TECHNICAL NOTE

Birgit Herber,[1] *Ph.D. and Kurt Herold,*[1] *Ph.D.*

DNA Typing of Human Dandruff

REFERENCE: Herber B, Herold K. DNA typing of human dandruff. J Forensic Sci 1998, 43(3):648–656.

ABSTRACT: Dandruff particles contain a considerable portion of nucleated cells within the aggregates of nuclei-free corneocytes. The nuclei could be partially degraded due to epidermal differentiation processes. To test the suitability of DNA from dandruff for forensic application, a study of 35 subjects and two crime cases has been carried out using different STR polymorphisms. In 90% of the samples DNA genotyping could be easily performed indicating that DNA from dandruff is at least suitable for STR analysis. DNA quantity per dandruff particle varies extremely within a range from 0.8 to 16.6 ng DNA for the tested subjects. Genotyping was also possible for a single dandruff particle if DNA extraction volume had been reduced. In mixed samples from dandruff coextracted with bloodstains or semen stains dandruff genotype was detectable in some cases.

These results demonstrate the relevance of dandruff in forensic casework either as an additional sample or as a source of inadvertent contamination.

KEYWORDS: forensic science, DNA typing, dandruff, polymerase chain reaction, short tandem repeats, HUMTH01, HUMVWA, HUMFES, HUMFGA, AMELOGENIN

Desquamation is a continous process of losing the outer layer of keratinized cells of the dead skin. Normally, shedding of corneocytes is imperceptible for most people. In others, excessive scaling results in flakes, clusters of cells which become visible to the naked eye. Scaling affects mainly the scalp where it is called dandruff. But it could also extend to other areas of the body.

Dandruff consists of epidermal cells with different degrees of keratinization indicating a disorder of the normal epidermopoesis. Higher mitotic rates within the basal membrane of the epidermis and accelerated transition time of keratinocytes within the epidermis have been determined as pathophysiological changes in respect to dandruff (1). Insufficient time is given for a complete keratinization and differentiation of keratinocytes. This results in nucleated horn cells (parakeratosis).

Dandruff could occur without evidence of other skin disease but is also common as side effect of several dermatoses such as psoriasis and seborrheic dermatitis (2). Possibly due to the fact that dandruff is a subpathological as well as a clinical symptom, its etiology still remains unclear but is extensively discussed in literature (for reviews, see 3–5). Histological studies indicate a microlocal inflammation in the upper dermis but inflammation is clinically not evident (6,7). The involvement of microorganisms in dandruff etiology (notably yeast of the genus *Pityrosporum*), first proposed by Malassez in 1874, has since been investigated by many researchers (8–11).

Based on the variety of theories, different therapeutical concepts have been developed leading to a considerable number of medicated anti-dandruff shampoos with anti-mycotic, antibiotic, antimitotic, anti-inflammatory, or keratolytic agents. Despite of all these efforts, dandruff is still a widespread problem among the normal healthy population. Although reliable statistical data do not exist, it has been estimated by experienced clinicians that about 20% of the population are suffering from dandruff.

As a result of a crime case with a mask from which no suitable stains of saliva or hair roots except dandruff were available, we had the idea to test dandruff for forensic application. Since PCR amplification of STRs has been introduced as an instrument of forensic casework together with appropriate extraction procedures, even minor amounts of biological samples could be utilized for genetic analysis and the list of DNA-poor sources is still increasing (12).

In this paper we examine the feasibility of DNA typing of human dandruff. A study of 35 subjects and two objects from crime cases is presented. Morphological studies are performed to confirm the presence of nuclei-containing cells within the cell aggregations of dandruff. The DNA quantity per dandruff particle is determined for a variety of samples from several subjects. Different STR loci are tested to ensure the suitability of the genomic DNA extracted from dandruff as template molecule. In addition, detection limits are estimated by titrating the DNA extract from a variable number of dandruff particles from different subjects. Finally, the risk of contaminations introduced by dandruff particles into other typical stains of evidence is evaluated.

Materials and Methods

Microscopic Examination

Dandruff particles were fixed in ethanol and acetic acid (3:1) as described by Clarke 1981 (13). During incubation time, suspension was vortexed vigorously for 5–6 times to separate aggregates into smaller parts or single cells. Finally cells were pelleted by centrifugation and the fixation fluid was removed. Further cytologic examination was carried out by two different approaches:

[1]Molecular biology specialist and biology specialist, respectively, Department of Biology, State Criminal Office of Hessen, Wiesbaden, Germany.

