G	18	2	2	Index2	F	NA	NA	50	66
T8993G	18	2	3	Index3	M	8	99	50	66

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	18	2	4	Brother	M	0	99	50	66
T8993G	19	1	1	Greatgrandmother's sister	F	NA	0	NA	67
T8993G	19	1	2	Greatgrandmother	F	NA	36	NA	67
T8993G	19	2	1	Grandmother's cousin	M	NA	NA	0	67
T8993G	19	2	2	Grandmother's cousin	F	NA	0	0	67
T8993G	19	2	3	Grandmother's cousin	F	NA	0	0	67
T8993G	19	2	4	Grandmother's cousin	F	NA	0	0	67
T8993G	19	2	5	Grandmother's cousin	M	NA	NA	0	67
T8993G	19	2	6	Grandmother	F	NA	NA	36	67
T8993G	19	2	7	Grandmother's brother	M	NA	NA	36	67
T8993G	19	2	8	Grandmother's sister	F	NA	35	36	67
T8993G	19	2	9	Grandmother's brother	M	50	68	36	67
T8993G	19	2	10	Grandmother's sister	F	NA	0	36	67
T8993G	19	2	11	Grandmother's brother	M	NA	NA	36	67
T8993G	19	3	1	Mother	F	37	54	NA	67
T8993G	19	3	2	Aunt	F	34	78	NA	67
T8993G	19	3	3	Aunt	F	0.25	NA	NA	67
T8993G	19	3	4	Uncle	M	17	NA	NA	67
T8993G	19	3	5	Uncle	M	1	NA	NA	67
T8993G	19	3	6	Uncle	M	20	80	NA	67
T8993G	19	3	7	Mother's cousin	M	NA	NA	35	67

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	19	3	8	Mother's cousin	M	NA	40	35	67
T8993G	19	3	9	Mother's cousin	F	NA	44	35	67
T8993G	19	3	10	Mother's cousin	F	NA	NA	0	67
T8993G	19	4	1	Sister	F	NA	41	54	67
T8993G	19	4	2	Index1	M	2	NA	54	67
T8993G	19	4	3	Index2	M	0.6	NA	54	67
T8993G	20	1	1	Greatgrandmother	F	NA	0	NA	68
T8993G	20	2	1	Grandmother's sister	F	NA	NA	0	68
T8993G	20	2	2	Grandmother's sister	F	NA	NA	0	68
T8993G	20	2	3	Grandmother's sister	F	NA	0	0	68
T8993G	20	2	4	Grandmother's sister	F	NA	0	0	68
T8993G	20	2	5	Grandmother's sister	F	NA	0	0	68
T8993G	20	2	6	Grandmother	F	NA	0	0	68
T8993G	20	2	7	Grandmother's brother	M	NA	0	0	68
T8993G	20	2	8	Grandmother's sister	F	NA	0	0	68
T8993G	20	2	9	Grandmother's brother	M	NA	NA	0	68
T8993G	20	3	1	Mother's cousin	F	NA	NA	NA	68
T8993G	20	3	2	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	3	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	4	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	5	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	6	Mother's cousin	M	NA	NA	NA	68

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	20	3	7	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	8	Mother's cousin	F	NA	NA	NA	68
T8993G	20	3	9	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	10	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	11	Mother's cousin	M	NA	NA	NA	68
T8993G	20	3	12	Mother's cousin	F	NA	NA	NA	68
T8993G	20	3	13	Mother's cousin	F	NA	NA	0	68
T8993G	20	3	14	Mother's cousin	M	NA	NA	0	68
T8993G	20	3	15	Mother's cousin	F	NA	NA	0	68
T8993G	20	3	16	Mother's cousin	M	NA	NA	0	68
T8993G	20	3	17	Mother's cousin	M	NA	NA	0	68
T8993G	20	3	18	Mother's cousin	M	NA	NA	0	68
T8993G	20	3	19	Mother's cousin	M	NA	NA	0	68
T8993G	20	3	20	Mother's cousin	F	NA	NA	0	68
T8993G	20	3	21	Mother	F	NA	38	0	68
T8993G	20	3	22	Aunt	F	NA	0	0	68
T8993G	20	3	23	Aunt	F	NA	0	0	68
T8993G	20	3	24	Uncle	M	NA	0	0	68
T8993G	20	3	25	Mother's cousin	M	NA	NA	0	68
T8993G	20	3	26	Mother's cousin	M	NA	NA	0	68
T8993G	20	4	1	Relative	F	NA	NA	NA	68
T8993G	20	4	2	Relative	F	NA	NA	NA	68
T8993G	20	4	3	Relative	M	NA	NA	NA	68

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	20	4	4	Relative	M	NA	NA	NA	68
T8993G	20	4	5	Relative	F	NA	NA	NA	68
T8993G	20	4	6	Relative	F	NA	NA	NA	68
T8993G	20	4	7	Relative	F	NA	NA	NA	68
T8993G	20	4	8	Relative	F	NA	NA	NA	68
T8993G	20	4	9	Miscarriaged relative	NA	NA	NA	NA	68
T8993G	20	4	10	Relative	M	NA	NA	NA	68
T8993G	20	4	11	Brother	M	NA	44	38	68
T8993G	20	4	12	Index	M	1.25	92	38	68
T8993G	20	4	13	Miscarriaged cousin	NA	NA	NA	0	68
T8993G	21	1	1	Grandmother	F	46	0	NA	69
T8993G	21	2	1	Mother	F	20	20	0	69
T8993G	21	3	1	Index	M	4	88	20	69
T8993G	21	3	2	Sister	F	0.33	78	20	69
T8993G	22	1	1	Grandmother	F	NA	0	NA	70
T8993G	22	2	1	Uncle	M	NA	0	0	70
T8993G	22	2	2	Uncle	M	NA	0	0	70
T8993G	22	2	3	Uncle	M	NA	0	0	70
T8993G	22	2	4	Uncle	M	NA	0	0	70
T8993G	22	2	5	Aunt	F	NA	0	0	70
T8993G	22	2	6	Uncle	M	NA	0	0	70
T8993G	22	2	7	Mother	F	NA	0	0	70
T8993G	22	3	1	Sister	F	NA	0	0	70

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	22	3	2	Index	F	NA	74	0	70
T8993G	23	1	1	Uncle	M	NA	48	NA	71
T8993G	23	1	2	Mother	F	NA	54	NA	71
T8993G	23	1	3	Uncle	M	NA	NA	NA	71
T8993G	23	2	1	Sister	F	NA	64	54	71
T8993G	23	2	2	Sister	F	NA	NA	54	71
T8993G	23	2	3	Sister	F	NA	0	54	71
T8993G	23	2	4	Index	F	0.5	NA	54	71
T8993G	24	1	1	Mother	F	NA	0	NA	72
T8993G	24	2	1	Sister	F	NA	0	0	72
T8993G	24	2	2	Index	F	2	NA	0	72
T8993G	25	1	1	Grandmother	F	NA	10	NA	73
T8993G	25	2	1	Aunt	F	NA	50	10	73
T8993G	25	2	2	Mother	F	NA	52	10	73
T8993G	25	2	3	Uncle	M	NA	0	10	73
T8993G	25	2	4	Aunt	F	NA	0	10	73
T8993G	25	3	1	Cousin	F	NA	NA	50	73
T8993G	25	3	2	Index	F	1.17	NA	52	73
T8993G	26	1	1	Mother	F	NA	0	NA	74
T8993G	26	2	1	Index	F	0.67	NA	0	74
T8993G	27	1	1	Greatgrandmother	F	NA	NA	NA	75
T8993G	27	2	1	Grandmother	F	NA	0	NA	75
T8993G	27	2	2	Grandmother's brother	M	NA	NA	NA	75

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	27	2	3	Grandmother's brother	M	NA	NA	NA	75
T8993G	27	2	4	Grandmother's brother	M	NA	NA	NA	75
T8993G	27	2	5	Grandmother's brother	M	NA	NA	NA	75
T8993G	27	2	6	Grandmother's brother	M	NA	NA	NA	75
T8993G	27	2	7	Grandmother's brother	M	NA	NA	NA	75
T8993G	27	2	8	Grandmother's brother	M	NA	NA	NA	75
T8993G	27	2	9	Grandmother's sister	F	NA	NA	NA	75
T8993G	27	2	10	Grandmother's sister	F	NA	NA	NA	75
T8993G	27	2	11	Grandmother's sister	F	NA	NA	NA	75
T8993G	27	2	12	Grandmother's sister	F	NA	NA	NA	75
T8993G	27	3	1	Aunt	F	NA	NA	0	75
T8993G	27	3	2	Mother	F	36	0	0	75
T8993G	27	4	1	Cousin	F	NA	NA	NA	75
T8993G	27	4	2	Index	M	NA	95	0	75
T8993G	28	1	1	Grandmother	F	NA	0	NA	75
T8993G	28	2	1	Mother	F	34	65	0	75
T8993G	28	2	2	Aunt	F	NA	NA	0	75
T8993G	28	3	1	Index	F	NA	NA	65	75
T8993G	28	3	2	Cousin	M	NA	NA	NA	75
T8993G	29	1	1	Grandmother	F	NA	19	NA	75

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	29	2	1	Mother	F	24	66	19	75
T8993G	29	2	2	Uncle	M	NA	78	19	75
T8993G	29	2	3	Aunt	F	NA	76	19	75
T8993G	29	2	4	Aunt	F	NA	58	19	75
T8993G	29	2	5	Miscarriage aunt	NA	NA	NA	19	75
T8993G	29	2	6	Deceased	NA	NA	NA	19	75
T8993G	29	2	7	Deceased	NA	NA	NA	19	75
T8993G	29	2	8	Deceased	NA	NA	NA	19	75
T8993G	29	3	1	Index	F	NA	95	66	75
T8993G	30	1	1	Grandmother	F	NA	0	NA	75
T8993G	30	2	1	Uncle	M	NA	NA	0	75
T8993G	30	2	2	Mother	F	32	30	0	75
T8993G	30	3	1	Index	M	9	83	30	75
T8993G	31	1	1	Mother	F	29	0	NA	75
T8993G	31	2	1	Index	M	NA	NA	0	75
T8993G	31	2	2	Sister	F	13	0	0	75
T8993G	31	2	3	Sister	F	9	NA	0	75
T8993G	32	1	1	Greatgrandmother	F	NA	0	NA	75
T8993G	32	2	1	Grandmother's sister	F	NA	0	0	75
T8993G	32	2	2	Grandmother's brother	M	NA	0	0	75
T8993G	32	2	3	Grandmother's sister	F	NA	0	0	75
T8993G	32	2	4	Grandmother's brother	F	NA	NA	0	75

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	32	2	5	Grandmother's brother	F	NA	NA	0	75
T8993G	32	2	6	Grandmother	F	NA	NA	0	75
T8993G	32	3	1	Mother's cousin	M	NA	NA	0	75
T8993G	32	3	2	Mother's cousin	M	NA	NA	0	75
T8993G	32	3	3	Mother's cousin	F	NA	0	0	75
T8993G	32	3	4	Mother's cousin	F	NA	0	0	75
T8993G	32	3	5	Mother's cousin	F	NA	0	0	75
T8993G	32	3	6	Mother's cousin	F	NA	0	0	75
T8993G	32	3	7	Mother's cousin	F	NA	0	0	75
T8993G	32	3	8	Aunt	F	NA	0	NA	75
T8993G	32	3	9	Aunt	F	NA	0	NA	75
T8993G	32	3	10	Mother	F	NA	55	NA	75
T8993G	32	3	11	Aunt	F	NA	0	NA	75
T8993G	32	4	1	Cousin	F	NA	0	0	75
T8993G	32	4	2	Cousin	M	NA	NA	0	75
T8993G	32	4	3	Cousin	M	NA	NA	0	75
T8993G	32	4	4	Cousin	M	NA	NA	0	75
T8993G	32	4	5	Index1	M	NA	85	55	75
T8993G	32	4	6	Miscarriaged sibling	NA	NA	NA	55	75
T8993G	32	4	7	Miscarriaged sibling	NA	NA	NA	55	75
T8993G	32	4	8	Sister	F	3	65	55	75
T8993G	32	4	9	Cousin	F	NA	NA	0	75
T8993G	32	4	10	Miscarriaged cousin	NA	NA	NA	0	75

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	33	1	1	Grandmother	F	NA	0	NA	36
T8993G	33	2	1	Mother	F	NA	11	0	36
T8993G	33	2	2	Aunt	F	NA	NA	0	36
T8993G	33	2	3	Uncle	M	NA	NA	0	36
T8993G	33	2	4	Aunt	F	NA	1	0	36
T8993G	33	2	5	Uncle	M	NA	NA	0	36
T8993G	33	2	6	Aunt	F	NA	NA	0	36
T8993G	33	2	7	Miscarriaged	Fetus	NA	NA	0	36
T8993G	33	2	8	Miscarriaged	Fetus	NA	NA	0	36
T8993G	33	3	1	Index	M	NA	79	11	36
T8993G	33	3	2	Sister	F	NA	59	11	36
T8993G	33	3	3	Sister	F	NA	7	11	36
T8993G	33	3	4	Sister	F	NA	5	11	36
T8993G	33	3	5	Cousin	F	NA	2	1	36
T8993G	33	3	6	Cousin	M	NA	NA	1	36
T8993G	33	4	1	Nephew	M	NA	NA	7	36
T8993G	33	4	2	Niece	F	NA	NA	7	36
T8993G	33	4	3	Son of the cousin	M	NA	NA	2	36
T8993G	33	4	4	Miscarriaged	Fetus	NA	NA	2	36
T8993G	33	4	5	Son of the cousin	M	NA	NA	2	36
T8993G	33	4	6	Miscarriaged	Fetus	NA	NA	2	36
T8993G	34	1	1	Grandmother	F	NA	NA	NA	36
T8993G	34	2	1	Mother	F	NA	30	NA	36

