MUTATION

Somatic Mutation Study of Hair Roots in an Individual

By Michael L. Silvia[1], David A. Pomposini[2], Han Na Rhee[3] and Susan A. Greenspoon[3]
[1]Virginia Commonwealth University, Richmond, VA, [2]Virginia Department of Forensic Science, Norfolk, VA, [3]Virginia Department of Forensic Science, Richmond, VA

The individual studied is a somatic mosaic because multiple DNA profiles were present within the body, with the findings inconsistent with a chimera.

INTRODUCTION

Human hair development is a rapid process. While in the growth stage, no other tissue except bone marrow has as high a rate of mitotic activity as the hair root bulb (1). One group of follicle progenitor stem cells derived from the ectoderm can give rise to up to 3 follicle buds (Figure 1). At 16–20 weeks the follicles begin producing hairs. Follicular material clinging to a forcibly pulled hair is usually of ectodermal origin. The head hair growth phase occurs over 3–5 years, and the rest phase, approximately 3 months. The pubic hair growth phase occurs over 4–7 months, and the rest phase lasts from 6–9 months (1). Therefore, a forcibly pulled head hair is likely in growth phase, while a forcibly pulled pubic hair is likely in rest phase.

A high rate of mitosis can increase the opportunity for mutations to occur. A somatic mutation is any permanent change in the sequence of genomic DNA in a somatic cell rather than in the germline. A mosaic is an individual or tissue with at least two cell lines differing in genotype or karyotype derived from a single zygote, while a chimera is an individual composed of cells derived from two genetically different zygotes (2). The data presented in this study provides evidence for mosaicism in multiple pubic hair roots and a single head hair root from a single individual.

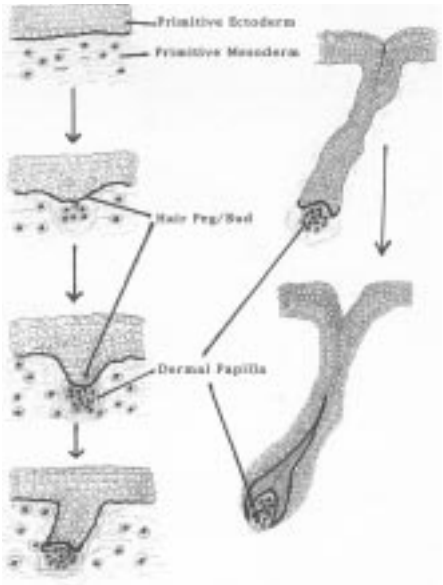

Figure 1. Hair follicle development. Hair follicle development during embryogenesis and fully formed containing a growing hair (1).

HAIR ROOT SAMPLES

Hairs (head, 143; pubic, 47) were collected from one donor by forceful pulling, and 0.5–0.7cm pieces were cut from the root end for DNA extraction. Samples were processed as described for the Tissue and Hair Extraction Kit (3), except that a modified proteinase K digestion buffer with a high concentration of DTT was used. A Biomek®2000 Automation Workstation (Beckman Coulter) carried out the remainder of the extraction as previously described (4).

The AluQuant®Human DNA Quantitation System[f,g] was used as recommended by the manufacturer (5). DNA samples were amplified using the PowerPlex®16 BIO System[b–e]. Reduced-volume reactions (12.5µl) were amplified for 31 rather than 32 PCR cycles (6,7). Manufacturer's recommendations were followed for the monoplex reactions (8). Amplified samples were separated on a 3% NuSieve®agarose product gel prior to polyacrylamide gel electrophoresis (PAGE) to estimate PAGE sample loading. PAGE was performed using a 6% PAGE-PLUS™ (Amresco) polyacrylamide gel run for 2 hours at 60W. PAGE gels were analyzed using the FMBIO®II Fluorescence Imaging System (MiraiBio) with the FMBIO®Analysis and STaRCall™ software programs.

RESULTS AND DISCUSSION

A triallelic pattern at D18S51 was identified and confirmed by re-amplification of a pulled head hair root sample with the PowerPlex®16 BIO System (Figure 2, Panel A). An aberrant 28,30 D21S11 genotype from a pulled pubic hair root sample was also identified (data not shown; normal genotype 29,30). An aberrant 11,12 genotype at CSF1PO for four pulled pubic hairs was confirmed by re-amplification with the PowerPlex®16 BIO System (Figure 2, Panel B) and by monoplex amplification of two of four samples with DNA extract remaining (Figure 3). The primers for the PowerPlex®16 BIO System and those for the *GenePrint*®Fluorescent Monoplex STR System, CSF1PO

