University Teaching Hospital Münster
Institute of Legal Medicine
- Director: Univ.-Prof. Dr. med. Bernd Brinkmann -

Natural radioactivity and human mitochondrial DNA mutations in Kerala (India)

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ABSTRACT

Natural radioactivity and human mitochondrial DNA mutations in Kerala (India)

Lucy Forster

The coast of Kollam in the south Indian state of Kerala contains the world's highest level of natural radioactivity in a densely populated area. It is widely debated whether this causes measurable genetic effects. Saliva was sampled from a total of 1012 individuals from 248 native families (covering 802 mtDNA transmissions). Three quarters of the samples were taken from the radioactive peninsula, and one quarter from 3km-distant, non-radioactive islands as a control population. In order to determine whether the maternal germline mutation rate is accelerated by the radiation, the control region of their mitochondrial DNA was sequenced and screened for mutations between mothers and their offspring. Both point mutations and homopolymeric length changes were found, and in each mutation case maternity was confirmed with a probability of >99% by typing nine autosomal loci. This dissertation reveals four main results: (1) Mothers with new mtDNA mutations in their saliva sample pass the mutant DNA to their children, demonstrating that saliva samples are ideal for tracing germline mtDNA mutations. (2) The families living in the radioactive area have significantly ($p<0.01$) more new point mutations than the control families. Insertion/deletion changes are also significantly increased, but it is uncertain how many of these changes are true DNA mutations. (3) The new mutations primarily affect nucleotide positions that have been hypervariable in the past 60,000 years of human mtDNA evolution. (4) Importantly for medical, forensic, and evolutionary genetics, none of the point mutations attained 100% fixation in any individual, within the two, three or four generations screened in this study. This finding largely explains the perceived discrepancy between "evolutionary" and "pedigree" mtDNA mutation rates.

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ZUSAMMENFASSUNG

Natürliche Radioaktivität und humane mitochondriale DNA Mutationen in Kerala (Indien)

Lucy Forster

Die Küste von Kollam im südindischen Staat Kerala beherbergt die weltweit höchste natürliche radioaktive Strahlung in einem dichtbesiedelten Gebiet. Es ist umstritten, ob diese Begebenheit eine meßbare genetische Auswirkung hat. Speichelproben wurden von 1012 Individuen aus 248 eingeborenen Familien entnommen, womit 802 mtDNA-Transmissionen erfaßt wurden. Drei Viertel dieser Proben stammten von der radioaktiven Halbinsel, und ein Viertel stammte von 3km entfernten, nichtradioaktiven Inseln und diente als Vergleichspopulation. Um festzustellen, ob die maternale Keimbahnmutationsrate durch die radioaktive Strahlung beschleunigt wird, wurde die Kontrollregion der mitochondrialen DNA sequenziert und auf Mutationsunterschiede zwischen Müttern und ihren Kindern überprüft. Sowohl Punktmutationen als auch homopolymerische Längenunterschiede wurden gefunden, und in jedem Mutationsfall wurde die Mutterschaft mit einer Wahrscheinlichkeit von >99% bestätigt, indem neun autosomale Loci typisiert wurden. Diese Dissertation ergibt vier Hauptresultate: (1) Mütter mit neuen mtDNA Mutationen in ihrer Speichelprobe vererben die mutierte DNA an ihre Kinder; dieses beweist, daß Speichelproben geeignet sind, um Keimbahn-mtDNA-Mutationen zu verfolgen. (2) Die Familien, die im radioaktiven Gebiet leben, haben signifikant ($p<0.01$) mehr neue Punktmutationen als die Vergleichsfamilien. Insertions-/Deletionsveränderungen sind auch signifikant erhöht, allerdings ist es unsicher, wie viele dieser Veränderungen echte DNA Mutationen darstellen. (3) Die Neumutationen sind hauptsächlich an Nukleotidpositionen anzutreffen, die sich bereits im Laufe der letzten 60000 Jahre als hypervariabel erwiesen haben. (4) Von Bedeutung für die medizinische, forensische und evolutionäre Genetik ist die Beobachtung, daß keine der Punktmutationen in einem Individuum zu 100% fixiert wurde innerhalb der zwei, drei oder vier Generationen, die in dieser Studie erfaßt wurden. Dieser Befund erklärt größtenteils die mutmaßliche Diskrepanz zwischen “evolutionären” und “familiären” mtDNA Mutationsraten.

Contents

Introduction

Chromosome lesions and cancer are well-known macroscopic results of ionising radiation (Rydberg 2001). Current research is therefore focussing on the effects of radiation on the DNA sequence itself and has recently revealed an intriguing multigenerational destabilisation in repetitive DNA loci in the germline of irradiated mice (Dubrova et al. 2000). In humans, similar long-term experimental irradiation and monitoring for point mutations across generations would be impractical and potentially unethical, so for this dissertation, advantage was taken of the unique natural setting in the south Indian state of Kerala. There, a coastal peninsula contains the world's highest levels of natural radioactivity in a densely populated area. This is due to the local abundance of monazite, a mineral containing 8–10.5% thorium dioxide. The radioactive strip on the peninsula measures only 10km by 1km, but supports a population of several thousand, whose traditional occupation is fishing (Grüneberg et al. 1966). Historical records reveal that the strip and its human population have existed for many centuries (Appendix 1). Radioactive and non-radioactive areas in the peninsula are easily distinguished by their colours: the radioactive sand is black, while the non-radioactive sand is white (Figure 1), which greatly simplifies the choice of sampling areas for radioactive and non-radioactive (control) population areas.

The biologically effective radiation dose received by the coastal population is 10000–12000μSv per year, approximately 10 times greater than the worldwide average. Studies in Kerala on rat morphology (Grüneberg et al. 1966), Down's syndrome (Kochupillai et

Figure 1: Radioactive black sand and non-radioactive white sand in Puthenthura, Kerala. Thousands of traditional fishing families have lived here for generations, exposed to the highest levels of natural radiation in the world. The radioactive sand contains monazite (cerium phosphate with ThO_2 inclusions) and appears black because it is associated with ilmenite. Monazite is washed down from the Western Ghats into the Arabian Sea, and is then deposited along approximately 10km of the sand bar shown in this photograph (Grüneberg et al. 1966).

al. 1976, Sundaram 1977), chromosomal aberrations (Cheriyan et al. 1999), congenital malformations (Jaikrishan et al. 1999), and cancer (Krishnan Nair et al. 1999) could not conclusively reveal significant abnormalities in residents, and direct DNA sequencing has never been performed in intergenerational radiation studies in Kerala or indeed elsewhere in the world.

In this PhD thesis, the non-coding mtDNA control region of 1012 Keralese individuals from 248 families was sequenced in order to directly determine whether lifelong exposure to high levels of natural radiation increases the mutation rate, and if so, whether its effects differ from long-term evolutionary mutational change. Mitochondrial DNA is inherited maternally and is therefore simple to trace within pedigrees; another technical advantage is the high copy number of several thousand per cell. In addition to the intrinsic medical, forensic, and evolutionary interest in mtDNA (Marchington et al. 1998, Anslinger et al. 2001, Pfeiffer et al. 2001, Lagerström-Fermér et al. 2001) the mtDNA control region is ideal for radiological studies for two reasons; first, the normal mtDNA control region mutation rate is high enough (Parsons et al. 1997) to provide mutations even in the control pedigrees; second, mutational hotspots are known in detail from evolutionary mtDNA studies, allowing a comparison between prehistoric DNA sequence evolution and current radiation-associated mtDNA evolution.

Subjects and Methods

Sampling

Saliva samples were obtained with informed consent from 746 healthy individuals from 180 families (spanning 600 mtDNA transmissions) living in the high-radiation seashores of Puthenthura (8°57.2′N 76°31.8′E), Neendakara (8°56.8′N 76°32.1′E), and Chavara (8°57.8′N 76°31.8′E). The samples were mainly taken from families living between the highway and sea, where the radioactivity is highest (Grüneberg et al. 1966). Furthermore, 266 control individuals (from 68 families spanning 202 mtDNA transmissions) were sampled 3km to the southeast from low-radiation islands off Mukkad (8°55.4′N 76°33.4′E), namely Fatima, Kanakkan, Puthen, and Arulappan islands. Nine of the families were sampled from Mukkad itself and nine from Saktikulamkara (8°55.4′N 76°32.5′E). The sampling locations are indicated in Figure 2.

Individuals were selected for sampling if they passed the criteria of lifelong residence (or residence of at least 20 years) of themselves and of their maternal ancestors at the sampling location, in order to exclude migrants between high- and low-radiation areas in our study. In practice, few such recent or ancestral migrants were encountered. The inhabitants of the high- and low-radiation areas are phenotypically, culturally, and linguistically indistinguishable, and the same major mtDNA branches were found in the radiation and in the control areas (Figure 3 and Table 1).

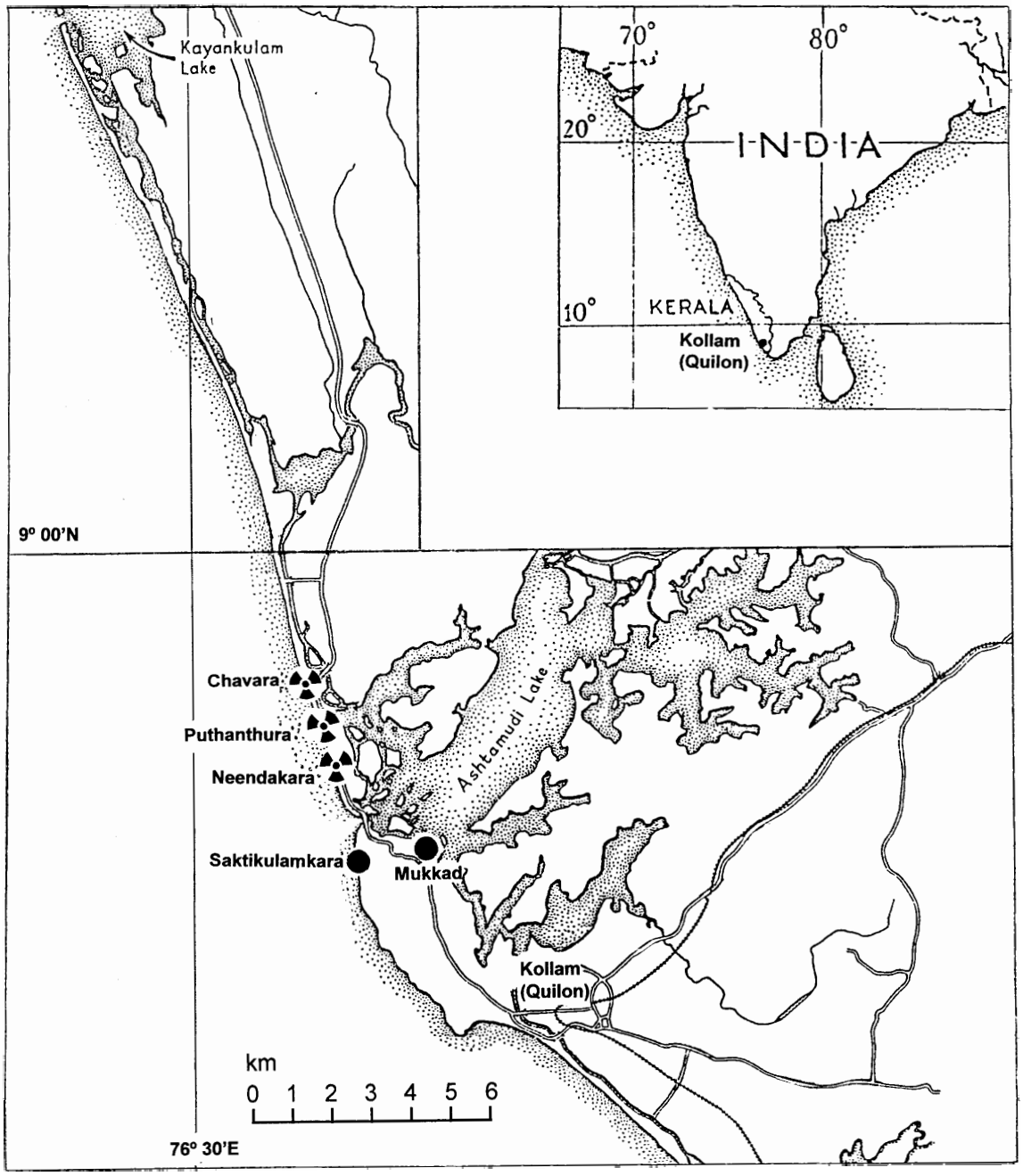

Figure 2: High-radiation and low-radiation localities sampled in this study.
The radioactivity in the peninsula increases from Kayankulam Lake to Ashtamudi Lake, with a peak around Chavara. The control samples were taken mainly from four lake islands off Mukkad, and partly from the white sand (i.e. non-radioactive) seashore of Saktikulamkara. The bridge across the mouth of Ashtamudi Lake was built in the 1920s. The district capital Kollam (formerly Quilon) is shown for orientation. This map is based on the ones published by Grüneberg et al. (1966); however, their original maps are incorrect, so the map presented here has been amended by consulting high-resolution colonial maps available in Cambridge University Library.

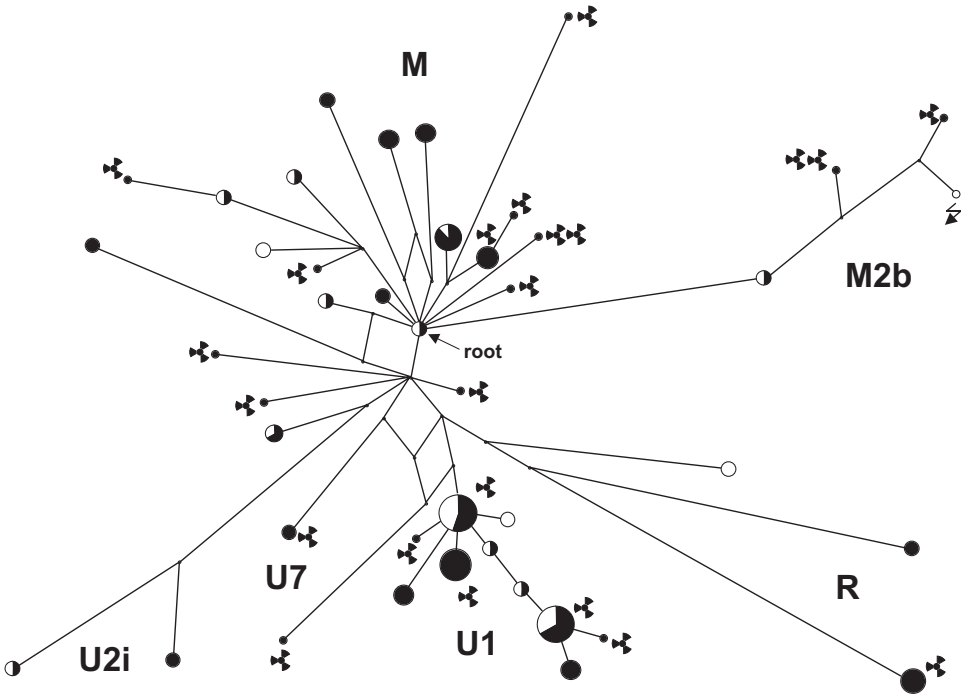

Figure 3: Skeleton network of Keralese mtDNA. Black shading in the circles indicates samples from the high-radiation area, and white shading those from the low-radiation area. Links represent mutations and circles mtDNA types, the circle size corresponding to the number of families with that type. Mutant family mtDNA types in the high- and low-radiation areas are marked by radiation symbols and a flash symbol, respectively. The root and the mtDNA groups M, U etc. are indicated in accordance with Kivisild et al. (1999). The network encompasses 203 out of the total of 248 families, by selecting mtDNA types occurring more than once and adding the mutant family mtDNA types. It was calculated with Network 3.110 (http://www.fluxus-engineering.com) by using sequentially the RM option with $r = 2$ and the MJ option with $\varepsilon = 1$, followed by post-processing to remove non-parsimonious reticulations in M2b. The mtDNA sequence considered ranged from nps 15990–16391 and from nps 35–465. Length polymorphisms around np16189 and np309 were disregarded, as was variation at nps 152 and 195. Other hypervariable positions were assigned half weights: nps 16129, 16189, 16311, 16362, and 146, and a transversion at np16318 was assigned triple weight.

Table 1: mtDNA clades and radiation-associated mutations

clade*	control absolute	control percent	radioactive absolute	radioactive percent	mutations
M	4	6%	24	13%	5
M1	0	0%	10	6%	
M2b	4	6%	4	2%	4**
M3	1	1%	1	1%	
M4	2	3%	10	6%	2
M5	1	1%	3	2%	
R	2	3%	15	8%	2
R1	0	0%	1	1%	
U1	46	68%	79	44%	5
U2i	3	4%	13	7%	
U7	0	0%	5	3%	2
other	5	7%	15	8%	3
Total	**68**	**100%**	**180**	**100%**	**23**

*clades as defined by Kivisild et al. 1999
**includes the single mutation in the controls

The residents in both areas are mainly Hindu (non-Brahmin) who nevertheless eat fish. The average sampling time depth is 2.9 generations in both areas (Appendix 2). Generation times were derived from Appendix 3. The average mtDNA generation times (measured at birth rather than at conception) in the high- and low-radiation areas are also very similar: the sample generation time (based on all sampled mothers) is 25.0 (SD 6.1) and 26.5 (SD 7.2) years, respectively, and the long-term generation time (based on dead mothers and post-menopausal mothers >55 years old) is 29.7 (SD 7.4) and 31.6 (SD 7.8) years, respectively. The Keralese mtDNA generation time of about 30 years may appear high, but is identical to the pre-industrial Danish, north German, French Canadian and Icelandic averages (Forster 1996, Tremblay and Vézina 2000, Sigurðardóttir et al. 2000).

Radiation dose estimation

In general, the bulk of natural radioactive dose received by soft tissues such as the gonads derives from three sources: terrestrial, dietary, and cosmic radiation, altogether amounting to an average of 1100μSv per year in Germany (Deutscher Bundestag 1988) for example. Lung dose may well be considerable, but can be disregarded as the focus is on the gonad dose. In Chavara, Puthanthura and Neendakara the terrestrial radioactive dose (γ-radiation emitted in the ^{232}Th series) has been measured by personal dosimetry and averages 9000μSv, 8000μSv, and 6500μSv, respectively (coastal sections 6-1, 6-2, and 6-3 in Gopal-Ayengar et al. 1972, Sunta 1993). Gross dietary radiation caused by ^{232}Th (and measured by its daughter nuclide ^{228}Ra) is 162pCi per day in the high-radiation peninsula (Mistry et al. 1970, Paul et al. 1982), but the corresponding biological dietary dose is not available in the literature. Therefore the conversion was calculated as follows. Eisenbud and Gesell (1997) give the conversion factor for ^{226}Ra from μCi per time unit to rem (1 rem = 10^{-2}Sv) per time unit as 25, which we take as an approximation for ^{228}Ra after multiplying by a factor of 5/2.2 to include the α decays of the daughter nuclides of the short-lived ^{220}Rn, whose half-life of 56 seconds is not long enough to allow diffusion out of the body. However, this conversion factor would refer only to bone dose (radium as an alkaline earth is preferentially deposited in bone). According to ICRP (1973), the soft tissue receives about 10% of whole body radium, thus the bone dose was divided by 10 and a soft tissue biological dose of about 3000μSv per year was obtained. Dietary ^{40}K intake (Mistry et al. 1970) contributes another 250μSv according to the ^{40}K conversion factor (Eisenbud and Gesell 1997). Cosmic radiation at equatorial latitudes contributes about 350μSv at sea level (UN 1962). In summary, the gonadal dose in the three high-radiation localities would amount to 10000–12000μSv per person per year, approximately 10 times greater than in monazite-free areas.

DNA typing

Within each family, only samples covering the maximum number of transmissions were sequenced (for example in the family shown in Figure 4, only individuals I.1, II.3, III.1, and III.2 were initially screened). When mutations were observed, all family members were sequenced.

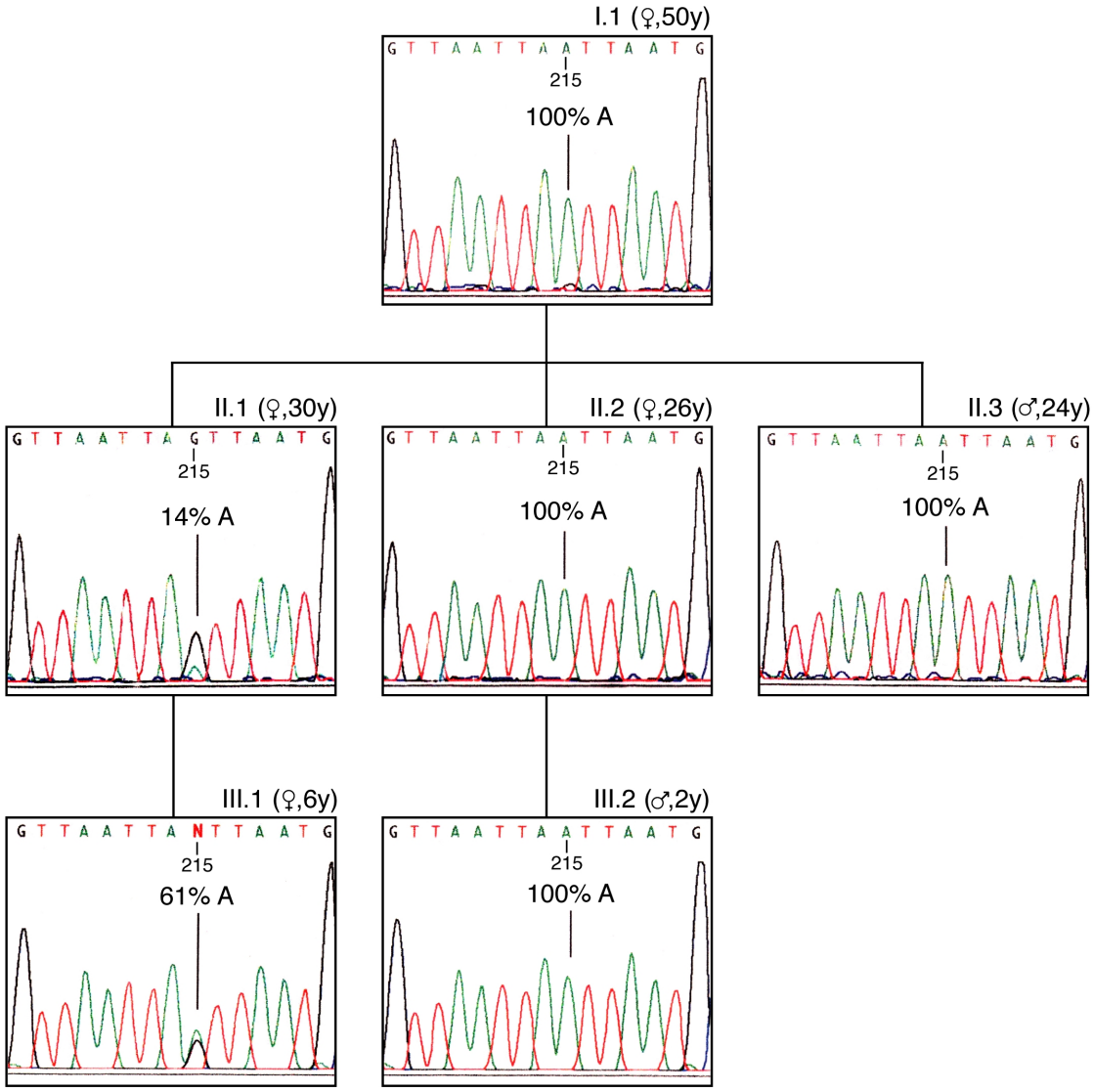

Figure 4: Inheritance of a new mtDNA point mutation. Each member of this family (number 184) was born and lived exclusively in the high-radiation peninsula of coastal Kerala, India. The percentages of the new nucleotide at np215 were determined by gravimetrically comparing the reduction of the A-peak area relative to a neighbouring reference peak in this forward sequencing reaction. Analogous evaluation of the reverse sequencing reactions (not shown) yielded similar values, both agreeing with counts of bacterially cloned mtDNA amplicons.

DNA was extracted from the dried saliva by the chelex method. Nucleotide positions (nps) 15971 to 00484 (numbering as in Anderson et al. 1981) were amplified by PCR using primers F15971 and R00484, and then sequenced as detailed in the following two protocols.

PCR amplification using F15971 and R00484
This protocol produces a 1100bp PCR-amplicon at nps 15971-00484 on human mtDNA.

Reaction Mix

2 µL	template (10 ng/µL)
1.5 µL	$MgCl_2$ (50 mM)
1 µL	BSA (2 µg/µL)
2.5 µL	10x buffer
2 µL	dNTPs (10 mM)
0.1 µL	primer F15971 (100pmol/µL)
0.1 µL	primer R00484 (100pmol/µL)
0.25 µL	Taq polymerase (Eurogentec)
H_2O ad 25µL	

Thermocycling
Biometra Thermocycler
4°C for 10 mins while loading samples from ice and heating lid
94°C – 30 sec

Then 32 cycles of
94°C – 20 secs
and either
56°C – 12 sec
72°C – 90 secs

Final extension
72°C – 30 min
Then hold at 4–10°C

F15971: 5'-TTA ACT CCA CCA TTA GCA CC-3'
R00484: 5'-TGA GAT TAG TAG TAT GGG AG-3'

The amplicon was purified by excision from agarose gel after electrophoresis. This purification step ensured a low baseline in the following sequencing reaction, performed with the Perkin Elmer Big Dye Terminator kit using F15971 and R00484 as well as R16410 (5'-GAG GAT GGT GGT CAA GGG AC-3') and F00015 (5'-CAC CCT ATT AAC CAC TCA CG-3'), as detailed in the following protocol.

MtDNA Sequencing using F15971 and R00484

This protocol produces an approximately 750-nucleotide sequence from a 1100bp PCR-amplicon at nps 15971-00484 on human mtDNA.

F15971 Reaction Mix (Forward sequence)

4 µL	template (10 ng/µL)
4 µL	Big Dye version2
1 µL	primer F15971 (7 pmol/µL) (i.e. 1:14 dilution of 100 µL stock)
H_2O ad 20 µL	

F15971 Thermocycling

Use thin 0.2 or 0.5mL tube

25 cycles of
96°C – 15 secs
50°C – 5 secs
60°C – 120 secs

Then hold at 4–10°C

R00484 Reaction Mix (Reverse sequence)

4 µL	template (10 ng/µL)
4 µL	Big Dye version2
1 µL	primer F15971 (10 pmol/µL) (i.e. 1:10 dilution of 100 µL stock)
H_2O ad 20 µL	

R00484 Thermocycling

Use thin 0.2 or 0.5mL tube

25 cycles of
96°C – 15 secs
58°C – 30 secs
58°C – 120 secs

Then hold at 4–10°C

F15971:	5'-TTA ACT CCA CCA TTA GCA CC-3'
R00285:	5'-GTT ATG ATG TCT GTG TGG AA-3'
R16410:	5'-GAG GAT GGT GGT CAA GGG AC-3'
F00015:	5'-CAC CCT ATT AAC CAC TCA CG-3'

Occasionally primers F00015, R16410 and R00285 were used, in the same conditions.

