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Investigation of mtDNA heteroplasmy discordance between mother and child

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Abstract. Maternal mitochondrial DNA (mtDNA) heteroplasmy discordances were investigated by means of mtDNA hypervariable region 1 sequencing using hair shaft and blood samples obtained from eight mother–offspring pairs. Despite mtDNA's inheritance through the maternal line, heteroplasmic discrepancies appear to exist. Results also suggest that heteroplasmic mutations may be more common in hair than in blood.

Keywords: mtDNA; Heteroplasmy; Mother–offspring pair; Hair shaft; Blood

1. Introduction

Mitochondrial DNA (mtDNA) sequencing has been validated for personal identifications in forensics analysis [1]. In recent years, improved DNA extraction methods and increased sequencing sensitivity have made possible the detection of heteroplasmic point mutations. While the results of one previous study suggest that heteroplasmy in the mtDNA control region is inherited and remains stable throughout life, other studies appear to indicate discrepancies among different samples obtained from a single individual or from maternally related individuals [2]. This study sought to analyze the mtDNA sequences of maternal lineages and to compare hair shaft and blood samples from individuals belonging to the same maternal lineage.

2. Material and methods

2.1. Samples

Hair shaft and blood samples were taken from eight mother–offspring pairs. Before DNA extraction from hair shaft samples, we provided the contamination by the serial washing procedure of water and ethanol. Moreover, all experiments were performed according to ISFG guidelines concerning mtDNA treatment to minimize DNA contamination.

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2.2. DNA extraction

DNA was extracted from hair and blood by the silica beads method, and mtDNA control region 1 (position 15997–16401) was amplified by PCR using the following primers: 5′-FAM-CACCATTAGCACCCAAAGCT-3′ (15997) and 5′-CTTTGGAGTTG-CAGTTGATG-3′ (16401).

2.3. Sequencing

BigDye Terminator Ver.3 was used as recommended by the manufacturer, and sequencing was performed in a 310 Genetic Analyzer. Analysis was performed with Sequence Analysis Ver.3.4.1.

3. Results

Heteroplasmic transitions were found among four of eight mother–offspring pairs, as shown in Table 1. For mother–offspring pairs who did not demonstrate heteroplasmy, mutation positions were found to correspond perfectly. In heteroplasmic point mutations, a heteroplasmic T/C transition at position 16189 was detected in three mother–offspring pairs, while a heteroplasmic G/A transition at position 16129 was detected in pair 2. In pair 4, a heteroplasmic T/C transition at position 16189 was observed only in the hair sample from the child. Although a heteroplasmic transition at 16189 was observed in both the mother's hair and blood samples, the proportions of the two nucleotides differed between hair and blood samples. In pairs 1, 3, and 4, length heteroplasmy was detected in all samples from both the mother and child in a C-stretch region, but a non-heteroplasmic

Table 1
Heteroplasmic positions found in the HV1 of mtDNA obtained from the four mother–offspring pairs

Base position		HV1			
Reference sequence		16129	16189	16189.1	16189.2
			T	–	–
Pair 1	mother's hair shaft		C>T	C/–	
	mother's blood		C<T	C/–	
	child's hair shaft		C>T	C/–	
	child's blood		C>>(T)	C/–	
Pair 2	mother's hair shaft	G/A			
	mother's blood	A			
	child's hair shaft	A			
	child's blood	A			
Pair 3	mother's hair shaft		C>T	C/–	C/–
	mother's blood		C>>(T)	C/–	C/–
	child's hair shaft		C>>(T)	C/–	C/–
	child's blood		C<T	C/–	C/–
Pair 4	mother's hair shaft		C	C	C/–
	mother's blood		C	C/–	–
	child's hair shaft		C>T	C/–	–
	child's blood		C	C/–	–

mutation consisting of a single cytosine insertion after position 16189 was detected only in a mother's hair sample of pair 4.

4. Discussion

MtDNA sequencing has been used as a tool for identifying individuals from minute quantities of DNA obtained from matter such as hair and bone [1]. However, the possibility of heteroplasmy has generally not been considered when considering whether two samples originate from the same maternal line. Our results indicated that discrepancies in heteroplasmy appear to occur despite maternal inheritance of mtDNA.

An earlier study reported no differences in intraindividual heteroplasmic point mutations in mtDNA sequences between hair and blood. The increasing sophistication and discriminating power of analysis has led to several recent reports that indicate extreme variability in the levels of heteroplasmy among hair roots from the same individual [3]. As with our study, heteroplasmic inconsistencies were found in the hair shaft and blood samples from the same individuals, suggesting that heteroplasmic mutations may occur more frequently in hair shafts than in blood.

These results suggest the need for careful consideration of heteroplasmy in mtDNA analyses and the need for multiple references and carefully scrutiny for the presence of heteroplasmy.

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