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	34	3	1	Sister	F	NA	NA	30	36
T8993G	34	3	2	Sister	F	NA	32	30	36
T8993G	34	3	3	Index	F	NA	82	30	36
T8993G	34	3	4	Brother	M	NA	NA	30	36
T8993G	34	3	5	Sister	F	NA	95	30	36
T8993G	34	3	6	Brother	M	NA	81	30	36
T8993G	34	4	1	Niece	F	NA	NA	32	36
T8993G	34	4	2	Nephew	M	NA	NA	32	36
T8993G	34	4	3	Nephew	M	NA	NA	32	36
T8993G	35	1	1	Greatgrandmother	F	NA	NA	NA	36
T8993G	35	2	1	Grandmother's sister	F	NA	NA	NA	36
T8993G	35	2	2	Grandmother's sister	F	NA	NA	NA	36
T8993G	35	2	3	Grandmother's sister	F	NA	NA	NA	36
T8993G	35	2	4	Grandmother's sister	F	NA	NA	NA	36
T8993G	35	2	5	Grandmother's sister	F	NA	NA	NA	36
T8993G	35	2	6	Grandmother	F	NA	NA	NA	36
T8993G	35	3	1	Mother	F	NA	0	NA	36
T8993G	35	3	2	Uncle	M	NA	NA	NA	36
T8993G	35	3	3	Uncle	M	NA	NA	NA	36
T8993G	35	3	4	Uncle	M	NA	NA	NA	36
T8993G	35	3	5	Uncle	M	NA	NA	NA	36
T8993G	35	3	6	Aunt	F	NA	NA	NA	36
T8993G	35	4	1	Miscarraiged sibling	Fetus	NA	NA	0	36

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	35	4	2	Index	M	NA	NA	0	36
T8993G	36	1	1	Greatgrandmother	F	NA	0	NA	36
T8993G	36	2	1	Grandmother	F	NA	17	0	36
T8993G	36	2	2	Grandmother's sister	F	NA	NA	0	36
T8993G	36	3	1	Mother	F	NA	51	17	36
T8993G	36	3	2	Mother's cousin	F	NA	0	NA	36
T8993G	36	4	1	Index	M	NA	NA	51	36
T8993G	36	4	2	Son	M	NA	NA	51	36
T8993G	36	4	3	Son	M	NA	89	51	36
T8993G	37	1	1	Grandmother	F	NA	NA	NA	36
T8993G	37	2	1	Aunt	F	NA	NA	NA	36
T8993G	37	2	2	Mother	F	NA	0	NA	36
T8993G	37	3	1	Sister	F	NA	NA	0	36
T8993G	37	3	2	Index	F	NA	NA	0	36
T8993G	38	1	1	Grandmother	F	NA	NA	NA	36
T8993G	38	2	1	Mother	F	NA	0	NA	36
T8993G	38	2	2	Aunt	F	NA	NA	NA	36
T8993G	38	2	3	Uncle	M	NA	NA	NA	36
T8993G	38	3	1	Sister	F	NA	0	0	36
T8993G	38	3	2	Miscarriaged sibling	Fetus	NA	NA	0	36
T8993G	38	3	3	Index	F	NA	80	0	36
T8993G	39	1	1	Grandmother	F	NA	NA	NA	36
T8993G	39	2	1	Mother	F	NA	0	NA	36

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	39	2	2	Aunt	F	NA	NA	NA	36
T8993G	39	3	1	Brother	M	NA	NA	0	36
T8993G	39	3	2	Index	F	NA	96	0	36
T8993G	40	1	1	Grandmother	F	NA	NA	NA	36
T8993G	40	2	1	Uncle	M	NA	NA	NA	36
T8993G	40	2	2	Uncle	M	NA	NA	NA	36
T8993G	40	2	3	Uncle	M	NA	NA	NA	36
T8993G	40	2	4	Uncle	M	NA	NA	NA	36
T8993G	40	2	5	Uncle	M	NA	NA	NA	36
T8993G	40	2	6	Uncle	M	NA	NA	NA	36
T8993G	40	2	7	Aunt	F	NA	NA	NA	36
T8993G	40	2	8	Uncle	M	NA	NA	NA	36
T8993G	40	2	9	Mother	F	NA	6	NA	36
T8993G	40	2	10	Aunt	F	NA	NA	NA	36
T8993G	40	2	11	Aunt	F	NA	NA	NA	36
T8993G	40	2	12	Aunt	F	NA	0	NA	36
T8993G	40	3	1	Cousin	F	NA	NA	NA	36
T8993G	40	3	2	Cousin	Fetus	NA	NA	NA	36
T8993G	40	3	3	Cousin	NA	NA	NA	NA	36
T8993G	40	3	4	Cousin	NA	NA	NA	NA	36
T8993G	40	3	5	Brother	M	NA	NA	6	36
T8993G	40	3	6	Index	M	NA	93	6	36
T8993G	40	3	7	Cousin	Fetus	NA	NA	0	36

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	40	3	8	Cousin	Fetus	NA	NA	0	36
T8993G	41	1	1	Mother	F	NA	0	NA	36
T8993G	41	2	1	Brother	M	NA	NA	0	36
T8993G	41	2	2	Sister	F	NA	NA	0	36
T8993G	41	2	3	Index	F	NA	NA	0	36
T8993G	42	1	1	Index	F	42	50	NA	76
T8993G	42	2	1	Son	M	22	75	50	76
T8993G	42	2	2	Son	M	21	75	50	76
T8993G	42	2	3	Son	M	NA	NA	50	76
T8993G	43	1	1	Grandmother	F	58	NA	NA	77
T8993G	43	2	1	Mother	F	40	20	NA	77
T8993G	43	2	2	Aunt	F	NA	NA	NA	77
T8993G	43	2	3	Uncle	M	28	90	NA	77
T8993G	43	2	4	Aunt	F	NA	NA	NA	77
T8993G	43	2	5	Uncle	M	NA	NA	NA	77
T8993G	43	3	1	Sister	F	NA	NA	20	77
T8993G	43	3	2	Brother	M	21	90	20	77
T8993G	43	3	3	Sister	F	19	90	20	77
T8993G	43	3	4	Index	M	17	NA	20	77
T8993G	43	3	5	Cousin	F	15	90	NA	77
T8993G	43	3	6	Cousin	F	6	90	NA	77
T8993G	44	1	1	Mother	F	NA	19	NA	78
T8993G	44	2	1	Index1	F	NA	91	19	78

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	44	2	2	Index2	F	NA	85	19	78
T8993G	45	1	1	Mother	F	NA	0	NA	78
T8993G	45	2	1	Index	M	NA	86	0	78
T8993G	46	1	1	Mother	F	29	30	NA	79
T8993G	46	2	1	Index	M	NA	72	30	79
T8993G	47	1	1	Grandmother	F	NA	0	NA	26
T8993G	47	2	1	Mother	F	NA	20	0	26
T8993G	47	3	1	Index1	M	NA	88	20	26
T8993G	47	3	2	Index2	F	NA	76	20	26
T8993G	48	1	1	Grandmother	F	NA	NA	NA	80
T8993G	48	2	1	Mother	F	27	41	NA	80
T8993G	48	2	2	Aunt	F	NA	NA	NA	80
T8993G	48	2	3	Uncle	M	NA	NA	NA	80
T8993G	48	2	4	Uncle	M	NA	NA	NA	80
T8993G	48	3	1	Kindred	NA	NA	NA	41	80
T8993G	48	3	2	Sister	F	NA	90	41	80
T8993G	48	3	3	Index	M	2.5	90	41	80
T8993G	48	3	4	Brother	M	NA	95	41	80
T8993G	48	3	5	Cousin	NA	NA	NA	NA	80
T8993G	48	3	6	Cousin	NA	NA	NA	NA	80
T8993G	48	3	7	Cousin	NA	NA	NA	NA	80
T8993G	48	3	8	Cousin	NA	NA	NA	NA	80
T8993G	49	1	1	Mother	F	NA	NA	NA	81

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	49	2	1	Index1	F	NA	0	NA	81
T8993G	49	2	2	Index2	F	NA	17	NA	81
T8993G	49	3	1	Child	Fetus	NA	NA	0	81
T8993G	49	3	2	Child	Fetus	NA	NA	0	81
T8993G	49	3	3	Child	Fetus	NA	NA	0	81
T8993G	49	3	4	Son	M	NA	100	17	81
T8993G	49	3	5	Child	Fetus	NA	0	17	81
T8993G	50	1	1	Index	F	NA	0	NA	81
T8993G	50	2	1	Son	M	NA	90	0	81
T8993G	50	2	2	Child	Fetus	NA	NA	0	81
T8993G	50	2	3	Child	Fetus	NA	NA	0	81
T8993G	51	1	1	Index	F	NA	30	NA	81
T8993G	51	2	1	Son	M	NA	NA	30	81
T8993G	51	2	2	Son	M	NA	NA	30	81
T8993G	51	2	3	Child	Fetus	NA	NA	30	81
T8993G	52	1	1	Index	F	NA	55	NA	81
T8993G	52	2	1	Daughter	F	NA	NA	55	81
T8993G	52	2	2	Daughter	F	NA	NA	55	81
T8993G	52	2	3	Child	Fetus	NA	NA	55	81
T8993G	53	1	1	Index	F	NA	65	NA	81
T8993G	53	2	1	Daughter	F	NA	NA	65	81
T8993G	53	2	2	Daughter	F	NA	NA	65	81
T8993G	53	2	3	Child	Fetus	NA	NA	65	81

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	54	1	1	Mother	F	NA	NA	NA	81
T8993G	54	2	1	Brother	M	NA	NA	NA	81
T8993G	54	2	2	Brother	M	NA	NA	NA	81
T8993G	54	2	3	Brother	M	NA	NA	NA	81
T8993G	54	2	4	Brother	M	NA	NA	NA	81
T8993G	54	2	5	Sister	F	NA	NA	NA	81
T8993G	54	2	6	Index1	F	NA	75	NA	81
T8993G	54	2	7	Index2	F	NA	30	NA	81
T8993G	54	2	8	Sister	F	NA	NA	NA	81
T8993G	54	3	1	Child	Fetus	NA	NA	75	81
T8993G	54	3	2	Child	Fetus	NA	NA	75	81
T8993G	54	3	3	Son	M	NA	NA	30	81
T8993G	54	3	4	Child	Fetus	NA	NA	30	81
T8993G	54	3	5	Child	Fetus	NA	NA	30	81
T8993G	55	1	1	Greatgrandmother of Index1-3	F	NA	NA	NA	82
T8993G	55	2	1	Grandmother of Index1-3's brother	M	NA	NA	NA	82
T8993G	55	2	2	Grandmother of Index1-3's brother	M	NA	NA	NA	82
T8993G	55	2	3	Grandmother of Index1-3's brother	M	NA	NA	NA	82
T8993G	55	2	4	Grandmother of Index1-3's sister	F	NA	NA	NA	82
T8993G	55	2	5	Grandmother of Index1-3's sister	F	NA	NA	NA	82

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	55	2	6	Grandmother of Index1-3	F	NA	NA	NA	82
T8993G	55	2	7	Grandmother of Index1-3's sister	F	NA	NA	NA	82
T8993G	55	3	1	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	2	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	3	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	4	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	5	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	6	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	7	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	8	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	9	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	10	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	11	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	12	Mother of Index1	F	NA	90	NA	82
T8993G	55	3	13	Mother of Index2	F	NA	96	NA	82
T8993G	55	3	14	Mother of Index3	F	NA	60	NA	82

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	55	3	15	Aunt of Index1-3	F	NA	NA	NA	82
T8993G	55	3	16	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	17	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	18	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	19	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	20	Mother of Index1-3's cousin	F	NA	NA	NA	82
T8993G	55	3	21	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	22	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	23	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	24	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	25	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	3	26	Mother of Index1-3's cousin	M	NA	NA	NA	82
T8993G	55	4	1	Index1	M	1.83	98	90	82
T8993G	55	4	2	Sister of Index1	F	NA	NA	90	82
T8993G	55	4	3	Sister of Index1	F	NA	NA	90	82
T8993G	55	4	4	Sister of Index2	F	NA	44	96	82
T8993G	55	4	5	Index2	M	NA	97	96	82

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
T8993G	55	4	6	Brother of Index2	M	NA	49	96	82
T8993G	55	4	7	Sister of Index3	F	NA	NA	60	82
T8993G	55	4	8	Index3	M	NA	94	60	82
T8993G	55	4	9	Brother of Index3	M	NA	NA	60	82
T8993G	55	4	10	Cousin	F	NA	NA	NA	82
T8993G	55	4	11	Cousin	M	NA	NA	NA	82
T8993G	55	4	12	Cousin	M	NA	NA	NA	82
T8993G	55	4	13	Cousin	NA	NA	NA	NA	82
T8993G	55	4	14	Cousin	NA	NA	NA	NA	82
T8993G	56	1	1	Mother	F	NA	80	NA	83
T8993G	56	2	1	Index	F	0.42	100	80	83
A8344G	1	1	1	Sister of the grandmother	F	NA	NA	NA	84
A8344G	1	1	2	Grandmother	F	NA	NA	NA	84
A8344G	1	2	1	Cousin of the mother	F	NA	NA	NA	84
A8344G	1	2	2	Mother	F	NA	NA	NA	84
A8344G	1	2	3	Aunt	F	NA	NA	NA	84
A8344G	1	2	4	Aunt	F	NA	NA	NA	84
A8344G	1	2	5	Uncle	M	NA	NA	NA	84
A8344G	1	3	1	Index	F	NA	NA	NA	84
A8344G	1	3	2	Cousin	M	NA	NA	NA	84
A8344G	1	3	3	Cousin	F	NA	NA	NA	84
A8344G	2	1	1	Greatgrandmother	F	NA	NA	NA	85
A8344G	2	2	1	Grandmother	F	64	NA	NA	85