Received 5 Feb. 1997; and in revised form 22 Sept. and 3 Dec. 1997; accepted 5 Dec. 1997.

1. Fixed cells were embedded in Eukitt (Riedel de Haen, Germany) or resuspended in 0.1% (v/v) Triton X-100 (Fluka, Switzerland) or stained with methyl blue (14) and directly examined by phase contrast microscopy. 2. Fixed cells were stained for 30 min in a heated carmine acetic acid solution (14). Additional vortexing for several times should provide further disaggregation of dandruff particles. Staining solution was removed and remaining acetocarmine was washed out 2 times with ethanol. After embedding the cells in Eukitt, examination could take place by normal light microscopy.

Extraction of DNA

With clean forceps 1 to 10 dandruff particles were directly transferred from the substrate into a 1.5 mL microcentrifuge tube (Eppendorf, Germany), followed by a brief centrifugation for pelleting the dandruff particles at the bottom of the tube. Extraction of DNA was carried out using the Chelex method according to Walsh et al. (15) with minor modifications: 200 μL Chelex (ReadyAmp™ Genomic DNA Purification System, Pharmacia, Germany) were supplemented with 3 μL of 20 mg/mL proteinase K (Boehringer Mannheim, Germany), 6 μL of 1 M dithiothreitol (DTT, Boehringer Mannheim) and 10 μL of 10% (v/v) Tween 20 (Fluka, Switzerland). Addition of Tween 20 is recommended to provide wetting of the partly hydrophobic surface of dandruff particles. Samples should not be vortexed to keep the particles at the bottom of the tube and immersed in the solution. After incubation on a shaker at 56°C overnight dandruff particles were usually completely dissolved. Lysis of cells was completed by vigorously vortexing, boiling at 100°C for 10 min in a thermoblock and vortexing again. Chelex beads were separated from supernatant containing DNA by centrifugation at high speed for 5–10 min.

DNA Quantitation

DNA from a variety of individual dandruff extracts was quantitated using the ACES™ 2.0$^+$ Human DNA Quantitation System (Gibco BRL, Gaithersburg, USA) as described by Budowle et al. (16) with minor modifications. The method is based on probe hybridization to a human alpha satellite locus (D17Z1), which is alkaline phosphatase labeled for luminol-based chemiluminiscent detection.

Ten μL of each DNA sample was added to 100 μL of spotting buffer (0.5 M NaCl, 0.5 M NaOH) and incubated for 5 min at room temperature to denature the DNA. DNA standards of the following quantities: 40, 20, 10, 4, 2, 1, 0.4, 0.2, 0.1, 0.04 ng of K562 control DNA, provided by the kit, were prepared in the same manner as well as blank samples containing no DNA. The prewetted nylon membrane (NY 12 N, Schleicher & Schüll, Dassel, Germany) was placed in a slot blot vacuum chamber (Gibco BRL, Gaithersburg, USA) and the entire volume of each sample was loaded into separate wells. Under a low air pressure (12 in. Hg) samples were pulled slowly through the membrane and each slot was rinsed afterwards with 400 μL of denaturing buffer. The membrane was air dried and baked at 80°C for 2 hours to crosslink DNA on membrane.

Hybridization of four membranes was carried out simultaneously in a shaking water bath by sequential incubation for 20 min at 50°C in 300 mL of prehybridization solution (0.5 M Na_2HPO_4, 0.1% SDS) and for 20 min at 50°C in 60 mL of hybridization buffer (0.5 M Na_2HPO_4, 0.1% SDS, 1% blocking reagent (Boehringer, Mannheim, Germany), containing 30 μL of the alkaline phosphatase labeled D17Z1 probe. The membranes were washed two times for 12 min in 500 mL wash buffer I (10 mM Na_2HPO_4, 0.1% SDS) at 50°C and two times for 5 min in 500 mL wash buffer II (0.1 M Maleic acid, 0.15 M NaCl, pH 7.5) at room temperature.

For detection of DNA, membranes were sprayed with Lumi-Phos Plus and covered in Saran Wrap. The excess of Lumi-Phos Plus was removed with a laboratory wipe. Exposure of membranes to Kodak RXS film (Kodak, Rochester, USA) was done for 22 to 46 hours at 32°C.

The quantity of sample DNA was determined by visual comparison of the sample signal intensity to a series of DNA standards of known concentrations which were blotted and cohybridized on every blot.

