TECHNICAL NOTE

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Gender Identification of Human Hair Using Fluorescence in Situ Hybridization

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ABSTRACT: Identification of the gender of hair represents relevant medicolegal evidence in criminal cases. The efficacy of Fluorescence In Situ Hybridization (FISH) using chromosome X and Y centromeric probes was tested to determine its ability to identify correctly the gender of hair. In this preliminary study, FISH correctly identified the gender of cells from hair as old as 26 days. The technique is accurate, rapid, sensitive, easily performed, and readily available. As a forensic laboratory technique, FISH shows great promise.

KEYWORDS: forensic science, gender determination, DNA, fluorescence in situ hybridization, hair, sex chromosomes, X chromosome, Y chromosome

In criminal cases, it is relevant to characterize forensically hair based on morphology, histology, cytology, and immunology. Furthermore, identification of the gender of a hair sample can be crucial in medicolegal cases. Determination of the sex of a hair has been done based on the presence or absence of sex chromatin (i.e., Barr bodies) in the cells of the hair root (1–5). More recently, molecular genetic techniques have been used to assist specifically in this endeavor (6,7).

Fluorescence In Situ Hybridization (FISH) represents a new advance in molecular genetic tests. In general, FISH has been successfully used on biological samples for clinical genetic and pathology testing. The technique uses nonradioactive fluorescently labeled chromosome specific DNA probes that can rapidly identify the presence of a chromosome(s), chromosome region(s), or gene(s) in cells. As a potential forensic test, FISH has been shown to detect accurately the presence of male epithelial cells in cervicovaginal smears obtained in instances of alleged rape and in a controlled study (8,9). FISH has also been shown to identify correctly the gender of two-week-old dried bloodstains (10).

We recently tested the ability of FISH to identify correctly the gender of hair in a forensic setting. The results of this study are presented and the potential for incorporating FISH evidence into forensic laboratory investigations is discussed.

Material and Methods

Samples

Hair samples were obtained randomly from seven male and three female autopsy cases. Hair was collected within 24 h of death. In each instance, hair was pulled (average of 18) and combed (average of three) from the head and pubic region. Each sample was separately placed into a clear plastic test tube and labeled with an identification number. Four samples were prepared for FISH within 24 h of collection. Six samples were maintained at room temperature (23–25°C) and not prepared for study for 21 to 26 days (average was 22.3).

Single slides were made from individual hairs from each site and collection method for each case in the following manner. Hair bulbs were allowed to rehydrate in 10 mL of isotonic buffered saline (pH = 6.8) overnight. The hair bulb was examined with a dissecting microscope and excised from its shaft using a sterile blade. The hair bulb was placed in a drop of 60% glacial acetic acid on a slide and gently teased apart with a sterile blade. A 22 by 22 mm No. 1 coverslip was placed over the teased bulb fragments and gentle pressure was applied (squash technique). The coverslip was removed by exposing the underside of the slide to liquid nitrogen and "popping off" the coverslip. This technique allowed individual cells to remain permanently fixed to the slide. The samples and slides were coded and blinded to the individual preparing the specimen and performing the analysis. Sterile gloves were worn throughout the handling, preparation, and testing to eliminate the potential for extracellular contamination.

Fluorescence In Situ Hybridization

The Spectrum CEP Direct Chromosome Enumeration System (VYSIS) using the X chromosome probe (alpha satellite DNA) direct labeled with Spectrum green and the Y chromosome probe