The sequences were analysed on an ABI Prism 310 Genetic Analyser, and the sequence was determined at least from nps15990-16390 and from nps35-465. Each sample was sequenced twice, except when a mutation was discovered, in which case the sample was re-extracted, re-amplified and sequenced a third and fourth time.

Mutation analysis

New point mutations manifest themselves as heteroplasmic nucleotides and were scored with the Sequence Navigator and then always rechecked manually. When heteroplasmy was observed, the proportions of both the mutant and the original nucleotide were determined gravimetrically using an internal reference peak area (Hühne et al. 1998). Each peak area was measured three times by threefold photocopying, enlarging and weighing. Furthermore, mutant and original nucleotide peak areas were always determined from forward and reverse sequencing reactions from two independent PCR amplifications; the error was usually found to be less than 15% (in one case it was 17%), nevertheless only peak area changes of more than 20% were considered as real changes to further minimise any risk of false positives. The gravimetric method relies on the low baseline of BigDye Terminator chemistry on the ABI 310 Genetic Analyser and was confirmed in the Freiburg laboratory by conventional bacterial cloning (Lutz et al. 2000) of 100 PCR amplicons for a sample (149.2) containing two heteroplasmic mutations at nps 144 and 152. In this sample, the two independent gravimetric measurements yielded 39% and 40% for 144C (cloning yielded 49% 144C), and 56% and 58% for 152C (cloning yielded 52% 152C). An alternative estimation of a peak area A can be calculated on the basis of the width $2x$ of the peak at half its height h:

$$A = \sqrt{\frac{\pi}{\ln 2}} xh$$

However, this approach was found to be less accurate than gravimetry.

If putative mtDNA point mutations were observed within families, maternity testing was performed using the nonaplex STR kit AmpFlSTR Profiler (PE Applied Biosystems) for all but two cases (in which the sample had been exhausted). For the numerous mtDNA insertion mutations, only selected families were subjected to maternity testing. The details are shown in Appendix 6. Maternity was accepted if the probability exceeded 99%.

Incidents of intergenerational change in DNA length at nps303–309 were also determined. In the Cambridge reference sequence, this tract comprises 7 cytosines, but worldwide, as in Kerala, around 50% or more of humans have 8 cytosines. As a rule, if there are 8 or more cytosines in this tract, then multiple length variants (7C, 8C, 9C, etc.) develop and co-exist in one individual, a state known as length heteroplasmy. The invariable thymine peak at np310 then appears as virtual multiple peaks, with the highest virtual thymine peak indicating the most common (modal) cytosine stretch length. Mutation events were screened at two levels: firstly, changes in the modal C allele length were scored as a change of mtDNA type, although such changes can be simply due to unequal inheritance of existing C alleles rather than indicative of a new insertion or deletion of a cytosine at the DNA

level. Nevertheless, this count is forensically relevant because a difference in modal length between two DNA samples might be considered a reason for excluding a maternal relationship between two such samples. Secondly, new mutations were counted if a mutation unambiguously occurred in the DNA itself, namely if a completely new C-allele was observed which was not present in the mother. In practice, this means the mother would be homoplasmic for one allele (i.e., the 7C allele or a shorter allele) and the child would have a different allele (a new allele, which is typically longer and heteroplasmic), or vice-versa. This count is only a lower limit for the real number of new C insertions or deletions, because the majority of newly generated alleles are probably masked by heteroplasmy, as explained in the Results.

Statistical methods

Phylogenetic analyses were carried out using the software package Network 3.111, available free at http://www.fluxus-engineering.com. The package comprises two distinct methods, reduced median networks and median-joining networks, both of which aim to reconstruct the evolutionary tree of a molecule, contained in a network which identifies and displays evolutionary parallelisms as reticulations. As is usual with large mtDNA control region data sets, it was necessary to downweight certain nucleotide positions known to be hypervariable (Figure 3).

Comparisons between new mutations observed in the radioactive and the control areas were evaluated with a χ^2 test, as available for example at http://www.psycho.uni-osnabrueck.de/ggediga/www/w3lib/2x2tab.htm.

Maternity probabilities were determined by the serology team in Münster (see Acknowledgements) by typing a set of nine STR loci, in cases where an mtDNA mismatch between a mother and her child was present, indicating either a mutation event or an unreported adoption. Fathers were generally not sampled, so the maternity probability was calculated solely on the STR results for mother and child as follows (Hummel 1971).

For Ch: a/b and M: a/a; or Ch: a/a and M: a/b

$$P = \frac{1}{1 + 2 \times f(a)}$$

For Ch: a/a and M: a/a

$$P = \frac{1}{1 + 1 \times f(a)}$$

For Ch: a/b and M: a/c

$$P = \frac{1}{1 + 4 \times f(a)}$$

For Ch: a/b and M: a/b

$$P = \frac{1}{1 + 4 \times \frac{f(a) \times f(b)}{f(a) + f(b)}}$$

where *f(a)*, *f(b)*, and *f(c)* are the population frequencies (for US Caucasoids, as given in the AmpFlSTR Profiler manual) of alleles a, b, and c. These probability formulae are for individual STR loci; they are converted into an overall probability for the nonaplex by multiplication :

$$P = \frac{1}{1 + p_1 \times p_2 \times ... \times p_9},$$

where each p_i is the second addend in the denominator of the appropriate individual probability formula above.

The maternity probability *P* (expressed as a percentage) states in how many cases out of 100 one would be correct in claiming the potential mother M to be the true mother of the child Ch. The implicit and impartial assumption is that half of the women analysed in maternity cases are not the mother of the child, which is a conservative assumption in the Kerala cases, as only 3 false mothers were found in 248 pedigrees (see Results).

Results

Observed adoptions

Originally, 179 families were sampled in the radioactive area. From this sample, maternity testing (Appendix 6) of families with "mutant" mtDNA revealed three undisclosed adoptions (97b.2, 215.2, 237.b3). The first two individuals were then excluded entirely from the pedigree mutational analyses. The third adoption (237.b3) was included as a separate family because that adopted individual had her own offspring. This yielded a total of 180 irradiated families (746 individuals spanning 600 transmissions) in the analysis, as reported in the Subjects and Methods section. Without this maternity testing, erroneous results would have been obtained, wrongly suggesting 25 full "mutations" instead of none. The fast mutation rates for mtDNA reported in other pedigree studies lacking maternity tests may therefore be excessive (e.g. Heyer et al. 2001, Sigurðardottir et al. 2000).

Observed point mutations

In the genetically related family members, 22 partial (heteroplasmic) mutations were observed in 600 high-radiation transmissions, and only 1 partial mutation in 202 low-radiation transmissions (Appendix 5), significantly more at $p<0.01$ (χ^2 test). An example of an mtDNA mutation passed down in a pedigree is shown in Figure 4. In the 22 high-radiation mutations, 18 families had 1 heteroplasmic position and 2 families had 2 heteroplasmies (one family at nps 144 and 152, and another family at nps 16189 and 16272). It is striking that only heteroplasmic mutations were observed, and that furthermore no mutation was observed to attain fixation in any individual even after sequencing up to 2 descendant generations. Previous pedigree studies yielding implausibly fast mtDNA mutation rates lack either maternity tests (see above) or an adequate differentiation between full and heteroplasmic mutations and may therefore tend towards overestimation. All mutations were heteroplasmic, in two cases even two heteroplasmies per individual was observed. Bacterial cloning (Appendix 7) of one of these individuals (149.2) revealed that in 100 clones of his mtDNA, 48 had the mother's mtDNA type 144C-152T, one clone had the type 144C-152C, and 51 clones had the type 144T-152C. Twenty-one of the clones contained additional point mutations, which may have occurred during the PCR/cloning process. It is thus possible that the single type 144C-152C may in fact be a cloning artifact. In conclusion, the two mutations probably happened on different mtDNA molecules in the maternal lineage, in agreement with the double-mutation case analysed by Howell et al. (1996).

The possibility can be excluded that mostly somatic mutations are being observed rather than germline mutations by analysing saliva, since in 12 out of 16 mutation cases where a sample from a descendant of the mutant mother is available, the mutant nucleotide was passed down heteroplasmically to the descendent in clearly detectable amounts (>20%). In the remaining 4 cases (families 80, 83, 84 and 190) the descendants are homoplasmic for one of the maternal nucleotides, so that transient somatic mutations cannot be ruled

Table 2: Ancient mutations in the Eurasian mtDNA tree and new mutations in Kerala families.

Nucleotide position (transitions unless specified)	Evolutionary mutations*	High-rad. mutations	Low-rad. mutations
152	16	3 (C/T)	
16189	12	3 (C/T)	
16362	12		
195 (10C&1A)	11	1 (C/T)	
146	10		
16129, 16311	6		
16093	5	2 (C/T)	
150, 200, 16304	5		
228	4	1 (A/G)	
16274, 16278, 16126	4		
189	3	1 (A/G)	
194	3	1 (C/T)	
93, 151, 199, 204, 16179, 16234, 16256, 16266, 16357, 16390	3		
16223	2	1 (C/T)	
207	2	1 (A/G)	
72, 73, 143, 153, 185, 188, 198, 225, 234, 239, 246, 247, 263, 16134, 16163, 16193	2		
16218, 16239, 16249, 16255, 16289, 16292, 16294, 16318 (G&T), 16319, 16325, 16356	2		
214	1	1 (A/G)	
291	1	1 (A/T)	
16291	1	1 (C/T)	
16320	1		1 (C/T)
55, 56T, 65.1T, 66T, 95C, 114del, 240, 249del, 250, 257, 285, 295, 16095, 16104,	1		
16111, 16162, 16166, 16172, 16174, 16176, 16181, 16183, 16186, 16187, 16188,	1		
16192, 16207, 16209, 16213, 16221, 16224, 16227, 16233, 16245, 16248, 16261,	1		
16263, 16265C, 16270, 16270.1C, 16280, 16286, 16288, 16290, 16295, 16296, 16298,	1		
16302, 16316, 16324, 16327A, 16335, 16336, 16344, 16346C, 16348, 16352, 16353,	1		
16354, 16366	1		
144	0	1 (C/T)	
215	0	3 (A/G)	
16272	0	1 (A/G)	
All other nucleotides within nps16093—16390 and within nps35—315	0		
Total mutations	**267**	**22**	**1**

*Evolutionary mutations are counted from mtDNA trees of Macaulay et al. (1999) and of Fig. 3.
Length changes at nps 16182/3 and 309 are disregarded.

out. It can furthermore be excluded that mtDNA branch-specific events are being observed, as the mtDNA profiles of the high- and low radiation areas are similar, and in any case the observed mutations are not restricted to any particular mtDNA branch (Table 1 and Figure 3).

The radiation-associated mtDNA mutations in this study share both hallmarks of mutations reconstructed in the evolutionary tree of Eurasian mtDNA (Table 2). Firstly, both evolutionary and radiation-associated mutations are strongly biased towards transitions. According to Table 2, within the mtDNA region considered here, evolutionary mutations consist of 95.5% transitions, 3.0% transversions, and 1.5% insertions/deletions. The radiation-associated mutations consist of 95.5% transitions and 4.5% transversions. Secondly, the evolutionary and radiation-associated mutations occur predominantly at the same nucleotide positions (Figure 5).

This visual impression can be quantified by comparing the nucleotide mutation rates. The average evolutionary mutation rate is 0.46 mutations per nucleotide position (267 mutations in 579 nucleotides of HV1 and HV2). If radiation-associated mutations were distributed randomly across the sequence, their mutation rate would be the same, namely 0.46. However, the radiation-associated mutations have hit nucleotide positions which mutate,

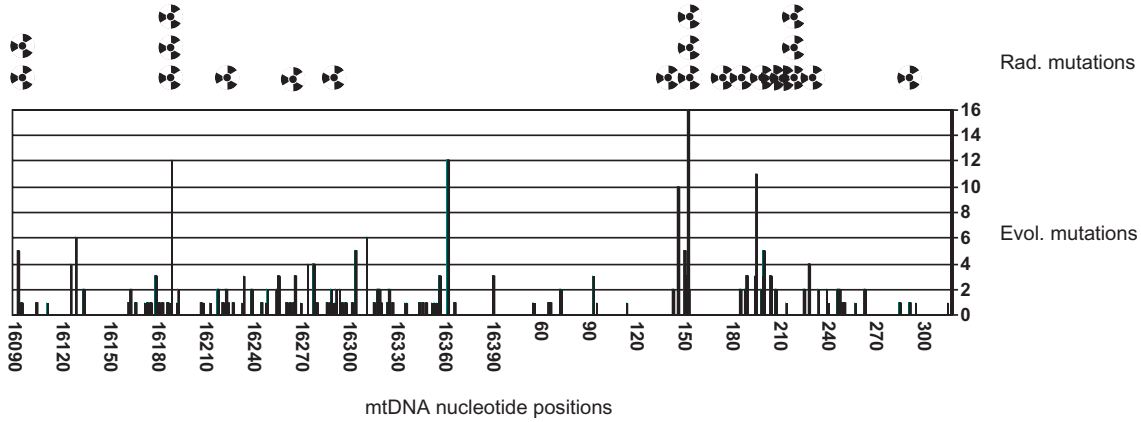

Figure 5: Ancient mutations in the Eurasian mtDNA tree and new mutations in Kerala families. The horizontal axis represents the 579 nucleotide positions of the mtDNA control region considered here (nps 16093–16390 and nps 35–315). The column heights are proportional to the absolute number of evolutionary mutations observed at each nucleotide position in a Eurasian mtDNA tree (Macaulay et al. 1999 and Figure 3). The radiation symbols mark the nucleotide positions observed to mutate in the families living in the high-radiation area of Kerala. Multiple mutations at a nucleotide position are indicated by a corresponding number of vertically placed radiation symbols. All values for this figure are taken from Table 2. A probable pedigree mutation at the evolutionary hotspot np146 in family 236 (Appendix 2) is omitted because the mutation is not clearly above the 20% threshold (Appendix 5).

on average, 12 times faster during evolution (mutation rate of 5.55, based on 122 evolutionary mutations at the 22 radiation-associated nucleotide positions). There are two striking exceptions to this rule: np16362 is an evolutionary hotspot (Table 2 and Figure 5) yet it has not been observed to mutate in the Kerala pedigrees. Conversely, np215 is rarely observed to mutate in evolutionary studies, yet in the Kerala pedigrees it has mutated three times, on three independent mtDNA evolutionary branches (Appendix 5).

Maternal age does not seem to be a major factor influencing the mutation probability, although the data available to assess this question are limited: in a total of 21 mutated pedigrees, a DNA sample from the non-mutant mother is available in only 7 cases (6 irradiated pedigrees and 1 control pedigree). The average age at conception for the 6 mutant irradiated mothers is 24.7 years, which is similar to the average irradiated sample generation time of 25.0 years. Indeed, 5 of the 6 mutant mothers are below this average age. The only mutant control mother was 27.25 years at conception of her mutated daughter, not remarkably different from the average control sample generation time of 26.5 years.

Observed insertion/deletion mutations

The cytosine stretch at nps303-309 (see the example in Figure 6) was screened for C-insertions/deletions. This region is unusual in the mtDNA genome because very often, different DNA lengths exist in one individual, making the mtDNA type heteroplasmic. Compare in Figure 6 the heteroplasmic mother I.1 with her homoplasmic daughter II.2. In this thesis it is confirmed that as a rule with few exceptions, length heteroplasmy occurs when there are more than 7 cytosines in the tract (see Table 3).

Table 3: mtDNA alleles at the C-tract nps303–309 in 250 unrelated Keralese.			
	High radiation	**Low radiation**	**notes**
3 C at nps303-309	0	1	homoplasmic
4 C at nps303-309	0	0	not observed
5 C at nps303-309	0	1	homoplasmic
6 C at nps303-309	8	0	homoplasmic
7 C at nps303-309	60	11	homoplasmic, except types 19, 109, 122, 183
8 C at nps303-309	63	31	***heteroplasmic***
9 C at nps303-309	48	24	***heteroplasmic***
10 C at nps303-309	3	0	***heteroplasmic***
Total	**182***	**68**	

*includes two adopted children not used for the pedigree study

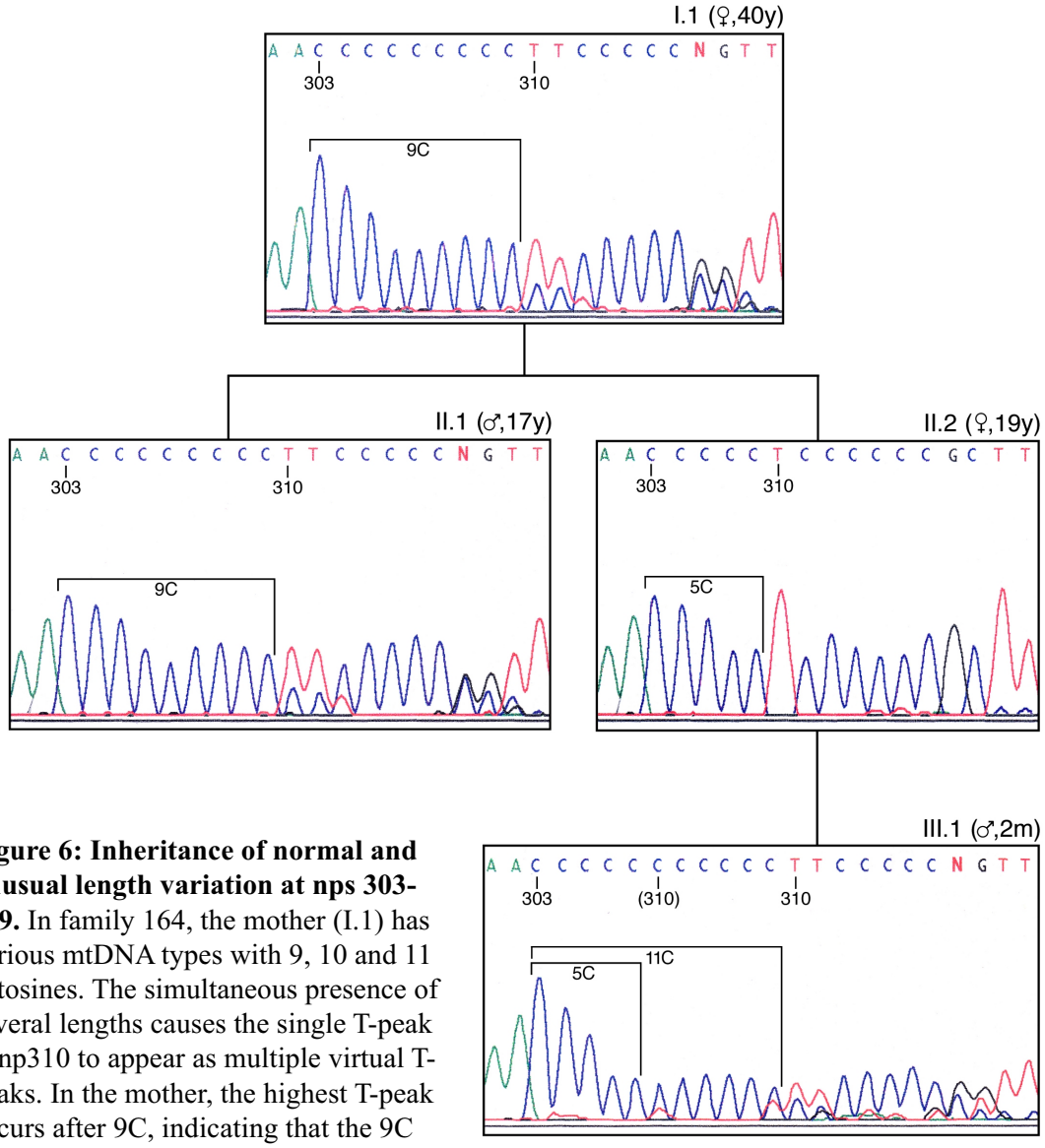

Figure 6: Inheritance of normal and unusual length variation at nps 303-309. In family 164, the mother (I.1) has various mtDNA types with 9, 10 and 11 cytosines. The simultaneous presence of several lengths causes the single T-peak at np310 to appear as multiple virtual T-peaks. In the mother, the highest T-peak occurs after 9C, indicating that the 9C allele is predominant. In one daughter (II.1), the proportions of length variants change slightly (the second virtual T peak, indicating a stretch of 10 cytosines, is now nearly as high as the first T peak). The other daughter (II.2) shows a remarkable deletion of 4 cytosines in her saliva sample, and no length heteroplasmy at all. The granddaughter (III.1) seems to have inherited the deletion as a minor variant, whereas most of her mitochondria have 11 cytosines. Her reverse sequence however does not show the minor variant.

When screening for new mutations at nps303-309 by comparing mothers and their daughters, it is important to distinguish two potential "mutation" mechanisms: "mutation" by drift and mutation by nucleotide insertion or deletion.

1. "Mutation" by drift is exemplified in Figure 6. The mother (I.1) has predominantly 9C in her mtDNA, but also some mtDNA molecules with 10C and 11C (as can be seen by the multiple minor T peaks at np310). One daughter (II.1) also has predominantly 9C, but in her the proportion of 10C has increased (the two T peaks are nearly the same height). If the proportion of 10C had increased even more, then 10C would have become the predominant molecule in the daughter (II.1) and, in forensic notation, the daughter would differ from the mother by a "mutation", even though an insertion or deletion has probably not taken place.

According to this forensic "mutational" notation, in 600 high-radiation transmissions, mothers and offspring differed in 35 cases by their modal C-length in the C-stretch between np303 and np309 (Appendix 2). Since in some cases the offspring's modal C differed from the mother's by not only a single nucleotide position but by two positions or even four positions, the total number of shifted C-positions in the irradiated families is 42 modal C shifts in 600 transmissions. In 202 low-radiation transmissions, only 5 modal C-length differences between mother and offspring were observed (see Table 4).

Table 4: Changes at the C-tract nps303-309 within Keralese pedigrees

	High-radiation	**Low-radiation**
1. Shifts in predominant allele (potential mutations)		
families witnessing allele shifts	24 of 180 families	5 of 68 families
children differing from their mother	35 in 600 transmissions	5 in 202 transmissions
differing by 1-nucleotide shifts	30 in 600 transmissions	5 in 202 transmissions
differing by 2-nucleotide shifts	4 in 600 transmissions	0 in 202 transmissions
differing by 3-nucleotide shifts	0 in 600 transmissions	0 in 202 transmissions
differing by 4-nucleotide shifts	1 in 600 transmissions	0 in 202 transmissions
total number of shifted C-positions	42 in 600 transmissions	5 in 202 transmissions
cytosines gained and lost after shift	30 gains, 11 losses, 1 ?	4 gains, 1 loss
2. Mutations affecting homoplasmy (definite mutations)		
	family 83: homo->hetero	no case observed
	family 164: see Figure 6	no case observed
	family 171a: homo->hetero	no case observed

This is an approximately 3-fold higher rate in the irradiated families, which at first glance appears significantly higher ($p<0.01$). However, more extensive DNA sequencing was performed in the high-radiation families (to explore the detailed inheritance of new point mutations as explained above) than in the low-radiation families. This extended sequencing revealed two length changes (shortening in the daughter and then lengthening in the granddaughter) in radioactive family 159, which need to be subtracted before comparing the irradiated families with the control families. The significance of the difference (40 changes in 600 transmissions versus 5 changes in 202 transmissions) then falls slightly below the 99% confidence level. Furthermore, the measurement of modal C changes cannot distinguish new mutations in the mtDNA from a shift in proportions of existing mtDNA alleles in a cell, for example due to a mitochondrial bottleneck during oogenesis. Conversely, even if a new C insertion or deletion mutation had really occurred, then such a mutation might be masked by the presence of an identical allele in the parent (for example in Figure 6, if the child II.1 had mutated a 9C-allele to a 10C-allele, this would remain invisible because the mother already has the 10C allele). For these two reasons, the mutations observed when measuring the modal C allele is an unresolvable combination of DNA mutation rate and intracellular drift "mutation" rate.

2. Mutation by nucleotide insertion/deletion is also exemplified in Figure 6 and Table 4. In order to detect true DNA insertions/deletions between mothers and offspring, it is necessary to restrict oneself to the visible appearance of new alleles in the offspring. Only three cases are available, all of these in the irradiated families. The most striking example is daughter II.2 in family 164 (Figure 6), who has only the allele 5C, clearly shorter than the shortest allele 9C detectable in the mother I.1. The loss of 4 cytosines at one stroke is remarkable and in fact unique in the Kerala sample, so maternity testing was performed to exclude the possibility of an adopted child. The maternity probability between grandmother and mother is 99.99%, and between the mother and daughter it is 99.38%, as determined by autosomal nonaplex typing. A second example is found in family 83 (a homoplasmic 7C-tract in the mother becomes a heteroplasmic 9C-tract in the daughter and in the granddaughter). The third and last example is family 171a, where a grandmother homoplasmic for 7C is ancestral to a granddaughter heteroplasmic for 7C, one of the four rare cases where the 7C allele is found to be heteroplasmic (Table 3).

It may be argued that the presence of 3 definite DNA mutations in the irradiated families versus zero definite DNA mutations in the low-radiation families supports the point mutation results above. However, inspection of Table 3 reveals that the high-radiation and the low radiation families differ considerably in their allele profile, with on average longer alleles in the low-radiation sample. There is therefore more opportunity to observe definite mutations in alleles of the irradiated families.

Compared to the point mutation survey above, the insertion/deletion analysis in this section has one minor and one major shortcoming. A minor concern is the question of maternity versus adoption. For many of the insertion/deletion changes, autosomal maternity testing was not performed. Nevertheless, the high genetic diversity of the combined HV1 and HV2 regions of mtDNA available for every sample in this study make it unlikely that any undiscovered adoption is present. A major concern in the insertion/deletion study is the inability to distinguish between somatic and germline mutations. The ancestral state of a length allele (7C, 8C, …) cannot be inferred from the evolutionary mtDNA tree because length alleles, unlike point mutations, have evolved back and forth too fast. With few exceptions, the families in this study are too small to follow an ancestral allele through at least two generations (to confirm that it is the germline type) and then to follow the mutated allele another two generations (to confirm that the mutated allele is in the germline). Only mutant families 86 and 164 happen to fulfil these requirements. In family 86, a germline mutation is confirmed, whereas in family 164 the complicated length mutations are probably at least partly explained by somatic mutation, pending further cloning experiments.

Cloning was indeed performed in another mutant individual. To estimate the in-vivo mutation speed of insertions/deletions at nps303-309, one individual (149.2, see Appendix 7) who was heteroplasmic at nps303-309 was measured by bacterial cloning. Four length variants were detected: 4% 7C, 75% 8C, 17% 9C, and 4% 10 C. This same individual had three different mtDNA types due to new mutations at positions 144 and 152 (discussed in the section *Observed point mutations*). No one-to-one correlation was seen between the three point mutation mtDNA types (144C-152T, 144T-152C, 144C-152C) and the 4 length variants. This may agree with the observation that nps303-309 insertions/deletions mutate faster than even the fastest point mutation np152 (Table 2).