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	2	2	2	Brother of the grandmother	M	NA	NA	NA	85
A8344G	2	3	1	Aunt/uncle	Fetus	NA	NA	NA	85
A8344G	2	3	2	Aunt/uncle	Fetus	NA	NA	NA	85
A8344G	2	3	3	Mother	F	NA	77	NA	85
A8344G	2	3	4	Uncle	M	NA	NA	NA	85
A8344G	2	3	5	Aunt	F	NA	75	NA	85
A8344G	2	4	1	Brother	M	17	53	77	85
A8344G	2	4	2	Index	M	15	82	77	85
A8344G	2	4	3	Sister	F	10	67	77	85
A8344G	2	4	4	Cousin	Fetus	NA	NA	75	85
A8344G	2	4	5	Cousin	F	NA	29	75	85
A8344G	2	4	6	Cousin	M	NA	74	75	85
A8344G	2	4	7	Cousin	M	NA	NA	75	85
A8344G	2	4	8	Cousin	M	NA	NA	75	85
A8344G	3	1	1	Greatgreatgrandmother	F	NA	NA	NA	24
A8344G	3	2	1	Brother of the greatgrandmother	M	NA	NA	NA	24
A8344G	3	2	2	Sister of the greatgrandmother	F	90	0	NA	24
A8344G	3	2	3	Brother of the greatgrandmother	M	NA	NA	NA	24
A8344G	3	2	4	Sister of the greatgrandmother	F	86	33	NA	24
A8344G	3	2	5	Brother of the	M	85	33	NA	24

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
				greatgrandmother					
A8344G	3	2	6	Greatgrandmother	F	83	10	NA	24
A8344G	3	3	1	Cousin of the grandmother	F	60	0	0	24
A8344G	3	3	2	Cousin of the grandmother	M	NA	NA	0	24
A8344G	3	3	3	Cousin of the grandmother	F	NA	NA	0	24
A8344G	3	3	4	Cousin of the grandmother	M	65	0	33	24
A8344G	3	3	5	Cousin of the grandmother	M	64	0	33	24
A8344G	3	3	6	Cousin of the grandmother	F	62	28	33	24
A8344G	3	3	7	Cousin of the grandmother	F	59	14	33	24
A8344G	3	3	8	Cousin of the grandmother	F	44	0	33	24
A8344G	3	3	9	Sister of the grandmother	F	NA	NA	10	24
A8344G	3	3	10	Brother of the grandmother	M	NA	NA	10	24
A8344G	3	3	11	Grandmother	F	59	73	10	24
A8344G	3	3	12	Brother of the grandmother	M	NA	NA	10	24
A8344G	3	3	13	Brother of the grandmother	M	48	0	10	24
A8344G	3	4	1	Cousin of the mother	F	42	0	28	24
A8344G	3	4	2	Cousin of the mother	M	NA	NA	28	24

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	3	4	3	Cousin of the mother	F	29	72	28	24
A8344G	3	4	4	Cousin of the mother	F	28	49	28	24
A8344G	3	4	5	Cousin of the mother	F	24	15	28	24
A8344G	3	4	6	Cousin of the mother	M	NA	NA	28	24
A8344G	3	4	7	Cousin of the mother	F	35	0	14	24
A8344G	3	4	8	Cousin of the mother	F	32	54	14	24
A8344G	3	4	9	Cousin of the mother	M	31	0	14	24
A8344G	3	4	10	Cousin of the mother	M	NA	NA	0	24
A8344G	3	4	11	Cousin of the mother	M	NA	NA	0	24
A8344G	3	4	12	Cousin of the mother	F	NA	NA	0	24
A8344G	3	4	13	Uncle	M	NA	NA	73	24
A8344G	3	4	14	Mother	F	37	72	73	24
A8344G	3	5	1	Index	M	16	88	72	24
A8344G	3	5	2	Brother	M	13	77	72	24
A8344G	3	5	3	Brother	M	5	74	72	24
A8344G	3	5	4	Brother	M	3	46	72	24
A8344G	4	1	1	Grandmother	F	70	43	NA	24
A8344G	4	2	1	Uncle	M	NA	NA	43	24
A8344G	4	2	2	Aunt	F	46	51	43	24
A8344G	4	2	3	Uncle	M	44	82	43	24
A8344G	4	2	4	Miscarriage	NI	NA	NA	43	24
A8344G	4	2	5	Mother	F	42	66	43	24
A8344G	4	2	6	Aunt	F	38	63	43	24

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	4	3	1	Cousin	F	NA	NA	51	24
A8344G	4	3	2	Cousin	F	NA	NA	51	24
A8344G	4	3	3	Cousin	F	NA	NA	51	24
A8344G	4	3	4	Index	M	23	80	66	24
A8344G	4	3	5	Brother	M	20	50	66	24
A8344G	4	3	6	Cousin	M	NA	NA	63	24
A8344G	4	3	7	Cousin	M	8	59	63	24
A8344G	5	1	1	Grandmother/greatgrandmother	F	NA	NA	NA	86
A8344G	5	2	1	Mother/grandmother	F	NA	NA	NA	86
A8344G	5	2	2	Aunt/Sister of the grandmother	F	NA	NA	NA	86
A8344G	5	2	3	Aunt/Sister of the grandmother	F	NA	NA	NA	86
A8344G	5	2	4	Aunt/Sister of the grandmother	F	NA	NA	NA	86
A8344G	5	2	5	Aunt/Sister of the grandmother	F	NA	NA	NA	86
A8344G	5	3	1	Brother/uncle	M	NA	NA	NA	86
A8344G	5	3	2	Brother/uncle	M	NA	NA	NA	86
A8344G	5	3	3	Brother/uncle	M	NA	NA	NA	86
A8344G	5	3	4	Index	F	70	NA	NA	86
A8344G	5	4	1	Index	M	50	NA	NA	86
A8344G	5	4	2	Index	F	43	NA	NA	86
A8344G	6	1	1	Mother/Grandmother	F	NA	NA	NA	86
A8344G	6	2	1	Index	F	66	NA	NA	86

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	6	2	2	Sister/Aunt	F	62	NA	NA	86
A8344G	6	3	1	Son/Brother	M	NA	NA	NA	86
A8344G	6	3	2	Index	M	NA	NA	NA	86
A8344G	7	1	1	Grandmother	F	NA	NA	NA	87
A8344G	7	2	1	Mother	F	39	72	NA	87
A8344G	7	2	2	Uncle	M	42	49	NA	87
A8344G	7	2	3	Uncle	M	NA	NA	NA	87
A8344G	7	3	1	Index	M	19	81	72	87
A8344G	7	3	2	Sister	F	NA	NA	72	87
A8344G	8	1	1	Greatgrandmother	F	NA	NA	NA	87
A8344G	8	2	1	Brother of the grandmother	M	NA	NA	NA	87
A8344G	8	2	2	Sister of the grandmother	F	NA	NA	NA	87
A8344G	8	2	3	Grandmother	F	NA	NA	NA	87
A8344G	8	2	4	Brother of the grandmother	M	NA	NA	NA	87
A8344G	8	2	5	Brother of the grandmother	M	NA	NA	NA	87
A8344G	8	2	6	Brother of the grandmother	M	NA	NA	NA	87
A8344G	8	2	7	Brother of the grandmother	M	NA	NA	NA	87
A8344G	8	2	8	Sister of the grandmother	F	NA	NA	NA	87
A8344G	8	2	9	Sister of the grandmother	F	NA	NA	NA	87

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	8	2	10	Sister of the grandmother	F	NA	NA	NA	87
A8344G	8	2	11	Sister of the grandmother	F	NA	NA	NA	87
A8344G	8	3	1	Uncle	M	NA	NA	NA	87
A8344G	8	3	2	Mother	F	65	49	NA	87
A8344G	8	3	3	Aunt	F	NA	NA	NA	87
A8344G	8	3	4	Aunt	F	NA	NA	NA	87
A8344G	8	3	5	Aunt	F	35	50	NA	87
A8344G	8	3	6	Uncle	M	35	NA	NA	87
A8344G	8	3	7	Aunt	F	NA	NA	NA	87
A8344G	8	3	8	Uncle	M	NA	NA	NA	87
A8344G	8	4	1	Index	F	28	62	49	87
A8344G	8	4	2	Brother	M	26	38	49	87
A8344G	8	4	3	Cousin	F	NA	NA	NA	87
A8344G	8	4	4	Cousin	F	NA	NA	NA	87
A8344G	8	4	5	Cousin	NI	NA	NA	NA	87
A8344G	8	4	6	Cousin	NI	NA	NA	NA	87
A8344G	8	4	7	Cousin	F	NA	NA	50	87
A8344G	8	4	8	Cousin	M	9	64	50	87
A8344G	8	4	9	Cousin	M	NA	NA	NA	87
A8344G	8	4	10	Cousin	M	NA	NA	NA	87
A8344G	8	5	1	Niece/Nephew	NI	NA	NA	NA	87
A8344G	8	5	2	Niece/Nephew	NI	NA	NA	NA	87

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	8	5	3	Niece/Nephew	NI	NA	NA	NA	87
A8344G	8	5	4	Niece/Nephew	NI	NA	NA	NA	87
A8344G	9	1	1	Mother	F	NA	NA	NA	87
A8344G	9	2	1	Brother/sister	NI	NA	NA	NA	87
A8344G	9	2	2	Brother/sister	NI	NA	NA	NA	87
A8344G	9	2	3	Brother/sister	NI	NA	NA	NA	87
A8344G	9	2	4	Brother/sister	NI	NA	NA	NA	87
A8344G	9	2	5	Sister	F	63	30	NA	87
A8344G	9	2	6	Index	F	59	70	NA	87
A8344G	9	3	1	Cousin of index's child	F	NA	NA	30	87
A8344G	9	3	2	Cousin of index's child	M	NA	NA	30	87
A8344G	9	3	3	Daughter	F	40	NA	70	87
A8344G	9	3	4	Son	M	38	75	70	87
A8344G	9	3	5	Son	M	NA	NA	70	87
A8344G	9	3	6	Daughter	F	40	69	70	87
A8344G	9	3	7	Son	M	37	57	70	87
A8344G	9	4	1	Niece	F	NA	NA	69	87
A8344G	9	4	2	Nephew	M	NA	NA	69	87
A8344G	10	1	1	Mother	F	55	84	NA	87
A8344G	10	2	1	Sister	F	35	0	84	87
A8344G	10	2	2	Brother	M	NA	NA	84	87
A8344G	10	2	3	Index	M	31	92	84	87
A8344G	10	2	4	Sister	F	27	94	84	87

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	10	3	1	Daughter of the sister	F	NA	NA	0	87
A8344G	10	3	2	Son of the sister	M	NA	NA	0	87
A8344G	11	1	1	Grandmother	F	NA	NA	NA	87
A8344G	11	2	1	Uncle	M	58	71	NA	87
A8344G	11	2	2	Aunt	F	NA	NA	NA	87
A8344G	11	2	3	Mother	F	59	62	NA	87
A8344G	11	3	1	Cousin	M	25	NA	NA	87
A8344G	11	3	2	Cousin	M	NA	NA	NA	87
A8344G	11	3	3	Cousin	M	NA	NA	NA	87
A8344G	11	3	4	Sister	F	NA	NA	62	87
A8344G	11	3	5	Index	M	22	87	62	87
A8344G	12	1	1	Mother	F	81	63	NA	88
A8344G	12	2	1	Index	F	55	53	63	88
A8344G	12	2	2	Sister	F	58	12	63	88
A8344G	12	2	3	Sister	F	NA	NA	63	88
A8344G	12	2	4	Sister	F	NA	NA	63	88
A8344G	12	3	1	Son	M	30	54	53	88
A8344G	12	3	2	Son	M	18	64	53	88
A8344G	12	3	3	Niece	F	25	15	12	88
A8344G	12	3	4	Niece	F	36	0	12	88
A8344G	12	3	5	Niece	F	21	24	12	88
A8344G	12	3	6	Nephew	M	NA	NA	NA	88
A8344G	12	4	1	Duaghter of niece	F	6	0	0	88

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	12	4	2	Duaghter of niece	F	12	22	0	88
A8344G	13	1	1	Grandmother	F	NA	NA	NA	89
A8344G	13	2	1	Uncle	M	NA	NA	NA	89
A8344G	13	2	2	Uncle	M	NA	NA	NA	89
A8344G	13	2	3	Uncle	M	NA	NA	NA	89
A8344G	13	2	4	Aunt	F	63	83	NA	89
A8344G	13	2	5	Mother	F	NA	NA	NA	89
A8344G	13	3	1	Cousin	F	41	72	83	89
A8344G	13	3	2	Cousin	F	36	76	83	89
A8344G	13	3	3	Cousin	F	NA	55	83	89
A8344G	13	3	4	Index	M	NA	NA	NA	89
A8344G	13	3	5	Sister	F	NA	0	NA	89
A8344G	13	4	1	Nephew	M	NA	0	0	89
A8344G	13	4	2	Nephew	M	NA	NA	0	89
A8344G	14	1	1	Index	F	NA	NA	NA	89
A8344G	14	2	1	Son	M	49	NA	NA	89
A8344G	14	2	2	Daughter	F	42	NA	NA	89
A8344G	15	1	1	Index	F	66	NA	NA	89
A8344G	15	2	1	Son	M	NA	NA	NA	89
A8344G	16	1	1	Mother	F	43	85	NA	89
A8344G	16	2	1	Brother	M	NA	NA	85	89
A8344G	16	2	2	Index	F	NA	56	85	89
A8344G	17	1	1	Grandmother/mother	F	NA	77.8	NA	90

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	17	2	1	Index	M	NA	77.7	77.8	90
A8344G	17	2	2	Uncle/brother	M	NA	1.4	77.8	90
A8344G	17	2	3	Aunt/sister	F	NA	NA	77.8	90
A8344G	17	2	4	Aunt/sister	F	NA	NA	77.8	90
A8344G	17	2	5	Index	F	NA	87.4	77.8	90
A8344G	17	2	6	Aunt/sister	F	NA	38.6	77.8	90
A8344G	17	2	7	Aunt/sister	F	NA	13.3	77.8	90
A8344G	17	3	1	Index	M	NA	50.7	87.4	90
A8344G	17	3	2	Index	F	NA	93.2	87.4	90
A8344G	17	3	3	Index	F	NA	88.6	87.4	90
A8344G	18	1	1	Greatgrandmother	F	NA	NA	NA	91
A8344G	18	2	1	Grandmother	F	NA	NA	NA	91
A8344G	18	3	1	Uncle	M	NA	NA	NA	91
A8344G	18	3	2	Mother	F	NA	NA	NA	91
A8344G	18	3	3	Uncle/aunt	NI	NA	NA	NA	91
A8344G	18	3	4	Uncle/aunt	NI	NA	NA	NA	91
A8344G	18	3	5	Uncle/aunt	NI	NA	NA	NA	91
A8344G	18	4	1	Index	F	NA	NA	NA	91
A8344G	18	4	2	Brother	M	NA	NA	NA	91
A8344G	18	4	3	Sister	F	NA	NA	NA	91
A8344G	18	4	4	Brother	M	NA	NA	NA	91
A8344G	18	4	5	Sister	F	NA	NA	NA	91
A8344G	18	5	1	Daughter	F	59	83	NA	91