DNA Fragment Amplification by PCR

PCR amplification was performed using a Perkin & Elmer TC1 thermal cycler in a final volume of 25 μL, containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP (GeneAmp, Perkin & Elmer, Langen, Germany), 1 μg bovine serum albumin (BSA, Pharmacia, Germany), 1 unit TaqPolymerase (Perkin & Elmer), 0.6 μM each Primer (Applied Biosystems, Weiterstadt, Germany). Aliquots of the Chelex extracted DNA (1 to 15 μL) were tested for different STR loci: HUMTH01 (17), HUMVWA (18), HUMFES (19), HUMFGA (20), and HUMAMELOGENIN (21). STR systems were used both as singleplex or duplex PCR (HUMTH01/AMELOGENIN; HUMVWA/HUMFES, HUMVWA/HUMFGA). Amplification conditions as standard protocol for all STR applications were: denaturation for 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 60°C for annealing, 1 min at 72°C for primer extension. Last cycle was finished by an elongation step of 10 min at 72°C.

Vertical Gel Electrophoresis

Amplified DNA fragments were separated by native polyacrylamide gel electrophoresis. The gels of 8% (w/v) 29:1 acrylamide: N,N-methylene bisacrylamide (Roth, Germany) with 320 × 180 × 0.8 mm of size were run in a continous 1× TBE buffer system using the BRL SA32 sequencing apparatus (Gibco BRL/Life Technologies GmbH, Eggenstein, Germany). The conditions for electrophoresis were set at constant power of 750 V. Electrophoresis was stopped when the Xylene-cyanol blue dye front had reached 20 to 24 cm distant from the top, depending on the STR amplification products which have to be separated. Multiplex or sequential loading of singleplex amplifications could be carried out under same electrophoretic conditions (22). Visualization of separated DNA fragments was performed by silver staining as described by Budowle et al. 1991 (23).

Induced Mixed Stains and Contamination Studies

The effect of dandruff contamination in a typical evidence stain such as bloodstain or semen stain using standard extraction protocols was evaluated.

One or two pieces of a bloodstain of 2 square millimeters in size were extracted together with 1, 2, 3, 5, or 10 dandruff particles each in 200 μL Chelex as described above except for the addition of proteinase K, DTT or Tween 20. The experimental set up for each amount of bloodstain and number of dandruff particles, respectively was done in triplicates.

In a second experiment, extraction of 2 μL and 10 μL of whole semen in the presence of 1, 2, 3, 5, or 10 dandruff particles each was performed as recommended by Walsh et al. 1991 (15) in 200 μL Chelex supplemented with 2 μL of 10 mg/ml proteinase K

and 7 μL 1 M DTT. The following extraction procedure was done as described above.

Dandruff samples derived from two different subjects. In the case of bloodstain extraction, the average DNA quantity per dandruff particle of one subject was 8.0 ng. In the case of semen stain extraction, dandruff particles from another subject were used with an average DNA amount of 6 ng per particle.

Five microliters of each extract from the bloodstain/dandruff mixed samples as well as 10 μL of each semen stain/dandruff mixed samples were analyzed using the PCR system HUMTH01. DNA types of the donors of all mixed stains were both heterozygous for the locus HUMTH01 resulting in four distinguishable alleles. Genetic typing of separate sample extracts from each dandruff donor and each stain was done as positive controls.

Results

Dandruff Sources

Among the 35 subjects of the present study were 17 women and 18 men, or 34 adults and one child, respectively. Three of the 35 subjects were related to other than the White Caucasian population (1 U.S. Black, 1 Algerian, 1 from India). Two of the 17 women had artificially dyed red hair. Two subjects, one man and one woman, were suffering from seborrhoeic dermatitis and psoriasis respectively, with severe desquamation as a side effect.

In addition, dandruff material from two different crime cases has been investigated: dandruff particles from a mask of an unknown suspect in a bank robbery case and from a tuft of hair found in a drain of a sink to identify a corpse in a murder case.

Morphology of Dandruff

Dandruff particles contain a considerable portion of nucleated epithelial cells (Fig. 1). The morphological structure of dandruff varies within a wide range in shape, size, composition, and color. In general, two types of dandruff could be classified: Type I (Fig. 2) represents relatively large particles of about 1–2 mm in diameter, easily visible by the naked eye, with a transparent (''exfoliative'') appearance and of soft consistency. Type II is characterized by a hard and compact structure, smaller in size, about 0.5 mm in diameter and often with a whitish color (Fig. 3). Even though most dandruff particles appear whitish and dry, color could depend on the presence of sebum which can be pathologically secreted in excess, e.g., in the case of seborrhoeic dermatitis. Dandruff particles then become yellowish and sticky, large aggregates will be formed.