Discussion

Radiological implications

This is the first comparison of evolutionary and radiation-associated heritable mtDNA point mutations, and thus it is difficult to compare with previous studies on radiation-associated mutational change. Perhaps the most closely related research in our context is a study (Dubrova et al. 1997) on the genetic effects of the Chernobyl fallout which exposed parents and their offspring in parts of Byelorussia to increased radiation levels since 26 April 1986. Although those authors investigated autosomal minisatellites rather than maternally inherited mtDNA, and although their initial exposure is much shorter and more intense than the constant natural radiation conditions in Kerala, the genetic effects seem to be comparable. The increased minisatellite length mutations in the Byelorussians were observed to fall within the normal allelic spectrum, hinting that it is the normal evolutionary mutation mechanism which is accelerated by radiation. MtDNA as a genetic system is well suited to confirm or reject this prediction, as evolutionary mutations can be reconstructed in detail in an mtDNA tree without the complex mutational dynamics that confound the phylogenetic analysis of autosomal minisatellites. As demonstrated, the mtDNA results presented here strongly support an acceleration of the evolutionary DNA mutation mechanism through radiation.

Comparison with a published pedigree study

Parsons et al. (1997) published one of the first studies to observe new mtDNA mutations in pedigrees, based partly on screening documented cell lines in tissue culture. Their main conclusion that new mutations occur at non-negligible frequencies is supported by the results of this thesis. However, at first glance there appears to be a discrepancy concerning the mtDNA mutation rate. The Parsons et al. (1997) study, which included reliable maternity testing, yielded 9 point mutations in 327 mtDNA transmissions (Parsons et al. 1997). In the present PhD thesis, only 1 point mutation was found in 202 control transmissions, and only 22 point mutations in 600 irradiated transmissions. The apparent discrepancy between studies is at least partly explained by the fact that Parsons et al. (1997) sequenced mother-child pairs, while in this thesis larger families were screened by omitting intermediate generations in the first instance. In other words, mutations which appear transiently in one generation, or indeed somatic mutations, are less likely to be included here. Indeed, such a transient mutation was found in this thesis project by chance when sequencing all members of family 55. Other factors contributing to the apparent discrepancy may be that Parsons et al. (1997) chose a lower cutoff level for mutations (here a mutation was considered reliable only if the mutant peak area exceeded 20%), and that the incidence of somatic mutations in the tissues used by Parsons et al. (1997), namely blood and cultured cell lines, had not been ascertained. Incidentally, none of the Keralese mutations, even when they are the majority type, penetrate to 100% in the 2-3 generations followed up. When calculating long-term ("evolutionary") mutation rates it is therefore not justified to assume that a

majority peak (e.g. 70%) will inevitably become a homoplasmic mutation and therefore may be counted as a full mutation (cf. Parsons 1997 vs. Lutz et al. 2000).

Forensic implications

New mutations can result in a genetic mismatch between a forensic stain and a suspect, or between a child and its parent or relative, which may be erroneously interpreted as proof of exclusion of identity/relatedness. This thesis demonstrates that new mtDNA mutations are not an insurmountable forensic problem, even in extreme conditions of natural radioactivity. First, new point mutations occurring in the previous generation are readily identifiable because they are always heteroplasmic (23 out of 23 cases). Second, new insertion mutations in the cytosine tract between nps303 and 309 are so frequent anyway even under low-radiation circumstances (5/202 transmissions) that they cannot be taken as strong evidence for or against genetic identity (in crime stain investigation) or relatedness (in maternity testing). If the C-tract differs by more than one cytosine between maternal relatives (only 5 out of 600 cases even in the rapidly mutating radioactive conditions) then a C-tract difference can be given a greater weight.

Future research

This study has used saliva samples and proved that saliva DNA traces germline inheritance very well, at least in the case of point mutations. Further forensic mtDNA research may focus on analysing mutant families on a variety of other tissues likely to be left at a scene of death or crime, or tissues commonly used in parentage testing.

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1990–1998 Medical studies at the University of Hamburg. Completed Ärztliche Vorprüfung (Pre-clinical examinations, 15 March 1994). Completed first state exam, 21 March 1996. Completed second state exam, 21 March 1997. Completed third state exam and official approval for practising medicine as "Arzt im Praktikum" on 17 June 1998.

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Appendix 1: Historical geography of the strip

The following text is cited from Grüneberg et al. (1966), with the Malayalam corrected by Lucy Forster.

The Malabar (Kerala) coast has been a place of trade since ancient times. Phoenicians, Jews and Arabs were trading with Kerala before the Greeks and Romans, and the ancestors of the 'Black Jews' of Malabar probably settled there in Solomon's era. The Elder Pliny (who wrote about A.D.75) and, about ten years later, the anonymous author of Periplus Maris Rubri (probably a Greek merchant living in Egypt who had travelled to India himself (McCrindle 1879) refer to the pepper trade with the Malabar coast, the south-west monsoon (called hippalos after the Greek pilot who first observed it) and the backwater system, which made safe navigation along the coast possible even during the monsoon season. Unfortunately, the identification of some of the places mentioned in these accounts presents difficulties, and it must remain conjectural to what extent they refer to the particular part of the coastline with which we are here concerned.

During the next 1300 years Quilon (in Malayalam *Kollam,* from *kulam,* pool or pond) was the chief city and port of the Malabar coast, not only doing considerable trade in goods produced locally and from Ceylon and the east coast of India, but also serving as a centre of trade between the Arabs and the Chinese. In the early centuries of the Christian era Kollam was known to the Arabs as *Mali*, and to the Chinese as *Mahlai* — probably from the indigenous word *mala*, meaning 'hill' (from Sanskrit *malaya*), which was specifically applied to the southern end of the Western Ghats. The Arabs did not know the meaning of the word but they called the place, or island as they imagined it, *Mali* or *Kulam-Mali*. Later the Persian suffix -bar was added, so the Arabs were the first to use the name Malabar — Land of Mali — afterwards adopted by the Portuguese and then by the British (Nainer 1942).

The accounts of Arab and Chinese writers are not informative on the geography of the region until the 14th century. In 1343, the great Moroccan traveller, Abū Abdullah Muhammad, commonly known as Ibn Battūta, journeyed by backwater down the Malabar coast. He was at that time in the service of the Sultan of Delhi (Muhammad bin Tughluq), who had sent him as his ambassador to China when his *kakam* (small junk), after other ships had been wrecked in a storm, left Calecut without him:

"I was told that the kakam must call and anchor at the port of Kawlam [Quilon]. Hence I resolved to travel up to Kawlam- a distance of ten days journey from Calecut whether one travels by land or by river. I travelled by river and hired a Muslim porter to carry my carpet. When Indians travel by this river they disembark in the evening and pass the night in the villages lying along the bank;

then they return to the ship on the morrow. We used to do the same. On the ship there was no Muslim except the one I had hired. He used to drink with the infidels after we had landed and used to quarrel with me and this augmented my unhappiness. On the fifth day of our journey we came to Kanjarkara [probably Vanji, i.e. Tiruvanjikulam, or Cranganore]. It lies high on a hill and is inhabited by the Jews who have their own chief and pay taxes to the Sultan of Quilon. All the trees which are to be found along by this river are Canella or Brazilwood (*Caesalpina* spp) trees, which are used as fuel. We used to light fires of that wood to cook our meals in the course of that journey. On the tenth day we came to the city of Kawlam. It is one of the most beautiful places in the country of Malabar with magnificent bazaars… Of the whole country of Malabar this city of Kawlam lies nearest to China, and to it travel the Chinese for the most part. Here Muslims are respected."

From Hussain (1953) *and Lee* (1829)

Ibn Battūta later remarks that Quilon was the greatest port he knew, save for Zaiton (Ts'üen-chow), and Marco Polo (who visited Quilon in 1294) thought likewise. In the account quoted above, we have definite evidence of the backwater system between Calecut and Quilon with 'villages lying along the bank'. The last stretch of his journey must have been past the strip, for there is no possible water-way further inland connecting the Ashtamudi Lake to the backwaters further north.

Neither Ibn Battūta nor any previous Arab travellers mention coconut plantations along the Malabar coast. Ibn Battūta gives a detailed and accurate description of the life-history and exploitation of the coconut in the Maldives, but in the account of his travels along the backwaters he says that all the trees were canella. However, both Friar Jordanus and Friar Oderic, who visited Quilon about ten years before Ibn Battūta, describe the coconut palm, and Friar Jordanus (Yule 1863) mentions the use of coir: 'From the rind of that fruit is made the twine with which they stitch their boats together in those parts' — as they do still today. So it is possible that the coconut, though grown there, was not yet the dominant vegetation along the coast in the 14th century. Malabar folklore suggests that the coconut was introduced in 'recent' times from Ceylon by the Izhava caste. Coconut cultivation and toddy drawing are still the hereditary occupations of the Izhavas, who are traditionally believed to have come from Ceylon (Achyuta Menon 1911, Nagam Aiya 1906), and some authorities have suggested a similar origin for the fisherman castes, the Mukkuvans and Marakkans (Achyuta Menon 1911). A majority of the people living on the strip today belong to one of these castes, or are descended from them; so it is possible that these people, with their thatched palm huts and coconut trees, have settled here only during the Christian era, perhaps during the last thousand years. The Mukkuvans are first referred to by the Portuguese at the beginning of the 16th century.

The breaking of the Arab-Mediterranean monopoly of the spice trade following Vasco da Gama's famous journey in 1498 to Malabar via the Cape of Good Hope led, among other

things, to a more detailed documentation of the Malabar coast. The strength of the Arabs in Calecut forced the Portuguese to establish their stations further south, in Cochin, Quilon and Kayankulam. On his second journey, in 1502, Vasco da Gama visited Quilon at the request of its queen, who did not wish all the trade to go through Cochin. Gaspar Corrêa, Vasco's chronicler, writes:

"She had in her kingdom pepper enough to fill twenty ships each year…. For the greater quantity of pepper which went to Cochym, the merchants bought it in this kingdom of Coulão [Quilon], and carried it in boats to Cochym by rivers which flow inside the country."

And, after the agreement was signed,

"There went on board with them the Queen's minister, who took the ships to a river called Calle Coulão [Kayankulam] which was five leagues from the Port."

From Stanley (1869)

The first passage quoted shows that the backwater was in use at that time, and the second that the Kayankulam bar was open. So the strip was fully isolated from the mainland. Two travellers not long afterwards visited the region; in 1505 Ludovico di Varthema travelled from Calecut by river — 'the most beautiful river I have ever seen' — and arrived at 'Cacolon' (Kayankulam). After leaving Kayankulam he came to 'Colon' (Quilon), a distance of twenty leagues (Badger, 1863). Nine years later Duarte Barbosa travelled by the same route:

"…Having passed this place [Porca] the kingdom of Coulam commences, and the first town is called Cayncolam in which dwell many gentiles [Hindus], Moors, and Indian Christians of the doctrine of St. Thomas. There is much pepper in this place of which there is much exportation… Further along the same course towards the south is a great city and good seaport which is named Coulam..."

From Stanley (1865)

The most accurate account of Malabar of that time is contained in the Suma Oriental of Tomé Pires, written in Malacca between 1512 and 1515. Pires describes many of the castes, including the Mukkuvan and Izhava castes:

"The whole country is thickly populated …In one part of this land of Malabar there are large rivers, deep in some places and shallow in others, which make it strong, and where they fish, where they can go in "tones", to wit, from Panane [Ponnani] to Coulam [Quilon].The other part of Malabar is dry and easy to travel over by land, but in this part [you have to go] in "tones catures". There are countless palm trees and arecas along the coast of Malabar; but they do not extend for more than a league and a half inland, or two leagues at the most."

Pires also lists Caya-Coulam and Coulam amongst the "inhabited seaports where there are ships" and briefly describes their respective kingdoms (Cortesão 1944). The Portuguese accounts show that in the early 16th century the Malabar coast was much as it is today. The Neendakara-Kayankulam strip had its present boundaries, though it may not itself have been a continuous spit at that time. However, there is not as yet any direct information concerning places on the strip.

These begin to be mentioned with the establishment of European settlements, notably in some Dutch sources of the 17th century (e.g. Captain John Nieuhoff; see Churchill and Churchill 1704). Prominent among those mentioned early is Kovilthottam (in Malayalam meaning temple garden), where an elegant Portuguese church dedicated to St Andrew is still in existence; it can be traced back to 1581. The village of Neendakara certainly existed in the early 18th century, and presumably very much earlier. Owing to limitations of space, no detailed discussion can be given here of the history of these places.

It was mentioned above that the strip may not always have been a single entity from Neendakara to Kayankulam. The reason for this surmise is mainly linguistic.

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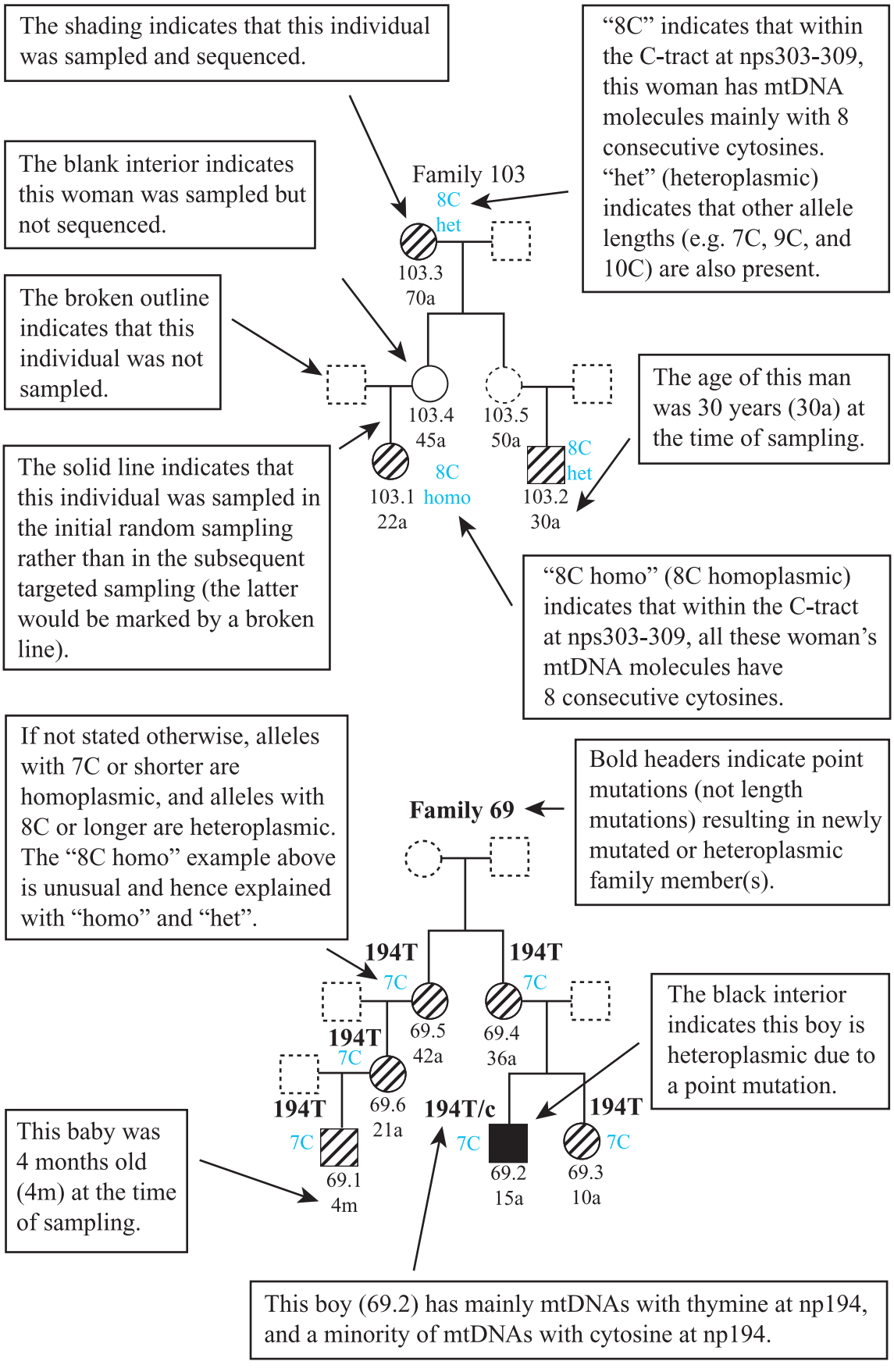
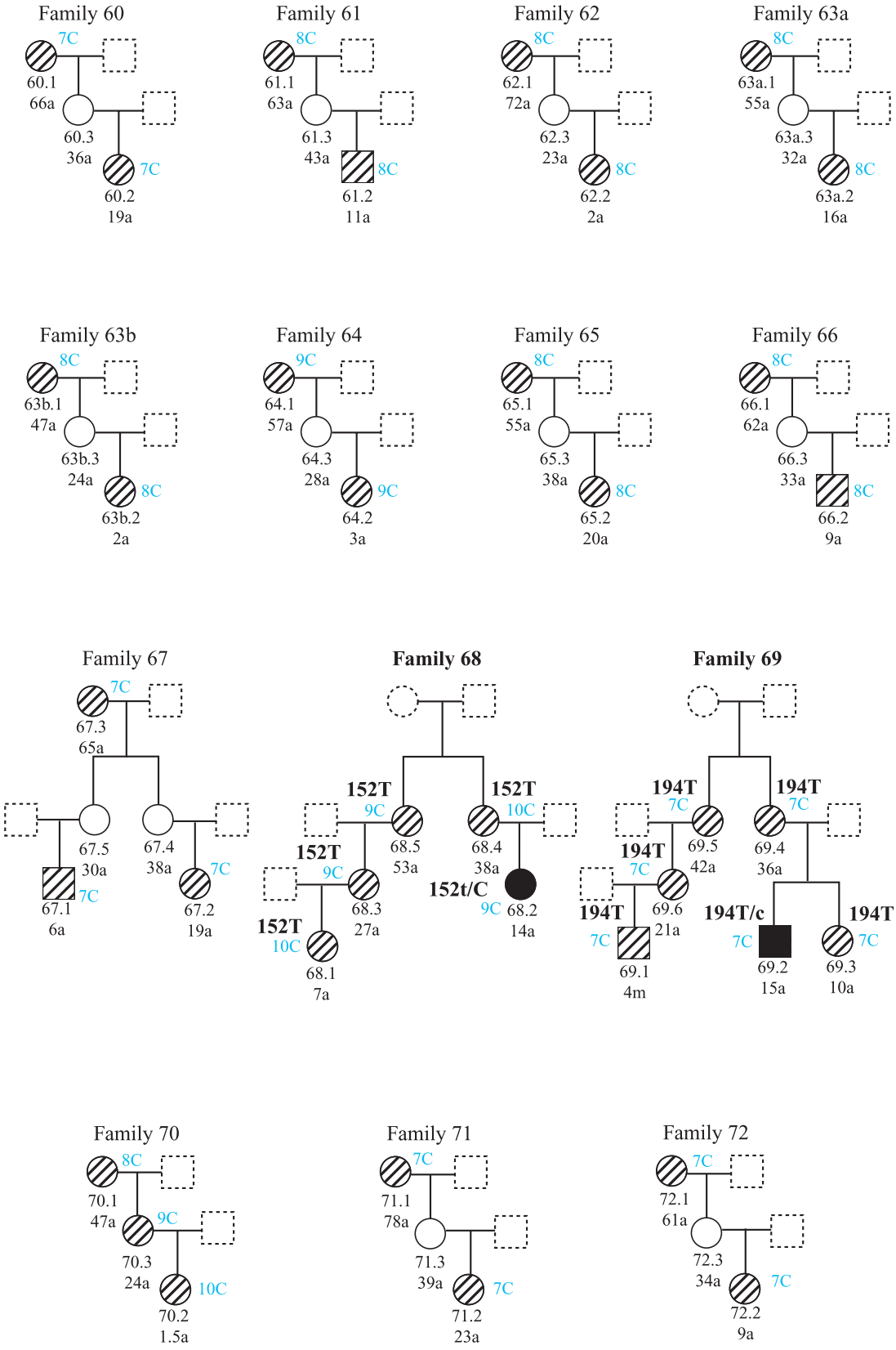
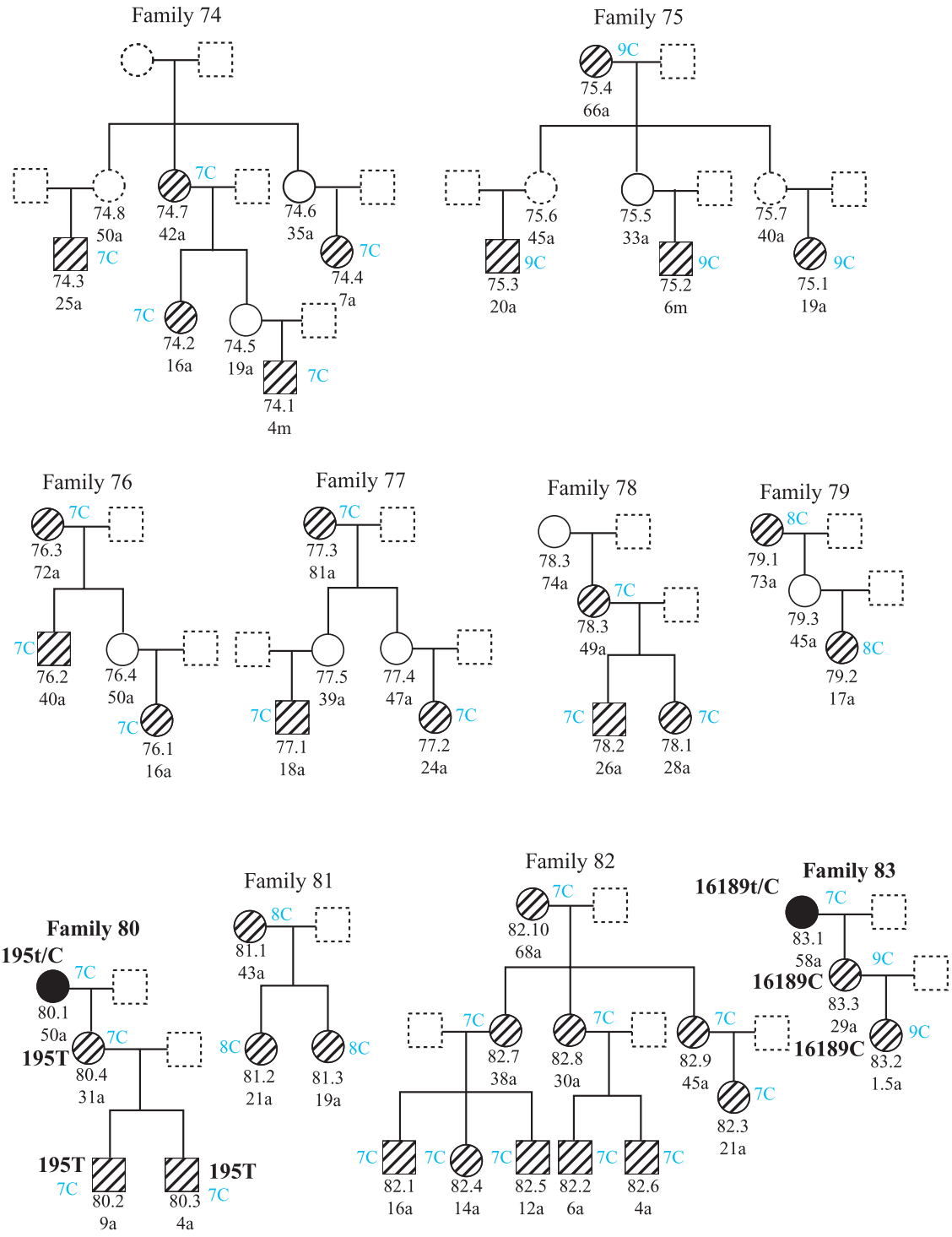
Appendix 2: Pedigrees of sampled families.

