AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 133:847–853 (2007)

mtDNA From Hair and Nail Clarifies the Genetic Relationship of the 15th Century Qilakitsoq Inuit Mummies

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KEY WORDS Greenland; ancient DNA; familial relationship

ABSTRACT The 15th century Inuit mummies excavated at Qilakitsoq in Greenland in 1978 were exceptionally well preserved and represent the largest find of naturally mummified specimens from the Arctic. The estimated ages of the individuals, their distribution between two adjacent graves, the results of tissue typing, and incomplete STR results led researchers to conclude that the eight mummies formed two distinct family groups: A grandmother (I/5), two daughters (I/3, I/4), and their two children (I/1, I/2) in one grave, and two sisters (II/6, II/8) and a daughter (II/7) of one of them in the other. Using mtDNA from hair and nail, we have reanalyzed the mummies. The results allowed the unambiguous assignment of each of the mummies to one of three mtDNA haplogroups: A2b (I/5); A2a (I/2, I/3, II/6, II/8); A2a-311 (I/1, I/4, II/7), excluded some of the previous relations, and pointed to new ones. I/5 is not the grandmother/mother of the individuals in Grave I, and she is not maternally related to any of the seven other mummies; I/3 and I/4 are not sisters and II/7 is neither the daughter of II/6 nor of II/8. However, I/1 may be the child of either I/4 or II/7 and these two may be sisters. I/2 may be the son of I/3, who may be the daughter of either II/6 or II/8, and these two may be sisters. The observation of haplogroups A2a and A2b amongst the 550-year-old Inuit puts a lower limit on the age of the two lineages in Greenland. Am J Phys Anthropol 133:847–853, 2007.

In 1972 the mummified bodies of eight Eskimos were discovered in a natural tomb among the rocks at Qilakitsoq in the Uummannaq Municipality on the west coast of Greenland. They were excavated in 1978. Although the burial was subsequently dated by C^{14} analysis to approximately A.D. 1460 (Tauber, 1989), the climatic conditions have been extremely favorable to the preservation of the bodies, which were essentially naturally freeze-dried. In particular, exceptionally well-preserved soft tissue, including internal organs, was present on the mummies (Hart Hansen, 1989), as was hair from both the humans, and on the garments and hides they had with them (Hansen et al., 1989; Møller, 1989). Although all the mummies were found within a single cave (rock covered ledge), the specimens had been positioned into two stacks of bodies, with a 1-m separation between the two. The first stack, known as Grave I, contained five mummies, while the second, Grave II, contained the remaining three. Based on the subsequent archaeological analyses of the find circumstances and burial circumstances, the mummies likely represent a group of people who died more or less at the same time (Andreasen, 1989), and represent the single largest find of mummified specimens from the Arctic.

Of the eight mummies found, the clothing and physical examination of the bodies revealed the adult mummies to be exclusively female, while the two infants are believed, based on the clothing alone, to be male. Within the stack of bodies that represents Grave I, the order of bodies was as follows: uppermost the first infant, ~6 months of age (designated I/1); then a second infant, ~4–4½ years of age (I/2); a female of probably 20–25 years of age (I/3); a female of ~25–30 years of age (I/4); and lowest was the mummy of a female, ~40–50 years of age (I/5). Grave II consisted of three adult females: uppermost a female of about 50 years of age (II/6); then a female of about 18–21 years of age (II/7); and finally a female of 50 years of age (II/8) (Jørgensen, 1989; Pedersen and Jakobsen, 1989).

This article contains Supplementary Material available at http://www.interscience.wiley.com/jpages/0002-9483/suppmat

Grant sponsor: University of Arizona.

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Received 13 September 2006; accepted 29 January 2007

DOI 10.1002/ajpa.20602
Published online 11 April 2007 in Wiley InterScience (www.interscience.wiley.com).

The exceptional preservation of the samples, and their curious provenance has naturally led to interest in the genetic relationships (if any) between the specimens—indeed earlier tests on the samples were among the first archaeological samples to be studied genetically. In an initial study, thigh muscle was HLA typed from the specimens, and the results led the authors to conclude that the stacks of bodies formed two distinct families (Hansen and Gürtler, 1983). This knowledge, plus the ages of the specimens thus led the authors to later argue that one family is buried in each tomb, and that a possible interpretation of the HLA results is that tomb I contained a grandmother (I/5), two daughters (I/3 and I/4), and one (two) grandchild(ren) (I/1 and/or I/2). Tomb II contained two sisters (II/6 and II/8) and a young woman (II/7) who may have been the daughter of either of them. Theoretically, the three eldest women could be sibs (Hansen, 1989).