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	18	5	2	Daughter	F	50	78	NA	91
A8344G	18	5	3	Nephew	M	46	83	NA	91
A8344G	18	5	4	Niece	F	NA	NA	NA	91
A8344G	18	6	1	Grandson	M	35	87	83	91
A8344G	18	6	2	Gradndaughter	F	30	77	83	91
A8344G	18	6	3	Gradndaughter	F	25	66	78	91
A8344G	18	6	4	Gradndaughter	F	21	56	78	91
A8344G	18	6	5	Great-nephew	M	22	74	NA	91
A8344G	18	7	1	Greatgrandson	M	NA	NA	77	91
A8344G	18	7	2	Greatgranddaughter	F	2	33	77	91
A8344G	19	1	1	Grandmother	F	NA	NA	NA	92
A8344G	19	2	1	Mother	F	73	38	NA	92
A8344G	19	2	2	Uncle	M	NA	NA	NA	92
A8344G	19	3	1	Brother	M	45	75	38	92
A8344G	19	3	2	Index*	F	45	91	38	92
A8344G	19	4	1	Son	M	0.83	NA	91	92
A8344G	19	4	2	Son	M	4	NA	91	92
A8344G	20	1	1	Grandmother	F	NA	NA	NA	93
A8344G	20	2	1	Uncle	M	NA	86	NA	93
A8344G	20	2	2	Mother	F	NA	87	NA	93
A8344G	20	2	3	Aunt	F	NA	75	NA	93
A8344G	20	2	4	Aunt	F	NA	87	NA	93
A8344G	20	3	1	Brother	M	NA	79	87	93

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	20	3	2	Index	F	36	87	87	93
A8344G	20	3	3	Cousin	F	NA	76	87	93
A8344G	20	3	4	Cousin	F	NA	NA	87	93
A8344G	20	3	5	Cousin	F	NA	NA	87	93
A8344G	21	1	1	Mother	F	NA	NA	NA	94
A8344G	21	2	1	Index	F	55	NA	NA	94
A8344G	21	2	2	Sister	F	52	NA	NA	94
A8344G	21	2	3	Brother	M	50	NA	NA	94
A8344G	21	2	4	Sister	F	43	67	NA	94
A8344G	21	3	1	Son	M	NA	NA	NA	94
A8344G	21	3	2	Daughter	F	NA	68	NA	94
A8344G	21	3	3	Daughter	F	NA	70	NA	94
A8344G	21	3	4	Son	M	NA	NA	NA	94
A8344G	21	3	5	Cousin	F	NA	NA	NA	94
A8344G	21	3	6	Cousin	M	NA	90	NA	94
A8344G	21	3	7	Cousin	M	NA	73	67	94
A8344G	21	3	8	Cousin	M	NA	68	67	94
A8344G	22	1	1	Mother	F	NA	NA	NA	94
A8344G	22	2	1	Index	F	56	NA	NA	94
A8344G	22	3	1	Daughter	F	NA	40	NA	94
A8344G	22	3	2	Son	M	NA	42	NA	94
A8344G	23	1	1	Greatgrandmother	F	NA	NA	NA	26
A8344G	23	2	1	Grandmother	F	NA	16	NA	26

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	23	2	2	Sister of the grandmother	F	NA	14	NA	26
A8344G	23	2	3	Brother of the grandmother	M	NA	NA	NA	26
A8344G	23	2	4	Brother of the grandmother	M	NA	NA	NA	26
A8344G	23	3	1	Aunt	F	NA	4	16	26
A8344G	23	3	2	Uncle	M	NA	NA	16	26
A8344G	23	3	3	Uncle	M	NA	30	16	26
A8344G	23	3	4	Aunt	F	NA	0	16	26
A8344G	23	3	5	Aunt	F	NA	0	16	26
A8344G	23	3	6	Mother	F	NA	62	16	26
A8344G	23	3	7	Aunt	F	NA	0	16	26
A8344G	23	4	1	Cousin	F	NA	5	0	26
A8344G	23	4	2	Cousin	F	NA	0	0	26
A8344G	23	4	3	Cousin	M	NA	0	0	26
A8344G	23	4	4	Cousin	F	NA	0	0	26
A8344G	23	4	5	Cousin	M	NA	10	0	26
A8344G	23	4	6	Cousin	F	NA	0	0	26
A8344G	23	4	7	Index	F	NA	NA	62	26
A8344G	23	4	8	Index	F	NA	NA	62	26
A8344G	23	4	9	Index	F	NA	65	62	26
A8344G	23	4	10	Sister	F	NA	54	62	26
A8344G	23	4	11	Cousin	M	NA	0	0	26
A8344G	23	4	12	Cousin	F	NA	0	0	26

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	23	4	13	Cousin	F	NA	0	0	26
A8344G	23	4	14	Cousin	F	NA	5	0	26
A8344G	24	1	1	Great-grandmother	F	65	10	NA	95
A8344G	24	2	1	Grandmother	F	39	20	10	95
A8344G	24	3	1	Mother	F	22	40	20	95
A8344G	24	3	2	Aunt	F	17	30	20	95
A8344G	24	3	3	Uncle	M	15	40	20	95
A8344G	24	4	1	Sister	F	4	30	40	95
A8344G	24	4	2	Index	M	1.83	100	40	95
A8344G	25	1	1	Greatgrandmother	F	NA	NA	NA	96
A8344G	25	2	1	Grandmother	F	NA	NA	NA	96
A8344G	25	2	2	Brother of the grandmother	M	NA	NA	NA	96
A8344G	25	2	3	Sister of the grandmother	F	NA	NA	NA	96
A8344G	25	2	4	Sister of the grandmother	F	NA	NA	NA	96
A8344G	25	2	5	Sister of the grandmother	F	NA	NA	NA	96
A8344G	25	2	6	Sister of the grandmother	F	NA	60	NA	96
A8344G	25	2	7	Sister of the grandmother	F	NA	NA	NA	96
A8344G	25	3	1	Uncle	M	NA	NA	NA	96
A8344G	25	3	2	Uncle	M	NA	NA	NA	96
A8344G	25	3	3	Mother	F	NA	0	NA	96

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	25	3	4	Uncle	M	NA	NA	NA	96
A8344G	25	3	5	Mother's cousin	M	NA	NA	NA	96
A8344G	25	3	6	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	7	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	8	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	9	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	10	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	11	Mother's cousin	M	NA	NA	NA	96
A8344G	25	3	12	Mother's cousin	M	NA	NA	NA	96
A8344G	25	3	13	Mother's cousin	F	NA	0	NA	96
A8344G	25	3	14	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	15	Mother's cousin	M	NA	NA	NA	96
A8344G	25	3	16	Mother's cousin	F	NA	NA	NA	96
A8344G	25	3	17	Mother's cousin	M	NA	NA	60	96
A8344G	25	3	18	Mother's cousin	F	NA	68	60	96
A8344G	25	3	19	Mother's cousin	F	NA	NA	60	96
A8344G	25	3	20	Mother's cousin	F	NA	NA	NA	96
A8344G	25	4	1	Index	M	50	70	0	96
A8344G	25	4	2	Brother	M	NA	0	0	96
A8344G	25	4	3	Cousin	F	NA	NA	NA	96
A8344G	25	4	4	Cousin	M	NA	NA	NA	96
A8344G	25	4	5	Cousin	F	NA	NA	NA	96
A8344G	25	4	6	Cousin	F	NA	NA	NA	96

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	25	4	7	Cousin	M	NA	NA	NA	96
A8344G	25	4	8	Cousin	M	NA	NA	NA	96
A8344G	25	4	9	Cousin	M	NA	NA	NA	96
A8344G	25	4	10	Cousin	M	39	31	NA	96
A8344G	25	4	11	Cousin	F	NA	NA	0	96
A8344G	25	4	12	Cousin	F	NA	NA	0	96
A8344G	25	4	13	Cousin	M	NA	NA	NA	96
A8344G	25	4	14	Cousin	F	NA	NA	NA	96
A8344G	25	4	15	Cousin	F	NA	NA	NA	96
A8344G	25	4	16	Cousin	M	22	46	68	96
A8344G	25	4	17	Cousin	M	NA	NA	68	96
A8344G	25	4	18	Cousin	F	NA	NA	68	96
A8344G	25	4	19	Cousin	F	NA	NA	NA	96
A8344G	25	4	20	Cousin	F	NA	NA	NA	96
A8344G	25	4	21	Cousin	F	NA	NA	NA	96
A8344G	25	5	1	Niece	F	NA	NA	NA	96
A8344G	25	5	2	Nephew	M	NA	NA	NA	96
A8344G	25	5	3	Nephew	M	NA	NA	NA	96
A8344G	25	5	4	Nephew	M	NA	NA	NA	96
A8344G	25	5	5	Niece	F	NA	NA	NA	96
A8344G	25	5	6	Niece	F	NA	NA	NA	96
A8344G	25	5	7	Nephew	M	NA	NA	NA	96
A8344G	25	5	8	Nephew	M	NA	NA	NA	96

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	25	5	9	Niece	F	NA	NA	NA	96
A8344G	25	5	10	Nephew	M	NA	NA	NA	96
A8344G	25	5	11	Niece	F	NA	NA	NA	96
A8344G	25	5	12	Nephew	M	NA	NA	NA	96
A8344G	25	5	13	Nephew	M	NA	NA	NA	96
A8344G	25	5	14	Niece	F	NA	NA	NA	96
A8344G	25	5	15	Niece	F	NA	NA	NA	96
A8344G	25	5	16	Nephew	M	NA	NA	NA	96
A8344G	26	1	1	Mother	F	35	50	NA	97
A8344G	26	1	2	Uncle	M	NA	20	NA	97
A8344G	26	1	3	Aunt	F	NA	25	NA	97
A8344G	26	2	1	Brother	M	10	65	50	97
A8344G	26	2	2	Index	M	9	75	50	97
A8344G	27	1	1	Mother	F	NA	NA	NA	98
A8344G	27	2	1	Brother	M	NA	70	NA	98
A8344G	27	2	2	Index	M	66	80	NA	98
A8344G	28	1	1	Mother	F	NA	25	NA	99
A8344G	28	2	1	Index	M	22	33	25	99
A8344G	29	1	1	Grandmother	F	NA	NA	NA	100
A8344G	29	2	1	Uncle	M	NA	50	NA	100
A8344G	29	2	2	Aunt	F	NA	69	NA	100
A8344G	29	2	3	Mother	F	42	32	NA	100
A8344G	29	3	1	Cousin	M	NA	3	69	100

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A8344G	29	3	2	Cousin	M	NA	74	69	100
A8344G	29	3	3	Cousin	M	NA	32	69	100
A8344G	29	3	4	Sister	F	NA	15	32	100
A8344G	29	3	5	Index	F	NA	90	32	100
A8344G	29	3	6	Sister	F	NA	7	32	100
A8344G	29	3	7	Brother	M	14	5	32	100
A8344G	30	1	1	Mother	F	NA	NA	NA	101
A8344G	30	2	1	Index	M	50	69	NA	101
A8344G	30	2	2	Index	M	46	75	NA	101
A8344G	30	2	3	Index	M	39	75	NA	101
A8344G	30	2	4	Sister	F	NA	NA	NA	101
A8344G	30	2	5	Brother	M	NA	NA	NA	101
A8344G	31	1	1	Mother	F	NA	NA	NA	102
A8344G	31	2	1	Sister	F	12	NA	NA	102
A8344G	31	2	2	Index	M	7	80	NA	102
A8344G	32	1	1	Greatgrandmother/Greatgreatgrandmother	F	NA	NA	NA	103
A8344G	32	2	1	Grandmother/Greatgrandmother	F	NA	NA	NA	103
A8344G	32	2	2	Brother of the grandmother/greatgrandmother	M	NA	NA	NA	103
A8344G	32	2	3	Sister of the grandmother/greatgrandmother	F	NA	NA	NA	103
A8344G	32	2	4	Sister of the	F	NA	NA	NA	103

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
				grandmother/greatgrandmother					
A8344G	32	3	1	Uncle/Brother of the grandmother	M	NA	NA	NA	103
A8344G	32	3	2	Uncle/Brother of the grandmother	M	NA	NA	NA	103
A8344G	32	3	3	Mother/grandmother	F	NA	NA	NA	103
A8344G	32	3	4	Cousin of the mother/grandmother	F	NA	NA	NA	103
A8344G	32	3	5	Cousin of the mother/grandmother	M	NA	NA	NA	103
A8344G	32	3	6	Cousin of the mother/grandmother	M	NA	NA	NA	103
A8344G	32	4	1	Index	F	63	46	NA	103
A8344G	32	4	2	Brother/Uncle	M	NA	NA	NA	103
A8344G	32	5	1	Daughter/Sister	F	NA	0	46	103
A8344G	32	5	2	Index	M	43	58	46	103
A8344G	32	5	3	Index	M	43	44	46	103
A8344G	32	6	1	Cousin	F	NA	NA	NA	103
A3243G	1	1	1	Mother	F	NA	NA	NA	5
A3243G	1	2	1	Sister	F	55	14	NA	5
A3243G	1	2	2	Index	F	54	24	NA	5
A3243G	1	2	3	Index	M	45	NA	NA	5
A3243G	1	3	1	Niece	F	26	14	14	5
A3243G	1	3	2	Niece	F	NA	NA	14	5
A3243G	1	3	3	Nephew	M	NA	NA	14	5