Explanation of symbols in example families 103 and 69:



High-radiation families

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7C
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16a
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74.6
35a
7C
74.4
7a
7C
74.1
4m
Family 75
9C
75.4
66a
75.6
45a
9C
75.3
20a
75.5
33a
9C
75.2
6m
75.7
40a
9C
75.1
19a
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7C
76.3
72a
7C
76.2
40a
76.4
50a
7C
76.1
16a
Family 77
7C
77.3
81a
77.5
39a
7C
77.1
18a
77.4
47a
7C
77.2
24a
Family 78
78.3
74a
7C
78.3
49a
7C
78.2
26a
7C
78.1
28a
Family 79
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79.1
73a
79.3
45a
8C
79.2
17a
Family 80
195t/C
7C
80.1
50a
195T
7C
80.4
31a
195T
7C
80.2
9a
80.3
4a
195T
7C
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8C
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43a
8C
81.2
21a
8C
81.3
19a
Family 82
7C
82.10
68a
7C
82.7
38a
7C
82.8
30a
7C
82.9
45a
7C
82.3
21a
7C
82.1
16a
7C
82.4
14a
7C
82.5
12a
7C
82.2
6a
7C
82.6
4a
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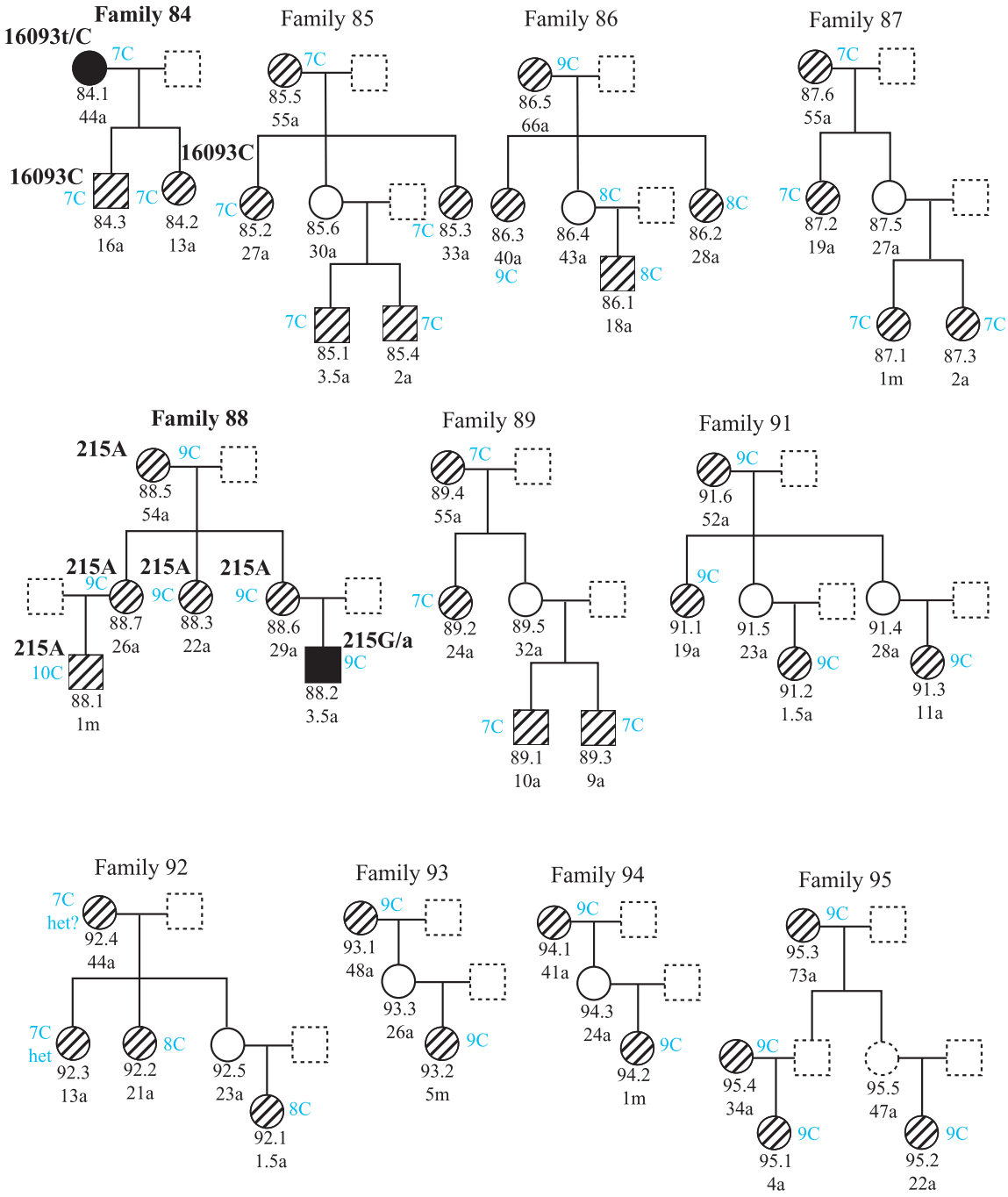
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16093C
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27a
85.6
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7C
85.3
33a
7C
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7C
85.4
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86.3
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9C
8C
86.4
43a
8C
86.1
18a
8C
86.2
28a
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55a
7C
87.2
19a
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27a
7C
87.1
1m
7C
87.3
2a
Family 88
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88.5
54a
215A
9C
88.7
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215A
9C
88.3
22a
215A
9C
88.6
29a
215G/a
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3.5a
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88.1
1m
Family 89
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89.4
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7C
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7C
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10a
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89.3
9a
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19a
91.5
23a
9C
91.2
1.5a
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9C
91.3
11a
Family 92
7C
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92.4
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92.3
13a
8C
92.2
21a
92.5
23a
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92.1
1.5a
Family 93
9C
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93.3
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93.2
5m
Family 94
9C
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94.2
1m
Family 95
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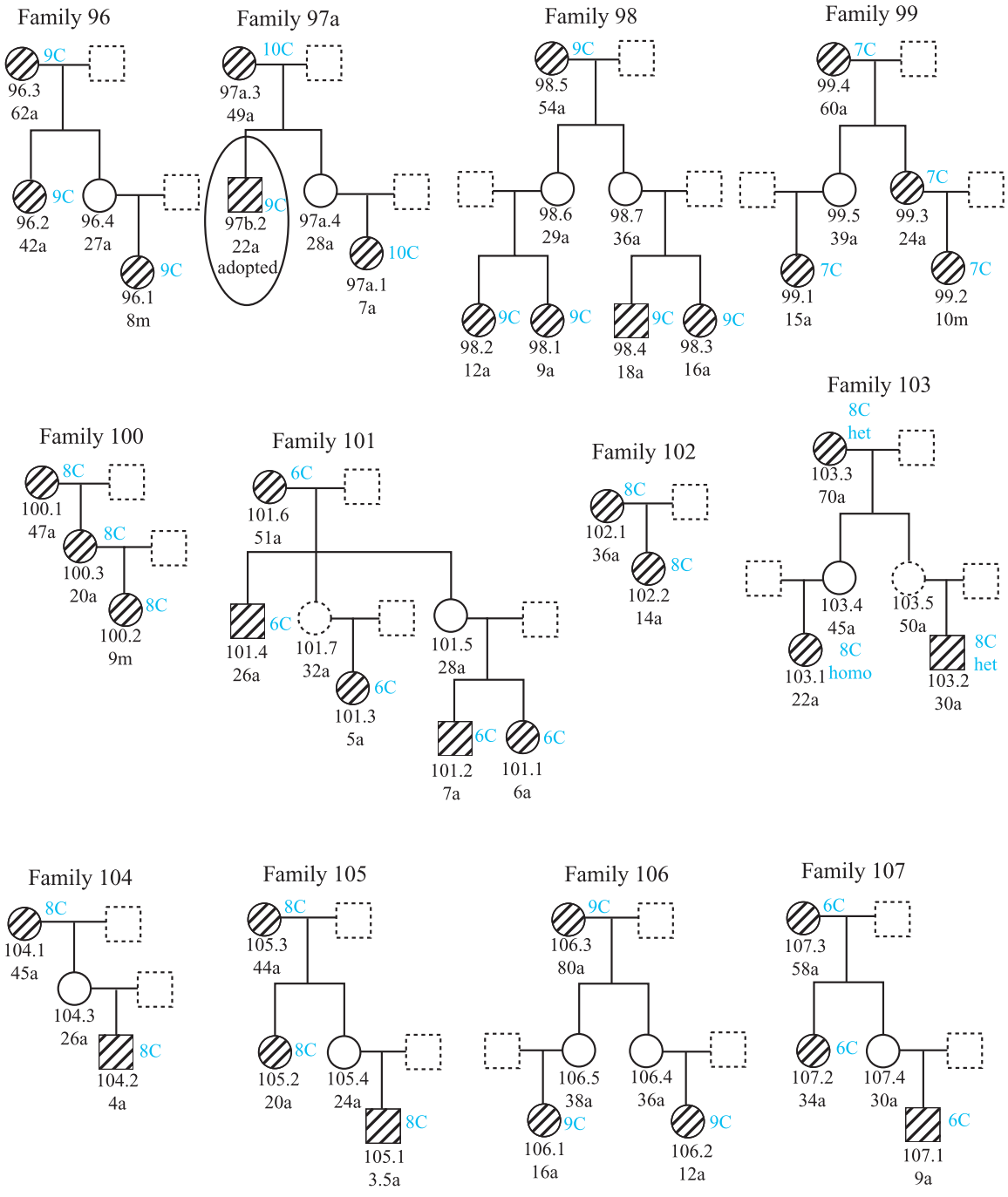
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97b.2
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10C
97a.1
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98.7
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98.2
12a
9C
98.1
9a
9C
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9C
98.3
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7a
6C
101.1
6a
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102.2
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103.4
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103.5
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8C
103.1
22a
homo
8C
het
103.2
30a
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45a
104.3
26a
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104.2
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8C
105.2
20a
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24a
8C
105.1
3.5a
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9C
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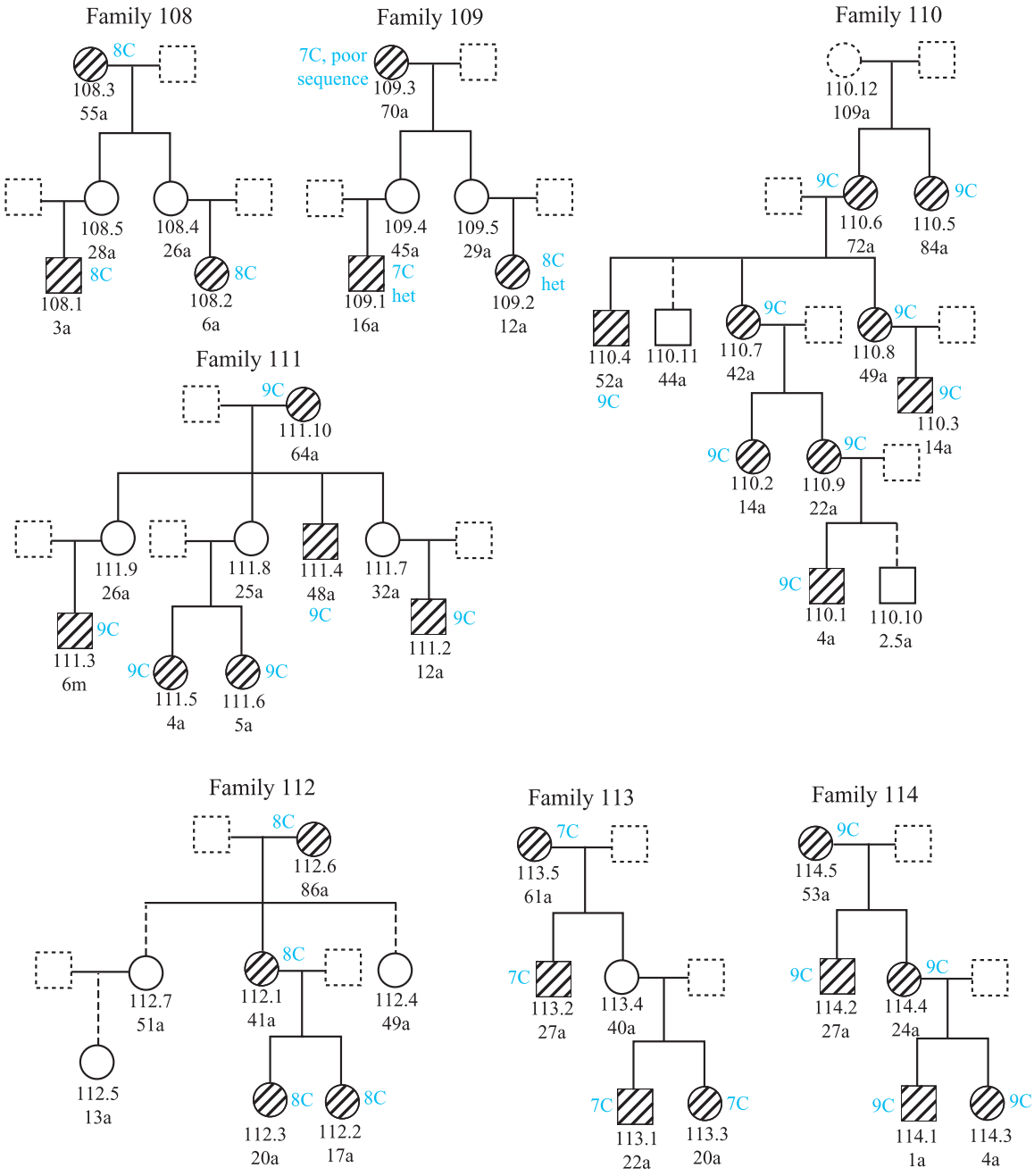
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7C het
109.1
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109.5
29a
8C het
109.2
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110.8
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9C
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4a
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9C
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9C
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32a
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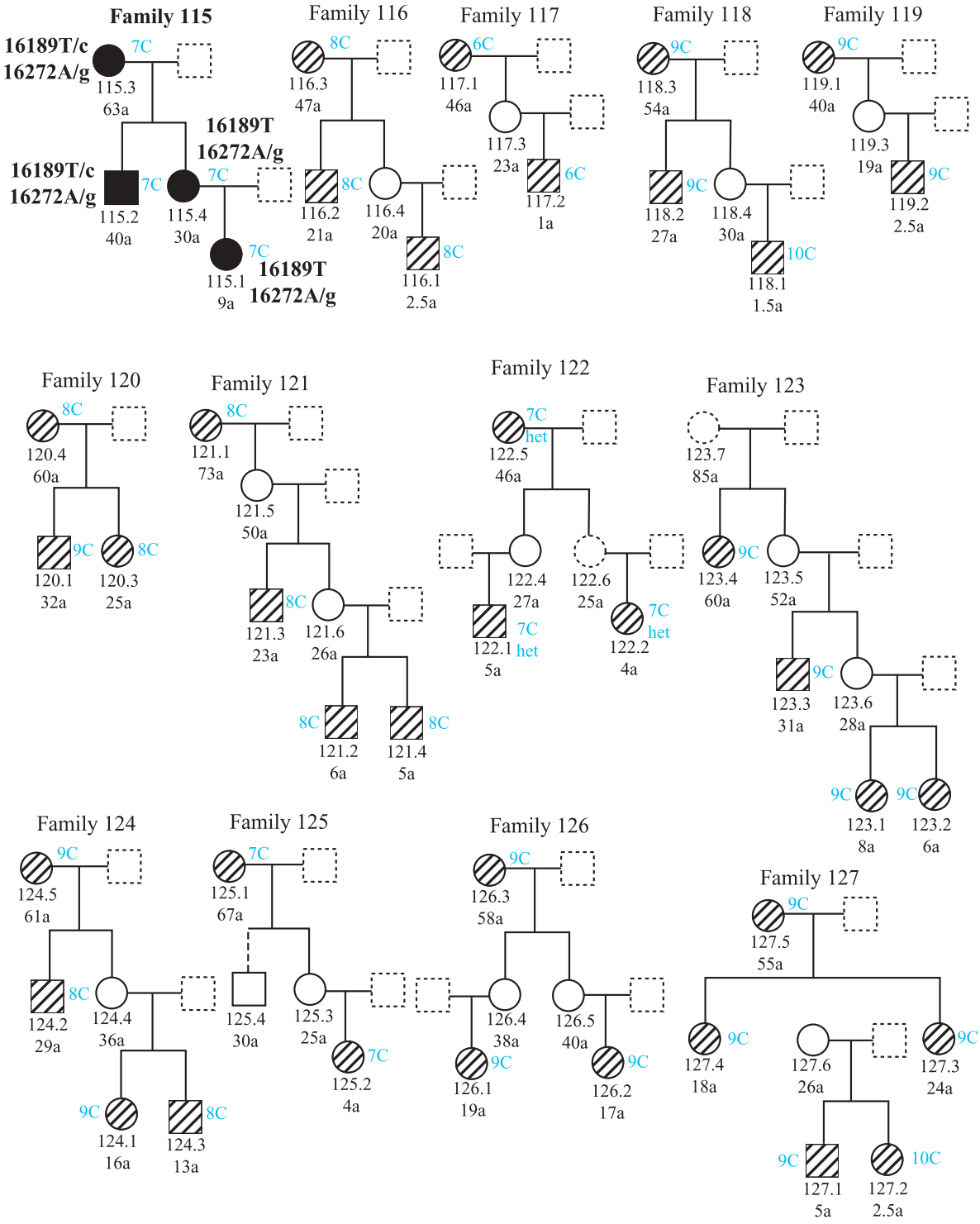
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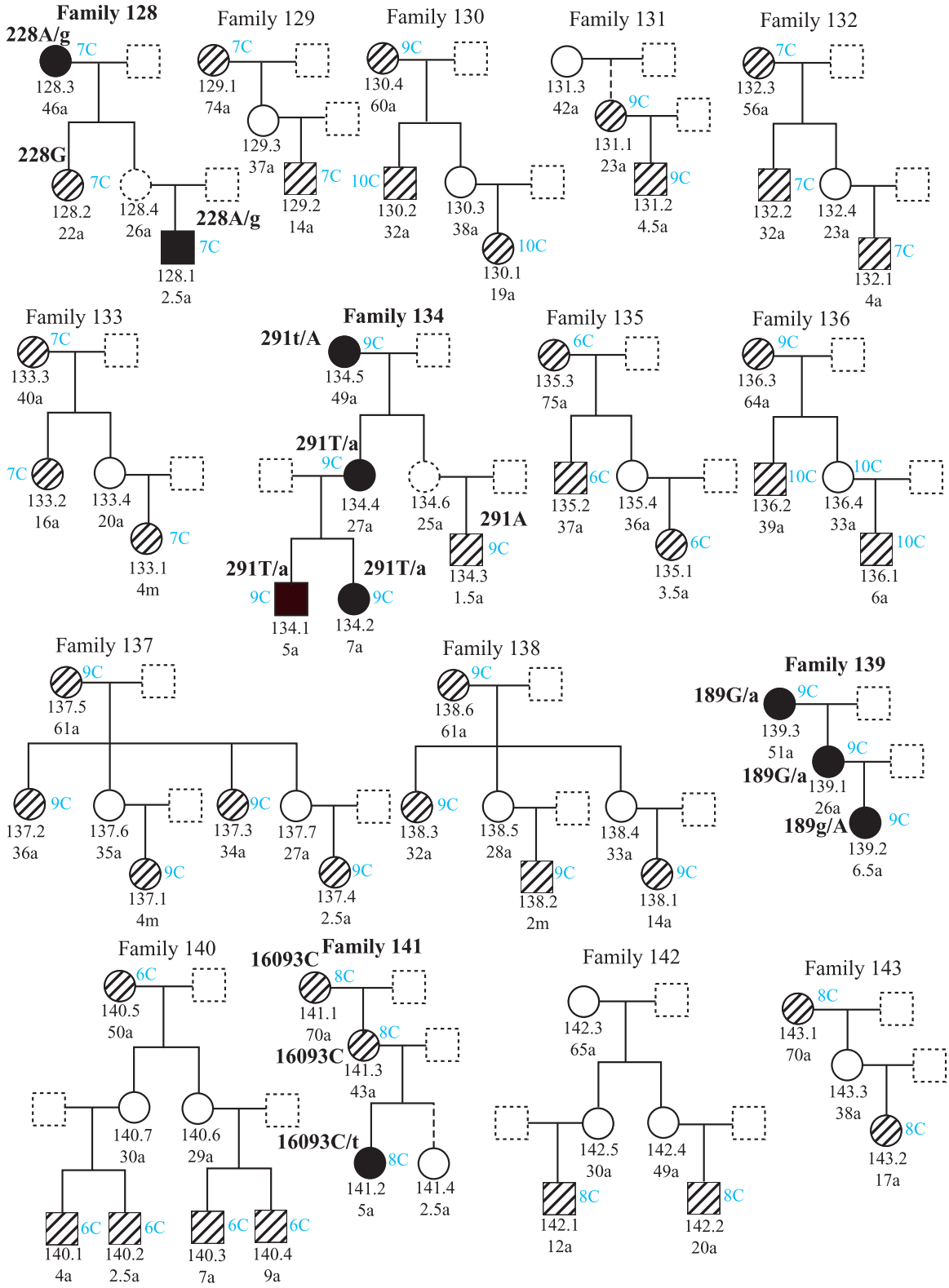
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189g/A
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141.4
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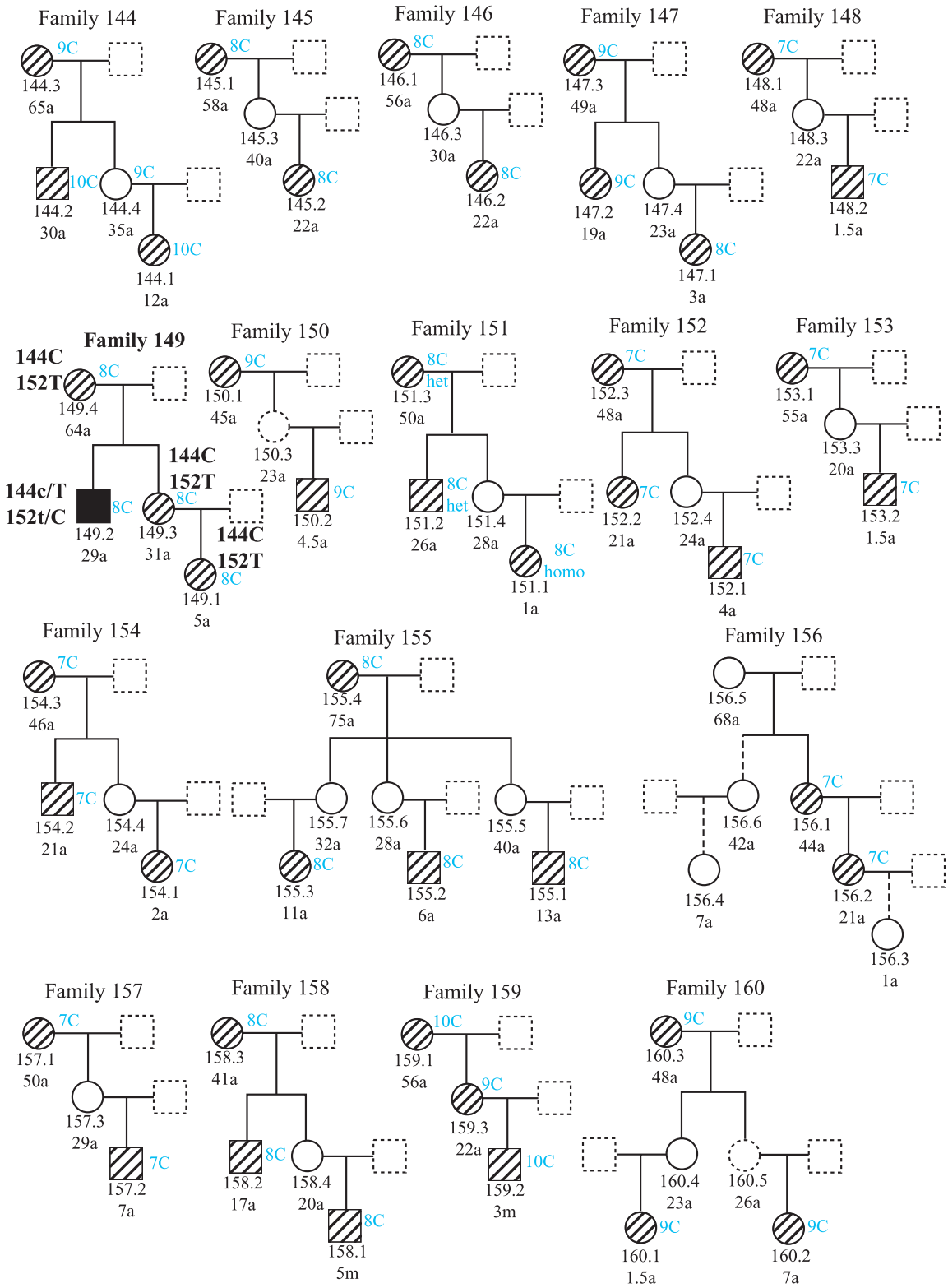
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144.4
35a
10C
144.1
12a
Family 145
8C
145.1
58a
145.3
40a
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145.2
22a
Family 146
8C
146.1
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146.3
30a
8C
146.2
22a
Family 147
9C
147.3
49a
9C
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23a
8C
147.1
3a
Family 148
7C
148.1
48a
148.3
22a
7C
148.2
1.5a
Family 149
144C
152T
8C
149.4
64a
144c/T
152t/C
8C
149.2
29a
144C
152T
8C
149.3
31a
144C
152T
8C
149.1
5a
Family 150
9C
150.1
45a
150.3
23a
9C
150.2
4.5a
Family 151
8C
het
151.3
50a
8C
het
151.2
26a
151.4
28a
8C
homo
151.1
1a
Family 152
7C
152.3
48a
7C
152.2
21a
152.4
24a
7C
152.1
4a
Family 153
7C
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55a
153.3
20a
7C
153.2
1.5a
Family 154
7C
154.3
46a
7C
154.2
21a
154.4
24a
7C
154.1
2a
Family 155
8C
155.4
75a
155.7
32a
8C
155.3
11a
155.6
28a
8C
155.2
6a
155.5
40a
8C
155.1
13a
Family 156
156.5
68a
156.6
42a
156.4
7a
7C
156.1
44a
7C
156.2
21a
156.3
1a
Family 157
7C
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50a
157.3
29a
7C
157.2
7a
Family 158
8C
158.3
41a
8C
158.2
17a
158.4
20a
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158.1
5m
Family 159
10C
159.1
56a
9C
159.3
22a
10C
159.2
3m
Family 160
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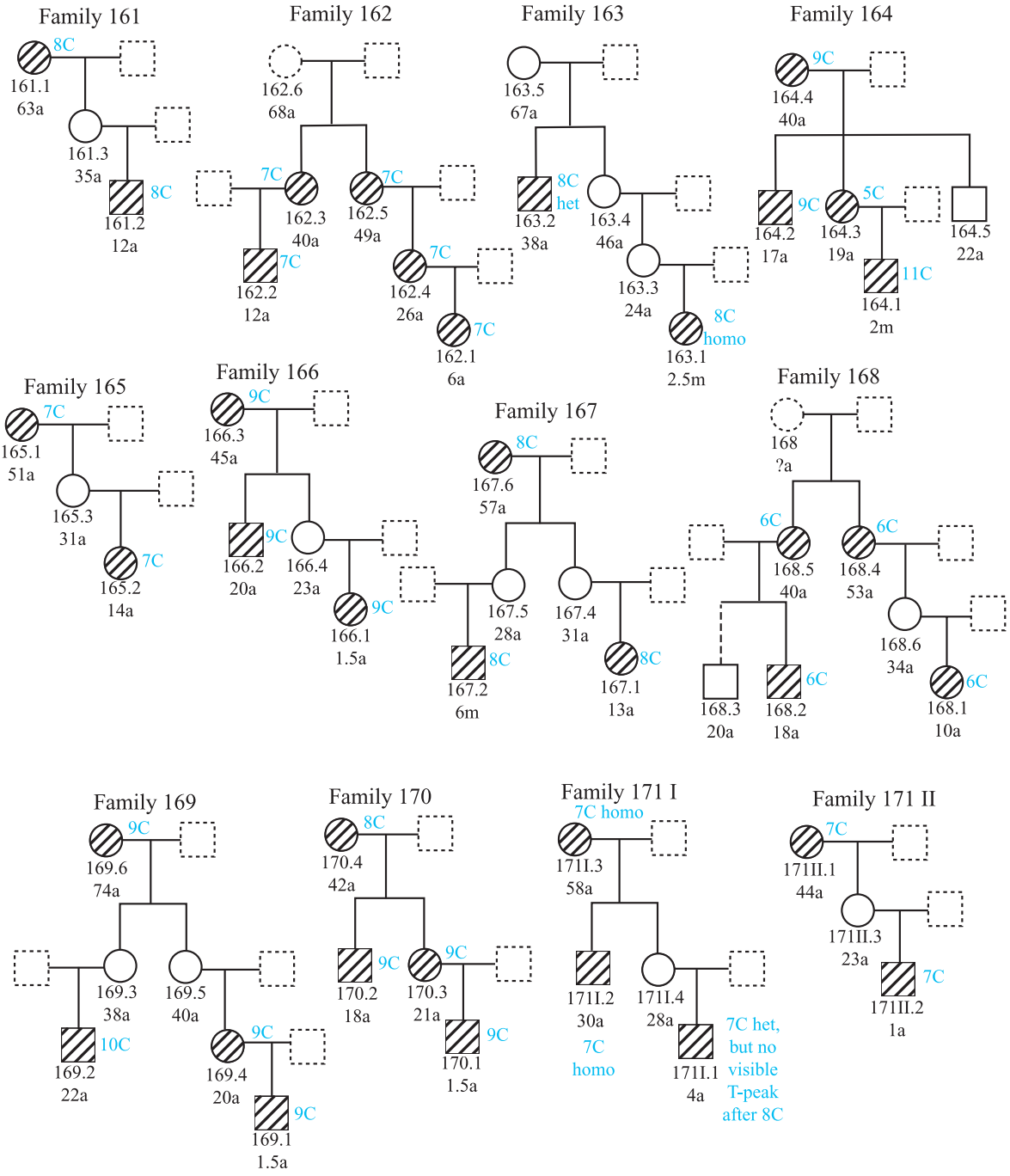
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Family 162
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162.3
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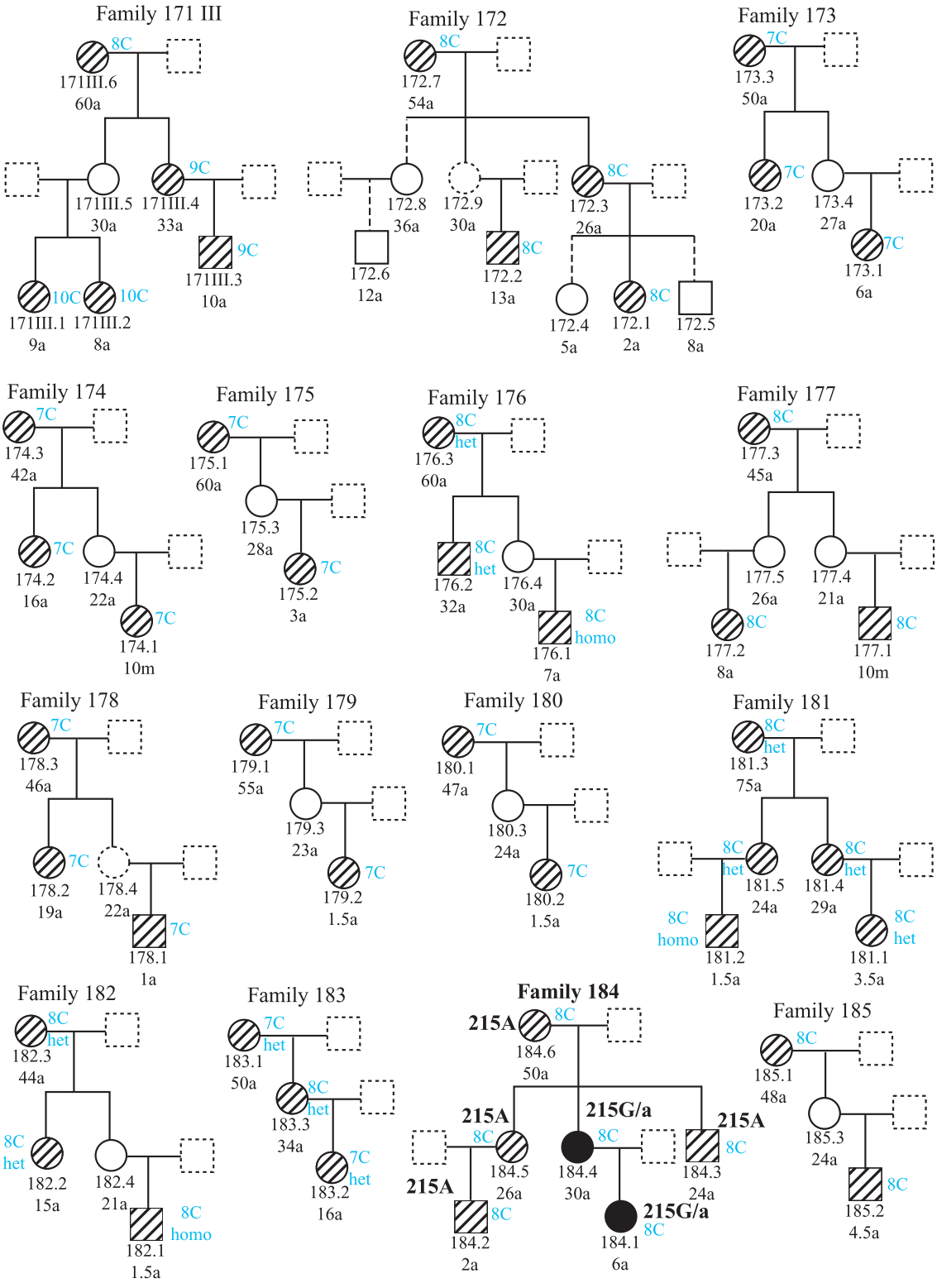
7C
162.5
49a
7C
162.2
12a
7C
162.4
26a
7C
162.1
6a
Family 163
163.5
67a
8C
het
163.2
38a
163.4
46a
163.3
24a
8C
homo
163.1
2.5m
Family 164
9C
164.4
40a
9C
164.2
17a
5C
164.3
19a
164.5
22a
11C
164.1
2m
Family 165
7C
165.1
51a
165.3
31a
7C
165.2
14a
Family 166
9C
166.3
45a
9C
166.2
20a
166.4
23a
9C
166.1
1.5a
Family 167
8C
167.6
57a
167.5
28a
8C
167.2
6m
167.4
31a
8C
167.1
13a
Family 168
168
?a
6C
168.5
40a
6C
168.4
53a
168.6
34a
6C
168.1
10a
168.3
20a
6C
168.2
18a
Family 169
9C
169.6
74a
169.3
38a
169.5
40a
10C
169.2
22a
9C
169.4
20a
9C
169.1
1.5a
Family 170
8C
170.4
42a
9C
170.2
18a
9C
170.3
21a
9C
170.1
1.5a
Family 171 I
7C homo
171I.3
58a
171I.2
30a
7C
homo
171I.4
28a
171I.1
4a
7C het,
but no
visible
T-peak
after 8C
Family 171 II
7C
171II.1
44a
171II.3
23a
7C
171II.2
1a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30a
171III.4
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9C
171III.3
10a
10C
10C
171III.1
9a
171III.2
8a
Family 172
8C
172.7
54a
172.8
36a
172.9
30a
172.3
26a
8C
172.6
12a
172.2
13a
172.4
5a
172.1
2a
8C
172.5
8a
Family 173
7C
173.3
50a
7C
173.2
20a
173.4
27a
7C
173.1
6a
Family 174
7C
174.3
42a
7C
174.2
16a
174.4
22a
7C
174.1
10m
Family 175
7C
175.1
60a
175.3
28a
7C
175.2
3a
Family 176
8C
het
176.3
60a
8C
het
176.2
32a
176.4
30a
8C
homo
176.1
7a
Family 177
8C
177.3
45a
177.5
26a
177.4
21a
8C
177.2
8a
8C
177.1
10m
Family 178
7C
178.3
46a
7C
178.2
19a
178.4
22a
7C
178.1
1a
Family 179
7C
179.1
55a
179.3
23a
7C
179.2
1.5a
Family 180
7C
180.1
47a
180.3
24a
7C
180.2
1.5a
Family 181
8C
het
181.3
75a
8C
het
181.5
24a
181.4
29a
8C
het
8C
homo
181.2
1.5a
8C
het
181.1
3.5a
Family 182
8C
het
182.3
44a
8C
het
182.2
15a
182.4
21a
8C
homo
182.1
1.5a
Family 183
7C
het
183.1
50a
8C
het
183.3
34a
7C
het
183.2
16a
Family 184
215A
8C
184.6
50a
215A
8C
184.5
26a
215A
184.2
2a
215G/a
8C
184.4
30a
215G/a
8C
184.1
6a
215A
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184.3
24a
Family 185
8C
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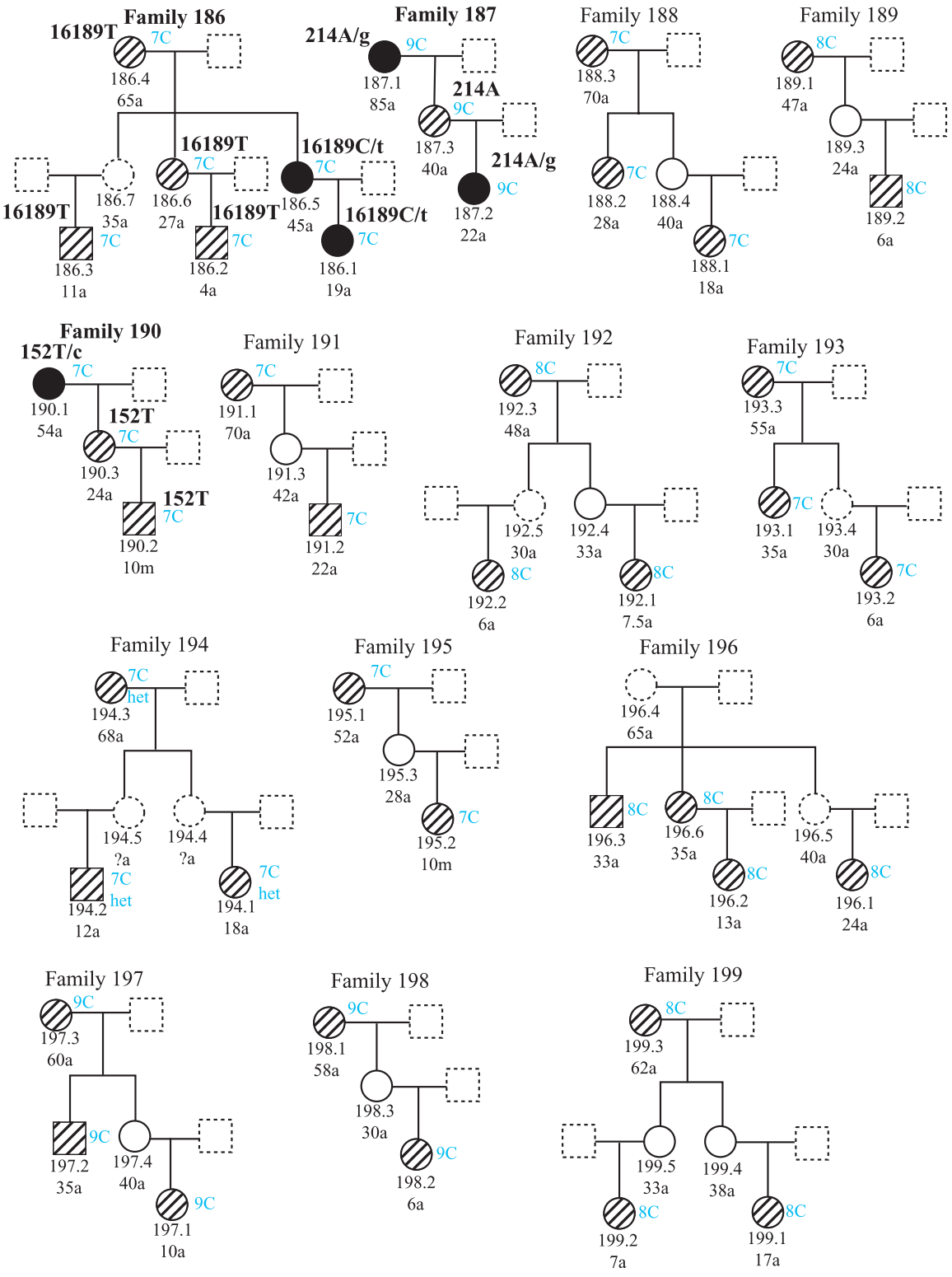
48a
185.3
24a
8C
185.2
4.5a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7C
186.4
65a
16189T
7C
186.6
27a
16189T
7C
186.2
4a
16189C/t
7C
186.5
45a
16189C/t
7C
186.1
19a
16189T
186.7
35a
16189T
7C
186.3
11a
Family 187
214A/g
9C
187.1
85a
214A
9C
187.3
40a
214A/g
9C
187.2
22a
Family 188
7C
188.3
70a
7C
188.2
28a
188.4
40a
7C
188.1
18a
Family 189
8C
189.1
47a
189.3
24a
8C
189.2
6a
Family 190
152T/c
7C
190.1
54a
152T
7C
190.3
24a
152T
7C
190.2
10m
Family 191
7C
191.1
70a
191.3
42a
7C
191.2
22a
Family 192
8C
192.3
48a
192.5
30a
192.4
33a
8C
192.2
6a
8C
192.1
7.5a
Family 193
7C
193.3
55a
7C
193.1
35a
193.4
30a
7C
193.2
6a
Family 194
7C
het
194.3
68a
194.5
?a
194.4
?a
7C
het
194.2
12a
7C
het
194.1
18a
Family 195
7C
195.1
52a
195.3
28a
7C
195.2
10m
Family 196
196.4
65a
8C
196.3
33a
8C
196.6
35a
196.5
40a
8C
196.2
13a
8C
196.1
24a
Family 197
9C
197.3
60a
9C
197.2
35a
197.4
40a
9C
197.1
10a
Family 198
9C
198.1
58a
198.3
30a
9C
198.2
6a
Family 199
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62a
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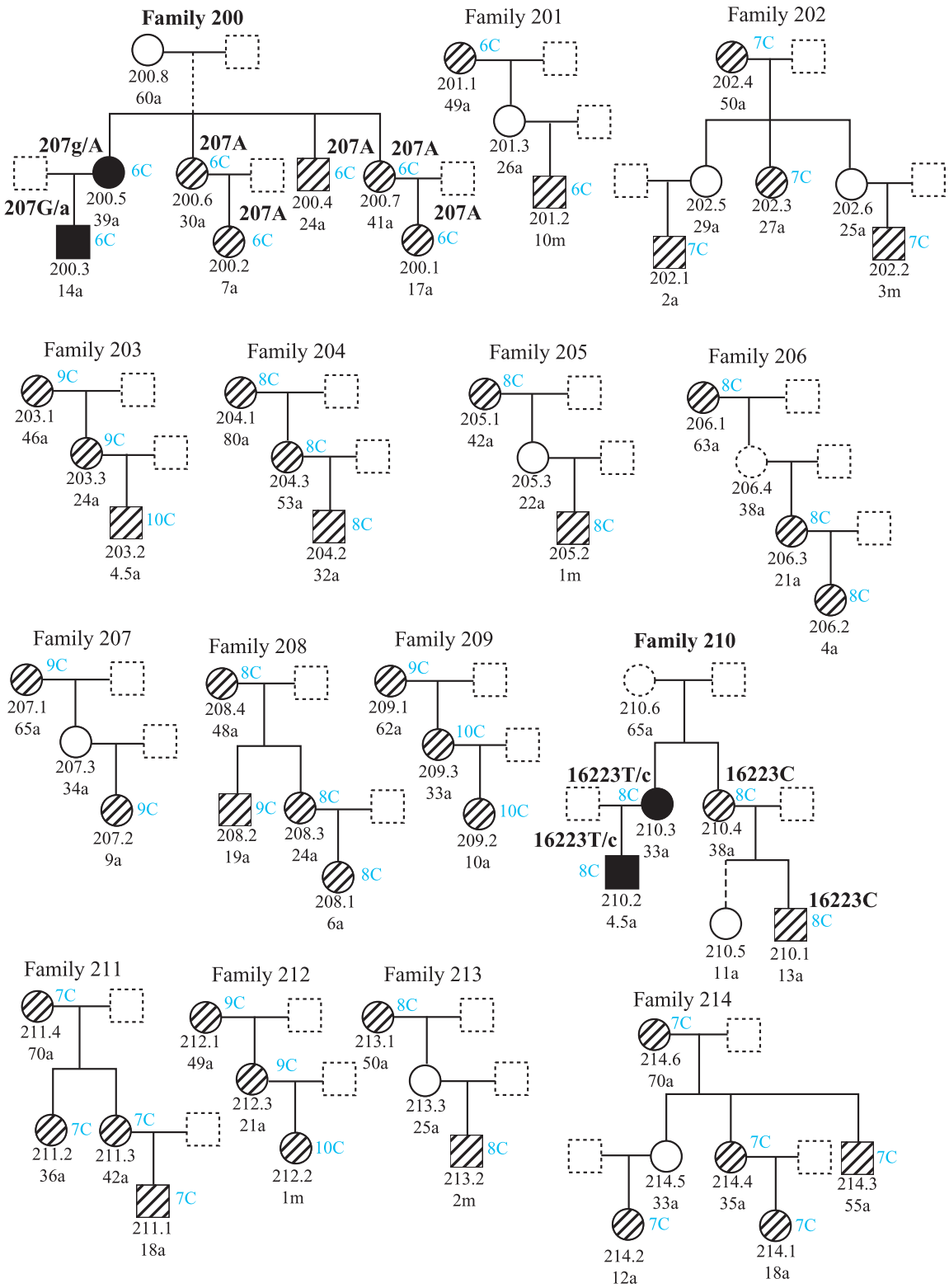
199.4
38a
8C
199.2
7a
8C
199.1
17a