With the development of more advanced genetic techniques further tests were performed on the samples to determine both the quality of the DNA in the mummies, and to try and confirm the genetic relationship. Thuesen and Engberg (1990) tested skin and bone samples from I/5 using probes for both Alu-repeats and α-repeats. Although the authors reported that DNA was present in the samples, they interestingly reported that it was both degraded and contaminated with actinomycete DNA. Despite this report, however, a third study attempted STR analyses on the samples, to confirm the genetic relationship (Simonsen et al., 2003). In combination with the original HLA results the authors of this study concluded a more complex relationship than originally thought: the elderly woman I/5 might be the mother of I/3 and the grandmother of I/2; she cannot be a full sibling of the other two elderly women II/6 and II/8, and she is not the mother of I/4. Neither is I/4 the mother of I/2. The two elderly women, II/6 and II/8, may be sisters. II/6 is not the mother of I/4. II/8 can be the mother of II/7. Neither of the two women, II/6 and II/8, can be the mothers of any of the younger individuals in Grave I (I/3, I/4, I/2 or I/1).

As with many high profile archaeological specimens that were discovered and prepared/conserved in the time before widespread DNA analyses, these human specimens have previously undergone a large amount of handling by both genetically European and Inuit people, under conditions that were not designed to protect the specimens from DNA cross contamination by the handlers. An increasing number of studies have demonstrated that DNA contamination in this way presents a serious challenge to aDNA studies, often leading to the generation of erroneous results that are derived from the contaminant DNA as opposed to the ancient specimen (cf. Richards et al., 1995; Handt et al., 1996; Hofreiter et al., 2000; Kolman and Tuross, 2000; Gilbert et al., 2005b, 2006a; Malmström et al., 2005; Sampietro et al., 2006). In addition to the degree of handling exposure, contamination has been linked to sample specific issues such as specimen porosity, therefore naturally porous tissues such as bones, teeth, and conceivably mummified tissue may often rapidly become permeated with contaminant DNA. One potential solution that has been proposed is the use where possible of less porous, keratinized tissues such as hair shaft and nail, as both have been shown to have contamination resistant properties (Tahir and Watson, 1995; Wilson et al., 1995; Jehaes et al., 1998; Anderson et al., 1999; Gilbert et al., 2006b), and hair at least has been used as sources of aDNA in specimens that date back over 64,500 years (Gilbert et al., 2004).

Therefore, in light of what is now known about the problems and persistence of contaminant sources of DNA, the previous reports of the degraded state of the DNA, and the contradictory findings of the first genetic studies, a number of questions arise about the relationships—particularly whether the mummies are indeed related, and if so, how. In an attempt to help resolve these issues, we report here the results of new mtDNA analyses that we have undertaken on hair and nail samples.

MATERIALS AND METHODS

Samples

Although one previous study has undertaken mtDNA analyses on bone and skin samples from mummy I/5 (Nielsen et al., 1994), in this study, to minimize sample destruction, we opted to use keratinous tissues. Head hair is present in large quantities on all the mummies, and nail samples are available for some specimens. Multiple hair shafts were sampled from each mummy, a number of which were sent to both DNA research teams (Department of Ecology and Evolutionary Biology, University of Arizona (AZ), and Institute of Forensic Medicine, University of Copenhagen (CPH)) to enable independent replication. In addition, nail specimens were taken and analyzed from mummies I/5 and II/7 (see Table 1 and Supplementary Table S.1 for details).

Chemicals, reagents, PCR- and centrifuge tubes, pipette tips

All chemicals and reagents were of analytical grade or the highest purity available. PCR tubes and microcentrifuge tubes for extracts and primers were free of human DNA as guaranteed by the manufacturer ("PCR-Clean" or "Biopure" tubes, Eppendorf). Pipette tips were aerosol resistant and certified pure and free of DNA (Molecular BioProducts). Water was molecular biology grade deionized, autoclaved, and filtered (8 kDa molecular weight cutoff).

Sample preparation and DNA extraction

Preparation and extraction of samples, and PCR reaction setup was carried out in laboratories dedicated to ancient DNA work. Both laboratories are designed along "Clean-lab" guidelines, and as such are situated in locations that are physically separated from that where DNA analyses on modern and PCR-amplified samples are performed. Staffs entering the laboratory were equipped with full body suits, hairnets, filter-containing facemasks, and gloves; furthermore, staffs followed a strict regime that ensures that they cannot work in the clean laboratory if they have been already working with amplified or high concentration DNA that day. As a further precaution, the laboratory is fitted with positive, filtered airflow, and is irradiated with $\lambda = 254$ nm ultraviolet light whenever not in use.