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	1	3	4	Niece	F	20	42	14	5
A3243G	1	3	5	Son	M	NA	NA	24	5
A3243G	1	3	6	Daughter	F	NA	NA	24	5
A3243G	2	1	1	Grandmother	F	64	0	NA	5
A3243G	2	2	1	Uncle	M	41	30	0	5
A3243G	2	2	2	Mother	F	39	40	0	5
A3243G	2	2	3	Aunt	F	30	28	0	5
A3243G	2	2	4	Aunt	F	25	52	0	5
A3243G	2	3	1	Index	M	18	75	40	5
A3243G	2	3	2	Sister	F	14	69	40	5
A3243G	2	3	3	Cousin	M	NA	NA	28	5
A3243G	2	3	4	Twin cousin	M	NA	NA	28	5
A3243G	2	3	5	Twin cousin	M	NA	NA	28	5
A3243G	3	1	1	Mother	F	46	NA	NA	5
A3243G	3	2	1	Index	M	16	NA	NA	5
A3243G	3	2	2	Brother	M	12	NA	NA	5
A3243G	4	1	1	Mother	F	27	28	NA	5
A3243G	4	2	1	Brother	M	NA	NA	28	5
A3243G	4	2	2	Index	F	6	81	28	5
A3243G	4	2	3	Brother	M	NA	NA	28	5
A3243G	4	2	4	Sister	F	NA	NA	28	5
A3243G	4	2	5	Index	F	3	85	28	5
A3243G	5	1	1	Grandmother	F	NA	NA	NA	5

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	5	2	1	Mother	F	69	14	NA	5
A3243G	5	2	2	Aunt	F	NA	NA	NA	5
A3243G	5	2	3	Aunt	F	59	28	NA	5
A3243G	5	3	1	Index	F	46	43	14	5
A3243G	5	3	2	Brother	M	NA	NA	14	5
A3243G	5	3	3	Half-sib brother	M	38	0	14	5
A3243G	5	3	4	Cousin	M	NA	NA	NA	5
A3243G	5	3	5	Cousin	M	NA	NA	NA	5
A3243G	5	3	6	Cousin	M	38	22	28	5
A3243G	5	3	7	Cousin	M	31	17	28	5
A3243G	5	3	8	Cousin	F	32	28	28	5
A3243G	6	1	1	Sister	F	30	NA	NA	5
A3243G	6	1	2	Index	F	38	NA	NA	5
A3243G	7	1	1	Mother	F	47	14	NA	5
A3243G	7	2	1	Index	F	13	59	14	5
A3243G	8	1	1	Mother	F	35	0	NA	5
A3243G	8	2	1	Index	F	11	61	0	5
A3243G	9	1	1	Mother	F	NA	24	NA	5
A3243G	9	2	1	Index	F	8	NA	24	5
A3243G	10	1	1	Mother	F	63	NA	NA	104
A3243G	10	2	1	Sister	F	54	14	NA	104
A3243G	10	2	2	Sister	F	53	NA	NA	104
A3243G	10	2	3	Index	M	43	24	NA	104

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	10	3	1	Niece	F	25	14	14	104
A3243G	10	3	2	Niece/nephew	Fetus	NA	NA	14	104
A3243G	10	3	3	Niece	F	18	42	14	104
A3243G	10	3	4	Nephew	M	26	NA	NA	104
A3243G	10	3	5	Niece	F	22	NA	NA	104
A3243G	11	1	1	Grandmother	F	64	2	NA	104
A3243G	11	2	1	Uncle	M	41	30	2	104
A3243G	11	2	2	Mother	F	39	40	2	104
A3243G	11	2	3	Aunt	F	37	28	2	104
A3243G	11	2	4	Aunt	F	25	52	2	104
A3243G	11	3	1	Index	M	19	75	40	104
A3243G	11	3	2	Sister	F	15	59	40	104
A3243G	11	3	3	Cousin	M	NA	NA	28	104
A3243G	11	3	4	Cousin	M	NA	NA	28	104
A3243G	11	3	5	Cousin	M	NA	NA	28	104
A3243G	12	1	1	Grandmother	F	50	6	NA	105
A3243G	12	2	1	Sister	F	29	3	6	105
A3243G	12	2	2	Brother	M	NA	NA	6	105
A3243G	12	2	3	Brother	M	25	25	6	105
A3243G	12	2	4	Sister	F	23	33	6	105
A3243G	12	2	5	Index	M	17	NA	6	105
A3243G	12	3	1	Niece	F	7	18	3	105
A3243G	12	3	2	Niece	F	3	0	3	105

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	13	1	1	Mother	F	48	11	NA	106
A3243G	13	2	1	Sister	F	NA	NA	11	106
A3243G	13	2	2	Index	F	24	32	11	106
A3243G	13	2	3	Sister	F	20	19	11	106
A3243G	13	2	4	Brother	M	19	21	11	106
A3243G	13	2	5	Brother	M	17	27	11	106
A3243G	14	1	1	Mother	F	54	40	NA	108
A3243G	14	2	1	Index	M	NA	NA	40	108
A3243G	14	2	2	Sister	F	27	NA	40	108
A3243G	15	1	1	Grandmother	F	85	40	NA	108
A3243G	15	2	1	Mother	F	58	60	40	108
A3243G	15	2	2	Uncle	M	55	60	40	108
A3243G	15	3	1	Index	M	27	90	60	108
A3243G	15	3	2	Brother	M	21	70	60	108
A3243G	16	1	1	Grandmother	F	NA	NA	NA	7
A3243G	16	2	1	Mother	F	NA	NA	NA	7
A3243G	16	2	2	Aunt/Uncle	NI	NA	NA	NA	7
A3243G	16	2	3	Aunt/Uncle	NI	NA	NA	NA	7
A3243G	16	2	4	Aunt/Uncle	NI	NA	NA	NA	7
A3243G	16	2	5	Aunt/Uncle	NI	NA	NA	NA	7
A3243G	16	2	6	Aunt/Uncle	NI	NA	NA	NA	7
A3243G	16	2	7	Aunt/Uncle	NI	NA	NA	NA	7
A3243G	16	3	1	Index	F	47	14	NA	7

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	16	3	2	Sister	F	42	10	NA	7
A3243G	16	3	3	Sister	F	NA	NA	NA	7
A3243G	16	3	4	Brother	M	NA	NA	NA	7
A3243G	16	4	1	Son	M	18	49	14	7
A3243G	16	4	2	Daughter	F	21	43	14	7
A3243G	16	4	3	Niece/Nephew	NI	NA	NA	NA	7
A3243G	16	4	4	Niece/Nephew	NI	NA	NA	NA	7
A3243G	16	4	5	Niece/Nephew	NI	NA	NA	NA	7
A3243G	16	4	6	Niece/Nephew	NI	NA	NA	NA	7
A3243G	16	4	7	Niece/Nephew	NI	NA	NA	NA	7
A3243G	17	1	1	Grandmother	F	NA	NA	NA	7
A3243G	17	2	1	Mother	F	NA	NA	NA	7
A3243G	17	2	2	Aunt	F	49	17	NA	7
A3243G	17	3	1	Sister	F	32	23	NA	7
A3243G	17	3	2	Index	F	27	27	NA	7
A3243G	17	3	3	Sister	F	25	18	NA	7
A3243G	17	3	4	Cousin	F	NA	NA	17	7
A3243G	17	3	5	Cousin	F	21	36	17	7
A3243G	17	3	6	Cousin	M	18	NA	17	7
A3243G	17	4	1	Nephew	M	1	NA	23	7
A3243G	18	1	1	Grandgrandmother	F	NA	NA	NA	7
A3243G	18	2	1	Grandmother	F	67	0	NA	7
A3243G	18	2	2	Brother of the grandmother	M	65	NA	NA	7

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	18	2	3	Sister of the grandmother	F	NA	NA	NA	7
A3243G	18	3	1	Mother	F	40	25	0	7
A3243G	18	3	2	Aunt	F	NA	NA	0	7
A3243G	18	3	3	Aunt	F	37	0	0	7
A3243G	18	4	1	Sister	F	21	36	25	7
A3243G	18	4	2	Index	M	18	59	25	7
A3243G	18	4	3	Cousin	NI	14	NA	NA	7
A3243G	18	4	4	Cousin	NI	NA	NA	NA	7
A3243G	18	4	5	Cousin	NI	NA	NA	NA	7
A3243G	18	4	6	Cousin	NI	NA	NA	0	7
A3243G	18	4	7	Cousin	NI	NA	NA	0	7
A3243G	18	4	8	Cousin	NI	NA	NA	0	7
A3243G	18	5	1	Son	M	NA	NA	36	7
A3243G	19	1	1	Mother	F	NA	NA	NA	7
A3243G	19	2	1	Sister	F	NA	NA	NA	7
A3243G	19	2	2	Brother	M	53	37	NA	7
A3243G	19	2	3	Index	F	56	91	NA	7
A3243G	19	2	4	Brother	M	NA	NA	NA	7
A3243G	19	3	1	Daughter	F	34	83	91	7
A3243G	19	3	2	Daughter	F	32	78	91	7
A3243G	20	1	1	Grandmother	F	NA	NA	NA	7
A3243G	20	2	1	Aunt	F	NA	NA	NA	7
A3243G	20	2	2	Uncle	M	58	8	NA	7

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	20	2	3	Aunt	F	59	0	NA	7
A3243G	20	2	4	Aunt	F	NA	NA	NA	7
A3243G	20	3	1	Cousin	F	NA	NA	NA	7
A3243G	20	3	2	Cousin	F	NA	NA	NA	7
A3243G	20	3	3	Sister	F	28	28	NA	7
A3243G	20	3	4	Index	M	29	43	NA	7
A3243G	20	3	5	Brother	M	31	NA	NA	7
A3243G	20	4	1	Niece	F	8	46	28	7
A3243G	20	4	2	Niece	F	6	50	28	7
A3243G	21	1	1	Greatgrandmother	F	NA	NA	NA	7
A3243G	21	2	1	Grandmother	F	NA	NA	NA	7
A3243G	21	2	2	Sister of the grandmother	F	NA	NA	NA	7
A3243G	21	2	3	Sister of the grandmother	F	NA	NA	NA	7
A3243G	21	3	1	Uncle	M	NA	NA	NA	7
A3243G	21	3	2	Mother	F	NA	NA	NA	7
A3243G	21	3	3	Uncle	M	NA	NA	NA	7
A3243G	21	3	4	Aunt	F	NA	NA	NA	7
A3243G	21	3	5	Aunt	F	NA	NA	NA	7
A3243G	21	3	6	Aunt	F	NA	NA	NA	7
A3243G	21	3	7	Aunt	F	NA	NA	NA	7
A3243G	21	4	1	Sister	F	NA	NA	NA	7
A3243G	21	4	2	Sister	F	NA	NA	NA	7

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	21	4	3	Sister	F	54	11	NA	7
A3243G	21	4	4	Sister	F	49	15	NA	7
A3243G	21	4	5	Index	M	43	NA	NA	7
A3243G	21	4	6	Cousin	F	52	12	NA	7
A3243G	21	4	7	Cousin	M	NA	NA	NA	7
A3243G	21	4	8	Cousin	M	58	4	NA	7
A3243G	21	4	9	Cousin	M	NA	NA	NA	7
A3243G	21	5	1	Niece	F	33	15	11	7
A3243G	21	5	2	Niece	F	30	11	11	7
A3243G	21	5	3	Niece	F	28	8	11	7
A3243G	21	5	4	Niece	F	27	24	15	7
A3243G	21	5	5	Nephew	M	24	31	15	7
A3243G	21	5	6	Cousin	M	26	13	12	7
A3243G	21	5	7	Cousin	M	26	13	12	7
A3243G	22	1	1	Grandmother	F	NA	NA	NA	7
A3243G	22	2	1	Brother	M	41	28	NA	7
A3243G	22	2	2	Index	M	NA	NA	NA	7
A3243G	22	2	3	Sister	F	48	19	NA	7
A3243G	22	3	1	Nephew	M	15	59	19	7
A3243G	22	3	2	Niece	F	8	NA	19	7
A3243G	23	1	1	Mother	F	38	0	NA	7
A3243G	23	2	1	Index	M	18	53	0	7
A3243G	24	1	1	Mother	F	60	0	NA	7

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	24	2	1	Index	M	35	19	0	7
A3243G	25	1	1	Mother	F	NA	14	NA	7
A3243G	25	2	1	Index	M	10	62	14	7
A3243G	26	1	1	Mother	F	60	0	NA	7
A3243G	26	2	1	Index	F	31	NA	0	7
A3243G	27	1	1	Mother	F	31	31	NA	7
A3243G	27	2	1	Index	NI	11	58	31	7
A3243G	28	1	1	Mother	F	35	10	NA	7
A3243G	28	2	1	Index	M	9	53	10	7
A3243G	29	1	1	Mother	F	47	10	NA	7
A3243G	29	2	1	Index	F	18	58	10	7
A3243G	30	1	1	Index	F	60	13	NA	7
A3243G	30	1	2	Sister	F	58	17	NA	7
A3243G	31	1	1	Mother	F	NA	NA	NA	6
A3243G	31	2	1	Index	F	44	17	NA	6
A3243G	31	3	1	Son	M	24	31	17	6
A3243G	31	3	2	Daughter	F	18	44	17	6
A3243G	31	3	3	Son	M	16	14	17	6
A3243G	32	1	1	Greatgrandmother	F	NA	NA	NA	6
A3243G	32	2	1	Grandmother	F	70	22	NA	6
A3243G	32	2	2	Brother of the grandmother	M	NA	NA	NA	6
A3243G	32	2	3	Sister of the grandmother	F	NA	NA	NA	6