Family 200
200.8
60a
207g/A
207G/a
200.5
39a
6C
200.3
14a
6C
207A
200.6
30a
6C
207A
200.2
7a
6C
200.4
24a
6C
207A
200.7
41a
6C
207A
200.1
17a
6C
Family 201
201.1
49a
6C
201.3
26a
201.2
10m
6C
Family 202
202.4
50a
7C
202.5
29a
202.1
2a
7C
202.3
27a
7C
202.6
25a
202.2
3m
7C
Family 203
203.1
46a
9C
203.3
24a
9C
203.2
4.5a
10C
Family 204
204.1
80a
8C
204.3
53a
8C
204.2
32a
8C
Family 205
205.1
42a
8C
205.3
22a
205.2
1m
8C
Family 206
206.1
63a
8C
206.4
38a
206.3
21a
8C
206.2
4a
8C
Family 207
207.1
65a
9C
207.3
34a
207.2
9a
9C
Family 208
208.4
48a
8C
208.2
19a
9C
208.3
24a
8C
208.1
6a
8C
Family 209
209.1
62a
9C
209.3
33a
209.2
10a
10C
Family 210
210.6
65a
16223T/c
16223T/c
8C
210.2
4.5a
8C
210.3
33a
8C
16223C
210.4
38a
8C
210.5
11a
16223C
210.1
13a
8C
Family 211
211.4
70a
7C
211.2
36a
7C
211.3
42a
7C
211.1
18a
7C
Family 212
212.1
49a
9C
212.3
21a
9C
212.2
1m
10C
Family 213
213.1
50a
8C
213.3
25a
213.2
2m
8C
Family 214
214.6
70a
7C
214.5
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214.1
18a
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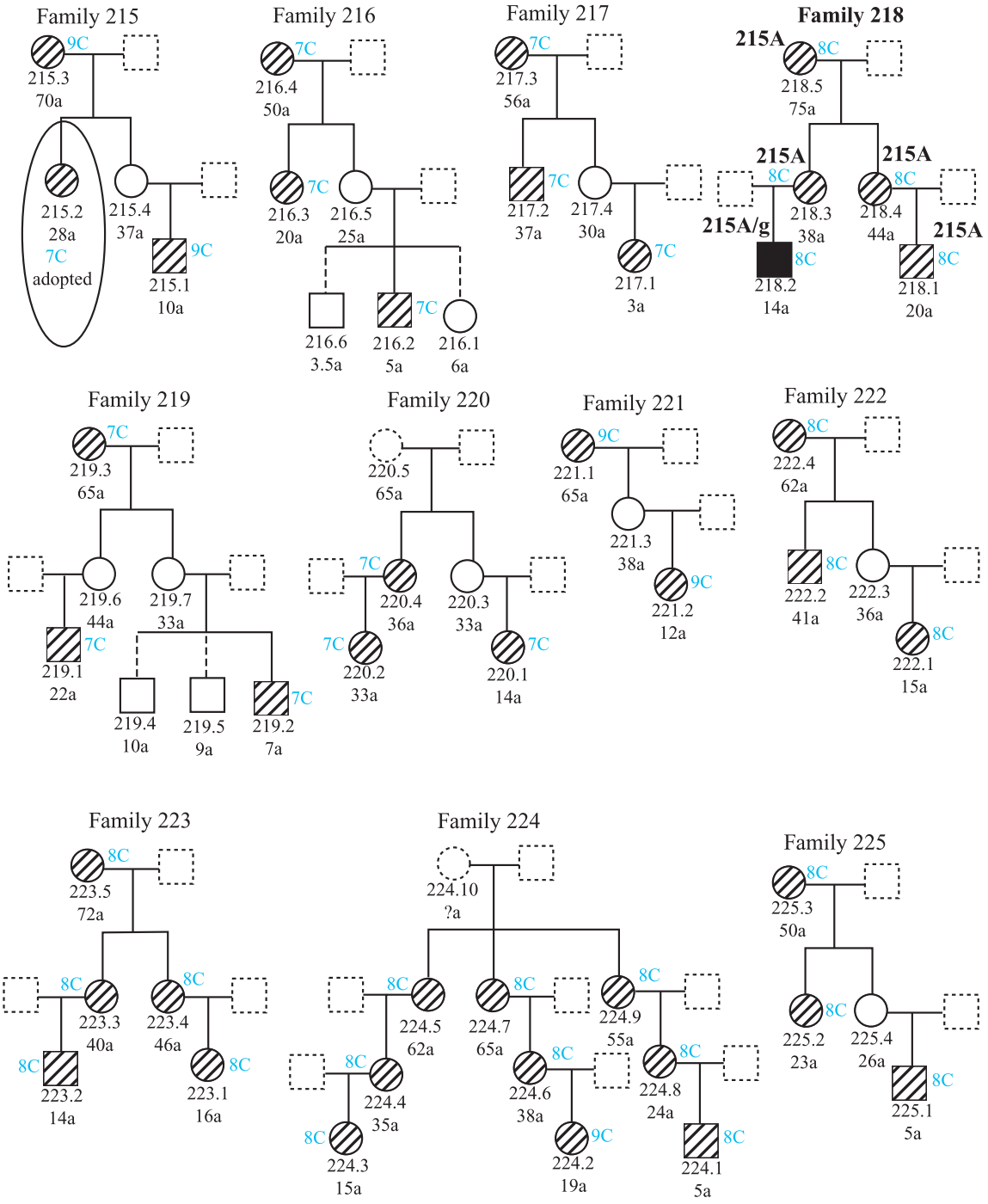
214.3
55a
7C


