

More evidence for non-maternal inheritance of mitochondrial DNA?

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ABSTRACT

Background: A single case of paternal co-transmission of mitochondrial DNA (mtDNA) in humans has been reported so far.

Methods: To find potential instances of non-maternal inheritance of mtDNA we searched published medical case studies (of single patients) for irregular mtDNA patterns by contrasting the given haplotype information for different clones or tissues to the worldwide mtDNA database as known to date – a method that has proven to be robust and reliable for the detection of flawed mtDNA sequence data.

Results: We found more than 20 studies reporting clear-cut instances with mtDNAs of different ancestries in single individuals. By way of example, we reviewed here a few cases from the recent medical literature, which, at face value, might therefore be taken as evidence for paternal inheritance of mtDNA or recombination.

Conclusions: Multiple types (or recombinant types) of quite dissimilar mitochondrial DNA, from different parts of the known mtDNA phylogeny, are frequently reported in single individuals. In view of re-analyses and corrigenda of forensic mtDNA data, however, we assert that the phenomenon of mixed or mosaic mtDNA can solely be ascribed to contamination and sample mix-up.

Abbreviations: mtDNA, mitochondrial DNA; HVS-I, first hypervariable segment; HVS-II, second hypervariable segment

INTRODUCTION

In the past few years some exciting claims have been made about the mode of inheritance of mtDNA as well as role of mtDNA in the pathogenesis of several human diseases. However, the early attempts to demonstrate that mtDNA would undergo recombination in populations were based on misread data^{1,2} or flawed data and unjustified premises about the mutational process,^{3,4} or biased data collection, inadequate and misapplied statistics, technical and logical errors.⁵⁻⁹ The observation that paternally inherited mtDNA was found in the muscle tissues of a Danish patient suffering from a mitochondrial myopathy¹⁰ is most remarkable but seems to remain a singular phenomenon so far, which was neither confirmed in another lab nor could be found in other cases of sporadic myopathies.¹¹⁻¹³ Most recently,¹⁴ the first attempt was reported claiming to have demonstrated in vivo mtDNA recombination in the singular muscle tissue of the Danish myopathy patient.¹⁰ One way of searching for further potential instances of abnormal inheritance of mtDNA is to dig into the rich literature of medical genetics, where mtDNA information is being generated on a case by case basis and recorded mutation by mutation, but – alas – without taking the emerging mtDNA phylogeny into account.¹⁵

METHODS

In our examination we in principle employed all mtDNA sequences published so far (*viz.* >2000 coding-region and >30,000 partial control-region sequences). The complete sequences can be hierarchically organized in a worldwide mtDNA phylogeny, the major branches (clades) of which are referred to as haplogroups, encoded in a hierarchical way.¹⁶⁻²⁰

RESULTS

PATERNAL mtDNA IN KLINEFELTER'S SYNDROME?

A case of abnormal mtDNA inheritance, which has apparently not been taken much notice of in the scientific community, was reported in cases of Klinefelter's syndrome,²¹ where a possible interaction of the sex chromosome and mtDNA was hypothesized. In that study, mtDNA was analysed in eight Klinefelter males, seven from the USA and one from Japan, for the variation in the first hypervariable segment (HVS-I) and the second hypervariable segment (HVS-II) of the mtDNA control region. Most astonishingly, all seven US samples turned out to have identical HVS-I&II sequences outside the long C-stretches (which, however, is highly improbable for unrelated "African-American" individuals) and even shared the same two heteroplasmies; see our Table 1, no.1. Puzzlingly, the Japanese Klinefelter individual (Table 1, no.2) had almost the same array of mutations, but his mother (Table 1, no.3) showed a number of different mutations, especially in HVS-II.

Table 1 mtDNA sequences reported by Oikawa et al. (2002)

No.	Haplotype ^a
1	G16042A G16060K G16089K G16208T G16384A C80T A126C A263G
2	G16042A G16060K G16089K G16208T G16384A C80G A126C A263G
3	G16042A G16060T G16089T G16384C C80T C120G T223G A227G T254G A263G C299G

^a Relative to the revised Cambridge Reference Sequence. rCRS; Andrews et al. (1999); regions 16024–16400, 30–400; long C-stretches disregarded; K = G/T

