*J Forensic Sci,* Sept. 2002, Vol. 47, No. 5
Paper ID JFS2001398_475
Available online at: www.astm.org

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# Correlation of Microscopic and Mitochondrial DNA Hair Comparisons

**ABSTRACT:** Expert opinions regarding the microscopic comparison of human hairs have been accepted routinely in courts for decades. However, with the advent of mitochondrial DNA (mtDNA) sequencing, an assessment can be made of the association by microscopic hair comparisons in casework between a questioned hair and reference hairs from an individual. While each method can be used separately, the two analytical methods can be complementary and together can provide additional information regarding source association. Human hairs submitted to the FBI Laboratory for analysis between 1996 and 2000 were reviewed. Of 170 hair examinations, there were 80 microscopic associations; of these, only nine were excluded by mtDNA. Importantly, 66 hairs that were considered either unsuitable for microscopic examinations or yielded inconclusive microscopic associations provided mtDNA results. Only six hairs did not provide sufficient mtDNA, and only three yielded inconclusive results. Consistency was observed in exculpatory results with the two procedures. This study demonstrates the utility of microscopic hair examinations and the strength of combining microscopic analysis with mtDNA sequencing.

**KEYWORDS:** forensic science, microscopic hair comparisons, mitochondrial DNA, significance

The microscopic comparison of morphological characteristics of human hairs has been accepted both scientifically and legally for decades. The advent of mitochondrial DNA (mtDNA) sequencing provides an additional test in the repertoire for assessing source association between a questioned hair and an individual. Neither the microscopic nor molecular analysis alone, or together, enables absolute positive identification; together, however, these methods can be complementary examinations. For example, mtDNA typing can often distinguish between hairs from different sources although they have similar morphological characteristics (or insufficient characteristics); in contrast, hair morphology comparisons can often distinguish between samples from different individuals that are maternally related, where mtDNA analysis is uninformative.

Likewise, the addition of mtDNA analysis, because of its discriminating power and its objectivity, provides for the assessment of the performance of microscopic hair comparisons. Human hair examinations in the FBI Laboratory that were subjected to both a microscopical comparison and to mtDNA analysis between 1996 and 2000 were reviewed. The results of the microscopic comparisons were categorized as a positive association (i.e., cannot exclude), a negative association (i.e., can exclude), an inconclusive result (i.e., insufficient information to render an interpretation), or no exam (i.e., insufficient or unsuitable sample to attempt an examination). All microscopic human hair comparisons that result in a positive association are then reviewed by a second qualified examiner. If the results between the two examiners concur, the questioned hair and a known sample of blood, saliva, or hair are subjected to mtDNA analysis. Hairs that are not suitable for microscopic comparison, such as body hairs, may still be analyzed for mtDNA on a case-by-case basis. The possible results of the mtDNA analysis are that the sequences between the questioned hair and the known sample are concordant, the sequences differ, the results are inconclusive, or that insufficient mtDNA exists for an analysis.

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Received 30 Nov. 2001; and in revised form 12 March 2002; accepted 22 March 2002; published 14 Aug. 2002.

## Microscopic Hair Comparison Protocols

The general procedure for forensic hair examinations has been described previously (1,2). The material is first examined to determine whether or not it is a hair. If the material is identified as a hair, a taxonomic characterization is attempted. Distinguishing characteristics of human and animal hairs have been known for centuries (Hooke first reported on this to the Royal Society in 1663 (2)). The earliest known forensic report on animal hairs was made in 1837 (3) and the fundamental method is still employed by mammologists, anthropologists, and forensic scientists (for example, see 4–6). For animal hairs, usually the genus can be specified and some hairs allow for a finer taxonomic distinction.

If the hair is human in origin, then body area, race, and suitability are assessed. The main body areas where hair appears are the head (scalp, eyes, and face), the pubic region, the auxiliary (underarm) regions, the chest, and the limbs. Hairs that reside in areas between these main regions may have some combination of the traits of more than one area and are termed "transitional" hairs.

Race (or major population group) of the donor of the hair is considered based on hair form, hair cross-section, pigmentation patterns, and overall appearance (2,7). Three main categories are used for racial estimation: Caucasian (or European ancestry), Negroid (or African ancestry), and Mongoloid (or Asian ancestry). If a mixture of racial characteristics is such that no one population groupís characteristics predominate, the hair may be termed "mixed racial" or unclassifiable for population origin.