At the AZ laboratory, the samples were initially decontaminated through bathing in 5% commercial bleach solution (0.5% hypochlorite), following previously published guidelines (Gilbert et al., 2004, 2006b), and subsequently DNA was extracted from 2 to 20 cm lengths,

TABLE 1. Samples studied and summary of results

Mummy	Tissue	mtDNA (copies/μl)	mtDNA D-loop substitutions (16064–16405) relative to rCRS (Andrews et al., 1999)	Haplogroup assigment
I/1	Hair	215	111, 192, 223, –, 290, 311, 319, 362	A2a-311
I/2	Hair	3,630	111, 192, 223, –, 290, –, 319, 362	A2a
I/3	Hair	775	111, 192, 223, –, 290, –, 319, 362	A2a
I/4	Hair	208	111, 192, 223, –, 290, 311, 319, 362	A2a-311
I/5	Hair	81	111, –, 223, 265, 290, –, 319, 362	A2b
	Nail	2,367	111, –, 223, 265, 290, –, 319, 362	A2b
II/6	Hair	297	111, 192, 223, –, 290, –, 319, 362	A2a
II/7	Hair	44	111, 192, 223, –, 290, 311, 319, 362	A2a-311
	Nail	380	111, 192, 223, –, 290, 311, 319, 362	A2a-311
II/8	Hair	2,601	111, 192, 223, –, 290, –, 319, 362	A2a

following Gilbert et al. (2004). For full sample details see Table 1 and Supplemental Table S.1. As an internal control for the results, at least two extractions were performed on different samples for each individual, each at least 6 months apart. The extracts were stored at −20°C.

At the CPH laboratory, hair shafts (2–38 cm lengths, dependent on sample availability) were cut into ~2-cm pieces and submersed in 5% commercial bleach for 10 min. The bleach was removed with a pipette and the hair pieces were rinsed through consecutive washings with water, 99% ethanol, and water. The hair pieces were incubated in 200 μl 10 mM Tris/HCl, 140 mM NaCl, 3 mM $CaCl_2$, 50 mM DTT, 1% SDS, 0.1 mg Proteinase K, pH 8.0 at 55°C. The hair was usually dissolved after 20 min. Nail samples were thinly sliced into small samples (0.5–1.0 mg), then decontaminated, rinsed, and digested as earlier. The resulting 200 μl hair/nail extracts were purified using the silica based QIAamp DNA mini kit (Qiagen, Venlo, The Netherlands) and eluted with 2 × 100 μl deionized, autoclaved water. The purified DNA was finally concentrated by centrifugation on a 30-kDa filter (Microcon, Millipore) and recovered from the filter with 80 μl autoclaved, deionized, and filtered water. The extracts were stored at −20°C in 20 μl aliquots.

PCR and cloning

The near complete mtDNA HVR1 region was PCR amplified from all DNA positive extractions using a number of different primer pairs that produce amplicons of between 135–141 bp (CPH) and 136–394 (AZ) (Table 2). In CPH 12.5 μl PCR reactions were performed containing 2.5 μl DNA extract, 2.5 μl High-Fidelity PCR Buffer (Invitrogen), 2.5 mM $MgSO_4$, 0.1 mM of each dNTP, 0.8 μM of each primer, 0.5 U Platinum Taq High-Fidelity polymerase (Invitrogen). The polymerase was activated by heating at 94°C for 7 min, followed by 40 cycles of PCR (for details refer to Table 2). In AZ 25 μl PCR reactions were performed containing 1 μl DNA extract, 2.5 μl High-Fidelity PCR Buffer (Invitrogen), 2.5 mM $MgSO_4$, 0.2 mM of each dNTP, 1 μM of each primer, 0.2 U Platinum Taq High-Fidelity polymerase (Invitrogen). The reaction parameters were similar to those in CPH. In both laboratories PCR and extraction blanks were run at a 1:1 ratio.

The majority of the amplified products were cloned using the Topo TA kit (Invitrogen) or the pGEM® Easy Vector system (Promega). A small number of amplified products from AZ were direct sequenced without cloning.

DNA sequencing

Multiple clones were sequenced for each cloned PCR reaction (see Supplemental Tables S.2 and S.3) using conserved primers and the ABI Prism 310 DNA single capillary DNA analyzer and the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturers instructions. DNA sequences were aligned with the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999) and analyzed for postmortem damage induced miscoding lesions, and the presence of contaminant DNA sequences. If no evidence of contamination could be observed, the multiple PCR fragments from the individual extractions were assembled into consensus contigs.