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	32	2	4	Sister of the grandmother	F	NA	NA	NA	6
A3243G	32	3	1	Mother	F	44	31	22	6
A3243G	32	3	2	Uncle	M	NA	NA	22	6
A3243G	32	3	3	Uncle	M	NA	NA	22	6
A3243G	32	4	1	Index	M	18	38	31	6
A3243G	32	4	2	Sister	F	15	54	31	6
A3243G	33	1	1	Grandmother	F	NA	NA	NA	6
A3243G	33	2	1	Uncle	M	60	77	NA	6
A3243G	33	2	2	Mother	F	58	79	NA	6
A3243G	33	3	1	Index	M	33	79	79	6
A3243G	33	3	2	Sister	F	28	89	79	6
A3243G	34	1	1	Grandmother	F	NA	2.5	NA	109
A3243G	34	2	1	Mother	F	NA	NA	2.5	109
A3243G	34	2	2	Aunt	F	NA	4	2.5	109
A3243G	34	2	3	Aunt	F	NA	24	2.5	109
A3243G	34	2	4	Uncle	M	NA	3	2.5	109
A3243G	34	2	5	Uncle	M	NA	0	2.5	109
A3243G	34	2	6	Uncle	M	NA	27	2.5	109
A3243G	34	3	1	Index	M	13	45	NA	109
A3243G	34	3	2	Brother	M	16	29	NA	109
A3243G	34	3	3	Cousin	M	NA	27	4	109
A3243G	34	3	4	Cousin	F	NA	0	4	109
A3243G	34	3	5	Cousin	F	NA	NA	4	109

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	35	1	1	Grandmother	F	NA	NA	NA	110
A3243G	35	2	1	Aunt	F	NA	NA	NA	110
A3243G	35	2	2	Uncle	M	NA	NA	NA	110
A3243G	35	2	3	Aunt	F	NA	NA	NA	110
A3243G	35	2	4	Mother	F	NA	NA	NA	110
A3243G	35	2	5	Aunt	F	NA	NA	NA	110
A3243G	35	2	6	Aunt	F	NA	40	NA	110
A3243G	35	2	7	Aunt	F	NA	11	NA	110
A3243G	35	2	8	Aunt	F	NA	NA	NA	110
A3243G	35	2	9	Aunt	F	NA	11	NA	110
A3243G	35	2	10	Uncle	M	NA	NA	NA	110
A3243G	35	3	1	Cousin	F	NA	NA	NA	110
A3243G	35	3	2	Cousin	F	NA	NA	NA	110
A3243G	35	3	3	Cousin	M	NA	NA	NA	110
A3243G	35	3	4	Cousin	F	NA	NA	NA	110
A3243G	35	3	5	Cousin	M	NA	NA	NA	110
A3243G	35	3	6	Cousin	M	NA	NA	NA	110
A3243G	35	3	7	Cousin	M	NA	NA	NA	110
A3243G	35	3	8	Brother	M	NA	NA	NA	110
A3243G	35	3	9	Brother	M	NA	NA	NA	110
A3243G	35	3	10	Index	M	NA	31	NA	110
A3243G	35	3	11	Brother	M	NA	NA	NA	110
A3243G	35	3	12	Brother	M	NA	NA	NA	110

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	35	3	13	Brother	M	NA	NA	NA	110
A3243G	35	3	14	Sister	F	NA	NA	NA	110
A3243G	35	3	15	Brother	M	NA	NA	NA	110
A3243G	35	3	16	Cousin	F	NA	NA	NA	110
A3243G	35	3	17	Cousin	F	NA	NA	NA	110
A3243G	35	3	18	Cousin	F	NA	NA	NA	110
A3243G	35	3	19	Cousin	M	NA	NA	40	110
A3243G	35	3	20	Cousin	M	NA	27	40	110
A3243G	35	3	21	Cousin	M	NA	NA	40	110
A3243G	35	3	22	Cousin	M	NA	0	40	110
A3243G	35	3	23	Cousin	M	NA	24	11	110
A3243G	35	3	24	Cousin	M	NA	NA	11	110
A3243G	35	3	25	Cousin	M	NA	NA	11	110
A3243G	35	3	26	Cousin	M	NA	NA	11	110
A3243G	35	3	27	Cousin	F	NA	NA	NA	110
A3243G	35	3	28	Cousin	F	NA	NA	NA	110
A3243G	35	3	29	Cousin	F	NA	NA	NA	110
A3243G	35	3	30	Cousin	M	NA	NA	NA	110
A3243G	35	3	31	Cousin	M	NA	NA	NA	110
A3243G	35	3	32	Cousin	M	NA	NA	NA	110
A3243G	35	3	33	Cousin	F	NA	26	11	110
A3243G	35	3	34	Cousin	F	NA	56	11	110
A3243G	35	3	35	Cousin	F	NA	83	11	110

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	35	4	1	Nephew	M	NA	NA	NA	110
A3243G	35	4	2	Niece	F	NA	NA	NA	110
A3243G	35	4	3	Niece/nephew	Fetus	NA	NA	NA	110
A3243G	35	4	4	Nephew	M	NA	NA	NA	110
A3243G	35	4	5	Niece	F	NA	NA	NA	110
A3243G	35	4	6	Niece	F	NA	NA	NA	110
A3243G	35	4	7	Niece	F	NA	NA	NA	110
A3243G	35	4	8	Niece	F	NA	NA	NA	110
A3243G	35	4	9	Nephew	M	NA	NA	NA	110
A3243G	35	4	10	Nephew	M	NA	NA	NA	110
A3243G	35	4	11	Nephew	M	NA	NA	NA	110
A3243G	35	4	12	Niece	F	NA	NA	NA	110
A3243G	35	4	13	Niece	F	NA	NA	NA	110
A3243G	35	4	14	Niece	F	NA	NA	NA	110
A3243G	35	4	15	Niece	F	NA	NA	NA	110
A3243G	35	4	16	Niece	F	NA	75	26	110
A3243G	35	4	17	Niece/nephew	Fetus	NA	NA	56	110
A3243G	35	4	18	Niece/nephew	Fetus	NA	NA	56	110
A3243G	35	4	19	Niece/nephew	Fetus	NA	NA	56	110
A3243G	35	4	20	Nephew	M	NA	40	83	110
A3243G	35	4	21	Niece	F	NA	80	83	110
A3243G	36	1	1	Relative	F	NA	NA	NA	107
A3243G	36	2	1	Relative	F	NA	NA	NA	107

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	36	2	2	Relative	M	NA	NA	NA	107
A3243G	36	2	3	Relative	F	82	0	NA	107
A3243G	36	3	1	Mother	F	60	15	NA	107
A3243G	36	3	2	Relative	F	59	0	NA	107
A3243G	36	3	3	Relative	M	57	0	NA	107
A3243G	36	3	4	Relative	M	56	0	NA	107
A3243G	36	3	5	Relative	F	53	15	NA	107
A3243G	36	3	6	Relative	M	50	0	NA	107
A3243G	36	3	7	Relative	F	45	15	NA	107
A3243G	36	4	1	Index	M	38	0	15	107
A3243G	36	4	2	Sister	F	33	28	15	107
A3243G	36	4	3	Brother	M	NA	38	15	107
A3243G	37	1	1	Grandmother	F	65	5	NA	111
A3243G	37	2	1	Mother	F	43	20	5	111
A3243G	37	3	1	Index	M	18	30	20	111
A3243G	37	3	2	Sister	F	14	NA	20	111
A3243G	38	1	1	Grandmother	F	78	0	NA	112
A3243G	38	2	1	Uncle	M	45	0	0	112
A3243G	38	2	2	Aunt	F	NA	NA	0	112
A3243G	38	2	3	Uncle	M	NA	NA	0	112
A3243G	38	2	4	Mother	F	42	15	0	112
A3243G	38	2	5	Aunt	F	38	7	0	112
A3243G	38	3	1	Sister	F	20	0	15	112

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	38	3	2	Index	M	17	NA	15	112
A3243G	38	3	3	Brother	M	15	0	15	112
A3243G	38	3	4	Brother	M	12	0	15	112
A3243G	39	1	1	Mother	F	85	0	NA	112
A3243G	39	2	1	Brother	M	NA	NA	0	112
A3243G	39	2	2	Sister	F	63	0	0	112
A3243G	39	2	3	Brother	M	62	0	0	112
A3243G	39	2	4	Brother	M	58	0	0	112
A3243G	39	2	5	Brother	M	56	0	0	112
A3243G	39	2	6	Sister	F	52	0	0	112
A3243G	39	2	7	Brother	M	46	0	0	112
A3243G	39	2	8	Index	F	42	8	0	112
A3243G	40	1	1	Mother	F	53	8	NA	113
A3243G	40	2	1	Index	F	30	35	8	113
A3243G	40	2	2	Brother	M	24	NA	8	113
A3243G	40	2	3	Brother	M	23	42	8	113
A3243G	40	3	1	Son	M	10	49	35	113
A3243G	40	3	2	Son	M	7	49	35	113
A3243G	40	3	3	Son	M	4	72	35	113
A3243G	41	1	1	Mother	F	NA	5.1	NA	114
A3243G	41	2	1	Sister	F	NA	0	5.1	114
A3243G	41	2	2	Index	M	14	11.8	5.1	114
A3243G	42	1	1	Grandmother	F	NA	NA	NA	115

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	42	2	1	Mother	F	NA	NA	NA	115
A3243G	42	3	1	Sister	F	68	NA	NA	115
A3243G	42	3	2	Sister	F	65	6	NA	115
A3243G	42	3	3	Sister	F	61	5	NA	115
A3243G	42	3	4	Index	F	56	12	NA	115
A3243G	42	4	1	Niece	F	31	NA	NA	115
A3243G	42	4	2	Niece	F	29	NA	NA	115
A3243G	42	4	3	Nephew	M	35	4	NA	115
A3243G	42	4	4	Nephew	M	31	13	NA	115
A3243G	42	4	5	Niece	F	28	9	NA	115
A3243G	42	4	6	Niece	F	25	17	NA	115
A3243G	42	4	7	Son	M	30	20	12	115
A3243G	42	4	8	Daughter	F	28	27	12	115
A3243G	42	4	9	Daughter	F	26	17	12	115
A3243G	42	5	1	Niece	F	2	NA	27	115
A3243G	42	5	2	Niece	F	1	NA	27	115
A3243G	42	5	3	Nephew	M	1	NA	17	115
A3243G	43	1	1	Grandgrandmother	F	NA	NA	NA	115
A3243G	43	2	1	Sister of the grandmother	F	NA	NA	NA	115
A3243G	43	2	2	Brother of the grandmother	M	NA	NA	NA	115
A3243G	43	2	3	Brother of the grandmother	M	NA	NA	NA	115
A3243G	43	2	4	Brother of the	M	NA	NA	NA	115

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
				grandmother					
A3243G	43	2	5	Sister of the grandmother	F	NA	NA	NA	115
A3243G	43	3	1	Uncle	M	NA	NA	NA	115
A3243G	43	3	2	Uncle	M	NA	NA	NA	115
A3243G	43	3	3	Aunt	F	NA	NA	NA	115
A3243G	43	3	4	Uncle	M	NA	NA	NA	115
A3243G	43	3	5	Aunt	F	NA	NA	NA	115
A3243G	43	3	6	Aunt	F	NA	NA	NA	115
A3243G	43	3	7	Mother	F	66	NA	NA	115
A3243G	43	3	8	Uncle	M	NA	NA	NA	115
A3243G	43	3	9	Uncle	M	NA	NA	NA	115
A3243G	43	3	10	Uncle	M	NA	NA	NA	115
A3243G	43	3	11	Uncle	M	NA	NA	NA	115
A3243G	43	3	12	Uncle	M	NA	NA	NA	115
A3243G	43	3	13	Uncle	M	NA	NA	NA	115
A3243G	43	3	14	Aunt	F	NA	NA	NA	115
A3243G	43	4	1	Brother	M	NA	NA	NA	115
A3243G	43	4	2	Index	F	34	34	NA	115
A3243G	43	4	3	Sister	F	NA	NA	NA	115
A3243G	43	5	1	Son	M	2	NA	34	115
A3243G	43	5	2	Daughter	F	0	NA	34	115
A3243G	44	1	1	Grandgrandmother	F	NA	NA	NA	115
A3243G	44	2	1	Grandmother	F	NA	NA	NA	115

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	44	2	2	Brother of the grandmother	M	NA	NA	NA	115
A3243G	44	2	3	Sister of the grandmother	F	NA	NA	NA	115
A3243G	44	2	4	Sister of the grandmother	F	NA	NA	NA	115
A3243G	44	3	1	Mother	F	69	4	NA	115
A3243G	44	4	1	Twin sister	F	43	NA	4	115
A3243G	44	4	2	Twin sister	F	43	NA	4	115
A3243G	44	4	3	Index	F	41	18	4	115
A3243G	44	4	4	Sister	F	31	NA	4	115
A3243G	44	5	1	Nephew	M	10	NA	NA	115
A3243G	44	5	2	Nephew	M	13	NA	NA	115
A3243G	44	5	3	Niece	F	9	NA	NA	115
A3243G	44	5	4	Daughter	F	11	NA	18	115
A3243G	44	5	5	Nephew	F	6	NA	NA	115
A3243G	45	1	1	Grandmother	F	NA	NA	NA	116
A3243G	45	2	1	Mother	F	36	43	NA	116
A3243G	45	2	2	Uncle	M	NA	NA	NA	116
A3243G	45	2	3	Aunt	F	NA	NA	NA	116
A3243G	45	2	4	Aunt	F	NA	NA	NA	116
A3243G	45	2	5	Aunt	F	NA	NA	NA	116
A3243G	45	2	6	Aunt	F	NA	NA	NA	116
A3243G	45	2	7	Aunt	F	NA	NA	NA	116
A3243G	45	3	1	Brother	M	10	49	43	116

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	45	3	2	Index	M	16	68	43	116
A3243G	45	3	3	Cousin	F	NA	NA	NA	116
A3243G	45	3	4	Cousin	F	NA	NA	NA	116
A3243G	45	3	5	Cousin	M	NA	NA	NA	116
A3243G	45	3	6	Cousin	M	NA	NA	NA	116
A3243G	45	3	7	Cousin	M	NA	NA	NA	116
A3243G	46	1	1	Grandmother	F	78	NA	NA	117
A3243G	46	2	1	Aunt	F	NA	NA	NA	117
A3243G	46	2	2	Aunt	F	61	NA	NA	117
A3243G	46	2	3	Aunt	F	NA	NA	NA	117
A3243G	46	2	4	Uncle	M	NA	NA	NA	117
A3243G	46	2	5	Aunt	F	NA	NA	NA	117
A3243G	46	2	6	Aunt	F	81	NA	NA	117
A3243G	46	2	7	Aunt	F	NA	NA	NA	117
A3243G	46	2	8	Uncle	M	NA	NA	NA	117
A3243G	46	2	9	Mother	F	71	NA	NA	117
A3243G	46	3	1	Sister	F	49	28	NA	117
A3243G	46	3	2	Sister	F	46	18	NA	117
A3243G	46	3	3	Sister	F	44	31	NA	117
A3243G	46	3	4	Index	F	39	17	NA	117
A3243G	46	4	1	Nephew	M	26	25	18	117
A3243G	46	4	2	Nephew	M	22	30	18	117
A3243G	46	4	3	Nephew	M	NA	NA	31	117