Family 215
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70a
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adopted
215.4
37a
9C
215.1
10a
Family 216
7C
216.4
50a
7C
216.3
20a
216.5
25a
216.6
3.5a
7C
216.2
5a
216.1
6a
Family 217
7C
217.3
56a
7C
217.2
37a
217.4
30a
7C
217.1
3a
Family 218
215A
8C
218.5
75a
215A
8C
218.3
38a
215A/g
8C
218.2
14a
215A
8C
218.4
44a
215A
8C
218.1
20a
Family 219
7C
219.3
65a
219.6
44a
219.7
33a
7C
219.1
22a
219.4
10a
219.5
9a
7C
219.2
7a
Family 220
220.5
65a
7C
220.4
36a
220.3
33a
7C
220.2
33a
7C
220.1
14a
Family 221
9C
221.1
65a
221.3
38a
9C
221.2
12a
Family 222
8C
222.4
62a
8C
222.2
41a
222.3
36a
8C
222.1
15a
Family 223
8C
223.5
72a
8C
223.3
40a
8C
223.4
46a
8C
223.2
14a
8C
223.1
16a
Family 224
224.10
?a
8C
224.5
62a
8C
224.7
65a
8C
224.9
55a
8C
224.4
35a
8C
224.3
15a
8C
224.6
38a
9C
224.2
19a
8C
224.8
24a
8C
224.1
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50a
8C
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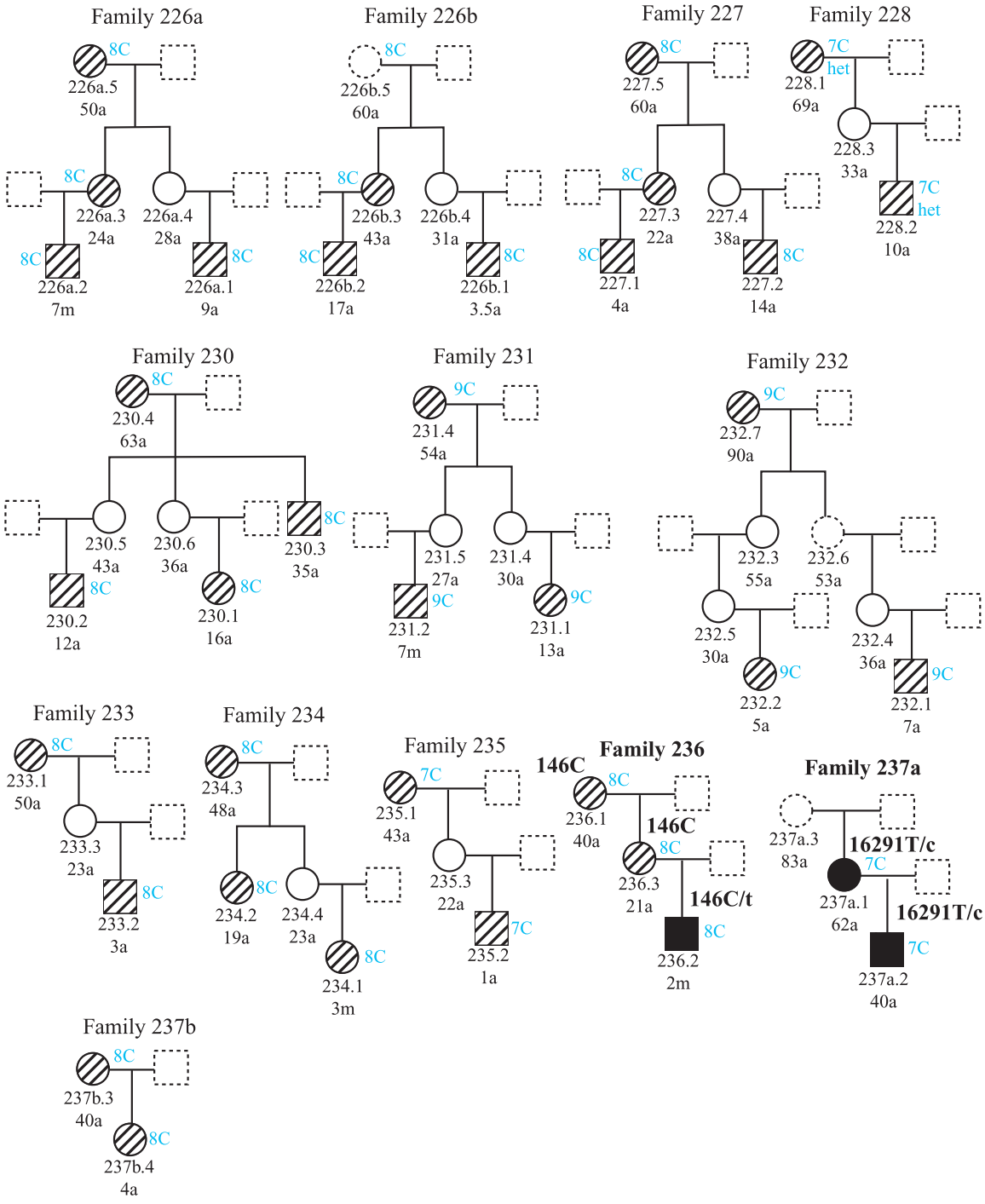
23a
225.4
26a
8C
225.1
5a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226a.2
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226a.1
9a
Family 226b
8C
226b.5
60a
8C
226b.3
43a
226b.4
31a
8C
226b.2
17a
8C
226b.1
3.5a
Family 227
8C
227.5
60a
8C
227.3
22a
227.4
38a
8C
227.1
4a
8C
227.2
14a
Family 228
7C
het
228.1
69a
228.3
33a
7C
het
228.2
10a
Family 230
8C
230.4
63a
230.5
43a
230.6
36a
8C
230.3
35a
8C
230.2
12a
8C
230.1
16a
Family 231
9C
231.4
54a
231.5
27a
231.4
30a
9C
231.2
7m
9C
231.1
13a
Family 232
9C
232.7
90a
232.3
55a
232.6
53a
232.5
30a
9C
232.2
5a
232.4
36a
9C
232.1
7a
Family 233
8C
233.1
50a
233.3
23a
8C
233.2
3a
Family 234
8C
234.3
48a
8C
234.2
19a
234.4
23a
8C
234.1
3m
Family 235
7C
235.1
43a
235.3
22a
7C
235.2
1a
Family 236
146C
8C
236.1
40a
146C
8C
236.3
21a
146C/t
8C
236.2
2m
Family 237a
237a.3
83a
16291T/c
7C
237a.1
62a
16291T/c
7C
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40a
Family 237b
8C
237b.3
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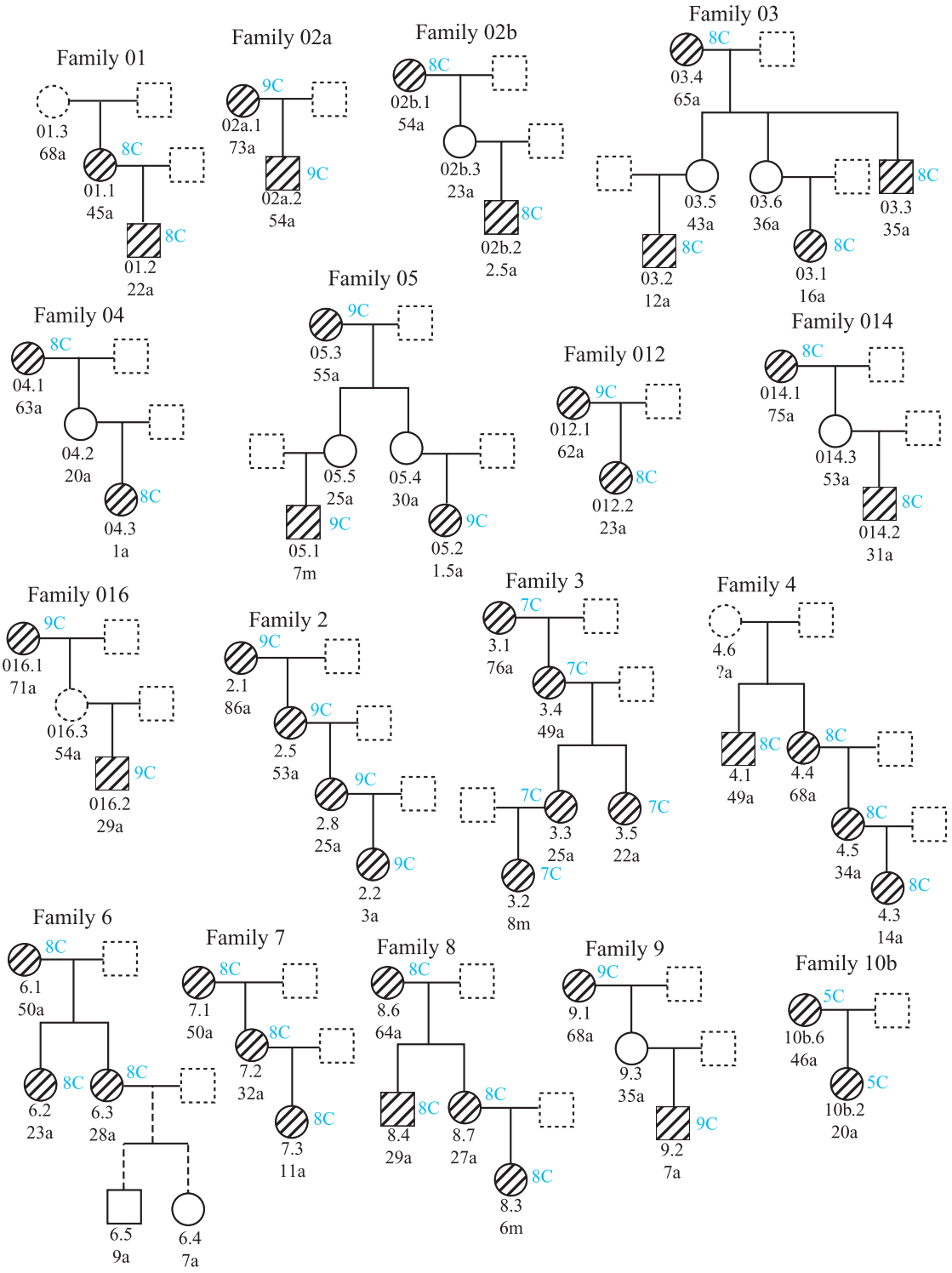
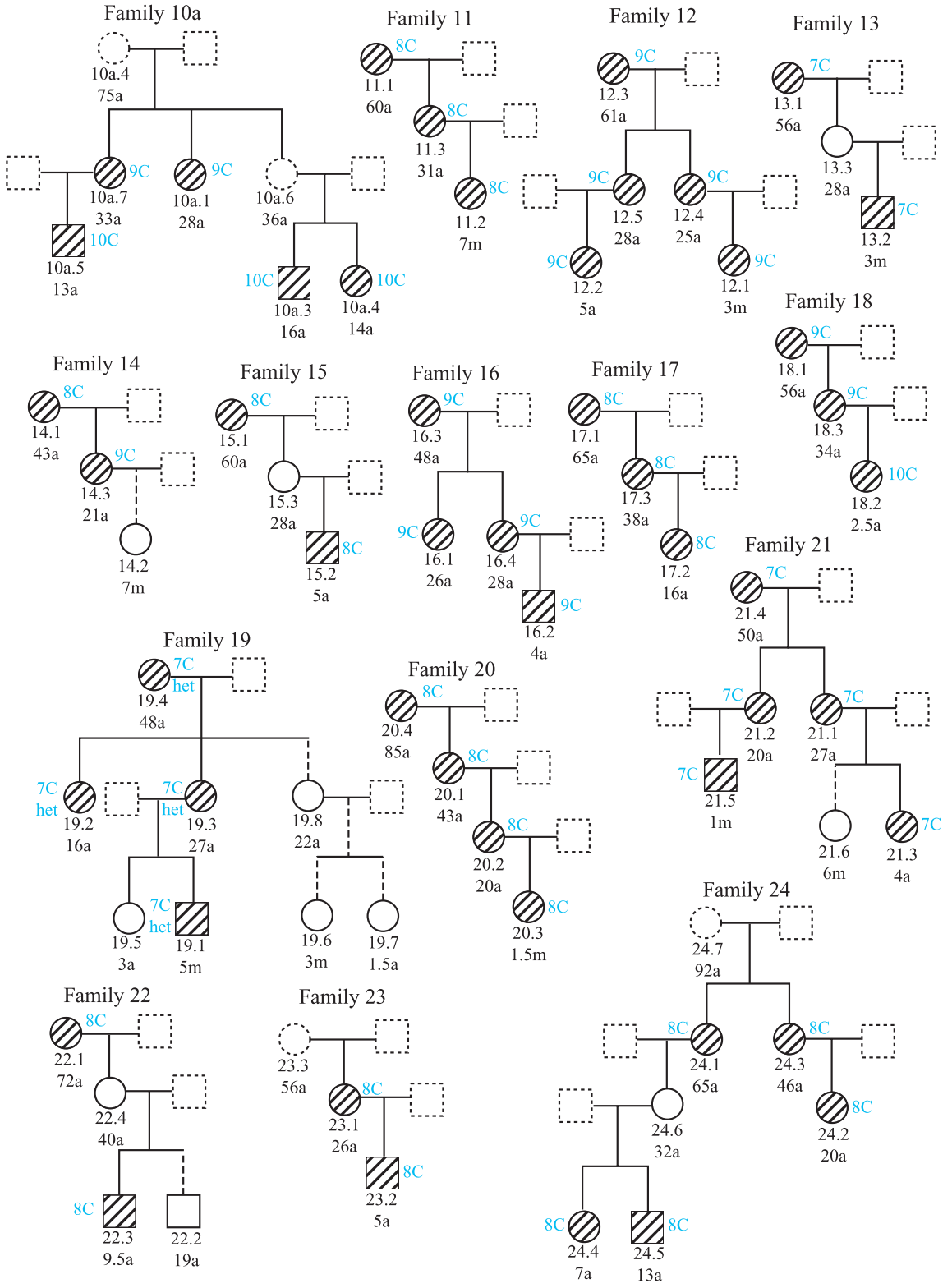
237b.4
4a

Low-radiation families

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10a.1
28a
10a.6
36a
10C
10a.5
13a
10C
10a.3
16a
10C
10a.4
14a
Family 11
8C
11.1
60a
8C
11.3
31a
8C
11.2
7m
Family 12
9C
12.3
61a
9C
9C
12.5
28a
9C
12.4
25a
9C
12.2
5a
9C
12.1
3m
Family 13
7C
13.1
56a
13.3
28a
7C
13.2
3m
Family 14
8C
14.1
43a
9C
14.3
21a
14.2
7m
Family 15
8C
15.1
60a
15.3
28a
8C
15.2
5a
Family 16
9C
16.3
48a
9C
16.1
26a
9C
16.4
28a
9C
16.2
4a
Family 17
8C
17.1
65a
8C
17.3
38a
8C
17.2
16a
Family 18
9C
18.1
56a
9C
18.3
34a
10C
18.2
2.5a
Family 19
7C
het
19.4
48a
7C
het
19.2
16a
7C
het
19.3
27a
19.8
22a
7C
het
19.5
3a
19.1
5m
19.6
3m
19.7
1.5a
Family 20
8C
20.4
85a
8C
20.1
43a
8C
20.2
20a
8C
20.3
1.5m
Family 21
7C
21.4
50a
7C
21.2
20a
7C
21.1
27a
7C
21.5
1m
21.6
6m
7C
21.3
4a
Family 22
8C
22.1
72a
22.4
40a
8C
22.3
9.5a
22.2
19a
Family 23
23.3
56a
8C
23.1
26a
8C
23.2
5a
Family 24
24.7
92a
8C
24.1
65a
8C
24.3
46a
24.6
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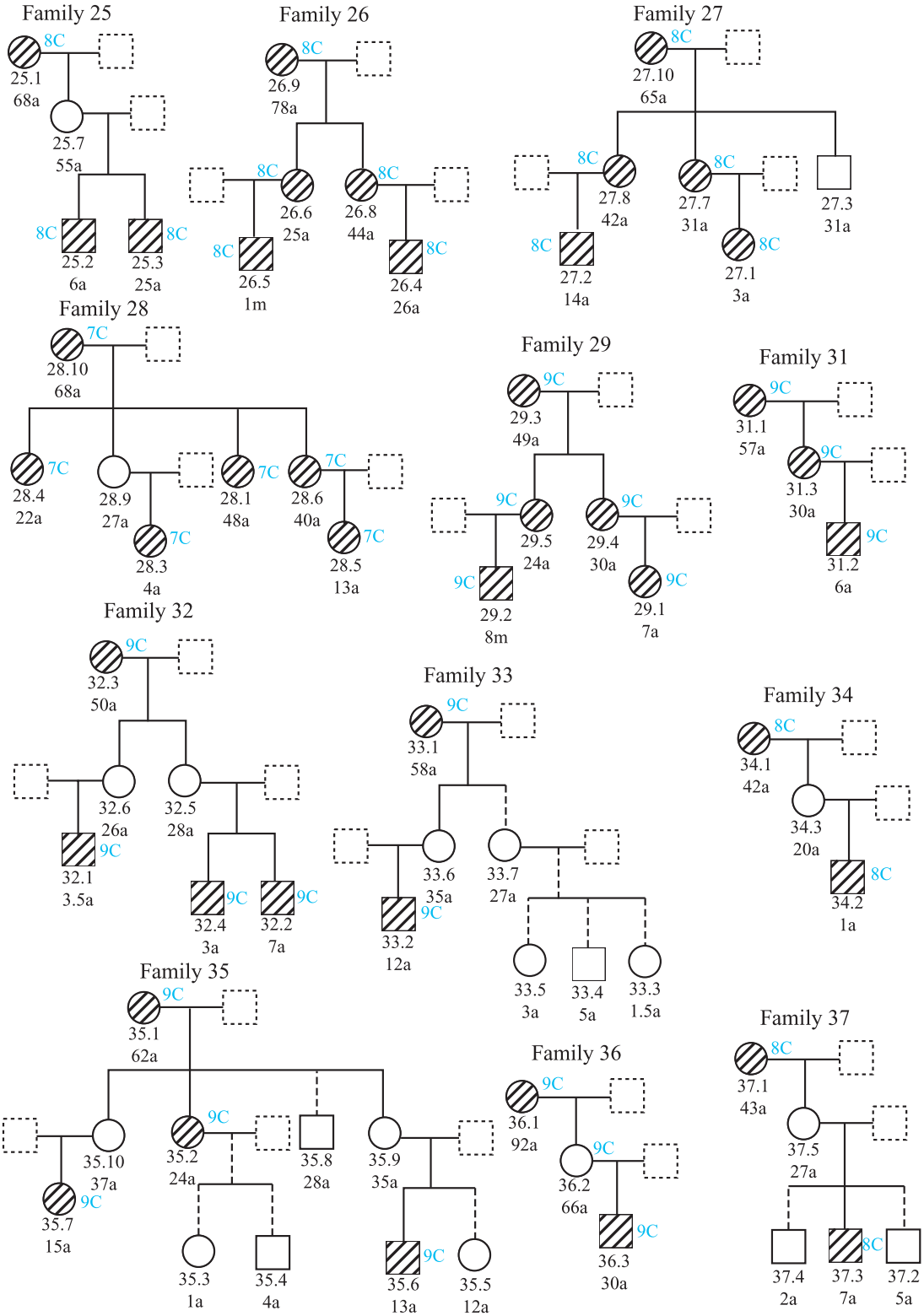
8C
24.2
20a
8C
24.4
7a
8C
24.5
13a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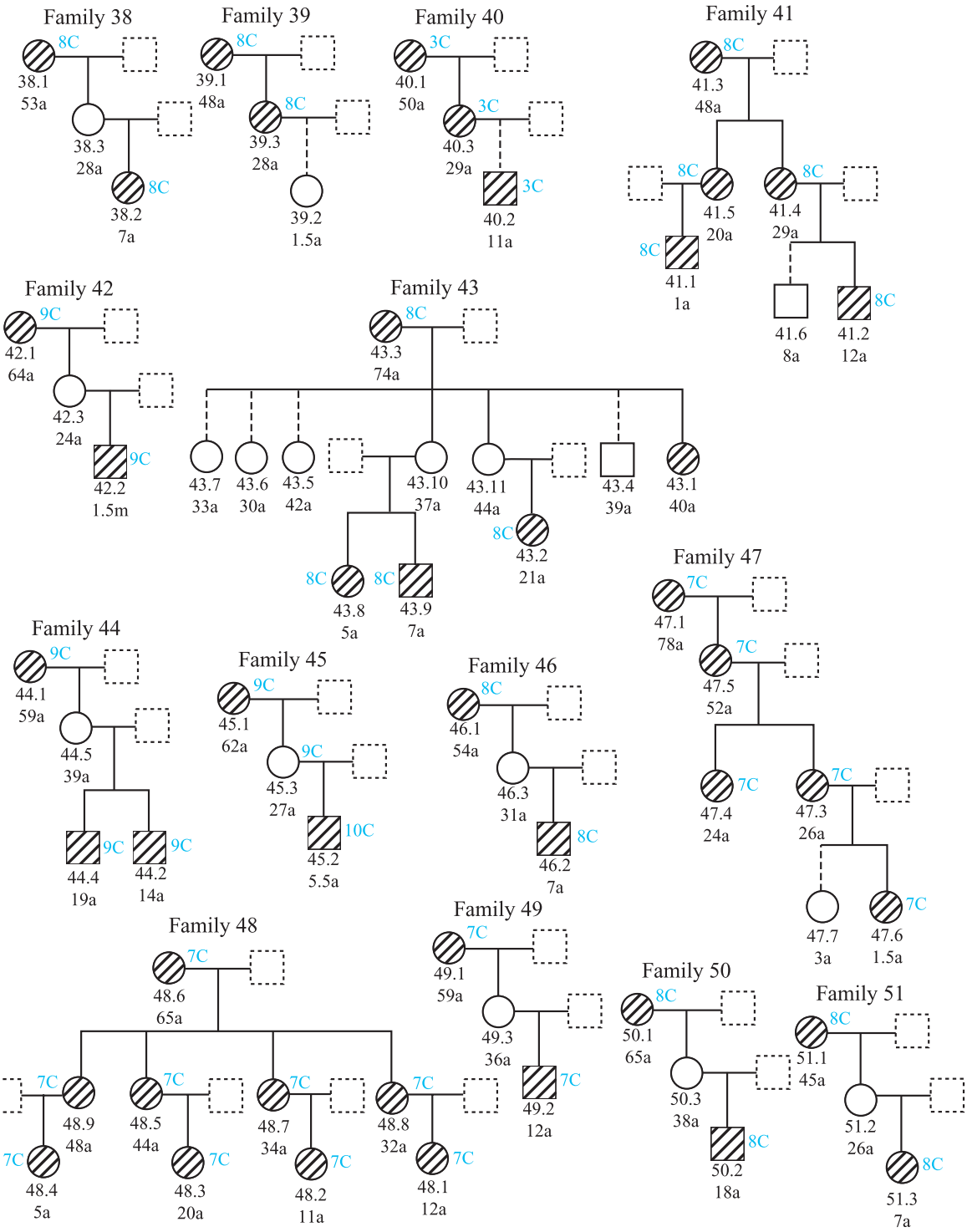
25.3 8C 25a
Family 26
26.9 8C 78a
26.6 8C 25a
26.8 8C 44a
26.5 8C 1m
26.4 8C 26a
Family 27
27.10 8C 65a
27.8 8C 42a
27.7 8C 31a
27.3 31a
27.2 8C 14a
27.1 8C 3a
Family 28
28.10 7C 68a
28.4 7C 22a
28.9 27a
28.1 7C 48a
28.6 7C 40a
28.3 7C 4a
28.5 7C 13a
Family 29
29.3 9C 49a
29.5 9C 24a
29.4 9C 30a
29.2 9C 8m
29.1 9C 7a
Family 31
31.1 9C 57a
31.3 9C 30a
31.2 9C 6a
Family 32
32.3 9C 50a
32.6 26a
32.5 28a
32.1 9C 3.5a
32.4 9C 3a
32.2 9C 7a
Family 33
33.1 9C 58a
33.6 35a
33.7 27a
33.2 9C 12a
33.5 3a
33.4 5a
33.3 1.5a
Family 34
34.1 8C 42a
34.3 20a
34.2 8C 1a
Family 35
35.1 9C 62a
35.10 37a
35.2 9C 24a
35.8 28a
35.9 35a
35.7 9C 15a
35.3 1a
35.4 4a
35.6 9C 13a
35.5 12a
Family 36
36.1 9C 92a
36.2 9C 66a
36.3 9C 30a
Family 37
37.1 8C 43a
37.5 27a
37.4 2a
37.3 8C 7a
37.2 5a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39.3
28a
39.2
1.5a
Family 40
3C
40.1
50a
3C
40.3
29a
3C
40.2
11a
Family 41
8C
41.3
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20a
8C
41.4
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8C
41.1
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41.6
8a
8C
41.2
12a
Family 42
9C
42.1
64a
42.3
24a
9C
42.2
1.5m
Family 43
8C
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74a
43.7
33a
43.6
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43.5
42a
43.10
37a
43.11
44a
43.4
39a
43.1
40a
8C
43.2
21a
8C
43.8
5a
8C
43.9
7a
Family 44
9C
44.1
59a
44.5
39a
9C
44.4
19a
9C
44.2
14a
Family 45
9C
45.1
62a
9C
45.3
27a
10C
45.2
5.5a
Family 46
8C
46.1
54a
46.3
31a
8C
46.2
7a
Family 47
7C
47.1
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7C
47.5
52a
7C
47.4
24a
7C
47.3
26a
47.7
3a
7C
47.6
1.5a
Family 48
7C
48.6
65a
7C
48.9
48a
7C
48.4
5a
7C
48.5
44a
7C
48.3
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7C
48.7
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7C
48.2
11a
7C
48.8
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7C
48.1
12a
Family 49
7C
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59a
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7C
49.2
12a
Family 50
8C
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65a
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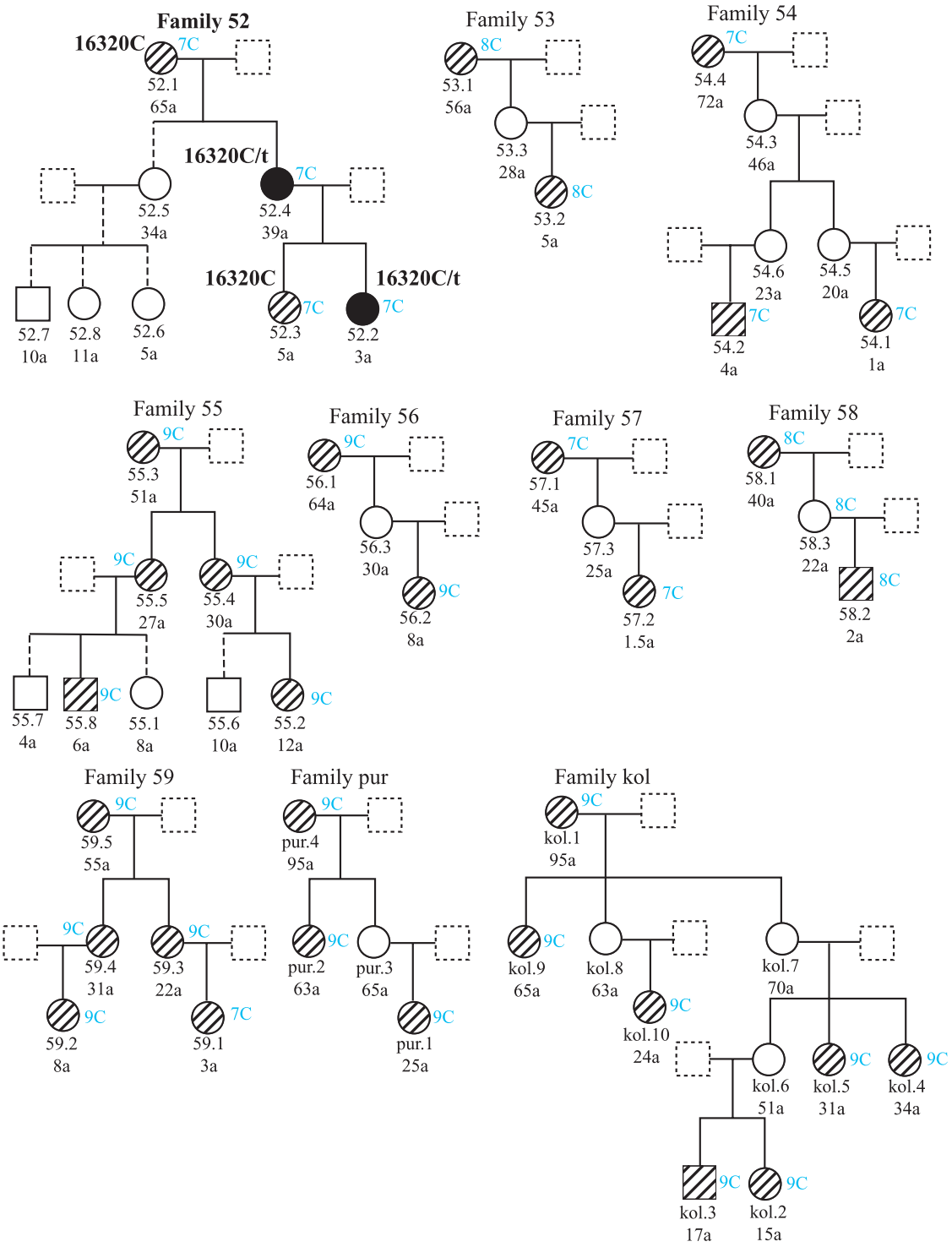
8C
51.3
7a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11a
52.6
5a
16320C
7C
52.3
5a
16320C/t
7C
52.2
3a
Family 53
8C
53.1
56a
53.3
28a
8C
53.2
5a
Family 54
7C
54.4
72a
54.3
46a
54.6
23a
54.5
20a
7C
54.2
4a
7C
54.1
1a
Family 55
9C
55.3
51a
9C
55.5
27a
9C
55.4
30a
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4a
9C
55.8
6a
55.1
8a
55.6
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9C
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56.3
30a
9C
56.2
8a
Family 57
7C
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45a
57.3
25a
7C
57.2
1.5a
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8C
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40a
8C
58.3
22a
8C
58.2
2a
Family 59
9C
59.5
55a
9C
59.4
31a
9C
59.3
22a
9C
59.2
8a
7C
59.1
3a
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9C
pur.4
95a
9C
pur.2
63a
pur.3
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9C
pur.1
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kol.9
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kol.8
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9C
kol.10
24a
kol.7
70a
kol.6
51a
9C
kol.5
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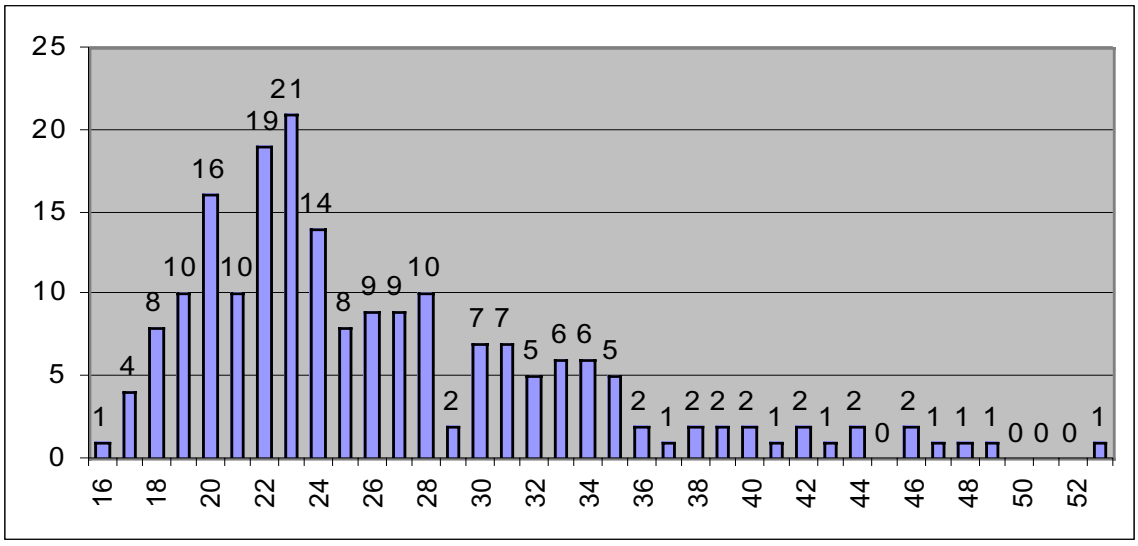
9C
kol.4
34a
9C
kol.3
17a
9C
kol.2
15a

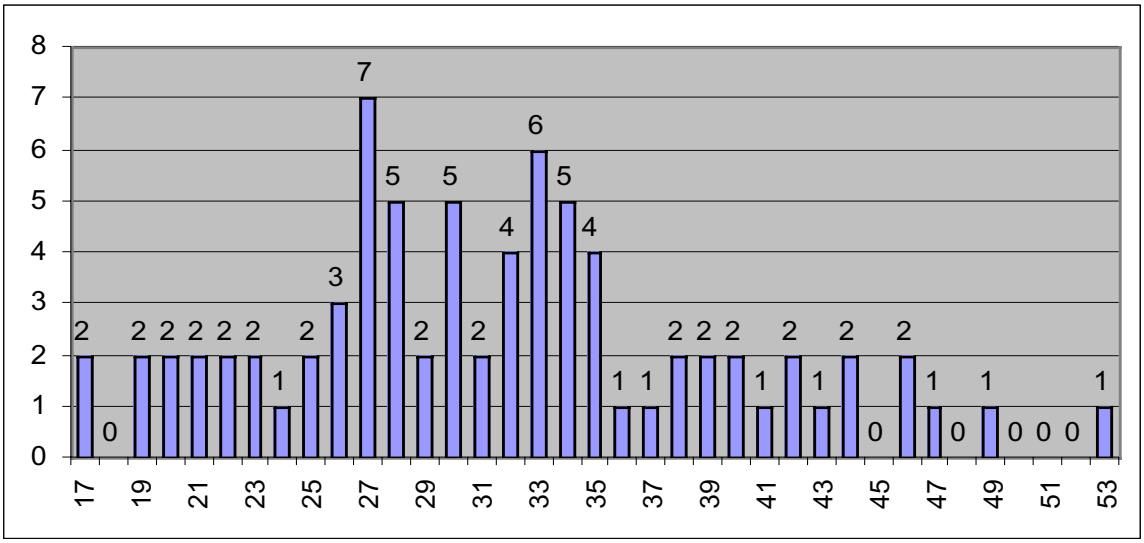
Appendix 3: Age distribution of mothers at birth of daughter

mtDNA generation times are given here as ages of mothers at the birth of their daughter. Sample sizes are from Forster et al. (2002), i.e. slightly smaller than elsewhere in this thesis. Ages were counted from birth rather than from conception. For both high-radiation and the low-radiation (control) areas, sample generation time (including all mothers) as well as long-term generation time were calculated. The latter includes only mothers who had "completed" their families and could not have further children to skew the average, i.e. mothers who were dead or who were >55 years old. Some dead mothers <55 years old have probably been deleted by applying this criterion, making the average generation time too old; on the other hand, the older women tend to underestimate their own age, making the generation time too young. These two sources of error may cancel to some extent.