Real time quantitative PCR analyses

The DNA content of the extracts was quantified by quantitative real time PCR (qPCR) using the ABI Prism 7000 Sequence Detection System and the TaqMan Universal PCR Master MIX (Applied BioSystems). Primers and probe for a mtDNA segment from nt 8294 to 8436 were as described by Andréasson et al. (2002). A mtDNA standard was produced by cloning a 302-bp segment containing the aforementioned 143-bp segment using the pGEM Easy Vector system (Promega). Cloned plasmids containing the mtDNA segment were purified using standard precipitation methods, electrophorized, and the plasmid band excised and purified using Wizard® SV (Promega). The plasmids were linearized by digestion with Rsa I overnight at 37°C. The concentration/number of copies/μl was estimated by measuring the absorbance at 260 nm and the preparation was diluted ~500-fold to yield a stem solution of 10^8 copies/5 μl. The final results reflect the approximate copy number of the 143-bp mitochondrial fragment within the extracts.

RESULTS AND DISCUSSION

Authenticity of results

Ancient DNA studies on human tissues are particularly problematic with regards to the generation of erroneous results due to sample contamination. Therefore it is recommended that authors provide sufficient information to support the authenticity of data (Gilbert et al., 2005a). We believe that the following information provides strong evidence to support the authenticity of our results:

1. mtDNA survival in old human hair and nail has been demonstrated previously (Anderson et al., 1999; Gil-

TABLE 2. Oligonucleotide primers used for DNA amplification

Primer pairs	Sequence (5′→3′)	Length of product (bp)	Annealing (°C)
L16063[a]	TTGGGTACCACCCAAGTAT	135	50 (40, 2.5)[b]
H16161[a]	GATGTGGATTGGGTTTTTA		
L16131[a,c]	CACCATGAATATTGTACGGT	136	50 (40, 2.5)
H16228[a,c]	TTGCAGTTGATGTGTGATAG		
L16225[a,c]	AAGTACAGCAATCAACCCTC	141	50 (40, 2.5)
H16325[a,c]	CTGTAATGTGCTATGTACGGTA		
L16307[a]	TACCCACCCTTAACAGTACA	136	50 (40, 2.5)
H16406[a]	TATTGATTTCACGGAGGA		
L16055[c]	GAAGCAGATTTGGGTACCAC	394	56 (40, 2.5)
H16410[c]	GCGGGATATTGATTTCACGG		
L16209[c]	CCATGCTTACAAGCAAG	187	56 (40, 2.5)
H16356[c]	GTCATCCATGGGGACGAGAA		

Number in primer name indicates position of 3′ nucleotide (Andrews et al., 1999).
[a] Primers used by the Copenhagen research group.
[b] Values within parentheses indicate cycles and extract (in μl), respectively.
[c] Primers used by the Arizona research group.

bert et al., 2004), and in this situation although the samples are older than those that have been previously used in DNA studies, they are still relatively young with regards to other human aDNA studies (≈600 years), and have been preserved under conditions that are optimal for DNA survival (cold and dry), thus rendering it likely that mtDNA will survive in the hairs.

2. Although the samples have been handled in the past, hair and nail has been demonstrated to be resistant to contamination (Tahir and Watson, 1995; Wilson et al., 1995; Jehaes et al., 1998; Anderson et al., 1999; Gilbert et al., 2006b), even when degraded, and in contrast to bone and teeth (Gilbert et al., 2006a) is relatively easy to successfully decontaminate (Gilbert et al., 2006b).
3. While some of the internal replicated DNA extracts did not yield amplifiable DNA, the sequence from each mummy was always generated from several different DNA extracts, in several cases from multiple tissues, using multiple overlapping independent PCR products, by two independent groups (see Supplemental Tables S.1, S.2, and S.3). This makes it unlikely that laboratory specific contamination, postmortem damage, heteroplasmy, or sequencing artifacts may have caused erroneous results.
4. The DNA levels within the samples are relatively low (Table 1 and supplemental information) in contrast to what would be expected from DNA from modern contaminant sources. Furthermore, the cloned sequences offer evidence of miscoding lesions that are believed to arise through DNA damage (Pääbo, 1989).
5. The generated sequences are phylogenetically plausible (see next section), fall within expected haplogroups, have a degree of diversity among them, and have never been previously generated in either laboratory.
6. The work was undertaken in dedicated aDNA labs, under strictly controlled conditions. No modern human DNA was used as controls.