The suitability of the hair for full microscopic comparison is then determined. If the hair is determined to be not suitable, then no further microscopic examination is performed. Hairs may not be suitable due to size, incompleteness, a lack of definable characteristics, or damage.

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EXHIBIT 1

If the hair is determined to be suitable for microscopic analysis, then it is compared against appropriate known hair samples. Head hairs and pubic hairs are typically suitable for comparison, but other hairs may also contain sufficient information for a full microscopic comparison. The main human hair characteristics are: race type, body area, color, length, tip, root, diameter, cuticle, scales, pigment, medulla, cortex, artificial treatment, damage, and special characteristics (2,7–9).

The results of a microscopic examination generally fall into three categories. First, the questioned hair can be determined to exhibit the same microscopic characteristics as the known hair samples. Given this result, the questioned hair cannot be excluded and could have originated from the person who supplied the known reference hairs. Second, the questioned hair may exhibit similarities to the known hair samples, but unexplainable differences also are observed. In this instance, no conclusion can be drawn about the origin of the hair. Finally, the questioned hair can be determined to be dissimilar to the known hairs and therefore could not be associated with the person who supplied the known reference sample. This interpretation scheme has been described in detail by Gaudette (10).

Hair characteristics are considered to be polygenic and continuously variable in their expression (2,11). If we consider a known sample to exist as an attenuated range of all possible characteristics, it becomes apparent that two known samples could coincide sufficiently for a single hair to exhibit the characteristics of more than one hair in the known samples. This is, however, rare (12,13). Microscopic comparison of hairs has never been considered a positive form of identification and, likewise, mtDNA does not lead to a unique identification of the donor.

The additional value of microscopy is that a large number of questioned hairs collected from the evidence can be examined quickly by microscopy and assessed, which minimizes the time and cost of mtDNA analyses. Many times features and traces of material on the hairs, unrelated to the issue of identity, can be rapidly ascertained from a microscopic examination, such as forceful removal, possible blood on the hairs, burning, crushing, "glass cuts," etc. In this way, the investigation can be furthered and valuable probative information that could not be gleaned from the sequence of the mtDNA is observed and preserved.

## Mitochondrial DNA

Sequence analysis of human mitochondrial DNA (mtDNA) extracted from a single hair shaft (1 to 2 cm in length) is a valid and reliable method (14,15). The mtDNA comprises less than 1% of total cellular DNA, but, in contrast to nuclear DNA, exists in high copy number in each cell. Thus, successful results from hair are more likely with mtDNA analysis than when employing other DNA typing strategies. The non-coding control region, approximately 1100 base pairs in length, contains two hypervariable regions, HVI and HVII. The hypervariable regions can be readily sequenced such that a high degree of information can be obtained for discriminating between maternally unrelated individuals. Maternally related individuals will share the same mtDNA type and generally cannot be differentiated using mtDNA alone. Also, some mtDNA sequences are more common than others. Therefore, mtDNA also is not considered a unique identifier.

## Materials and Methods

As of January 21, 2001, 170 microscopic hair examinations and their respective mtDNA results were available for review at the FBI Laboratory.

The individual items of evidence were processed separately in cleaned purpose-built rooms for trace evidence collection; the hairs were collected from the submitted items by picking, taping, scraping, vacuuming, or any combination of these methods. The debris was then sorted under a stereomicroscope and the hairs mounted separately from the fibers. The known hair sample was then viewed in transmitted light (brightfield) at final magnifications ranging from 40 to 250X. The hairs were examined and described, from root to tip, detailing the distinctive and/or significant microscopic characteristics (1,7). The questioned hair was then examined for suitability, body area, race, and other class traits. If the hair was deemed suitable, then a complete examination was made of that hair in comparison with the known sample(s) on a comparison microscope using transmitted light (brightfield). This enabled a point-by-point, side-by-side comparison of the microscopic characteristics in the known sample with those in the questioned hair, which is more reliable than a simple subjective determination of the properties of an object (16,17). A conclusion was then drawn as to whether the questioned hair could have come from the same person who supplied the known sample, could not have come from that person, or that similarities and unexplained differences precluded a conclusive interpretation. If the hairs were determined to be consistent with coming from an individual, and if the results were confirmed by a second qualified hair examiner, then the hair was prepared for submission to mtDNA analysis. Hairs that were not suitable for microscopic examination or that were excluded microscopically, however, may have been considered appropriate for mtDNA sequencing analysis and this is assessed on a case-by-case basis.