Note that the rCRS²² nucleotide at position 223 has been reported incorrectly as G (instead of T) and the “number of polymorphism” for the “normal Japanese” sample (of size 60) have been inverted for most positions in HVS-II.²¹ The high level of polymorphism (34/60) for position 227 is most implausible in view of the corresponding value (1/373) in two sets of Japanese data.^{23 24} In any case, most of the nucleotide positions listed in Table 1 were also claimed to be polymorphic (to various degrees) in the “normal Japanese”.²¹ This is extraordinary insofar as besides position 227 only 263 was found to be polymorphic in published Japanese data. Also worldwide, most of those positions are extremely conservative. In fact, the ten positions 16060, 16089, 16208, 16384, 80, 120, 126, 223, 254, and 299 were all found unvaried in the databases MITOMAP (<http://www.mitomap.org/>) and SWGDAM (<http://www.fbi.gov/hq/lab/fsc/backissu/april2002/miller1.htm>), except for one incorrectly recorded entry (16089) in MITOMAP, whereas 16042, 227, and 263 are known to be polymorphic to a very minor degree. In the Klinefelter data,²¹ the latter three positions show solely transitions but the former ten positions all show transversions (Table 1).

Some of these unexpected variants can be attributed to mis-interpretation of the sequence electropherograms, which were generated with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and AmpliTag FS DNA polymerase. This version of sequencing chemistry is known to produce the frequently observed phenomenon that G-peaks, 3´ adjacent to A-peaks, are displayed with significantly reduced peak height or may even vanish.²⁵ This applies to positions 16060 and 16208, which are directly located 3´ to adenosines (at 16059 and 16207). Depending on the background signal height these G positions may then have been difficult to assign, although reverse sequencing, when applied, would correctly identify such positions.

The variation in the region 16184-16197 which includes the C-stretch of HVS-I is also remarkable in these data: although the first seven sequences are identical in HVS-I outside this stretch, all nine individuals differ in this stretch, yielding a peculiar pattern of mutations never observed before. Similarly, the pattern in the region 303-317 covering the C-stretch(es) of HVS-II with novel mutations is unusual. The chromatograms would rather indicate that length heteroplasmies were not properly recognized and erroneously interpreted as homo- or heteroplasmic mutations in the C-stretch. This rather reflects additional sequencing problems associated with the C-stretch, so that these differences would not necessarily prove that different mtDNA samples had actually been amplified.

We are thus seeing a very clear pattern of artificial mutations, since transversions are relatively rare in human mtDNA, especially in HVS-II, and thus signpost phantom mutations.^{26 27} In summary, the Klinefelter data²¹ bear multiple artifacts and were likely the combined result of (1) biochemical problems (dye blobs, etc.), (2) poor quality and readability, and (3) sample mix-up or contamination. Whatever the specific causes of these patterns would be, these completely flawed data could not support statements such as “...we could conclude that strict maternal inheritance is disturbed in the generic of offspring with Klinefelter’s syndrome”.²¹

MULTIPLE SOMATIC MUTATIONS IN mtDNA?

In cancer research, the interest concerning mtDNA focuses on the question of whether somatic mutations accumulate in tumors. Recently, as many as 24 somatic mutations were observed in a single tumor of primary lung cancer (JHU_MITO #12, Supplementary Table 3²⁸), “providing additional evidence for a ‘mitochondrial DNA mutator’ phenotype”.²⁸ These mutations comprise four heteroplasmic and 20

homoplasmic mutations, 18 of which are, however, known to belong to an evolutionary pathway in the mtDNA phylogeny (Figure 1). This mutational path separates haplogroup (pre-V)2 mtDNA¹⁸ from a particular branch of haplogroup L3e3 mtDNA.²⁹⁻³¹ Note that the employed MitoChip tool²⁸ was only able to detect <80% of the mutations – but still sufficiently many to infer the pathway unambiguously. Clearly we are seeing here not somatic mutations (except possibly those four heteroplasmic mutations) but two mtDNA lineages stemming from different individuals that were however attributed to the same patient.