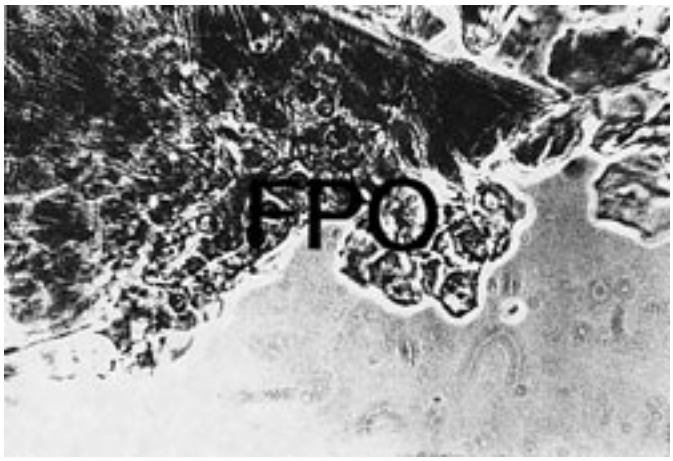

FIG. 1—*Parakeratotic keratinocytes in dandruff: Nucleated cells of epidermal origin (Staining with carmine acetic acid, ×400).*

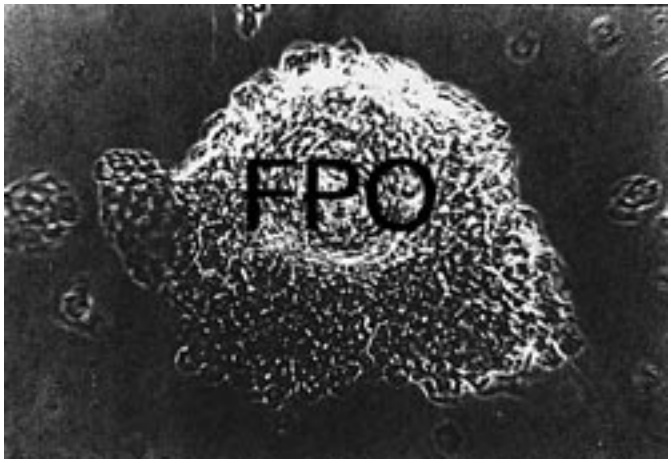

FIG. 2—*Dandruff of Type I: A broad rim of few cell layers surrounding a central area of denser cell layers typical for the exfoliative appearance (Methyl blue staining, ×160).*

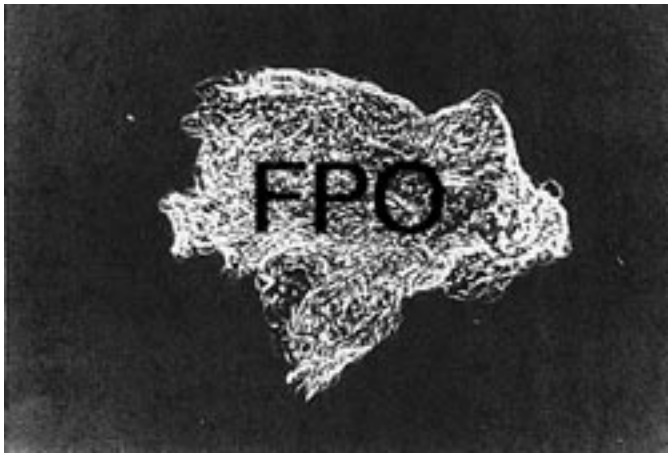

FIG. 3—*Dandruff of Type II with a compact structure. Few single cells could be discerned only at the edge of the dandruff particle (in Triton X-100 solution, ×160).*

Normal dandruff particles of Type II sometimes cannot be visually distinguished by eye from other amorphous structures with similar appearance, e.g., dust particles or grains of sand. An example of such an artifact is shown in Fig. 4. Staining with carmine acetic acid revealed no hints for intact cellular shapes or nuclei.

DNA Quantitation

To estimate the average amount of DNA per dandruff particle, a variety of samples with a known number of dandruff particles (between one and 13) extracted in 100 μL or 200 μL Chelex was quantitated by the hybridization method using a human specific probe (D17Z1) and chemiluminiscent detection as described in Materials and Methods.