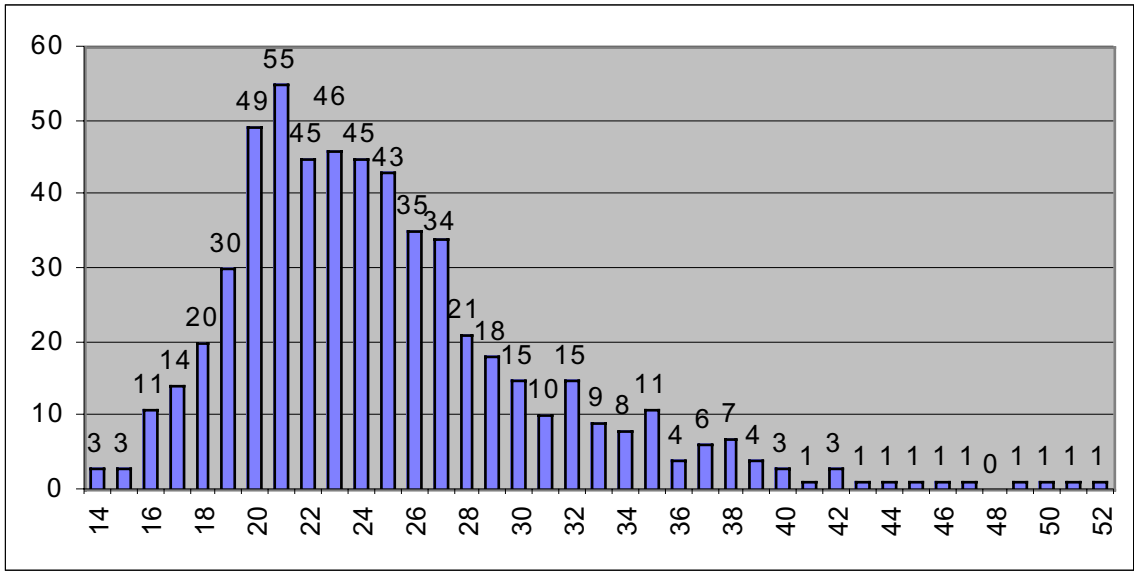
(a) Sample generation times in the control area (all mothers, $n = 198$). The youngest mother was 16, the oldest was 53. One mother aged 13 was considered implausible.



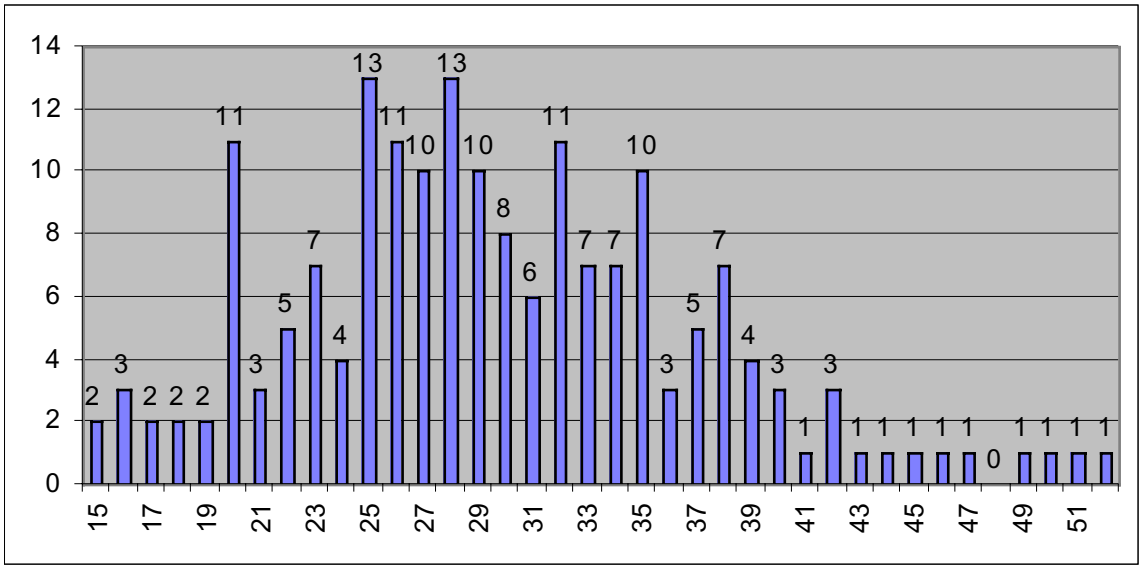
(b) Long-term generation times of completed families in the control area (post-menopausal and dead mothers, $n = 77$).



(c) Sample generation times in the radioactive area (all mothers, *n* = 577). The youngest mother was 14, the oldest was 52. One mother aged 12 was considered implausible.



(d) Long-term generation times of completed families in the radioactive area (post-menopausal and dead mothers, *n* = 182).



Appendix 4: DNA Sequences

Area	Family	mtDNA HV1 variation relative to Anderson et al. 1981								mtDNA HV2 variation relative to Anderson et al. 1981									Location	Longitude	Latitude	Sequence range
Low-rad	O1	16187.T	16223.T	16274.A	16319.A	16320.T				73.G	143.A	195.C	263.G	309.1C	315.1C	337.G	447.G		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16510 and n35- n465
Low-rad	O2a	16223.T	16519.C							73.G	263.G	309.1C	309.2C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16530 and n35- n465
Low-rad	O2b	16223.T	16266.T	16289.G						73.G	263.G	309.1C	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	O3	16051.G	16234.T							73.G	146.C	152.C	263.G	309.1C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	O4	16051.G	16162.G	16239.T	16289.G	16335.G	16352.C	16353.T	16519.N	73.G	146.C	195.C	225.A	234.G	263.G	309.1C	315.1C		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
Low-rad	O5	16223.T	16325.C	16357.C	16519.C					73.G	146.C	151.T	239.C	263.G	309.1C	309.2C	315.1C		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	O12	16129.A	16218.T	16309.G						263.G	309.1C	309.2C	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n35- n465
Low-rad	O14	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	O16	16223.T	16311.C							73.G	151.T	152.C	239.C	249.-	263.G	309.1C	309.2C	315.1C	Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16550 and n35- n465
Low-rad	2	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	3	16223.T	16231.C	16311.C	16362.C					73.G	263.G	315.1C	461.T						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
Low-rad	4	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	6	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	7	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	8	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	9	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	10a	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	10b	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	308.-	309.-	315.1C	456.T		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	11	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	12	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	13	16037.G	16187.T	16189.C	16223.T	16274.A	16319.A	16320.T	16362.C	73.G	143.A	195.C	198.T	263.G	315.1C	447.G			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	14	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	15	16129.A	16223.T	16519.C						73.G	199.C	263.G	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n35- n465
Low-rad	16	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	17	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	18	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	19	16129.A	16223.T							73.G	199.C	263.G	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	20	16129.A	16223.T	16264.T	16265.C	16519.C				73.G	263.G	309.1C	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
Low-rad	21	16051.G	16168.T	16249.C	16519.C					73.G	146.C	152.C	263.G	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n35- n465
Low-rad	22	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	23	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	24	16095.T	16193.T	16223.T						73.G	151.T	152.C	239.C	263.G	309.1C	315.1C			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n35- n465
Low-rad	25	16304.C	16524.R	16526.R						64.T	73.G	189.G	263.G	309.1C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
Low-rad	26	16095.T	16193.T	16223.T						73.G	151.T	152.C	239.C	263.G	309.1C	315.1C			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
Low-rad	27	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	28	16182.C	16183.C	16189.C	16249.C					73.G	146.C	263.G	285.T	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	29	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	263.G	285.T	309.1C	309.2C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	31	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	455.1T	456.T	Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	32	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	33	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	34	16000.C	16183.C	16189.C	16278.T	16319.A	16362.C			73.G	152.C	204.C	240.G	263.G	309.1C	315.1C			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	35	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	36	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	37	16000.C	16183.C	16189.C	16278.T	16319.A	16362.C			73.G	152.C	204.C	240.G	263.G	309.1C	315.1C			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	38	16069.T	16179.T	16223.T	16287.T	16301.T	16304.C	16342.C		73.G	146.C	263.G	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	39	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	40	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	306.-	307.-	308.-	309.-	315.1C 456.T	Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n17- n465
Low-rad	41	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	42	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	43	16069.T	16223.T	16287.T	16301.T	16304.C				73.G	146.C	263.G	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16440 and n35- n465
Low-rad	44	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	45	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	46	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	47	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	48	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	49	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	50	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	51	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	52	16037.G	16187.T	16189.C	16223.T	16319.A	16320.N	16362.C		73.G	143.A	195.C	198.T	263.G	315.1C	337.G	447.G		Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
Low-rad	53	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	54	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	315.1C						Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	55	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	56	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	57	16092.C	16166.G	16182.C	16183.C	16189.C	16249.C			73.G	146.C	263.G	285.T	315.1C					Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n13- n465
Low-rad	58	16166.G	16182.C	16183.C	16189.C	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T			Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	59	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	309.2C	315.1C				Kollam district, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Low-rad	pur	16182.C	16183.C	16189.C	16249.C					73.G	150.T	263.G	285.T	309.1C	309.2C	315.1C			Alleppey, Kerala, India	9,30 N	76,20 E	n15990- n16390 and n35- n465
Low-rad	kol	16182.C	16183.C	16189.C	16249.C					73.G	150.T	263.G	285.T	309.1C	309.2C	315.1C			Alleppey, Kerala, India	9,30 N	76,20 E	n15990- n16390 and n35- n465
High-rad	60	16093.C	16223.T							73.G	199.C	263.G	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	61	16182.C	16183.C	16189.C	16249.C					73.G	263.G	285.T	309.1C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	62	16129.A	16223.T							73.G	199.C	263.G	309.1C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465

Area	Family	mtDNA HV1 variation relative to Anderson et al. 1981	mtDNA HV2 variation relative to Anderson et al. 1981	Location	Longitude	Latitude	Sequence range
High-rad	63a	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	63b	16304.C	64.T 73.G 146.C 189.G 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	64	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	65	16051.G 16234.T 16362.C	73.G 146.C 152.C 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	66	16129.A 16223.T	73.G 199.C 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	67	16093.C 16223.T	73.G 199.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	68	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	69	16249.C 16318.T	73.G 93.G 194.T 246.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16477 and n35- n465
High-rad	70	16182.C 16183.C 16189.C 16249.C 16318.G 16362.C	73.G 263.G 285.T 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	71	16223.T	73.G 146.C 150.T 199.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	72	16051.G 16193.T 16278.T 16357.C	73.G 200.G 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	74	16126.C 16223.T 16519.C	73.G 204.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	75	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	76	16174.T 16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	77	16129.A 16179.T 16227.G 16245.T 16266.T 16278.T 16362.C	73.G 189.G 195.C 246.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16438 and n35- n465
High-rad	78	16129.A 16179.T 16227.G 16245.T 16266.T 16278.T 16362.C	73.G 189.G 195.C 246.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	79	16309.G 16318.T 16348.T 16362.C	73.G 151.T 152.C 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	80	16176.T 16223.T 16519.C	73.G 151.T 152.C 195.Y 239.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16524 and n35- n465
High-rad	81	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	82	16093.C 16223.T 16519.C	73.G 199.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	83	16166.G 16182.C 16183.C 16189.Y 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	84	16093.Y 16129.A 16172.C 16519.C	73.G 263.G 315.1C 338.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	85	16051.G 16162.G 16239.T 16289.G 16335.G 16352.C 16353.T	73.G 146.C 195.C 225.A 234.G 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16485 and n35- n465
High-rad	86	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	87	16223.T 16362.C	56.T 65.1T 66.T 73.G 146.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16446 and n35- n465
High-rad	88	16174.T 16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	89	16174.T 16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	91	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	92	16093.C 16129.A 16223.T 16311.C	73.G 215.G 263.G 309.1C 315.1C 318.C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16473 and n35- n465
High-rad	93	16093.C 16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	94	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	95	16093.C 16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	96	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	97a	16093.C 16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 309.3C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	97b	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	98	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 236.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	99	16111.T 16223.T	73.G 195.A 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16490 and n35- n465
High-rad	100	16126.C 16223.T 16362.C	73.G 200.G 204.C 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	101	16051.G 16182.C 16183.C 16189.C 16218.T 16227.G 16256.T 16278.T 16292.T	73.G 152.C 195.C 207.A 228.A 234.G 263.G 309.- 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	102	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	103	16223.T 16311.C	73.G 151.T 152.C 239.C 249.- 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16486 and n35- n465
High-rad	104	16292.T	73.G 263.G 309.1C 315.1C 373.G	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16483 and n35- n465
High-rad	105	16126.C 16223.T 16362.C 16519.C	73.G 200.G 204.C 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16527 and n35- n465
High-rad	106	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	107	16051.G 16182.C 16183.C 16189.C 16218.T 16227.G 16256.T 16278.T 16286.T 16292.T	73.G 152.C 195.C 207.A 228.A 234.G 263.G 309.- 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	108	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	109	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	110	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	111	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	112	16126.C 16223.T 16311.C	73.G 146.C 150.T 263.G 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16490 and n35- n465
High-rad	113	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	114	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	115	16048.A 16129.A 16189.Y 16218.T 16223.T 16272.R	73.G 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16489 and n35- n465
High-rad	116	16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	117	16182.C 16183.C 16189.C 16218.T 16227.G 16256.T 16278.T 16286.T 16292.T	73.G 152.C 188.G 195.C 207.A 228.A 234.G 263.G 309.- 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	118	16182.C 16183.C 16189.C 16249.C 16318.G 16362.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	119	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	120	16174.T 16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	121	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	122	16174.T 16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	123	16093.C 16129.A 16145.A 16182.C 16183.C 16189.C	73.G 150.T 263.G 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	124	16182.C 16183.C 16189.C 16249.C 16318.G 16362.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	125	16223.T 16287.T 16362.C	56.T 65.1T 66.T 73.G 146.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16540 and n35- n465
High-rad	126	16182.C 16183.C 16189.C 16249.C	73.G 263.G 285.T 309.1C 309.2C 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	127	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	128	16093.C 16223.T 16519.C	73.G 199.C 228.R 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16560 and n1- n465
High-rad	129	16093.C 16223.T 16519.C	73.G 199.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16539 and n1- n465
High-rad	130	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 106.- 107.- 108.- 109.- 110.- 111.- 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	131	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	132	16051.G 16168.T 16249.C	73.G 146.C 152.C 168.C 263.G 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	133	16181.G 16266.T 16304.C 16362.C 16519.C 16524.R	73.G 93.G 200.G 263.G 315.1C 453.C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	134	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 291.W 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	135	16051.G 16182.C 16183.C 16189.C 16218.T 16227.G 16256.T 16278.T 16286.T 16292.T	73.G 152.C 195.C 207.A 228.A 234.G 263.G 309.- 315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	136	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 309.3N 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	137	16166.G 16182.C 16183.C 16189.C 16249.C	73.G 146.C 263.G 285.T 309.1C 309.2C 315.1C 456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465

Area	Family	mtDNA HV1 variation relative to Anderson et al. 1981										mtDNA HV2 variation relative to Anderson et al. 1981											Location	Longitude	Latitude	Sequence range
High-rad	138	16069.T	16274.A	16318.T	16519.C							73.G	151.T	152.C	263.G	309.1C	309.2C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	139	16182.C	16183.C	16189.C	16249.C							73.G	189.R	263.G	285.T	309.1C	309.2C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	140	16051.G	16182.C	16183.C	16189.C	16218.T	16227.G	16256.T	16278.T	16286.T	16292.T	73.G	152.C	195.C	207.A	228.A	234.G	263.G	309.-	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	141	16093.C	16390.A	16519.C								73.G	146.C	195.C	263.G	309.1C	315.1C	456.T					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	142	16182.C	16183.C	16189.C	16249.C	16318.G	16362.C					73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	143	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	144	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	145	16051.G	16209.C	16239.T	16352.C	16353.T	16390.A					73.G	146.C	152.C	234.G	263.G	309.1C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	146	16223.T	16293.G	16325.C								55.1T	59.-	60.-	65.1T	73.G	186.T	263.G	309.1C	315.1C	319.C		Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	147	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	148	16093.C	16223.T									73.G	199.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	149	16187.T	16223.T	16274.A	16319.A	16320.T	16362.C	16519.C				73.G	143.A	146.C	195.C	198.T	263.G	309.1C	315.1C	337.G	447.G		Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	150	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	151	16183.G	16184.T	16223.T	16311.C	16519.C						73.G	150.T	195.C	198.T	207.A	263.G	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	152	16181.G	16266.T	16304.C	16362.C							73.G	93.G	200.G	263.G	315.1C	453.C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16458 and n35- n465
High-rad	153	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	315.1C	456.T						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	154	16223.T										55.1T	59.-	60.-	65.1T	66.T	73.G	153.G	183.G	263.G	315.1C	463.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16456 and n1- n465
High-rad	155	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	156	16187.T	16223.T	16231.C	16356.C	16362.C						73.G	263.G	315.1C	461.T								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	157	16129.A	16144.A	16223.T								73.G	152.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	158	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	159	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	309.2C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	160	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	309.2C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	161	16126.C	16223.T	16311.C								73.G	146.C	150.T	263.G	309.1C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	162	16223.T	16304.C	16519.C								73.G	199.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	163	16169.1C	16172.C	16182.C	16183.C	16189.C	16223.T	16274.A	16319.A	16320.T		73.G	152.C	182.T	195.C	263.G	309.1C	315.1C	447.G				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	164	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	165	16223.T	16266.T									73.G	199.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	166	16126.C	16223.T	16311.C	16519.C							73.G	146.C	150.T	263.G	309.1C	309.2C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16550 and n35- n465
High-rad	167	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	168	16051.G	16182.C	16183.C	16189.C	16218.T	16227.G	16256.T	16278.T	16286.T	16292.T	73.G	152.C	195.C	207.A	228.A	234.G	263.G	309.-	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	169	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	170	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	315.1C	456.T					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	171 I	16223.T										73.G	199.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16447 and n35- n465
High-rad	171 II	16174.T	16182.C	16183.C	16189.C	16249.C						73.G	263.G	285.T	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	171 III	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	309.2C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	172	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	315.1C	456.T					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	173	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	174	16051.G	16209.C	16239.T	16352.C	16353.T	16390.A					73.G	146.C	152.C	234.G	263.G	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16441 and n35- n465
High-rad	175	16111.T	16223.T									73.G	195.A	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	176	16051.G	16206.C	16271.C								73.G	263.G	309.1C	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16440 and n1- n465
High-rad	177	16126.C	16223.T	16362.C								73.G	200.G	204.C	263.G	309.1C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16437 and n35- n465
High-rad	178	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	179	16051.G	16193.T	16278.T	16357.C							73.G	263.G	315.1C									Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	180	16304.C	16311.C									150.T	263.G	315.1C									Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	181	16223.T										73.G	152.C	195.A	263.G	309.1C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16443 and n35- n465
High-rad	182	16292.T	16311.C									73.G	263.G	309.1C	315.1C	373.G							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16437 and n35- n465
High-rad	183	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	184	16069.T	16274.A	16318.T	16519.C							73.G	151.T	152.C	263.G	309.1C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16569 and n1- n465
High-rad	185	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	186	16093.C	16223.T									73.G	199.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	187	16223.T	16325.C	16357.C								73.G	146.C	151.T	152.C	153.G	214.R	239.C	263.G	309.1C	309.2C	315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16477and n35- n465
High-rad	188	16051.G	16093.A	16154.C	16206.C	16230.G	16311.C					73.G	154.C	195.C	263.G	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	189	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	190	16037.G	16187.T	16189.C	16223.T	16274.A	16319.A	16320.T	16362.C			73.G	114.T	143.A	152.Y	195.C	198.T	263.G	315.1C	337.G	447.G		Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	191	16182.C	16183.C	16189.C	16223.T	16311.C						73.G	94.A	146.C	263.G	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	192	16223.T	16231.C	16311.C	16362.C							73.G	263.G	309.1C	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	193	16093.C										73.G	199.C	263.G	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	194	16206.C	16248.T	16291.T								73.G	263.G	315.1C									Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	195	16093.C	16176.T	16206.C								73.G	263.G	315.1C									Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	196	16223.T	16325.C	16357.C								73.G	146.C	151.T	239.C	263.G	279.C	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	197	16111.T	16144.C	16223.T	16224.C	16311.C						73.G	228.A	234.G	263.G	309.1C	309.2C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	198	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	309.2C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	199	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	200	16051.G	16182.C	16183.C	16189.C	16218.T	16227.G	16256.T	16278.T	16286.T	16292.T	73.G	152.C	195.C	207.A	228.A	234.G	263.G	309.-	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	201	16051.G	16182.C	16183.C	16189.C	16218.T	16227.G	16256.T	16278.T	16286.T	16292.T	73.G	152.C	195.C	207.A	228.A	234.G	263.G	309.-	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	202	16051.G	16093.C	16186.T	16206.C							73.G	263.G	315.1C									Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	203	16182.C	16183.C	16189.C	16249.C	16271.C						73.G	263.G	285.T	309.1C	309.2C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	204	16126.C	16223.T	16311.C								73.G	146.C	150.T	189.G	263.G	309.1C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	205	16223.T	16247.G	16318.T								73.G	93.G	194.T	195.C	246.C	263.G	296.T	309.1C	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	206	16126.C	16223.T	16362.C								73.G	200.G	204.C	263.G	309.1C	315.1C						Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	207	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	208	16182.C	16183.C	16189.C	16249.C							73.G	263.G	285.T	309.1C	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	209	16166.G	16182.C	16183.C	16189.C	16249.C						73.G	146.C	263.G	285.T	309.1C	309.2C	309.3C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	210	16179.T	16223.Y	16294.T								73.G	263.G	309.1C	315.1C								Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465

Area	Family	mtDNA HV1 variation relative to Anderson et al. 1981						mtDNA HV2 variation relative to Anderson et al. 1981								Location	Longitude	Latitude	Sequence range
High-rad	211	16213.A	16223.T	16231.C	16356.C	16362.C		73.G	195.C	263.G	315.1C	461.T				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	212	16166.G	16182.C	16183.C	16189.C	16249.C		73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	213	16182.C	16183.C	16189.C	16249.C			73.G	263.G	285.T	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	214	16174.T	16182.C	16183.C	16189.C	16249.C		73.G	263.G	285.T	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	215.1	16093.C	16166.G	16182.C	16183.C	16189.C	16249.C	73.G	146.C	263.G	285.T	309.1C	309.2C	315.1C	456.T	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	215.2	16242.T	16292.T					73.G	263.G	315.1C	373.G					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	216	16051.G	16209.C	16239.T	16352.C	16353.T		73.G	146.C	152.C	234.G	263.G	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	217	16223.T	16362.C	56.T	65.1T	66.T		73.G	146.C	263.G	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	218	16086.C	16104.T	16223.T	16304.C	16357.C		73.G	93.G	95.C	199.C	263.G	309.1C	315.1C		Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	219	16174.T	16182.C	16183.C	16189.C	16249.C		73.G	263.G	285.T	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	220	16174.T	16182.C	16183.C	16189.C	16249.C		73.G	263.G	285.T	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	221	16266.T	16291.T	16304.C	16309.G	16325.C	16356.C	73.G	152.C	263.G	309.1C	309.2C	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	222	16051.G	16234.T					73.G	146.C	152.C	263.G	309.1C	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	223	16129.A	16223.T					73.G	199.C	263.G	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	224	16129.A	16223.T					73.G	199.C	263.G	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16474 and n1- n465
High-rad	225	16129.A	16223.T					73.G	199.C	263.G	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	226a	16129.A	16223.T					73.G	199.C	263.G	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	226b	16182.C	16183.C	16189.C	16249.C			73.G	263.G	285.T	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	227	16126.C	16223.T	16311.C				73.G	146.C	150.T	263.G	309.1C	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	228	16166.G	16182.C	16183.C	16189.C	16249.C		73.G	146.C	263.G	285.T	315.1C	456.T			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	230	16051.G	16234.T					73.G	146.C	152.C	263.G	309.1C	315.1C			Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	231	16223.T	16325.C	16357.C				73.G	146.C	151.T	239.C	263.G	309.1C	309.2C	315.1C	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	232	16309.G	16318.T	16348.T	16355.T	16362.C	16519.C	73.G	151.T	152.C	263.G	309.1C	309.2C	315.1C		Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16519 and n35- n465
High-rad	233	16223.T	16266.T	16289.G				73.G	263.G	309.1C	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	234	16069.T	16179.T	16223.T	16287.T	16301.T	16304.C	73.G	146.C	263.G	309.1C	315.1C				Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	235	16174.T	16182.C	16183.C	16189.C	16249.C		73.G	263.G	285.T	315.1C					Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	236	16051.G	16168.T	16249.C				73.G	146.C	263.G	285.T	309.1C	315.1C	456.T		Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
High-rad	237a	16291.Y	16519.C					263.G	315.1C							Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16519 and n35- n465
High-rad	237b	16187.T	16223.T	16274.A	16319.A	16320.T		73.G	143.A	195.C	263.G	309.1C	315.1C	337.G	447.G	Neendakara/Chavara, Kerala, India	8,53 N	76,36 E	n15990- n16390 and n35- n465
Sri Lanka	tam	16051.G	16086.C	16291.T	16297.C	16353.T	16390.A	73.G	146.C	234.G	263.G	309.1C	315.1C			Tamil, Jaffna, Sri Lanka	9,40 N	80,00 E	n15990- n16390 and n35- n465

Appendix 5: New Mutations

mutant person	mutant nucleotides		proportions of alleles within the person*	
Person 52.2	16320 C/T		C 69% & T 22%	
Person 52.4	16320 C/T		C 79% & T 21%	
Person 68.2	152 C/T		C 49% & T 44%	
Person 69.2	194 T/C		T 59% & C 31%	
Person 80.1	195 C/T		C 46% & T 43%	
Person 83.1	16189 C/T		C 71% & T 27%	
Person 84.1	16093 C/T		C 67% & T 34%	
Person 88.2	215 G/A		G 56% & A 31%	
Person 115.1	16189 T	16272 A/G	T 100% & C 0%	A c.90% & G c.10%
Person 115.2	16189 T/C	16272 A/G	T 76% & C 20%	A 84% & G ?%
Person 115.3	16189 T/C	16272 A/G	T 63% & C 40%	A 69% & G 36%
Person 115.4	16189 T	16272 A/G	T 100% & C 0%	A 98% & G ?%
Person 128.1	228 A/G		A 60% & G 26%	
Person 128.3	228 A/G		A 53% & G 39%	
Person 134.1 & 2	291 T/A		T ?% & A 20%	
Person 134.4	291 T/A		T ?% & A 19%	
Person 134.5	291 A/T		A 51% & T ?%	
Person 139.1 & 3	189 G/A		G 70% & A 15%	
Person 139.2	189 A/G		A 69% & G 17% (sic!)	
Person 141.2	16093 C/T		C 64% & T 39%	
Person 149.2	144 T/C	152 C/T	T ?% & C 40%	T 41% & C 57%
Person 184.1	215 A/G		A 61% & G 47%	
Person 184.4	215 G/A		G 91% & A 14% (sic!)	
Person 186.1	16189 C/T		C 85% & T 19%	
Person 186.5	16189 C/T		C 104% & T 13%	
Person 187.1	214 A/G		A 70% & G ?%	
Person 187.2	214 A/G		A 52% & G ?%	
Person 190.1	152 T/C		T 74% & C 38%	
Person 200.3	207 G/A		G 69% & A 22%	
Person 200.5	207 A/G		A 71% & G 20%	
Person 210.2 & 3	16223 T/C		T 66% & C 28%	
Person 218.2	215 A/G		A 74% & G 17%	
Person 236.2	146 C/T		C 79% & T 20%	
Person 237a.1	16291 T/C		T 67% & C 26%	
Person 237a.2	16291 T/C		T 49% & C 43%	

*Allele percentages often do not sum to exactly 100% due to measurement error (peak areas were measured independently from both nucleotides). Question marks signify that for some mutant nucleotides, no pure 100% peak was available for comparison in the chromatogram database; such nucleotides could thus not be quantified directly.