DNA sequences

Contemporary Inuit haplotypes from Greenland and North Canadian Kitikmeot populations, that have not been subject to admixture with Europeans, all exclusively into two haplotype clusters, A2 and D3 (Helgason et al., 2006). The ≈600-year-old mtDNA haplotypes in this study are no exception: all fall within Hg A2 (Helgason et al., 2006). However three distinct subgroups are represented in the data (Table 1). Four individuals (I/2, I/3, II/6, II/8) belong to the root form of A2a (16111c-t, 16192c-t, 16223c-t, 16290c-t, 16319g-a, 16362t-c) (Helgason et al., 2006), while three (I/1, I/4, II/7) belong to a common, derived form of A2a (in the following referred to as A2a-311) that includes a further transition at 16311t-c (Helgason et al., 2006). The last individual (I/5) belongs to the root group of the related haplogroup A2b, lacking the 16192 c-t, but with derived 16265a-g (Helgason et al., 2006). Intriguingly, this result is different to that published in the only previous study to examine mtDNA from these specimens (Nielsen et al., 1994), where the sequence to mummy I/5 was reported as containing the 16192 c-t transition (in addition to the 16111c-t transition), whereas the 16223c-t transition was not observed (the sequence was only generated between 16099 and 16255). However, for a number of reasons we argue that our results likely represent the true sequence. Firstly, the 16223c-t transition is part of the sequence motifs for haplogroups A and D (Richards et al., 2000), and contemporary Inuit populations belong exclusively to subgroups of A2 and D3, both containing the 16223c-t transition (Helgason et al., 2006). Secondly, our sequence was derived from multiple extractions and PCRs on the individual. Thirdly, the mummies are reported to likely be contaminated with modern DNA (cf. Nielsen et al., 1994). To deal with this we used hair and nail as DNA sources, which are much easier to decontaminate from exogenous sources of DNA (cf. Tahir and Watson, 1995; Wilson et al., 1995; Jehaes et al., 1998; Anderson et al., 1999; Gilbert et al., 2006b) than sources such as bone (cf. Richards et al., 1995; Handt et al., 1996; Hofreiter et al., 2000; Kolman and Tuross, 2000; Gilbert et al., 2005b, 2006a; Malmström et al., 2005; Sampietro et al., 2006). Lastly, we did not use any modern Inuit DNA in our study, and have never worked on modern Inuit DNA, unlike in the previous study (Nielsen et al., 1994) where modern Inuit DNA was used as a control. As such, we believe that the result of the previous study (Nielsen et al., 1994) has arisen due to contamination of the sample either prior to, or during the study.