Quantitation was done for 28 different sample extracts from a total of 20 subjects. A dilution series of human genomic DNA applied to the same membrane served as a standard for estimating the DNA content of each sample by visual comparison of the slot

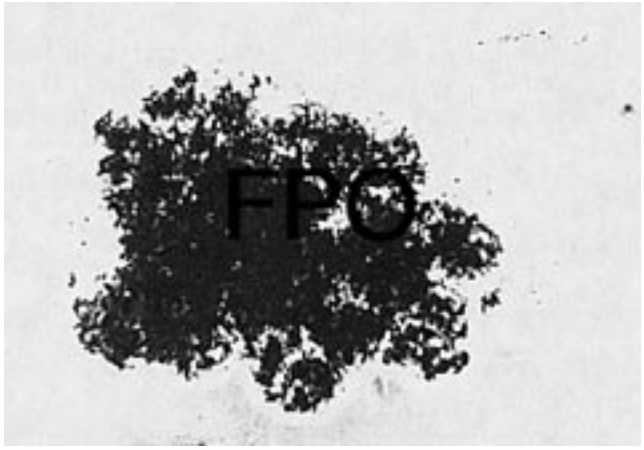

FIG. 4—*Artifical particle. No cellular structure was found (Carmine acetic acid staining, ×160).*

intensities. The minimum amount of DNA which could be detected after 46 hours exposed to film was 200 pg. Negative controls such as Chelex solution or spotting buffer without sample DNA show no detectable signals. Based on the quantity results for 10 μL of the tested samples (equivalent to 5% or 10% of the total extract volume), the amount of DNA per dandruff particle for each sample was calculated. The results given in Table 1 indicate that each dandruff particle can contain very different amounts of DNA within a wide range from 0.8 to 16.6 ng DNA per dandruff particle. The largest quantity of 133.3 ng DNA was found in dandruff particles of a subject suffering from severe psoriasis. Apart from this extremely high value, the average amount of a single dandruff particle is 4.7 ng DNA.

Despite the great variations of dandruff samples from different subjects, DNA content per dandruff particle from the same subject collected at the same time is less variable. This was demonstrated for two subjects by testing a serial extraction of dandruff particles (1, 3, 4, 5, and 10). Results of samples no. 16 a-c and 17 a-e are shown in Table 1. In both cases, mean deviation from the average quantity is 23%.

In addition, separate samples from two other subjects were collected at long time intervals of five to six months as indicated in Table 1. Dandruff particles were collected in winter, summer, and autumn of one year (sample no. 18 a + b, 19 a) and in spring of the following year (sample no. 19 b). DNA quantities of those samples show much greater variability than could be observed for samples collected at the same time from each individual subject (Table 1, samples no. 16 a-c and 17 a-e). A significant decrease of DNA content per dandruff particle of about 40% from winter to summer (sample 18 a + b) and of more than 55% from autumn to spring (sample 19 a + b) is evident for both subjects. These results indicate that DNA quantity of a dandruff particle differs not only between individual subjects but also depends on seasonal variations.

STR Analysis

Our first DNA typing of dandruff was performed using the STR system HUMTH01. DNA from 5 dandruff particles of each subject or stain was extracted in 200 μL Chelex as described above. PCR amplified fragments were separated and visualized by polyacrylamide gel electrophoresis and silver staining. The results for all 35 subjects and a stain from one crime case are shown in Fig. 5. DNA fragments of 31 samples could be easily detected, three samples showed weak signals but fragments could still be identified, two samples revealed no results at all. Those findings correspond to a rate of success of 90%. Only 6% of the samples failed in STR analysis. The DNA profiles were consistent with those of saliva samples, which served as control stains from the same 35 subjects (data not shown).

As a proof of the suitability of DNA from dandruff, additional STR loci were tested using PCR multiplex application for the STRs HUMVWA and HUMFES, HUMVWA and HUMFGA, or HUMTH01 and AMELOGENIN respectively. For that purpose 5 dandruff particles from each of 17 subjects and a stain from another crime case were assayed. The results of the VWA/FES multiplex analysis are shown in Fig. 6. In about 80% of the tested samples genotypes could be detected without any problems. Samples of lane #3 and #4 are mixed samples. Mixture happened inadvertently probably during collection of the samples from a black cardboard which was subsequently used by some subjects. Those kinds of