Appendix 6: Maternity testing results

Family	D3S1538	VWA	FGA	THO1	TPOX	CSF1PO	D5S818	D13S317	D7S820	ACTBP2	amelogenin	morph. sex	Probability
m 68.4	15/16	18/20	24/25	6	8/11	11/12	9/12	9/11	11		X	female	
c 68.2	15/16	16/20	21/25	6/9	11	11	12/13	8/9	11		X	female	0.9999
m 68.3	15/16	16	20/24	6/9	11/12	n.d.	11	9/11	8/11		X	female	
c 68.1	15/16	16/17	20/23	6/9	8/12	11/12	11/13	11/12	10/11		X	female	0.995093
m 69.4	17	15/16	20/25	6/9	8/9	12	12	8/11	7/8	17/18	X	female	
c 69.2	17	16	20/21	9	8	12	11/12	11/12	8	18/28.2	XY	male	0.99805
m 70.1	15/17	17	21/24	6/9.3	9/10	10/11	12	12	7/11		X	female	
c 70.3	15/17	17	21/22	6	10/12	10/12	11/12	12/13	7/8		X	female	0.9997
m 70.3	15/17	17	21/22	6	10/12	10/12	11/12	12/13	7/8		X	female	
c 70.2	15/16	17/20	22/24	6	8/10	10	11/12	8/13	7/8		X	female	0.9998
m 80.1	15	16/18	22/24	7/8	8/9	11	11/15	11/12	7/10		X	female	
c 80.4	15/17	16	22/25	8/9	8/12	11/12	11	11/12	7/8		X	female	0.997
m 83.1	insufficient DNA											female	
c 83.3	17/18	15/19	20/23	8/9	11	12	10/13	11/12	9/12		X	female	
m 84.1	16/18	18/19	23	9	8/9	12	11/13	11/14	10/12		X	female	
c 84.2	14/16	18/19	23	9	9	10/12	11/12	11/12	12		X	female	0.99787
m 86.5	14/15	16/17	19/23	6/9	8/9	10/12	10/12	10/14	8/12		X	female	
c 86.2	15/17	16/17	19/24	6/9	8/11	12	10/11	9/14	8		X	female	0.9988
m 86.5	14/15	16/17	19/23	6/9	8/9	10/12	10/12	10/14	8/12		X	female	
c 86.4	14/18	16/17	23	6	8	12	10/11	9/14	8		X	female	0.9996
m 88.6	15/16	16/18	24	6/9	11	10/11	11/13	11/13	7/11	16/33.2	X	female	
c 88.2	15/16	16	21/24	6	11	11/12	11	11/12	11	15/16	XY	male	0.99911
m 88.7	16	16/18	24	6/7	11	10/11	12/13	11/13	7/8		X	female	
c 88.1	16/18	15/16	20/24	6	9/11	11/12	12	12/13	7/12		X	male	0.9998
m 91.5	16/17	15/17	19/22	6/9	11	10/11	10/12	8/12	10/12	17/19	X	female	
c 91.2	17/18	17/19	19/24	6/9.3	11	n.d.	10/14	8/11	12	19/29.2	X	female	0.9992
m 91.4	16	17/19	21/22	9.3	10/12	10/11	10/12	12/13	10/13		X	female	
c 91.3	not determined											female	
m 92.4	15/17	14/17	24/27	6	8/11	10/11	11/12	11/12	10/12	20/30.2	X	female	
c 92.2	15	14/15	24/27	6/9.3	8/11	10/11	11/12	11/13	12	28.2/30.2	X	female	0.9779
m 92.4	15/17	14/17	24/27	6	8/11	10/11	11/12	11/12	10/12	20/30.2	X	female	
c 92.3	15/16	14/15	20/24	6/9.3	11	10/11	12	11	12		X	female	0,9968
m 92.5	15/17	14/15	20/24	6/9.3	n.d.	n.d.	11/12	11	n.d.	20/28.2	X	female	
c 92.1	17	15/19	20/27	7/9.3	8/9	10/12	11/13	11/12	10/12	28.2	X	female	0.99993
m 95.4	16/17	14/16	24	6/8	9/10	12/15	11/13	12	10/13		X	female	
c 95.1	15/16	14/16	21/24	6/8	8/9	12/15	9/11	12/13	7/10		X	female	0.9998
m 97.3	15	15/17	19/23	6/8	9/11	10/11	13/15	11/12	12	16/27.2	X	female	
c 97.2	16/18	17/18	23/24	6/9	8	12	11/13	11/12	9/11	19/28.2	XY	male	exclusion
m 97.4	15/17	14/17	19/25	8/9	8/11	10/11	13	8/12	11/12	18/27.2	X	female	
c 97.1	15/16	14/18	19/23	8/9.3	8/11	10/12	11/13	8	11/12	18/22.2	X	female	0.9998
m 115.3	17/19	14/17	22/25	6/8	9/11	n.d.	10/12	8/11	8/10	21.1/27.2	X	female	
c 115.4	17/18	14/17	20/22	6/8	11/12	13	12/13	11	10/11	21.1/31.2	X	female	0.9988
m 115.4	17/18	14/17	20/22	6/8	11/12	13	12/13	11	10/11	21.1/31.2	X	female	
c 115.1	17/18	16/17	22/27	6/9.3	10/12	10/13	12/13	11/14	11/12		X	female	0.9990
m 118.4	15/16	16/17	21/24	6/9.3	10/11	10/12	11/12	10/12	8/13		X	female	
c 118.1	16/17	17/18	20/21	6/9	8/10	10/12	11/13	9/12	8/10		XY	male	0.9156
m 120.4	15/16	14/19	20/21	7/9.3	11	11/12	13	11	7/11		X	female	
c 120.3	15/16	14/15	20	7/9.3	9/11	n.d.	13	11	11		X	female	0.9997
m 124.5	15/16	16/17	21/24	9	8/10	11/13	11/13	12/13	10/12		X	female	
c 124.2	insufficient DNA											male	
m 127.6	15/16	19	20/23	6/8	8/9	13	11	12	8/9		X	female	mutation
c 127.2	15/16	15/19	20/23	6/8	9	12/13	11/12	11	8/11		X	female	0.9342
m 128.3	insufficient DNA											female	
c 128.2	15/16	15/17	20/21	6	8/10	8/12	12/14	13	10/11		X	female	
m 130.4	14/17	17	20/25	8/9	8/11	11/12	10/12	12/13	8/11		X		
c 130.2	14/17	17	25	9/9.3	8/11	12	10/13	9/13	8/11		XY		0.9998
m 131.1	15	15/16	20/21	6/8	8	12	13/14	11/12	8/11		X	female	
c 131.2	not determined											male	
m 133.4	17	17/19	21/23	8/9	8/10	10/12?	12/13	12	10/12		X	female	
c 133.1	insufficient DNA											female	
m 134.5	15/16	14/15	20/24	6/9.3	8	12	12/14	11	8/11		X	female	

Family	D3S1538	VWA	FGA	THO1	TPOX	CSF1PO	D5S818	D13S317	D7S820	ACTBP2	amelogenin	morph. sex	Probability
c 134.4	16/18	14	22/24	6	8	9/12	12	11/12	10/11		X	female	0,99359
m 136.3	16/17	14/16	20	6/9	8	12	11/12	8	11/13		X	female	
c 136.2	17/18	16	20	6/9	8/11	12	12/13	8/10	11/13		XY	male	0,999745
m 139.1	12/17	17/18	22/24	6/9	11/12	11/12	13	8/12	7/11		X	female	
c 139.2	16/17	16/18	22/23	6	10/11	11	11/13	8	7/10		X	female	0.99893
m 141.3	15/16	17/19	21/25	9	11	12	9/11	8/12	8/10		X	female	
c 141.2	15/16	17/20	21/25	8/9	11	11/12	9/11	12	8/10		X	female	0.99976
m 144.3	16/17	15/18	20/25	8/9	10/12	10/11	11/12	11/12	8/11		X	female	
c 144.2	15/16	18	25/25.2	7/9	8/10	10/12	11/14	10/12	11		XY	male	0.9815
m 147.3	16/17	14/17	18/25	6/9	8/9	12	12/13	8/11	10/12		X	female	
c 147.4	16/17	14/17	23/25	6/9	9/10	11/12	10/13	8/11	7/10		X	female	0.9988
m 147.4	16/17	14/17	23/25	6/9	9/10	11/12	10/13	8/11	7/10		X	female	
c 147.1	16/17	14/16	20/23	6/9	8/10	11/12	10/11	11/12	7/11		X	female	0.9999
m 149.4	15/16	14/16	22/24	9/9.3	10/11	11/12	12	12	8/13		X	female	
c 149.2	15	14/16	21/24	9/9.3	11	12	11/12	12/14	11/13		XY	male	0.99963
m 159.1	18	18/20	20/25	9/9.3	8	11/15	11/12	8	8/9		X	female	
c 159.3	15/18	17/18	22/25	7/9.3	8/11	11/12	11	8/12	8/12		X	female	0.9723
m 164.4	16/17	19	24	6/9	11/12	11	10/11	9	11/13		X	female	
c 164.3	15/16	18/19	20/24	6/9	11/12	11/12	11/13	9/12	11		X	female	0.9999
m 164.3	15/16	18/19	20/24	6/9	11/12	11/12	11/13	9/12	11		X	female	
c 164.1	15/17	16/18	20/26	9	11	11/12	11	9/11	11/12		XY	male	0.9938
m 169.6	14/17	14/17	22/23	6/9	8/9	11/12	10/12	8/12	8/10		X	female	
c 169.3	14/17	14/19	23/24	6/8	8	11/12	11/12	8	8/12		X	female	0.9915
m 169.4	14/17	16/18	23/24	6/9	8/12	11/13	11/12	12	12		X	female	
c 169.1	16/17	16/18	21/24	6/9	11/12	11/13	11	8/12	12		XY	male	0.9999
m 170.4	16/17	16/18	23/24	8/9	8	11/12	11/12	11/12	8		X	female	
c 170.3	15/17	14/18	23/24	6/8	8/9	11	9/12	12	8		X	female	0.9976
m 171 III.6	16	17	20/22	8/9.3	8/12	10/12	13	8/12	11/12		X	female	
c 171 III.4	16/17	17	20/22	8	8	10/12	11/13	11/12	8/11		X	female	0.9984
m181.3	16	17/20	24	6	9/11	9/10	12	8/11	7/8		X	female	
c 181.4	16/17	18/20	20/24	6/7	9/11	9/13	10/12	8/11	7/10		X	female	0.9999
m 181.4	16/17	18/20	20/24	6/7	9/11	9/13	10/12	8/11	7/10		X	female	
c 181.1	15/16	17/18	20/24	6/9	8/11	9/12	10/12	8/11	7/8		X	female	0.9998
m 184.6	15/17	18	21/25	9	8/11	n.d.	11/12	9/12	7		X	female	
c 184.4	15/17	14/18	21/22	6/9	10/11	n.d.	11/12	8/9	7/9	21.2/36.2	X	female	0.9995
m 184.4	15/17	14/18	21/22	6/9	10/11	n.d.	11/12	8/9	7/9	21.2/36.2	X	female	
c 184.1	15/16	17/18	22/23	8/9	9/10	11/12	12	8/12	7/10	20/21.2	X	female	0.9999
m 186.4	16	18	20/24	6	9/11	10/12	12/13	7/11	12		X	female	
c 186.5	16/18	15/18	20/24	6	8/11	n.d.	11/12	11	11/12	20/29.2	X	female	0,996
m 186.5	16/18	15/18	20/24	6	8/11	n.d.	11/12	11	11/12	20/29.2	X	female	
c 186.1	16/18	15/16	24/26	6/8	11	n.d.	10/11	11	n.d.	29.2/31.2	X	female	0.9954
m 186.6	16/18	18	24	6	9	12	11/13	7/11	12/14	20/29.2	X	female	
c 186.2	16	16/18	24	6/8	8/9	11/12	12/13	7/11	10/14	18/29.2	XY	male	0.999998
m 187.1	15/16	14/18	22	8/9.3	9	10/12	10/11	8/12	7/8		X	female	
c 187.3	15/16	14/16	22/24	8/9.3	9/11	10/12	10/13	9/12	7/12		X	female	0.99993
m 187.3	15/16	14/16	22/24	8/9.3	9/11	10/12	10/13	9/12	7/12		X	female	
c 187.2	15/16	14/16	22/24	9/9.3	9/11	12	11/13	11/12	7/8		X	female	0.99922
m 190.1	16/17	14/18	21/22	6/9.3	8	10	11	9/12	9/14		X	female	
c 190.3	15/16	14/16	21/22	6/9.3	8/11	10	11	9/11	9		X	female	0.9994
m 200.5	insufficient DNA											female	
c 200.3	15/18	15	18/23	6	8/9	11/12	10/11	8/12	8		XY	male	
m 203.3	16	15/18	20/22	9.3	9/11	11/12	10/11	9/10	10/11		X	female	
c 203.2	16/17	15/17	20	8/9.3	9	10/12	9/10	9/11	7/10		X	female	0.9991
m 208.4	16/17	16/17	20/24	9/9.3	8/11	10/11	12	12/13	7/9		X	female	
c 208.2	16	16/18	20/24	6/9	8/11	10/11	12/13	11/13	8/9		XY	male	0.9974
m 210.3	14/16	17/18	22/25	7/9	8/11	10/13	9/11	8/12	8/12		X	female	
c 210.2	insufficient DNA											male	
m 212.1	17	15/16	23	7/9.3	9/11	10/13	11	8/11	8		X	female	
c 212.3	insufficient DNA											female	
m 215.3	16	18	21	8	8/11	10/12	11/12	12	7/11		X	female	
c 215.2	15/16	18	22/24	8/9	8/9	10/11	11/12	9	8/11		X	female	exclusion
m 218.3	not determined												
c 218.2	17/18	16	20/23	6/9	8/11	12/13	11/13	7	8		XY	male	
m 224.6	14/15	16/17	20/25	6/9	9/12	12/14?	11/12	8/10	10		X	female	
c 224.2	15/16	16/18	20/21	7/9	9/11	12?	12	8/11	10?		X	female	0.9992

Family	D3S1538	VWA	FGA	THO1	TPOX	CSF1PO	D5S818	D13S317	D7S820	ACTBP2	amelogenin	morph. sex	Probability
m 233.1	15/16	16	19/21	9/9.3	8/10	n.d.	10/13	11/12	10/12	25.2/29.2	X	female	
c 233.3	insufficient DNA											female	
m 234.4	16/18	16/18	24.2/25	6/9	9/11	11/12	10/11	8/11	11	17/18	X	female	
c 234.1	15/16	14/18	21/25	6	11	11/12	10/13	9/11	9/11	17/18	X	female	0.9995
sister 234.4	16/18	16/18	24.2/25	6/9	9/11	11/12	10/11	8/11	11	17/18	X	female	
sister 234.2	16	16/18	24/25	6/9	9/11	10/12	11/12	8/9	11	18/23.2	X	female	0.94564*
m 236.3	15/16	16/18	22/25	8	8/11	12	11/13	11	7/8		X	female	
c 236.2	15	16	22/25	7/8	8	12	11/12	9/11	8		XY	male	0.9984
m 237a.1	14/17	14/15	20	6/7	9/11	10/11	11/13	8/12	10/11		X	female	
c 237a.2	14/18	14/18	20/23	7/9.3	9/10	10/13	13	9/12	8/11		XY	male	0.9982
m 02a.1	15	14/16	20/25	7/9.3	9	12	10/13	12/14	10		X	female	
c 02a.2	15/16	16/17	19/25	9/9.3	9/10	10/12	13	11/12	10		XY	male	0.9960
m 014.3	15/16	16	22/25	6/9	8/10	11/12?	12	9/12	8/10		X	female	
c 014.2	15/17	16	22/24	6/7	8/11	11/12	12	9/11	8		XY	male	0.9968
m 2.8	15/16	16/18	22	8	8	10/12	11/12	8/9	8/13		X	female	
c 2.2	15/16	18	22/24	8/9	8/9	10/11	11/12	9	8/11		X	female	0.9985
m 8.6	16/17	15/18	21/22	8	8	11/12	11/12	9/12	10/13		X	female	
c 8.4	15/16	16/18	21/24	8	8	12	11/12	7/12	8/13		XY	male	0.9978
m 10a.7	15/17	16	19	6/9	11	11/12?	11/12	8/9	10/12		X	female	
c 10a.5	15/17	16	19/20	6/8	11	11	11/12	8/12	10/11		XY	male	0.9991
m 14.1	15/17	14/19	24/24.2	6/9	10/11	12/13	11/13	8	7/10		X	female	
c 14.3	15/17	14/15	24/25	7/9	11	n.d.	12/13	8/13	10/11		X	female	0.9968
m 18.3	15/16	17/19	23/24	6/8	8/11	11	11/12	9/12	10/12		X	female	
c 18.2	15/17	14/17	23/24	6/8	11	11	12	12	10/11		X	female	0.9965
m 27.7	17/18	16	20/23	9	11	12/13	11/12	7/9	9/12		X	female	
c 27.1	16/17	14/16	20/23	9	9/11	12	12	7/11	9/12		X	female	0.9999
m 27.10	15/16	16/19	23	6	8	12/13	11/12	10/12	8/12		X	female	
c 27.3	15/16	18/19	22/23	6/8	8/11	12	11	8/12	8		X	female	0.9921
m 52.1	16/19	18	20/24	6	8/11	11	11/13	8/11	10		X	female	
c 52.4	14/19	14/18	19/24	6	8/9	11/12	11	8/11	8/10		X	female	0.9996
m 55.3	15	16	19	6/9	8/10	12	11/12	9/12	10/12		X	female	
c 55.4	15	16	19/23	6/9	8/9	12	11/12	11/12	10		X	female	0.9992
m 57.3	15/17	18/19	23/24	9	9/11	12/13	12/13	8/14	8/13		X	female	
c 57.2	insufficient DNA											female	

*This value was calculated using the software of Prof. Max P. Baur (Institut f r Medizinische Biometrie, Informatik und Epidemiologie, Bonn).

Appendix 7: Bacterial cloning of doubly mutated mtDNA

One of the sampled individuals in the radioactive area of Kerala (individual 149.2 in Appendix 2) has two new mtDNA mutations in his saliva sample. Direct DNA sequencing displayed two heteroplasmic nucleotide positions at np 144 (c/T) and np 152 (t/C), and peak area measurements suggested that the nucleotide proportions were approximately 40/60 and 41/57 respectively (Appendix 5). As described in the following, bacterial cloning was performed to confirm these proportions, and to find out whether the two mutations were on the same mtDNA molecules or on different ones. First, native Pfu-Polymerase (Stratagene, Heidelberg) was used to amplify the mtDNA. As this polymerase creates blunt ends, 0.2µL AmpliTaq-DNA-polymerase (5 U/µL) (Applied Biosystems, Darmstadt, Germany) was added in the last step of the PCR (10 minutes at 72°C). The latter polymerase adds adenine residues, which are necessary for ligation with a thymine vector. The employed *E. coli* strain K12 is included as competent cells in the TOPO-TA-Cloning- Kit® 2.1 of the supplier Invitrogen (Karlsruhe, Germany). The strain designation is:
(F- mcrA *(mrr-hsdRMS-mcrBC)F80lacZ*(M15*(lacX74recA1araD139*(ara-leu)7697galUgalKrpsL(StrR)endA1nupG). Foreign DNA introduced into this strain is neither modified nor degraded by restriction. The F' episome of this strain contains the lacZ*M15 segment which codes for the peptide β-galactosidase. Thus, the successful introduction of DNA into the lacZ gene can be visually identified by offering β-galactosidase substrate to the bacterial colonies and selecting those colonies for DNA purification whose lacZ activity is destroyed and can therefore no longer transform white XGal substrate into blue product. (Experimental details courtesy of Dr. Sabine Lutz-Bonengel, Freiburg).

The following table lists the occurrence of the two point mutations and the length variation at nps303-309 in 100 clones (15 further clones failed as indicated by "n.d."). Furthermore, other point mutations are listed, which were probably introduced secondarily during the cloning and PCR procedure. For example, one such secondary mutation 307.T (clone 55) has occurred on a 9C allele, giving the following nucleotide sequence between np302 and np316: ACCCCTCCCCTCCCCCCG.

Appendix 7: Bacterial cloning of doubly mutated mtDNA *(cont.)*

clone	np144	np152	nps303–309	other nps	clone	np144	np152	nps303–309	other nps
1	T	C	8C		59	C	T	8C	
2	C	T	8C	210.G	60	T	C	9C	
3	T	C	8C		61	C	T	8C	
4	T	C	8C		62	C	T	8C	
5	C	T	9C		63	T	C	8C	
6	T	C	8C	70.T	64	T	C	9C	
7	C	T	8C	251.A	65	C	T	8C	
8	C	T	8C	65.C&305.T	66	C	T	8C	
9	C	T	8C	210.G	67	T	C	8C	
10	C	T	9C		68	n.d.	n.d.	n.d.	n.d.
11	T	C	8C		69	C	T	8C	
12	C	T	8C		70	T	C	8C	
13	C	T	9C		71	T	C	9C	
14	C	T	8C		72	T	C	9C	
15	T	C	8C		73	C	T	8C	225.T
16	T	C	8C	104.T	74	C	T	8C	
17	C	C	9C		75	C	T	8C	
18	T	C	9C		76	T	C	8C	
19	T	C	8C		77	T	C	8C	
20	T	C	8C	346.C	78	C	T	8C	
21	C	T	8C		79	T	C	7C	
22	T	C	8C		80	T	C	8C	
23	C	T	8C		81	C	T	8C	
24	T	C	8C		82	T	C	8C	
25	T	C	8C		83	T	C	10C	
26	T	C	8C	306.T	84	n.d.	n.d.	n.d.	n.d.
27	C	T	8C		85	n.d.	n.d.	n.d.	n.d.
28	C	T	10C		86	n.d.	n.d.	n.d.	n.d.
29	n.d.	n.d.	n.d.	n.d.	87	T	C	8C	
30	C	T	8C		88	n.d.	n.d.	n.d.	n.d.
31	C	T	8C		89	T	C	8C	
32	T	C	8C		90	T	C	8C	
33	C	T	8C		91	T	C	9C	
34	C	T	9C	189.G	92	n.d.	n.d.	n.d.	n.d.
35	C	T	8C		93	C	T	8C	
36	n.d.	n.d.	n.d.	n.d.	94	C	T	8C	
37	T	C	8C		95	T	C	8C	
38	C	T	7C		96	n.d.	n.d.	n.d.	n.d.
39	T	C	8C		97	T	C	10C	184.C
40	C	T	7C	291.G	98	T	C	8C	
41	T	C	8C		99	T	C	8C	324.G
42	C	T	8C		100	C	T	8C	345.A
43	T	C	9C	99.C	101	T	C	8C	
44	C	T	8C		102	C	T	8C	
45	T	C	8C	305.A	103	C	T	8C	
46	T	C	8C		104	T	C	8C	139.A
47	C	T	8C		105	n.d.	n.d.	n.d.	n.d.
48	C	T	9C		106	n.d.	n.d.	n.d.	n.d.
49	n.d.	n.d.	n.d.	n.d.	107	C	T	7C	
50	T	C	8C		108	C	T	8C	
51	T	C	8C	326.G	109	T	C	8C	
52	n.d.	n.d.	n.d.	n.d.	110	C	T	8C	
53	C	T	9C		111	C	T	8C	
54	T	C	8C		112	n.d.	n.d.	n.d.	n.d.
55	T	C	9C	307.T	113	n.d.	n.d.	n.d.	n.d.
56	C	T	9C		114	T	C	8C	
57	T	C	10C		115	C	T	8C	
58	C	T	9C	312.A					