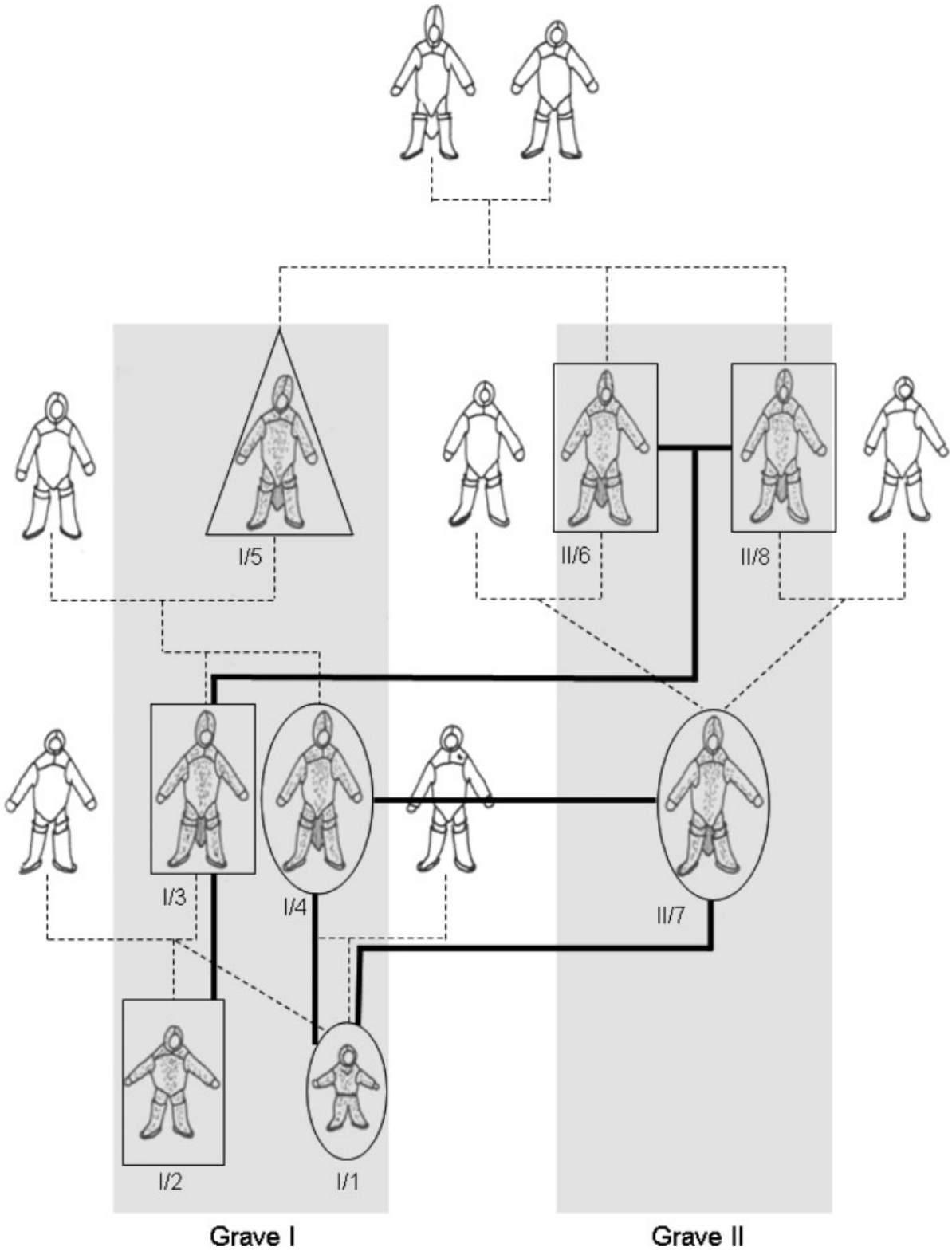

Fig. 1. Qilakitsoq mummies: the familial relationship between the mummies as previously believed, and as indicated under the results of this study. Figures within the shaded areas represent the five adult female and two male child mummies discovered in two adjacent tombs. Figures outside the shaded areas represent hypothetical fathers/husbands. Dotted lines show possible family relations as previously inferred from grave position, estimated age, tissue typing (Hansen and Gürtler, 1983; Hansen, 1989; Jørgensen, 1989; Pedersen and Jakobsen, 1989), and STR analysis (Simonsen et al., 2003). □, mtDNA lineage A2a; ○, lineage A2a-311; △, lineage A2b. Solid lines indicate possible maternal relations; absence of solid lines between mummies indicates that maternal relation is not possible. Figure modified from Hart Hansen et al. (1991).

Although Helgason et al. (2006) noted that A2a is common to modern W. and S. Greenland indigenous populations, while A2b is predominantly found in Kitikmeot and N. Greenland, both forms are found widely across the region. Thus based on the small size of our data set, nothing can be concluded from the results other than that the samples are characteristically Inuit.

The age of origin of subhaplogroups A2a and A2b in Greenland has been calculated by Helgason et al. (2006) using a variety of colonization models (i.e. manipulation of founder vs. source population). The observation of A2a and A2b within our specimens fits within the calculated origin times. For example, under the model of a combined Kitikmeot and Siberian source population, Helgason et al. (2006) calculated the origin of A2a in Greenland as 426 ± 159 years (based on pedigree mutation rate) or 1,160 ± 434 (using an evolutionary rate model). Similarly they calculated the age of A2b 376 ± 160 or 1,024 ± 435. The presence, therefore, of both haplogroups A2a and A2b in the Qilakitsoq mummies that are nearly 550 years old clearly sets a lower boundary for the ages, thus the calculated dates of Helgason et al. (2006) can be modified accordingly.

New insights into the relationships of the mummies

Based on the mtDNA sequence data generated in this study we can conclude that, in contrast to the original hypothesis of two distinct family groups divided into two distinct grave piles (Hansen and Gürtler, 1983), the Qilakitsoq mummies represent at least three different genetic maternal lineages that are mixed between the piles (Table 1), and that we name according to the observed haplogroups: lineage A2b (mummy I/5), lineage A2a (mummies I/2, I/3, II/6, II/8), and lineage A2a-311 (mummies I/1, I/4, II/7). As similar mtDNA sequences may be shared between many individuals that are not directly related, it is not possible, using the mtDNA data alone, to further resolve whether the members of clusters A2a and A2a-311 represent direct maternal relatives. However, when the mtDNA data is compared with the previous HLA and STR findings, some consistencies are found, which in addition to the information of the estimated age of deaths of the mummies provide some new information that helps both confirm and refute several of the previous putative relationships, and narrow down the genetic relationships between others (summarized in Fig. 1).

Lineage A2b. I/5 is maternally unrelated at a close genetic level to the other mummies. However, as some similarity was observed between I/5 with II/6 and II/8 in the previous STR and HLA analyses, it cannot be excluded that they share a father.