TABLE 1—*DNA quantity estimated by visual comparison with standard DNA.*

Sample	Quantity Result* (nanograms)	Number of Dandruff Particles/ Extracted in μL Chelex	Quantity per Dandruff part.† (nanograms)	Comments
1	0.5	5/200	2.0	
2	1.0	5/200	4.0	
3	1.5	5/200	6.0	
4	0.5	5/200	2.0	
5	0.5	5/200	2.0	
6	2.5	5/200	10.0	
7	0.5	5/200	2.0	
8	1.5	5/200	6.0	
9	1.0	5/200	4.0	
10	0.5	5/200	2.0	
11	0.5	5/200	2.0	
12	0.2	5/200	0.8	
13	1.5	5/200	6.0	
14	4.0	13/200	6.2	
15	4.0	10/200	8.0	
16 (a)‡	1.0	5/100	2.0	Titration
(b)‡	0.5	4/100	1.25	‘‘
(c)‡	0.5	3/100	1.7	’’
17 (a)‡	2.5	10/200	5.0	Titration
(b)‡	2.0	5/200	8.0	‘‘
(c)‡	1.5	4/200	7.5	’’
(d)‡	1.0	3/200	6.7	‘‘
(e)‡	0.5	1/100	5.0	’’
18 (a)‡	2.5	3/200	16.6	(February)‖
(b)‡	1.0	1/100	10.0	(July)‖
19 (a)‡	0.5	5/200	2.0	(September)‖
(b)‡	0.5	11/200	0.9	(March)‖
20	20.0	3/200	133.3§	severe Psoriasis
21–23	0	Chelex	0	(triplicates)¶
24	0	spotting buffer	0	

*The values in the 2nd column indicate the quantity of DNA in 10 μL of sample tested.

†The values in the right column (bold numbers) show the DNA content calculated for a single dandruff particle of each sample.

‡(a)–(e) indicate different dandruff extractions for the same subject.

§The value for sample no. 20 from a subject with severe psoriasis was not taken into account for the calculation of the average amount of DNA per dandruff particle.

‖Samples were collected at long time intervals. Months indicate the time of each sample collection.

¶Three different samples of Chelex were tested as negative controls.

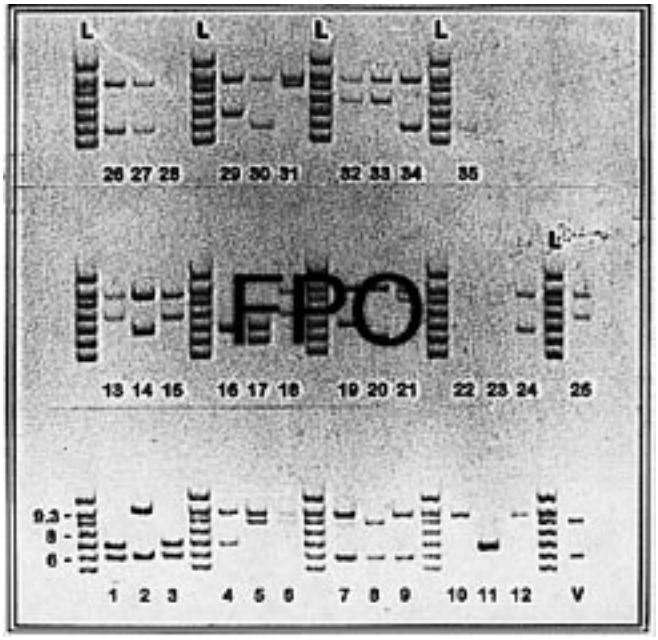

FIG. 5—*DNA analysis for the STR locus HUMTH01 of dandruff DNA derived from 35 subjects and one crime case (V). Five dandruff particles of each subject and a stain from crime scene were analyzed. The allelic ladder (L) represents the alleles 5, 6, 7, 8, 9, 9.3, and 11.*

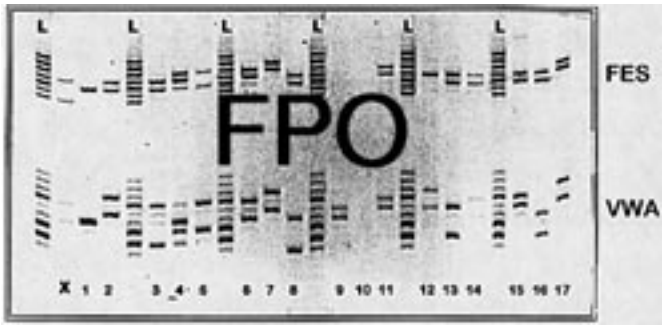

FIG. 6—*Multiplex DNA analysis for the STRs HUMVWA and HUMFES from dandruff of 17 subjects and one additional crime case (X). The allelic ladders for HUMVWA contain the alleles 13–21, for HUMFES the alleles 8–14.*

contamination could be eliminated after renewed extraction of then separately collected dandruff particles from each of the relevant subjects (for comparison see Fig. 5; subjects no. 19 and 25). PCR fragments of one sample, which failed previously for the HUMTH01 locus (subject no. 22), were also not amplified in both systems of the multiplex setup (lane #10). One sample showed a partial dropout for STR HUMFES but not for the HUMVWA locus (lane #9). This phenomenon could be occasionally observed in VWA/FES multiplex PCR when template DNA or other amplification conditions are not optimal.