The procedures for mtDNA sample preparation and analysis have been described previously (14,15) and therefore will not be reproduced here.

## Results and Discussion

The purpose of this study was to use mtDNA results to assess the performance of microscopic analyses. The comparison of results can be used to determine which method will yield more conclusive results and how often hair comparisons fail to include or exclude compared with mtDNA typing.

The general results of the 170 hair comparisons and subsequent mtDNA sequences are displayed in Table 1, and a breakdown of the results by method is in Table 2. Racial and body area data are listed in Table 3. For the microscopic comparisons, 58.2% of the analyses yielded conclusive results (80 associations and 19 exclusions). It should be noted that very few hairs that are microscopically excluded in typical casework are submitted for mtDNA analysis. Comparatively, 94.7% of the evidence hairs yielded conclusive results by mtDNA sequencing (97 concordant and 64 exclusions). The greater success for mtDNA typing compared with microscopic analyses is to be expected. Many morphological characteristics in an individualís hair are not discrete in appearance; they are expressed as a distribution. Thus, conclusive outcomes are

TABLE 1—*Results of microscopic and mitochondrial DNA analyses.*

| | Microscopic | Mitochondrial |
|---|---|---|
| Association | 80 | 97 |
| Inconclusive | 37 | 3 |
| Exclusion | 19 | 64 |
| No Exam | 34 | 6 |

TABLE 2—*Results of microscopic and mitochondrial DNA analyses by method.*

| Microscopic Results | Mitochondrial | | Results | | Totals |
|---|---|---|---|---|---|
| | Association | Inconclusive | Exclusion | Insufficient | |
| Association | 69 | 1 | 9 | 1 | 80 |
| Inconclusive | 15 | 1 | 20 | 1 | 37 |
| Exclusion | 0 | 1 | 17 | 1 | 19 |
| No Exam | 13 | 0 | 18 | 3 | 34 |
| Totals | 97 | 3 | 64 | 6 | 170 |

TABLE 3—*Racial and color assessments for head and pubic hairs (n = 139).*

| By Frequency | Grey | Red | Blond/Light Brown | Brown | Dark Brown/Black | Undetermined |
|---|---|---|---|---|---|---|
| Caucasian Head | 3 | 1 | 11 | 34 | 1 | 27 |
| Negroid Head | 0 | 0 | 0 | 5 | 14 | 11* |
| Caucasian Pubic | 0 | 3 | 3 | 8 | 0 | 3 |
| Mixed Racial Head | 0 | 0 | 0 | 0 | 2 | 3 |
| Mongoloid Head | 0 | 0 | 0 | 2 | 2 | 0 |
| Negroid Pubic | 0 | 0 | 0 | 1 | 1 | 0 |
| Undetermined Pubic | 0 | 0 | 0 | 1 | 0 | 1 |
| Undetermined Head | 0 | 0 | 0 | 0 | 0 | 1 |
| Mongoloid Pubic | 0 | 0 | 0 | 0 | 0 | 0 |
| Mixed Racial Pubic | 0 | 0 | 0 | 0 | 0 | 0 |

* One negroid head hair had been artificially treated to a blue color; no original color was visible.

less likely than that obtained with the discrete results of mtDNA sequencing. Furthermore, mtDNA sequencing is a very sensitive technique that requires only 1 to 2 cm of hair and often only a single reference is required for comparison purposes.

Prior to the advent of mtDNA analysis, hair morphology examinations that yielded inconclusive results may have provided little useful information. Of the 170 microscopic examinations that were made in this study, 37 (21.7%) were inconclusive as to association or exclusion and 34 (20%) were not suitable for examination. However, mtDNA sequencing provided information for 35 inconclusive and 31 insufficient hairs, 66 in total. Interestingly, the genetic information was exculpatory in slightly more than half of these "uninformative" hairs. Thus, when the evidence may be considered meaningful, mtDNA analysis may be performed on hairs that, after the microscopic comparison, fall into the inconclusive or no exam categories.