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	46	4	4	Niece	F	NA	NA	31	117
A3243G	46	4	5	Daughter	F	NA	NA	17	117
A3243G	47	1	1	Grandmother	F	NA	NA	NA	117
A3243G	47	2	1	Aunt	F	NA	NA	NA	117
A3243G	47	2	2	Mother	F	69	5	NA	117
A3243G	47	2	3	Aunt	F	NA	NA	NA	117
A3243G	47	2	4	Uncle	M	65	2	NA	117
A3243G	47	2	5	Aunt	F	NA	NA	NA	117
A3243G	47	3	1	Brother	M	36	23	5	117
A3243G	47	3	2	Index	F	34	38	5	117
A3243G	48	1	1	Grand grandmother	F	NA	NA	NA	117
A3243G	48	2	1	Sister of grandmother	F	NA	NA	NA	117
A3243G	48	2	2	Brother of grandmother	M	NA	NA	NA	117
A3243G	48	2	3	Sister of grandmother	F	NA	NA	NA	117
A3243G	48	2	4	Brother of grandmother	M	NA	NA	NA	117
A3243G	48	2	5	Twin sister of grandmother	F	NA	NA	NA	117
A3243G	48	2	6	Grandmother	F	76	3	NA	117
A3243G	48	2	7	Sister of grandmother	F	74	7	NA	117
A3243G	48	2	8	Sister of grandmother	F	NA	NA	NA	117
A3243G	48	2	9	Brother of grandmother	M	NA	NA	NA	117
A3243G	48	2	10	Sister of grandmother	F	57	NA	NA	117

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	48	2	11	Brother of grandmother	M	NA	NA	NA	117
A3243G	48	3	1	Cousin of the mother	F	NA	NA	NA	117
A3243G	48	3	2	Cousin of the mother	F	NA	NA	NA	117
A3243G	48	3	3	Mother	F	55	18	3	117
A3243G	48	3	4	Cousin of the mother	F	54	4	7	117
A3243G	48	3	5	Cousin of the mother	M	55	4	7	117
A3243G	48	3	6	Cousin of the mother	M	47	15	NA	117
A3243G	48	3	7	Cousin of the mother	M	45	16	NA	117
A3243G	48	3	8	Cousin of the mother	F	43	10	NA	117
A3243G	48	4	1	Index	M	30	45	18	117
A3243G	48	4	2	Sister	F	28	26	18	117
A3243G	48	4	3	Cousin	F	35	7	4	117
A3243G	48	4	4	Cousin	M	33	4	4	117
A3243G	48	4	5	Cousin	F	19	13	10	117
A3243G	49	1	1	Mother	F	NA	NA	NA	118
A3243G	49	2	1	Index	M	39	31	NA	118
A3243G	50	1	1	Index	F	52	16	NA	118
A3243G	50	2	1	Son	M	24	37	16	118
A3243G	51	1	1	Index	F	55	6	NA	118
A3243G	51	2	1	Son	M	25	44	6	118
A3243G	52	1	1	Grandmother	F	NA	NA	NA	119
A3243G	52	2	1	Mother	F	45	0	NA	119
A3243G	52	2	2	Aunt	F	NA	NA	NA	119

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	52	2	3	Uncle	M	NA	NA	NA	119
A3243G	52	2	4	Aunt	F	NA	NA	NA	119
A3243G	52	3	1	Index	M	21	36	0	119
A3243G	52	3	2	Sister	F	NA	NA	0	119
A3243G	52	3	3	Brother	M	NA	NA	0	119
A3243G	52	3	4	Cousin	M	NA	NA	NA	119
A3243G	52	3	5	Cousin	M	NA	NA	NA	119
A3243G	52	3	6	Cousin	F	NA	NA	NA	119
A3243G	52	3	7	Cousin	M	NA	NA	NA	119
A3243G	52	3	8	Cousin	M	NA	NA	NA	119
A3243G	53	1	1	Mother	F	NA	NA	NA	120
A3243G	53	2	1	Index	F	56	17.5	NA	120
A3243G	53	2	2	Index	M	44	22	NA	120
A3243G	53	2	3	Index	F	39	29	NA	120
A3243G	53	2	4	Index	F	37	16	NA	120
A3243G	53	3	1	Index	M	26	25	17.5	120
A3243G	54	1	1	Grandmother	F	NA	NA	NA	121
A3243G	54	2	1	Mother	F	47	23	NA	121
A3243G	54	2	2	Aunt	F	NA	NA	NA	121
A3243G	54	2	3	Aunt	F	40	0	NA	121
A3243G	54	2	4	Aunt	F	NA	NA	NA	121
A3243G	54	3	1	Index	M	27	58	23	121
A3243G	54	3	2	Sister	F	25	35	23	121

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	54	3	3	Brother	M	23	24	23	121
A3243G	54	3	4	Sister	F	8	66	23	121
A3243G	55	1	1	Mother	F	NA	NA	NA	122
A3243G	55	2	1	Brother	M	NA	NA	NA	122
A3243G	55	2	2	Brother	M	NA	NA	NA	122
A3243G	55	2	3	Index	M	12	NA	NA	122
A3243G	55	2	4	Brother	M	NA	NA	NA	122
A3243G	56	1	1	Mother	F	NA	NA	NA	122
A3243G	56	2	1	Sister	F	NA	NA	NA	122
A3243G	56	2	2	Brother	M	NA	NA	NA	122
A3243G	56	2	3	Brother	M	NA	NA	NA	122
A3243G	56	2	4	Index	F	52	NA	NA	122
A3243G	57	1	1	Grandmother	F	NA	NA	NA	122
A3243G	57	2	1	Mother	F	NA	NA	NA	122
A3243G	57	3	1	Index	M	10	54	NA	122
A3243G	57	3	2	Brother	M	NA	NA	NA	122
A3243G	57	3	3	Sister	F	NA	NA	NA	122
A3243G	58	1	1	Mother	F	NA	NA	NA	122
A3243G	58	2	1	Brother	M	NA	NA	NA	122
A3243G	58	2	2	Brother	M	NA	NA	NA	122
A3243G	58	2	3	Brother	M	NA	NA	NA	122
A3243G	58	2	4	Brother	M	NA	NA	NA	122
A3243G	58	2	5	Index	F	20	NA	NA	122

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	58	2	6	Index	F	28	20	NA	122
A3243G	59	1	1	Grandmother	F	NA	NA	NA	123
A3243G	59	2	1	Aunt	F	NA	NA	NA	123
A3243G	59	2	2	Mother	F	72	6	NA	123
A3243G	59	2	3	Aunt	F	NA	NA	NA	123
A3243G	59	2	4	Uncle	M	NA	NA	NA	123
A3243G	59	2	5	Aunt	F	NA	NA	NA	123
A3243G	59	2	6	Uncle	M	NA	NA	NA	123
A3243G	59	3	1	Sister	F	29	0	6	123
A3243G	59	3	2	Index	F	31	NA	6	123
A3243G	59	3	3	Sister	F	32	7	6	123
A3243G	59	3	4	Sister	F	34	30	6	123
A3243G	59	3	5	Sister	F	36	14	6	123
A3243G	59	3	6	Sister	F	NA	NA	6	123
A3243G	59	3	7	Brother	M	39	0	6	123
A3243G	59	3	8	Sister	F	NA	NA	6	123
A3243G	59	3	9	Brother	M	43	0	6	123
A3243G	59	3	10	Sister	F	39	2	6	123
A3243G	59	3	11	Sister	F	47	12	6	123
A3243G	59	3	12	Sister	F	48	0	6	123
A3243G	59	3	13	Cousin	F	NA	NA	NA	123
A3243G	59	3	14	Cousin	M	NA	NA	NA	123
A3243G	59	3	15	Cousin	M	NA	NA	NA	123

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	59	3	16	Cousin	F	28	0	NA	123
A3243G	59	4	1	Nephew	M	NA	NA	0	123
A3243G	59	4	2	Nephew	M	NA	NA	0	123
A3243G	59	4	3	Nephew	M	NA	NA	7	123
A3243G	59	4	4	Nephew	M	NA	NA	7	123
A3243G	59	4	5	Nephew	M	NA	NA	14	123
A3243G	59	4	6	Nephew	M	NA	NA	2	123
A3243G	59	4	7	Nephew	M	NA	NA	2	123
A3243G	59	4	8	Niece	F	24	0	0	123
A3243G	59	4	9	Nephew	M	28	0	0	123
A3243G	60	1	1	Mother	F	78	NA	NA	124
A3243G	60	2	1	Sister	F	47	NA	NA	124
A3243G	60	2	2	Sister	F	44	NA	NA	124
A3243G	60	2	3	Brother	M	41	NA	NA	124
A3243G	60	2	4	Index	F	37	13	NA	124
A3243G	60	2	5	Sister	M	NA	NA	NA	124
A3243G	61	1	1	Mother	F	60	NA	NA	124
A3243G	61	2	1	Sister	F	NA	NA	NA	124
A3243G	61	2	2	Brother	M	NA	NA	NA	124
A3243G	61	2	3	Sister	F	NA	NA	NA	124
A3243G	61	2	4	Index	M	23	1	NA	124
A3243G	61	2	5	Brother	M	NA	NA	NA	124
A3243G	62	1	1	Grandmother	F	NA	NA	NA	124

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	62	2	1	Mother	F	65	NA	NA	124
A3243G	62	3	1	Brother	M	NA	NA	NA	124
A3243G	62	3	2	Sister	F	40	NA	NA	124
A3243G	62	3	3	Index	F	38	14	NA	124
A3243G	62	3	4	Brother	M	NA	NA	NA	124
A3243G	63	1	1	Mother	F	NA	NA	NA	124
A3243G	63	2	1	Brother	M	NA	NA	NA	124
A3243G	63	2	2	Sister	F	NA	NA	NA	124
A3243G	63	2	3	Brother	M	NA	NA	NA	124
A3243G	63	2	4	Index	M	79	1	NA	124
A3243G	63	2	5	Sister	F	NA	NA	NA	124
A3243G	64	1	1	Mother	F	43	NA	NA	124
A3243G	64	2	1	Sister	F	40	NA	NA	124
A3243G	64	2	2	Sister	F	39	9	NA	124
A3243G	64	2	3	Index	M	35	5	NA	124
A3243G	64	2	4	Sister	F	32	4	NA	124
A3243G	65	1	1	Index	F	41	7.24	NA	125
A3243G	65	2	1	Son	M	15	11.7	7.24	125
A3243G	65	2	2	Son	M	NA	NA	7.24	125
A3243G	66	1	1	Mother	F	NA	0	NA	126
A3243G	66	2	1	Brother	M	NA	NA	0	126
A3243G	66	2	2	Brother	M	NA	29	0	126
A3243G	66	2	3	Index	F	22	28	0	126

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	66	3	1	Son	M	NA	53	28	126
A3243G	67	1	1	Mother	F	NA	20	NA	126
A3243G	67	2	1	Index	F	32	19	20	126
A3243G	67	2	2	Sister	F	NA	NA	20	126
A3243G	67	2	3	Index	F	27	17	20	126
A3243G	67	3	1	Daughter	F	NA	0	19	126
A3243G	67	3	2	Niece	F	NA	38	NA	126
A3243G	68	1	1	Mother	F	NA	NA	NA	126
A3243G	68	1	2	Aunt	F	NA	NA	NA	126
A3243G	68	2	1	Index	F	25	18	NA	126
A3243G	68	2	2	Cousin	M	NA	NA	NA	126
A3243G	68	2	3	Cousin	F	NA	NA	NA	126
A3243G	69	1	1	Grandmother	F	72	0	NA	127
A3243G	69	2	1	Mother	F	38	11	0	127
A3243G	69	2	2	Aunt	F	NA	NA	0	127
A3243G	69	2	3	Aunt	F	38	0	0	127
A3243G	69	2	4	Uncle	M	NA	NA	0	127
A3243G	69	2	5	Uncle	M	40	0	0	127
A3243G	69	2	6	Uncle	M	39	0	0	127
A3243G	69	3	1	Index	M	14	56	11	127
A3243G	69	3	2	Brother	M	8	65	11	127
A3243G	70	1	1	Grandmother	F	NA	NA	NA	128
A3243G	70	2	1	Mother	F	NA	NA	NA	128

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	70	2	2	Uncle	M	NA	NA	NA	128
A3243G	70	2	3	Aunt	F	NA	NA	NA	128
A3243G	70	2	4	Uncle	M	NA	NA	NA	128
A3243G	70	2	5	Uncle	M	NA	NA	NA	128
A3243G	70	3	1	Sister	F	46	25	NA	128
A3243G	70	3	2	Brother	M	NA	NA	NA	128
A3243G	70	3	3	Sister	F	43	20	NA	128
A3243G	70	3	4	Sister	F	41	24	NA	128
A3243G	70	3	5	Sister	F	40	27	NA	128
A3243G	70	3	6	Sister	F	39	27	NA	128
A3243G	70	3	7	Index	M	35	28	NA	128
A3243G	70	4	1	Niece	F	16	21	27	128
A3243G	70	4	2	Niece	F	14	33	27	128
A3243G	70	4	3	Niece	F	13	40	27	128
A3243G	70	4	4	Nephew	M	10	47	27	128
A3243G	70	4	5	Nephew	M	8	42	27	128
A3243G	71	1	1	Grandmother of the grandmother	F	NA	NA	NA	129
A3243G	71	2	1	Uncle of the grandmother	M	NA	NA	NA	129
A3243G	71	2	2	Aunt of the grandmother	F	NA	NA	NA	129
A3243G	71	2	3	Mother of the grandmother	F	NA	NA	NA	129
A3243G	71	3	1	Sister of the grandmother	F	NA	NA	NA	129