Lineage A2a. The results are consistent with infant I/2 being the son of I/3 who in turn is the daughter of II/6 or II/8; and that II/6 and II/8 are sisters with shared parents.

Lineage A2a-311. The baby I/1 is possibly the child of I/4 or II/7. However, as I/1 has not been previously analyzed for HLA or STR data, this relationship cannot be confirmed using the current knowledge, although the burial proximity to I/4 and the young age of the infant (1/2 year) suggest it is likely that I/4 is the true mother. I/4 and II/7 may be sisters. If so, their mother is not present among the mummies.

CONCLUSIONS

The exceedingly well-preserved Qilakitsoq mummies are among the most famous of the naturally preserved mummies discovered in recent history. Their discovery in the unusual setting of two clear piles of bodies has led to obvious interest as to the reason why. Previous studies have failed to answer this question, possibly due to either poor DNA survival or sample/extract contamination. As keratinized tissues have previously been demonstrated as a useful and reliable source of ancient mtDNA (Gilbert et al., 2004), this study therefore presented the interesting challenge of attempting to clarify the conflicting findings of the previous investigations using the minimally destructive techniques of mtDNA analyses on small hair and nail samples. The data both indicate that some family relationship probably exists between at least some of the specimens, but that the relationship is more complex than originally postulated—on the maternal side there are at least three distinct genetic lineages.

An obvious question is why our data disagrees with the previous findings. Although sample contamination is a tempting explanation, and likely explains the discrepancy with mummy I/5 in our study and the previous mtDNA study (Nielsen et al., 1994), a more simple explanation may be that the biomolecular preservation within the specimens was not sufficient for accurate HLA and STR typing to the resolution required to resolve the familial relationships. For example, we note that with the exception of mummy II/8, only partial STR profiles could be obtained in the STR investigation (Simonsen et al., 2003), a clear indicator of the poor quality of the nuDNA recovered. Further, the STR profiles reported showed a high degree of apparent homozygosity, which could simply be a result of allelic dropouts caused by the scarcity of template DNA molecules. Thus, if this is the case, then the STR results should naturally be regarded with some caution, and as such, our results represent the most reliable genetic indicators of the intraspecimen relationships currently available. However, as we demonstrate, mtDNA cannot answer the question of the relationships fully. Therefore it is to be hoped that, with the development of future techniques for manipulating degraded nuDNA, new opportunities will arise to help close the book on the genetic relationships of the Qilakitsoq mummies.

ACKNOWLEDGMENTS

The authors are indebted to the Greenland and Danish National Museums for facilitating the sampling of hair and nail specimens from the mummies.

LITERATURE CITED

Anderson TD, Ross JP, Roby RK, Lee DA, Holland MM. 1999. A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework. J Forensic Sci 44:1053–1056.

Andreasen C. 1989. The archaeology of Qilakitsoq. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 11–22.

Andreasson H, Gyllensten U, Allen M. 2002. Real-time DNA quantification of nuclear and mitochondrial DNA in forensic analysis. BioTechniques 33:402–411.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147.

Gilbert MT, Wilson AS, Bunce M, Hansen AJ, Willerslev E, Shapiro B, Higham TF, Richards MP, O'Connell TC, Tobin DJ, Janaway RC, Cooper A. 2004. Ancient mitochondrial DNA from hair. Curr Biol 14:R463–R464.

Gilbert MTP, Bandelt HJ, Hofreiter M, Barnes I. 2005a. Assessing ancient DNA studies. Trends Ecol Evol 20:541–544.

Gilbert MTP, Hansen AJ, Willerslev E, Turner-Walker G, Collins M. 2006a. Insights into the processes behind the contamination of degraded human teeth and bone samples with exogenous sources of DNA. Int J Osteoarchaeol 16:156–164.

Gilbert MTP, Menez L, Janaway RC, Tobin DJ, Cooper A, Wilson AS. 2006b. Resistance of degraded hair shafts to contaminant DNA. Forensic Sci Int 156:208–212.

Gilbert MTP, Rudbeck L, Willerslev E, Hansen AJ, Smith C, Penkman KEH, Prangenberg K, Nielsen-Marsh CM, Jans ME, Arthur P, Lynnerup N, Turner-Walker G, Biddle M, Kjolbye-Biddle B, Collins MJ. 2005b. Biochemical and physical correlates of DNA contamination in archaeological human bones and teeth excavated at Matera, Italy. J Archaeol Sci 32:785–793.

Handt O, Krings M, Ward RH, Pääbo S. 1996. The retrieval of ancient human DNA sequences. Am J Hum Genet 59:368–376.