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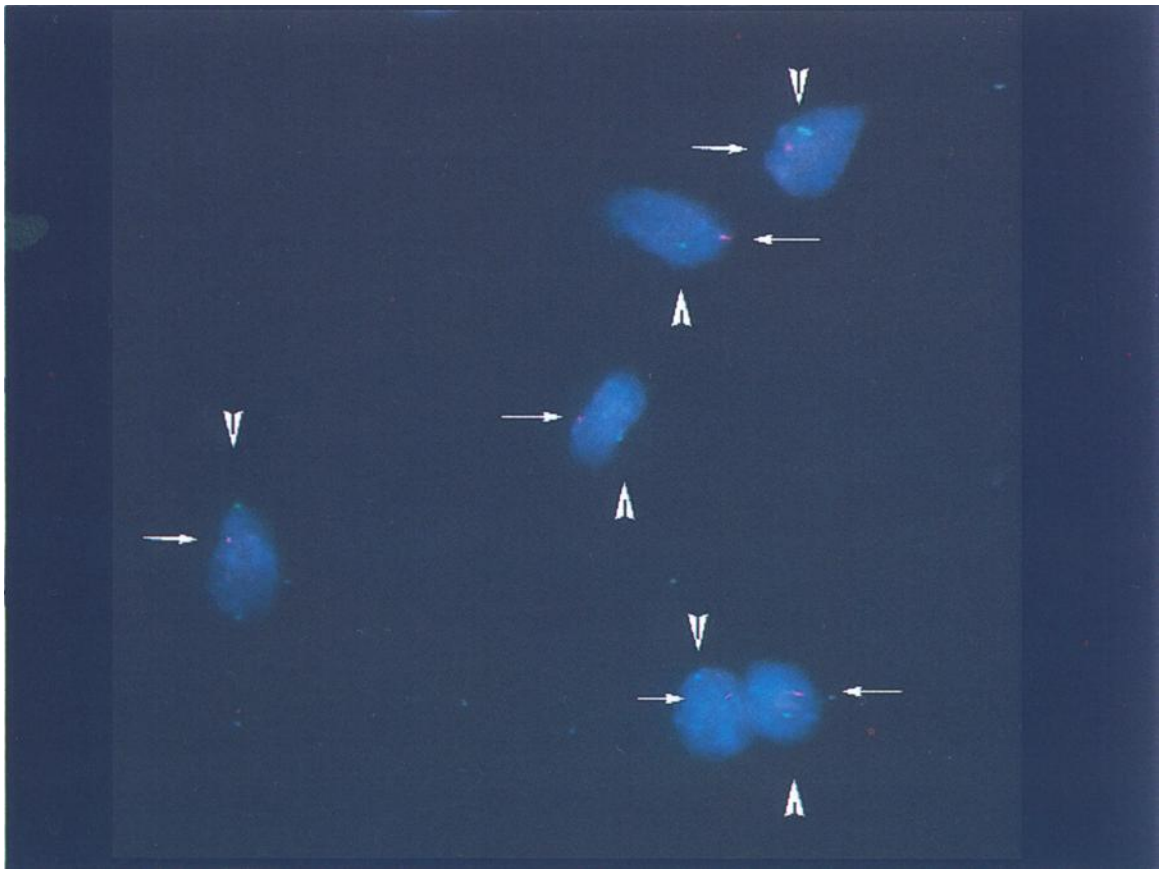

FIG. 1—*Cells obtained from male (XY) hair with the large arrow head showing a green signal (X chromosome) and the small arrow showing the presence of a red signal (Y chromosome).*

(satellite III DNA) labeled with Spectrum Orange were used in this study. The Y chromosome probe is highly specific for the repeated alphoid DNA located at the centromere of human chromosome Y, whereas X chromosome DNA probe hybridizes to the short repeats related to the AATGG in the pericentric heterochromatin of the X chromosome. Both X and Y chromosome specific probes were used together on each sample because the presence of the two probes rules out the possibility of nonspecific hybridization of either a X or Y probe alone.

The FISH methodology using the X and Y direct labeled probes was that described by Pettenati et al. (1995). Slides were dehydrated through a series of ethanol dilutions (70%/80%/90%100%) then denatured in 70% formamide/2XSSC at 75°C for 5 min followed by dehydration through an ethanol series. After allowing the slides to air dry, the DNA probes were denatured at 75°C for 5 min. The X and Y DNA hybridization mixture was added to each slide, then covered with a glass coverslip and sealed with rubber cement. Hybridization was allowed to occur by incubating the slides at 42°C for 1 h in a humidified chamber. The slides were post-washed in 2XSSC at 75°C for 2 min, followed a wash in 2XSSC/0.1% NP40 at room temperature for 1 min.