MUTATION

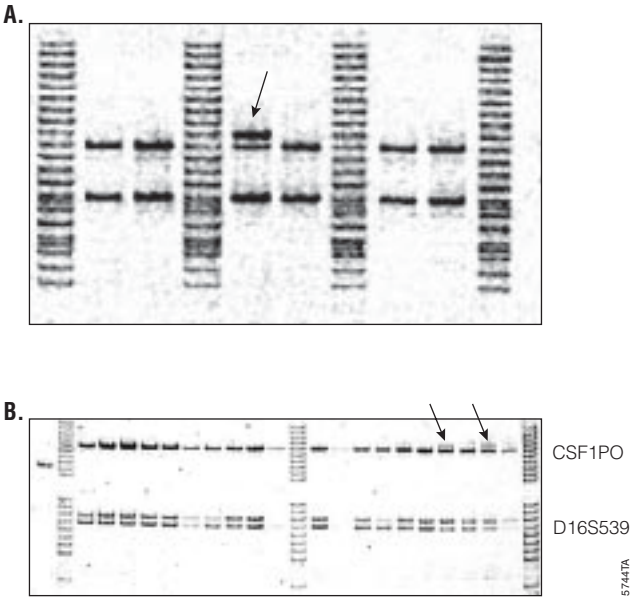

Figure 2. PowerPlex®16 BIO System gel images depicting aberrant banding patterns. Panel A. The arrow indicates the triallelic pattern observed at D18S51 for one head hair root. **Panel B.** The arrows indicate aberrant diallelic patterns at CSF1PO for two pubic hair root samples (normal genotype 11,11).

Figure 3. CSF1PO monoplex primer gel images depicting aberrant patterns reconfirmed. The arrows indicate aberrant diallelic patterns at CSF1PO for two pubic hair root samples.

Table 1. Observed Mutations in Hair Roots and Their Occurrence Rates. Three loci had potential somatic mutations observed: one at D18S51, one at D21S11 and four at CSF1PO. Rates were estimated by dividing the number of mutations by the total number of hairs typed.

	Total Attemped	PCR Product	Full Profile	Number of Mutations	Occurrence Rate
Head Hairs Total	169	147	143	1	
Hair Study 3	115	94	90	0	0.006993007
Hair Study 2	24	24	24	0	(D18S51 locus)
Hair Study 1	24	23	23	0	
Repeat Hair Study	6	6	6	1	
Pubic Hairs Total	71	54	47	5	0.085106383
Hair Study 3	54	45	41	5	(CSF1PO locus)
Hair Study 1	11	6	5	0	0.021276596
Repeat Hair Study	6	3	1	0	(D21S11 locus)

(Fluorescein), are of different and nonoverlapping sequences (9,10). A summary of the results is displayed in Table 1. The accuracy of the frequencies determined in this study could not be estimated due to the low sample numbers involved. The potential mutations occurred at only one locus per sample and never in combination.

CONCLUSION

Somatic mutation in the allelic repeat section is the most likely explanation for the anomalous patterns observed at D21S11, D18S51 and CSF1PO. Two nonoverlapping, independent primer sets for the CSF1PO locus produced the same genotyping result, making primer point mutation(s) unlikely at that locus. Similarly, primer point mutations are unlikely at D21S11 and D18S51 since they do not explain the aberrant patterns observed at those loci. Somatic mutations have a low frequency of occurrence, even in hair roots, which have a high mitotic rate. The individual studied is a somatic mosaic because multiple DNA profiles were present within the body, with the findings inconsistent with a chimera. Awareness of somatic mutation is important for forensic biology casework, since somatic mutation in hair roots has been reported (11). It may be informative to record aberrant patterns in casework to help estimate the frequencies of somatic mutations.

ACKNOWLEDGEMENTS

We gratefully acknowledge help from Dr. William Eggleston, Charles Linch and Eve Rossi from the Central Lab Forensic Biology section, and Bob McLaren from Promega Corporation.

REFERENCES

1. Linch, C.A., Whiting, D.A. and Holland, M.M. (2001) Human hair histogenesis for the mitochondrial DNA forensic scientist. *J. Forensic Sci.* **46**, 844–53.
2. Nussbaum, R.L., McInnes, R.R. and Willard, H.F. (2004) In: *Thompson & Thompson: Genetics in Medicine* 6th ed., revised, Saunders, Philadelphia, 407–8, 412.
3. *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* #TB307 Promega Corporation.
4. Greenspoon, S.A. *et al.* (2004) Application of the BioMek 2000 laboratory automation workstation and the DNA IQ system to the extraction of forensic casework samples. *J. Forensic Sci.* **49**, 29–39.
5. *AluQuant®Human DNA Quantitation System Technical Bulletin* #TB291, Promega Corporation.
6. Fregeau, C.J. *et al.* (2003) AmpFlSTR® Profiler Plus™ short tandem repeat DNA analysis of casework samples, mixture samples, and nonhuman DNA samples amplified under reduced PCR volume conditions (25 microL). *J. Forensic Sci.* **48**, 1014–34.
7. Leclair, B. *et al.* (2003) STR DNA typing: Increased sensitivity and efficient sample consumption using reduced PCR reaction volumes. *J. Forensic Sci.* **48**, 1001–13.
8. GenePrint®*Fluorescent STR System Technical Manual* #TMD006, Promega Corporation.
9. Masibay, A., Mozer, T.J. and Sprecher C. (2000) Promega Corporation reveals primer sequences in its testing kits. *J. Forensic Sci.* **45**, 1360–2.
10. Krenke, B.E. *et al*. (2002) Validation of a 16-locus fluorescent multiplex system. *J. Forensic Sci.* **47**, 773–85.
11. Clayton, T.M. *et al.* (2004) A genetic basis for anomalous band patterns encountered during DNA STR profiling. *J. Forensic Sci.* **49**, 1207–14.