Hansen HE. 1989. Possible family relationships revealed by determination of HLA-transplantation antigens. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 144–146.

Hansen HE, Gürtler H. 1983. HLA types of mummified Eskimo bodies from the 15th century. Am J Phys Anthropol 61:447–452.

Hansen JC, Toribara TY, Muhs AG. 1989. Trace metals in human and animal hair from the 15th century graves at Qilakitsoq compared with recent samples. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 161–168.

Hart Hansen JP. 1989. The mummies from Qilakitsoq—paleopathological aspects. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 69–82.

Hart Hansen JP, Meldgaard J, Nordqvist J. 1991. The Greenland mummies. London: British Museum Press.

Helgason A, Palsson G, Pedersen HS, Angulalik E, Gunnarsdottir ED, Yngvadottir B, Stefansson K. 2006. mtDNA variation in Inuit populations of Greenland and Canada: migration history and population structure. Am J Phys Anthropol 130:123–134.

Hofreiter M, Poinar HN, Spaulding WG, Bauer K, Martin PS, Possnert G, Pääbo S. 2000. A molecular analysis of ground sloth diet through the last glaciation. Mol Ecol 9:1975–1984.

Jehaes E, Gilissen A, Cassiman JJ, Decorte R. 1998. Evaluation of a decontamination protocol for hair shafts before mtDNA sequencing. Forensic Sci Int 94:65–71.

Jørgensen JB. 1989. Anthropology of the Qilakitso Qeskimos. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 56–57.

Kolman CJ, Tuross N. 2000. Ancient DNA analysis of human populations. Am J Phys Anthropol 111:5–23.

Malmström H, Stora J, Dalén L, Holmlund G, Götherström A. 2005. Extensive human DNA contamination in extracts from ancient dog bones and teeth. Mol Biol Evol 22:2040–2047.

Møller G. 1989. Eskimo clothing from Qilakitsoq. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 23–46.

Nielsen H, Engberg J, Thuesen I. 1994. DNA from Arctic human burials. In: Herrmann B, Hummel S, editors. Ancient DNA. New York: Springer-Verlag. p 122–140.

Pääbo S. 1989. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. Proc Natl Acad Sci USA 86:1939–1943.

Pedersen PO, Jakobsen J. 1989. Teeth and jaws of the Qilakitsoq mummies. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 112–130.

Richards M, Macaulay V, Hickey E, Vega E, Sykes B, Guida V, Rengo C, Sellitto D, Cruciani F, Kivisild T, Villems R, Thomas M, Rychkov S, Rychkov O, Rychkov Y, Golge M, Dimitrov D, Hill E, Bradley D, Romano V, Cali F, Vona G, Demaine A, Papiha S, Triantaphyllidis C, Stefanescu G, Hatina J, Belledi M, Di Rienzo A, Novelletto A, Oppenheim A, Nørby S, Al Zaheri N, Santachiara-Benerecetti S, Scozzari R, Torroni A, Bandelt HJ. 2000. Tracing European founder lineages in the near eastern mtDNA pool. Am J Hum Genet 67:1251–1276.

Richards MB, Sykes BC, Hedges REM. 1995. Authenticating DNA extracted from ancient skeletal remains. J Archaeol Sci 22:291–299.

Sampietro ML, Gilbert MTP, Lao O, Caramelli D, Lari M, Bertranpetit J, Lalueza-Fox C. 2006. Tracking down human contamination in ancient human teeth. Mol Biol Evol 23:1801–1807.

Simonsen B, Tonnesen E, Hansen HE. 2003. The Qilakitsoq mummies: family relations reexamined by DNA-profile. In: Lynnerup N, Andreasen C, Berglund J, editors. Mummies in a new millennium. Copenhagen: Danish Polar Center. p 130–134.

Tahir MA, Watson N. 1995. Typing of DNA HLA-DQα alleles extracted from human nail material using polymerase chain reaction. J Forensic Sci 40:634–636.

Tauber H. 1989. Age and diet of the mummified Eskimos from Qilakitsoq. In: Hart Hansen JP, Gulløv HC, editors. The mummies from Qilakitsoq: Eskimos in the 15th century. Kommissionen for Videnskabelige Undersøgelser i Grønland. p 137–138.

Thuesen I, Engberg J. 1990. Recovery and analysis of human genetic material from mummified tissue and bone. J Archaeol Sci 17:679–689.

Wilson MR, DiZinno JA, Polanskey D, Replogle J, Budowle B. 1995. Validation of mitochondrial DNA sequencing for forensic casework analysis. Int J Legal Med 108:68–74.