Multiplex application of STR genotyping was also tested for the systems VWA/FGA and HUMTH01/AMELOGENIN. In both cases positive results were obtained (data not shown).

Limits of Biological Material

To estimate the minimum dandruff material which is required for reliable STR genotyping, a titration study was performed. From three subjects, dandruff particles with an average DNA content of 1.6 ng (subject no. 24), 2.8 ng (no. 4), and 6.3 ng (no. 5) were collected separately for Type I (no. 4) or Type II (no. 5 and 24). Extractions of 2, 3, 4, 5, or 10 dandruff particles each were done in 200 μL Chelex. In two cases, DNA from a single dandruff particle was extracted in either 200 or 100 μL Chelex for direct comparison. In addition, titration of different aliquots of each DNA extract (1, 5, 15 μL) was carried out in the same experimental setup. Results are presented in Fig. 7. DNA genotyping could be performed with up to three dandruff particles using the standard extraction procedure. DNA amplification of the extract of two dandruff particles (subject no. 24) was also possible but of less efficiency. The intensity of visualized fragments corresponds with both, the number of dandruff particles and the amount of Chelex extract used in PCR amplification, indicating that the DNA quantity of each sample refers directly to the amount of biological material. These correlations were also confirmed by the results of DNA quantitation of one of the titration series (see Table 1). Samples no. 17 b-e correspond to subject no. 5, Fig. 7. The total amount of DNA in 10 μL of each sample from the same subject (Table 1, second column) increases according to the increasing number of extracted particles. No significant differences in suitability of DNA could be determined between dandruff of Type I and Type II. DNA analysis of one dandruff particle extracted in 200 μL Chelex revealed no evaluable profile (see Fig. 7, subject no. 24) or failed completely (subjects no. 4 and 5). However, if extraction volume has been reduced to 100 μL of Chelex, DNA profiles were consistent with the referring samples of extracts from two or more dandruff particles. The results demonstrate that DNA analysis could be performed even if only a single dandruff particle is to be analyzed.

Induced Mixed Stains and Contamination Studies

Sample mixtures of bloodstains or semen stains with an increasing number of dandruff particles in each extract were tested by genotyping to evaluate a possible risk of dandruff contamination in a typical evidence stain. Routine extraction protocols for bloodstains and semen stains, respectively were used. In addition, two different amounts of each stain coextracted with a various number of dandruff particles were tested to compare different DNA ratios of the mixed samples.

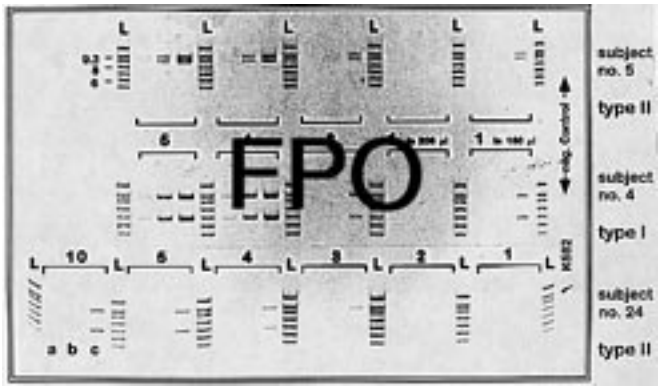

FIG. 7—*Titration study for estimating the minimal amount of dandruff material reliable for genotyping. Variable number of dandruff particles (1, 2, 3, 4, 5, 10) from three subjects (no. 4, 5, and 24) were tested with three different amounts of the Chelex extracts (a = 1 μL, b = 5μL, c = 15 μL). The STR system HUMTH01 was used; allelic ladder (L) consists of the alleles 5, 6, 7, 8, 9, 9.3, and 11. DNA from K562 cell line serves as positive control. Negative controls are indicated as well.*