Another recent paper³² aimed at demonstrating marked mtDNA heterogeneity among individual CD34⁺ clones from adult bone marrow. In particular, the predominant ‘aggregate’ HVS-I&II sequence of many clones from the bone marrow donor no. 2 actually matches (within 16024-16365 and 73-340) a sequence (listed as USA.CAU.000339 in the SWGDAM database) that evidently belongs to the North African haplogroup U6a1.¹⁷ One of the CD34⁺ clones from the same donor; however, gave a totally different sequence with another specific mutational motif (e.g. CB155 and FO156 from a Japanese data set²⁴ or KOR.ASN.000124 and JPN.ASN.000041 from the SWGDAM database), which qualifies this sequence as a member of the East Asian haplogroup D4c.³³ In another case,³⁴ the mtDNA sequence of a single CD34⁺ clone from cord blood donor no. 1 showed a pattern extremely distinct from the aggregate cord blood mtDNA and other CD34⁺ clones. Namely, the aggregate sequence and the deviant sequence harbor the motifs of two distinct Indian subhaplogroups of haplogroup M (A73G T146C T195A A263G T489C 522-523del 16166del C16223T T16519C versus A73G A153G A263G C463T T485C T489C C16223T; authors’ unpublished data). Although it was contended that “*to prevent DNA cross-contamination, special precautions were taken*”,³² it seems that these ‘precautions’ were not sufficient. Two-step nested PCR, as applied in these cases, bears an elevated risk of contaminant mtDNA to enter the process.

A study of mtDNA control region mutations in patients with esophageal squamous cell carcinoma³⁵ offers an interesting case of seeming recombinant mtDNA. The mtDNA found in the tumor sample of Case 20 (C16185T C16223T C16260T T16298C) clearly indicates that this is an East Asian haplogroup Z sequence,¹⁶ whereas the blood sample (C16256T C16270T A16399G) points to the European haplogroup U5a1.^{19,20} In contrast, no single discriminating mutation was recorded for HVS-II, despite the fact that the HVS-II mutation motif T152C 249d T489C would clearly separate those two haplogroups. Thus, we infer that either the blood mtDNA or the tumor mtDNA must constitute a recombinant type. The reported data³⁵ contain yet another case of totally different mtDNA lineages in one patient, and there are plenty of further cases from cancer research, where mixed or recombinant mtDNAs in single patients can be inferred in the same way as outlined above.

CONCLUSION

In summary, previous screening of mtDNA data of evolutionary and forensic studies has provided a rich record of obviously flawed or doubtful results that would directly impact upon the question whether mtDNA may occasionally be inherited non-maternally. Clear-cut artificial recombinants between separately amplified segments can easily be detected through focused database searches and phylogenetic analysis.³⁶ For instance, the SWGDAM database, a forensic mtDNA database that went online in 2002, contained at least six obvious instances of artificial recombination,^{36,37} only four of which have been corrected so far through two partial revisions of the database for transcription errors.^{38,39} This could point to the possibility

that some recombinants had been generated in the lab through sample mix-up or contamination and are therefore unrecognizable by mere rereading of sequencer outputs. The German database “D-Loop-BASE” (<http://www.d-loop-base.de/>) suffered from massive artificial recombination and all kinds of other problems,⁴⁰ so that it eventually had to go offline in early 2004.

Have we really discovered new cases of non-maternal inheritance of mtDNA? The Klinefelter case²¹ can be dismissed as the result of an obvious sequencing disaster. The other instances^{28 32 34} discussed above indeed point to different sources of the mtDNAs that were attributed to single patients. In one cancer patient,³⁵ one compound haplotype is even composed from HVS-I and HVS-II stemming from different mtDNAs. To explain these findings, however, one does not have to invoke novel mechanisms of mtDNA inheritance. The lesson learnt from forensic mtDNA databases offers a much more straightforward explanation – namely, the mechanism of laboratory artifacts.

The recent study¹⁴ about seeming recombination of mtDNA in the Danish patient should indeed give researchers the hint that recombination needs to be considered as a potential factor in their studies – but then *artificial* recombination should come to mind first. Among other questionable aspects of that study,¹⁴ we observe that the methodological approach employed (single molecule PCR) is in fact extremely prone to contamination (due to the minimal amounts of DNA that were used); moreover, the highly statistically significant mtDNA recombination hotspots B and C bound familiar mtDNA segments generated with standard primer pairs (as applied in human population genetics). Therefore, there is room here for much skepticism.

The cohorts of published artifacts in forensic databases^{27 36 37 40 41} suggest that the most natural reason for seeing totally different mtDNAs or mosaic compound haplotypes in a single individual is casual handling and mis-sequencing of samples in the laboratory. It seems that the chances for artificial recombination and the possibility of other systematic errors in mtDNA analyses are notoriously underestimated. With solid complete sequencing studies, there is increasing evidence that there is absolutely no sign of natural recombination.^{7 29} For example, the HVS-I&II status gave a good predictor for the coding region variation in the case of frequent HVS-I&II haplotypes, notwithstanding occasional single recurrent mutations in HVS-I&II.⁴² On the other hand, poor lab work executed on large parts of the coding region yielded very clear signals of artifacts.^{43 44} In summary, the point is that the mtDNA phylogeny mirrors the population history during thousands of years, a period which should be long enough to generate – if any – signal of recombination. Any population harbors phylogenetically distant mtDNA haplotypes, so that there is in general a ~50% chance to have such distant mtDNAs involved in a recombination event – whether it would be real or not. It is then evident that the frivolous statement “*recombination is difficult to detect in population genetic data, even if it is occurring at appreciable frequencies*”⁴⁵ is plain wrong.