Microscopic hair comparisons can exclude samples where the mtDNA sequences are the same, such as those from maternal relatives or unrelated people with the same mtDNA sequences. In contrast, there will be hairs that cannot be excluded due to a congruence of features. Of the 80 hairs that were microscopically associated, nine comparisons were excluded by mtDNA analysis. The class characteristics of these hairs are listed in Table 4. Many of these (4 or 44%) were defined as blond Caucasian head hairs. Blond hairs, by their coloration, have much less pigmentation than darker hairs. Because pigmentation is an important comparison characteristic, hairs with sparse or no pigmentation but from different sources could appear similar. These nine mtDNA exclusions should not be construed as a false positive rate for the microscopic method or a false exclusion rate for mtDNA typing: it displays the limits of the comparison of the hairs examined in this sample only and not for any hairs examined by any particular examiner in any one case. The microscopic comparison is not an absolute identification and therefore some small number of individual hairs that have a congruence of certain characteristics, even though they originate from separate individuals, may exist. Thus, in those rare instances where the same microscopic characteristics are exhibited in hairs from different individuals, the appropriate interpretation for the microscopic comparison is association. However, the data in Tables 3 and 4 indicate the type of hairs that are more and less likely to be informative, consistent with the conclusions of Gaudette (10).

TABLE 4—*Class descriptions of hairs excluded by mitochondrial DNA analysis.*

| Racial Estimate | Body Area | Color |
|---|---|---|
| Caucasian | Head | Blond |
| Caucasian | Head | Blond |
| Caucasian | Head | Blond |
| Caucasian | Head | Blond |
| Caucasian | Head | Brown |
| Caucasian | Head | None given |
| Caucasian | Pubic | Brown |
| Negroid | Head | Brown |
| Negroid | Head | Dark brown/black |

There were no apparent differences in the exclusions obtained with both analytical methods. Of the 19 microscopic exclusions, 17 were confirmed by mtDNA sequencing. The other two hairs provided inconclusive or insufficient results with mtDNA sequencing.

Thus, the exculpatory power of hair comparisons appears to be quite good. One could argue that this sample size is small and that many hairs that are excluded microscopically were not subjected to mtDNA typing. These microscopically excluded hairs in the current study were subjected to mtDNA typing for a variety of reasons, based largely on case circumstances. Given the consistency of exculpatory results between microscopic and mitochondrial examinations, the general assertion that microscopic hair comparisons are a reliable technique for exclusion is supported (10,12,13). Therefore, it seems reasonable to continue to routinely examine hairs microscopically because a microscopical comparison can reliably exclude hairs, which is a principle aim of the forensic comparison. When the hairs are not excluded, the power and complementary value of mtDNA can be exploited.

Another benefit to mtDNA analysis, especially on uninformative hairs, is the knowledge of population traits and, thus, quantification. Further, if a suspect or elimination sample is submitted many months or years after the crime was committed, the hairs may be of limited value for microscopic examination because of phenotypic changes; the mtDNA will not have changed, however.

## Conclusions

Both the microscopic and molecular analysis of hairs are useful in forensic investigations because both rely on independent types of information. The mtDNA sequences provide information about the genotype of the source individual, while the microscopic examination evaluates physical characteristics of an individualís hair in his/her environment (phenotype). Based upon the existing literature and the results of this study, when possible it is recommended that both microscopic and mtDNA analysis be used for analyzing hair evidence. It is important to realize that microscopy is not a "screening test" and mtDNA analysis is not a "confirmatory test." Both methods, or either, can provide probative information to an investigation: one is not superior to another as both analyze different characteristics. The only question left, then, as posed by Robertson (9, p. 127),

> ". . .Would appear to be to what extent preliminary microscopic examinations should be conducted prior to DNA analysis . . . it may well be the case that there will *be little if any reduction in the level of microscopic examination as it will be both necessary and desirable to eliminate as many questioned hairs as possible* and concentrate mtDNA analysis on only key hairs." (emphasis added)

We concur. The data in this study support the utility of both methods within the limits of the sample. In some cases, very lightly or very darkly pigmented hairs, for example, may not exhibit sufficient characteristics to exclude different sources. Microscopical comparisons of hairs with sufficient characteristics yield similar conclusions with that of hairs examined by mtDNA.

Clearly, additional work can be done to expand the utility of microscopic and mitochondrial hair examinations in forensic science. The two methods combined provide an additional level of information that provides greater accuracy than either alone.

*Acknowledgments*

The authors would like to thank Robert Fram, Harold Deadman, Joseph DiZinno, and Doug Deedrick for their comments on the manuscript.

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