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	71	3	2	Brother of the grandmother	M	NA	NA	NA	129
A3243G	71	3	3	Brother of the grandmother	M	NA	NA	NA	129
A3243G	71	3	4	Grandmother	F	NA	NA	NA	129
A3243G	71	3	5	Brother of the grandmother	M	NA	NA	NA	129
A3243G	71	3	6	Sister of the grandmother	F	NA	NA	NA	129
A3243G	71	3	7	Sister of the grandmother	F	NA	NA	NA	129
A3243G	71	4	1	Uncle	M	NA	NA	NA	129
A3243G	71	4	2	Aunt	F	NA	NA	NA	129
A3243G	71	4	3	Uncle	M	NA	NA	NA	129
A3243G	71	4	4	Uncle	M	NA	NA	NA	129
A3243G	71	4	5	Aunt	F	NA	NA	NA	129
A3243G	71	4	6	Aunt	F	NA	NA	NA	129
A3243G	71	4	7	Mother	F	64	57	NA	129
A3243G	71	4	8	Aunt	F	NA	NA	NA	129
A3243G	71	4	9	Aunt	F	NA	NA	NA	129
A3243G	71	5	1	Index	M	38	87	57	129
A3243G	71	5	2	Index	M	36	89	57	129
A3243G	71	5	3	Brother	M	NA	NA	57	129
A3243G	72	1	1	Grandmother/Mother	F	NA	NA	NA	129
A3243G	72	2	1	Aunt/sister	F	NA	NA	NA	129
A3243G	72	2	2	Uncle?brother	M	NA	NA	NA	129

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	72	2	3	Aunt/sister	F	NA	NA	NA	129
A3243G	72	2	4	Index/Mother	F	NA	5	NA	129
A3243G	72	3	1	Index	F	16	25	5	129
A3243G	72	3	2	Sister	F	15	NA	5	129
A3243G	73	1	1	Mother	F	NA	4	NA	26
A3243G	73	2	1	Brother	M	NA	7	4	26
A3243G	73	2	2	Index	M	NA	24	4	26
A3243G	73	2	3	Index	M	NA	18	4	26
A3243G	74	1	1	Mother	F	NA	11	NA	26
A3243G	74	2	1	Index	M	NA	56	11	26
A3243G	74	2	2	Index	M	NA	65	11	26
A3243G	75	1	1	Sister/Mother	F	NA	8	NA	26
A3243G	75	1	2	Index	M	NA	12	NA	26
A3243G	75	2	1	Index	M	NA	23	8	26
A3243G	76	1	1	Mother	F	NA	NA	NA	130
A3243G	76	2	1	Index	F	52	NA	NA	130
A3243G	76	2	2	Brother	M	NA	NA	NA	130
A3243G	76	2	3	Brother	M	NA	NA	NA	130
A3243G	76	2	4	Brother	M	NA	NA	NA	130
A3243G	76	2	5	Brother	M	NA	NA	NA	130
A3243G	76	2	6	Brother	M	NA	NA	NA	130
A3243G	76	2	7	Sister	F	NA	NA	NA	130
A3243G	76	2	8	Sister	F	NA	NA	NA	130

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	76	2	9	Sister	F	NA	NA	NA	130
A3243G	76	2	10	Sister	F	67	2	NA	130
A3243G	76	3	1	Daughter	F	19	44	NA	130
A3243G	76	3	2	Son	M	NA	29	NA	130
A3243G	76	3	3	Son	M	NA	26	NA	130
A3243G	76	3	4	Cousin	F	NA	4	NA	130
A3243G	76	3	5	Cousin	M	NA	NA	NA	130
A3243G	76	3	6	Cousin	M	NA	NA	NA	130
A3243G	76	3	7	Cousin	F	NA	4	NA	130
A3243G	76	3	8	Cousin	M	NA	NA	2	130
A3243G	76	3	9	Cousin	M	NA	NA	2	130
A3243G	76	4	1	Second-cousin	M	NA	NA	4	130
A3243G	76	4	2	Second-cousin	F	23	48	4	130
A3243G	77	1	1	Grandmother/Mother	F	NA	NA	NA	131
A3243G	77	1	2	Grandmother/Mother /Aunt	F	NA	NA	NA	131
A3243G	77	2	1	Sister/Aunt	F	NA	NA	NA	131
A3243G	77	2	2	Index	F	65	5	NA	131
A3243G	77	2	3	Index	F	65	5	NA	131
A3243G	77	2	4	Index	M	61	16	NA	131
A3243G	77	2	5	Brother/Uncle	M	NA	NA	NA	131
A3243G	77	2	6	Index	F	56	19	NA	131
A3243G	77	2	7	Index	M	55	19	NA	131
A3243G	77	2	8	Index	M	51	17	NA	131

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	77	2	9	Index	M	52	13	NA	131
A3243G	77	3	1	Index	M	46	28	NA	131
A3243G	77	3	2	Brother	M	NA	NA	NA	131
A3243G	77	3	3	Index	M	41	34	NA	131
A3243G	77	3	4	Brother	M	NA	NA	NA	131
A3243G	77	3	5	Son/Brother/Uncle	M	NA	NA	5	131
A3243G	77	3	6	Index	F	48	5	5	131
A3243G	77	3	7	Daughter/Sister/Aunt	F	NA	NA	5	131
A3243G	77	3	8	Index	F	44	5	5	131
A3243G	77	3	9	Daughter	F	NA	NA	5	131
A3243G	77	3	10	Son	M	NA	NA	5	131
A3243G	77	3	11	Daughter	F	NA	NA	5	131
A3243G	77	3	12	Index	M	26	45	19	131
A3243G	77	3	13	Index	F	30	42	19	131
A3243G	77	4	1	Index	M	17	8	5	131
A3243G	77	4	2	Daughter/Sister	F	NA	NA	5	131
A3243G	77	4	3	Son	M	NA	NA	5	131
A3243G	78	1	1	Index	F	26	63.15	NA	132
A3243G	78	2	1	Son	M	3.5	68.9	63.15	132
A3243G	78	2	2	Son	M	4	71.3	63.15	132
A3243G	79	1	1	Mother	F	41	13	NA	133
A3243G	79	2	1	Index	M	NA	47	13	133
A3243G	79	2	2	Brother	M	14	34	13	133

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	79	2	3	Sister	F	10	0	13	133
A3243G	80	1	1	Mother	F	NA	0	NA	135
A3243G	80	2	1	Sister	F	NA	25	0	135
A3243G	80	2	2	Index	F	NA	30	0	135
A3243G	80	3	1	Daughter	F	NA	51	30	135
A3243G	81	1	1	Mother	F	NA	NA	NA	136
A3243G	81	1	2	Uncle	M	NA	NA	NA	136
A3243G	81	2	1	Index	F	NA	0	NA	136
A3243G	81	2	2	Sister	F	NA	5	NA	136
A3243G	81	2	3	Sister	F	NA	5	NA	136
A3243G	81	2	4	Sister	F	NA	10	NA	136
A3243G	81	2	5	Sister	F	NA	NA	NA	136
A3243G	81	3	1	Son	M	NA	NA	0	136
A3243G	81	3	2	Daughter	F	0	NA	0	136
A3243G	81	3	3	Fetus	NI	0	NA	0	136
A3243G	81	3	4	Nephew	M	15	NA	5	136
A3243G	81	3	5	Niece/nephew	Fetus	NA	NA	5	136
A3243G	81	3	6	Twin-Nephew	M	11	NA	5	136
A3243G	81	3	7	Twin-Nephew	M	11	NA	5	136
A3243G	81	3	8	Nephew	M	NA	80	5	136
A3243G	82	1	1	Grandmother	F	NA	NA	NA	136
A3243G	82	2	1	Mother	F	NA	20	20	136
A3243G	82	2	2	Aunt	F	NA	NA	NA	136

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	82	2	3	Aunt	F	NA	NA	NA	136
A3243G	82	2	4	Uncle	M	NA	NA	NA	136
A3243G	82	3	1	Brother	M	NA	NA	NA	136
A3243G	82	3	2	Brother	M	NA	NA	NA	136
A3243G	82	3	3	Index	F	NA	0	NA	136
A3243G	82	4	1	Miscarriaged child	NI	NA	NA	NA	136
A3243G	82	4	2	Daughter	F	0	NA	NA	136
A3243G	83	1	1	Index	F	NA	0	NA	136
A3243G	83	2	1	Son	M	NA	NA	0	136
A3243G	83	2	2	Daughter	F	NA	NA	0	136
A3243G	83	2	3	Son	M	0.75	NA	0	136
A3243G	83	2	4	Child	NI	0	NA	0	136
A3243G	84	1	1	Mother	F	NA	NA	NA	136
A3243G	84	1	2	Aunt	F	NA	NA	NA	136
A3243G	84	2	1	Brother	M	NA	NA	NA	136
A3243G	84	2	2	Index	F	29	34	NA	136
A3243G	84	3	1	Daughter	F	0	NA	34	136
A3243G	84	3	2	Child	NI	0	NA	34	136
A3243G	85	1	1	Mother	F	NA	10	NA	136
A3243G	85	2	1	Index	F	24	21	10	136
A3243G	85	2	2	Brother	M	NA	5	10	136
A3243G	85	2	3	Sister	F	NA	NA	10	136
A3243G	85	3	1	Miscarriage child	NI	0	NA	21	136

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	85	3	2	Daughter	F	0	NA	21	136
A3243G	85	3	3	Son	M	0	NA	21	136
A3243G	86	1	1	Grandmother	F	NA	NA	NA	137
A3243G	86	2	1	Uncle	M	NA	NA	NA	137
A3243G	86	2	2	Aunt	F	NA	NA	NA	137
A3243G	86	2	3	Mother	F	NA	NA	NA	137
A3243G	86	3	1	Cousin	M	NA	NA	NA	137
A3243G	86	3	2	Cousin	M	45	0	NA	137
A3243G	86	3	3	Cousin	F	42	37.8	NA	137
A3243G	86	3	4	Brother	M	36	36.7	NA	137
A3243G	86	3	5	Index	M	28	58.1	NA	137
A3243G	86	4	1	Nephew	M	21	35.4	37.8	137
A3243G	87	1	1	Grandmother/mother	F	NA	NA	NA	139
A3243G	87	2	1	Index	M	NA	NA	NA	139
A3243G	87	2	2	Index	M	NA	10	NA	139
A3243G	87	2	3	Index	F	57	20	NA	139
A3243G	87	2	4	Index	M	55	10	NA	139
A3243G	87	2	5	Uncle/brother	F	NA	NA	NA	139
A3243G	87	3	1	Sibling	NI	NA	NA	20	139
A3243G	87	3	2	Index	M	32	15	20	139
A3243G	88	1	1	Grandmother	F	NA	NA	NA	141
A3243G	88	2	1	Mother	F	53	5	NA	141
A3243G	88	2	2	Aunt	F	46	10	NA	141

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	88	2	3	Aunt	F	NA	NA	NA	141
A3243G	88	2	4	Aunt	F	NA	NA	NA	141
A3243G	88	2	5	Uncle	M	NA	NA	NA	141
A3243G	88	2	6	Uncle	M	NA	NA	NA	141
A3243G	88	3	1	Index	F	33	15	5	141
A3243G	88	3	2	Sister	F	32	15	5	141
A3243G	88	3	3	Sister	F	21	10	5	141
A3243G	89	1	1	Mother	F	54	10	NA	141
A3243G	89	1	2	Aunt	F	NA	NA	NA	141
A3243G	89	1	3	Aunt	F	NA	NA	NA	141
A3243G	89	1	4	Aunt	F	NA	NA	NA	141
A3243G	89	1	5	Aunt	F	NA	NA	NA	141
A3243G	89	1	6	Aunt	F	NA	NA	NA	141
A3243G	89	2	1	Index	M	26	30	10	141
A3243G	90	1	1	Mother	F	53	8	NA	138
A3243G	90	2	1	Index	F	30	35	8	138
A3243G	90	2	2	Brother	M	NA	NA	8	138
A3243G	90	2	3	Brother	M	NA	NA	8	138
A3243G	90	3	1	Son	M	10	49	35	138
A3243G	90	3	2	Son	M	7	49	35	138
A3243G	90	3	3	Son	M	4	72	35	138
A3243G	91	1	1	Grandmother	F	65	52.8	NA	142
A3243G	91	2	1	Mother	F	47	30.8	52.8	142

Type of mutation	Family no.	Generation no.	Individual no.	Relationship[a]	Gender	Age at sampling	Heteroplasmy level	Mother's heteroplasmy level	References
A3243G	91	2	2	Aunt	F	42	15.6	52.8	142
A3243G	91	2	3	Uncle	M	41	20.2	52.8	142
A3243G	91	2	4	Aunt	F	38	6.6	52.8	142
A3243G	91	2	5	Uncle	M	36	22.9	52.8	142
A3243G	91	3	1	Sister	F	23	34.9	30.8	142
A3243G	91	3	2	Index	F	22	65.7	30.8	142
A3243G	91	3	3	Cousin	M	20	3.7	15.6	142
A3243G	91	3	4	Cousin	F	15	1.3	6.6	142
A3243G	92	1	1	Index	F	33	33	NA	140
A3243G	92	2	1	Son	M	13	58	33	140
A3243G	92	2	2	Son	M	9	55	33	140
A3243G	92	2	3	Son	M	8	67	33	140
A3243G	93	1	1	Mother	F	NA	0	NA	140
A3243G	93	2	1	Index	M	15	6	0	140
A3243G	94	1	1	Index	F	48	8	NA	134
A3243G	94	1	2	Brother	M	47	12	NA	134
A3243G	94	2	1	Son	M	28	23	8	134

Output (Acknowledge the Thailand Research Fund)

International Journal Publication -

Application:

1. The manuscript in the topic “Difference in mtDNA heteroplasmy inheritance between protein coding and tRNA gene mutations” will be submitted to American Journal of human genetics or Mitochondrion

2. The manuscript in the topic “The pedigree model of human mtDNA heteroplasmy inheritance: how can we use it to estimate recurrence risk” will be submitted to American Journal of human genetics or Mitochondrion

…..Others:

The result of this study was presented as a poster in the National genetic conference 2013.