It has been demanded that “special claims require special evidence”.⁴⁶ Non-maternal inheritance of mtDNA would certainly be very special. This entails that for every result that would call the dogma of maternal inheritance of mtDNA into question one would need independent extraction, amplification, and sequencing in another lab – quite in analogy to the situation with ancient DNA, where independent replication is requested.⁴⁷ This clearly holds for all cases of (seeming) paternal inheritance or blocks of multiple somatic mutations in patients. We agree that “*systematic haplotype analyses of large cohorts of patients with sporadic mtDNA mutations and healthy individuals are therefore warranted to unravel this enigma*”.⁴⁸ However, it appears to

be even more important to unravel the causes of laboratory artifacts that might lead to premature claims of mitochondrial association with certain diseases⁴⁹ or of sporadic non-maternal inheritance of mtDNA. Any attempt aiming to put recombination of mtDNA into the play as a natural cause should also explain why the worldwide mtDNA phylogeny faithfully mirrors the non-recombinant nature of this genome.

Conflict of interests: none declared.

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Figure legend

Figure 1 Coding-region sites distinguishing two particular lineages from haplogroups (pre-V)2 and L3e3 assigned to one individual (Maitra et al. 2004). Prefix h indicates heteroplasmy and a suffix designates a transversion.

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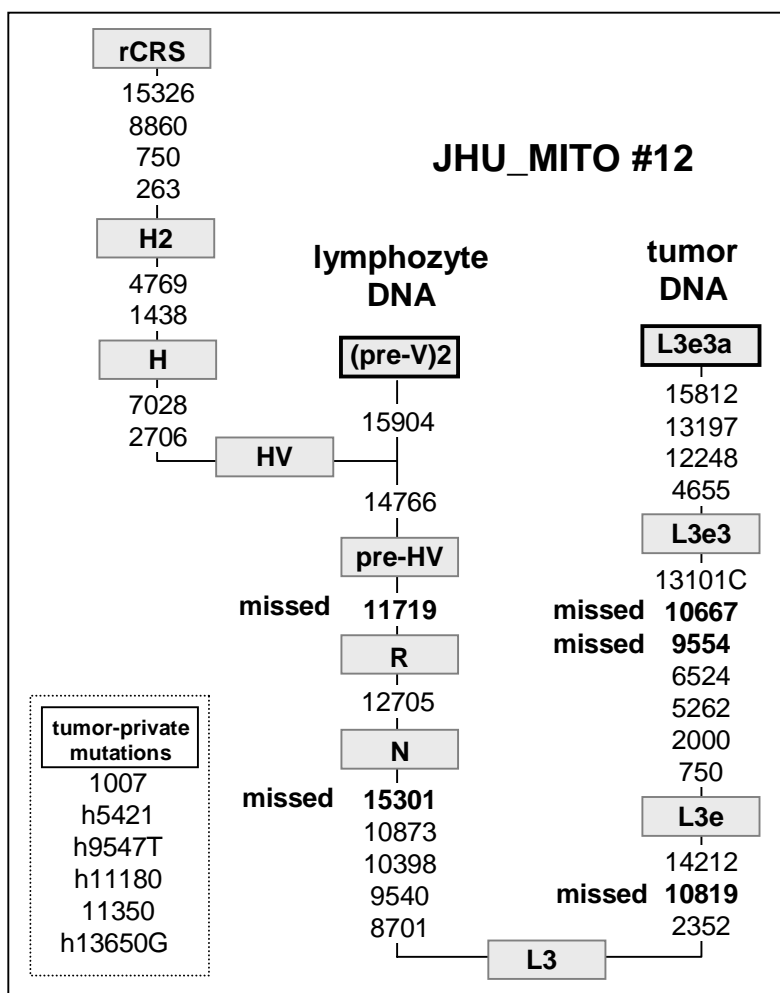


Figure 1