Cells were counterstained with DAPI (4,6-diamnio-2-phenylindole) and covered with a glass coverslip before microscopic analysis using a fluorescent microscope equipped with a triple band pass [Omega, N.H.]. Cells were easily visualized and only nonoverlapping cells with two clearly definable fluorescent signals were counted. Cells were scored as being of male origin if an orange fluorescent signal indicating the presence of the Y chromosome was identified within a nucleus as well as a fluorescent green X signal. Conversely, cells were scored as female if two green fluorescent signals were identified within a nucleus indicating the presence of two X chromosomes. At least one slide was examined from each site and collection method per case. Specimens were classified as rare positive (>2 or <5 scorable cells); positive (>5 scorable cells); nonviable negative (nonstaining, unhybridized cells); viable negative (stained but no fluorescent signals).

Results

The sex of the individual was correctly identified from at least two of the four collection sites in each case. FISH easily identified the presence of the number of copies of the X and Y chromosome signals (Fig. 1). Overall, results were available in 65% of the collections (n = 40) (Table 1). Samples obtained from a pubic pull yielded results in 100% of the cases although the combed samples provided a definitive result in about 50% of the cases. The age of the sample did not appear to affect final results. Results were available overall from 62.5% of the older samples as compared with 68.7% of the freshly prepared samples. Of the 26 positive results, 8 were classified as rare positive and 18 as positive. Conversely, there were 14 negative results with 11 being nonviable negative and 3 being viable negative. It was observed that fewer, morphologically intact, viable (those uptaking the counterstain) cells were available from the older samples.

TABLE 1—*Success of gender identification of hair in regards to the sites of origin and method of obtainment.*

	Head pull	Head comb	Pubic pull	Pubic comb	Total (%)
Male*(n = 3)	3	1	3	1	66.7
Female* (n = 3)	1	2	3	1	58.3
Male† (n = 4)	2	2	4	3	68.7
Total % (n = 10)	60	50	100	50	

*Samples prepared 21–26 days after collection.
†Samples prepared within 24 h of collection.

Discussion

Gender determination constitutes one of the more important steps in the identification analysis of forensic evidence. Hair as evidence is often an integral part of a court presentation. Classification and identification of hair is based on morphology, histology, cytology, and immunology. In addition, sex determination of hair has represented an important step in classification. In the past, gender identification of hair has been based primarily on the sex chromatin in the epithelial root sheath cells usually by the presence or absence of the Y chromosome body (1–5). However, interpreting this test can be difficult and is not without error (5).

More recently, molecular genetics techniques using Y-specific DNA probes have been used for gender determination of blood, saliva, semen, hair, bone, and skin (11–16). One of the newer molecular genetic techniques is FISH. This molecular cytogenetic technique uses nonradioactive fluorescent labeled DNA probe(s) specific for a chromosome or chromosomal region that are hybridized to their homologous regions in cells. Identification of a chromosome(s) is done by identifying the fluorescent signal(s) in cells and/or metaphase chromosome spreads under a fluorescent microscope. This technique is simple and rapid with a high sensitivity and specificity in detecting human chromosomes and/or chromosomal regions in both interphase and metaphase cells. Molecular cytogenetic techniques have already found important roles in clinical and research areas of cytogenetics, prenatal diagnosis, tumor biology, and gene mapping (17–19).

FISH has been used to identify rapidly and correctly the chromosome composition and hence sex using hair (20). This present study showed that FISH can forensically determine the gender of a hair sample. In each instance, the gender of the individual was correctly identified. This technique allows for individual hairs to be examined. Because results were not available with each individual hair or from each site consistently with the exception of the pubic pull, use of other techniques to isolate cells from the hair bulb may yield better results (20–22). In addition, other DNA tests could be performed on these hairs after FISH with the best results expected on the plucked hairs.

FISH is a simple, rapid laboratory technique that can use commercially available kits. The technique is highly sensitive and specific and can provide convincing additional evidence in forensic cases. The applicability of FISH in the forensic science laboratory has been already demonstrated (8–10). FISH will most likely see increased forensic use and application.

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