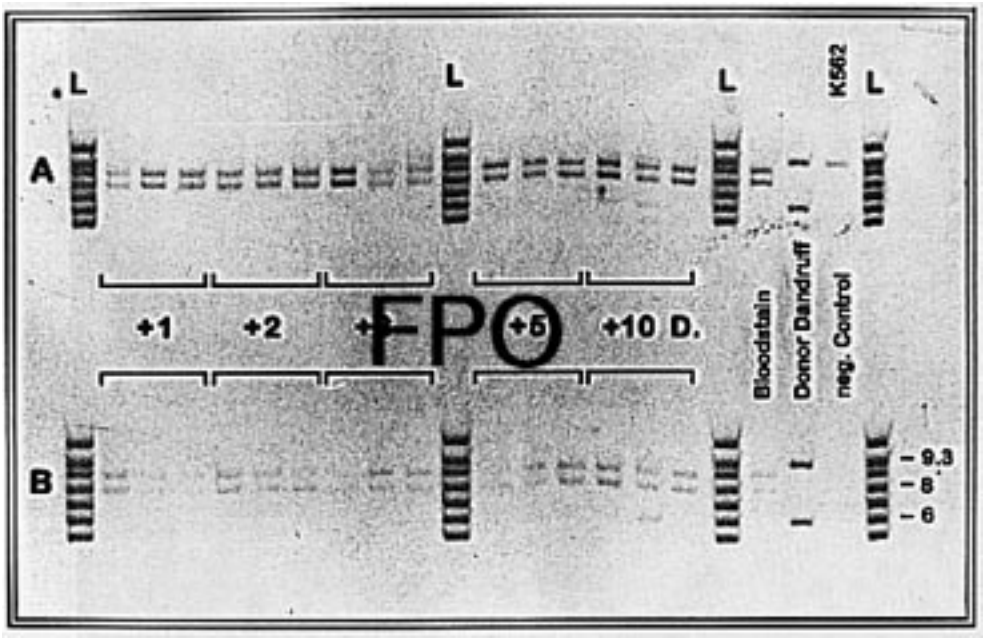

FIG. 8—*DNA analysis of induced mixed samples using the STR system HUMTH01. One (A) or two pieces (B) of a bloodstain (2 square millimeters in size) were mixed with 1, 2, 3, 5, and 10 dandruff particles from one subject. In addition, bloodstain and DNA from dandruff donor (saliva) were separately extracted and analyzed for comparison. HUMTH01 type of bloodstain is 8/9 and type of dandruff donor is 6/9.3. The alleles of the ladder (L) are 5, 6, 7, 8, 9, 9.3, and 11. K562 positive control and negative control are indicated.*

In the case of bloodstain samples, the contamination type of the dandruff donor could only be detected in samples mixed with 10 dandruff particles (Fig. 8). This seemed to be the same for both amounts of bloodstains tested (Fig. 8 A and B) but on the original gel a very faint band of allele 6 was also visible in one of the bloodstain samples of lower amount (two square milimeters) coextracted with 5 dandruff particles (panel B). These results show that dandruff DNA can be extracted and amplified to some extent even if only Chelex extraction solution is used, not supplemented with proteinase K, DTT, or Tween 20.

This was also proven by additional experiments extracting only dandruff particles in Chelex solution without any supplements. Dandruff genotyping from those samples was possible but was much less efficient compared to Chelex extracts supplemented with proteinase K and DTT (data not shown).

In mixtures of dandruff particles and semen stains extracted with Chelex in the presence of proteinase K and DTT it is more evident to detect genotypes from both donors (Fig. 9). The minimum portion of the contamination type was 3 to 5 dandruff particles depending on the DNA amount of primary type. Influences of the DNA ratio on the amplification efficiency of each genotype are clearly demonstrated. In mixed samples of 2 μL semen coextracted with 3, 5, or 10 dandruff particles each, contaminant type was of greater intensity than the primary type (Fig. 9A). On the other hand, in extracts from 10 μL semen, dandruff contamination type was not detected for 3 or less particles but clearly detectable for 5 or 10 particles coextracted (Fig. 9B).

Results presented here demonstrate that there is a considerable risk of dandruff contamination in typical stains from a crime scene. Using standard extraction protocols, this is more evident for semen stains than bloodstains. Level of contamination also depends on the DNA quantity per dandruff particle and on the DNA ratio in mixed samples. The average DNA amount of dandruff particles used for the contamination studies were with 8 ng and 6 ng, respectively, relatively high.

Discussion

Dandruff derives from the horny layer of the outer part of the skin which mainly consists of nuclei-free, keratinized corneocytes (24). Nevertheless, microscopic examination of dandruff particles clearly demonstrates the existence of solid nuclei containing cells within the aggregates. Those parakeratotic cells are assumed to be the result of an abnormal epidermopoesis with an incomplete terminal differentiation and keratinization. The proportion of nucleated keratinocytes versus non-nucleated corneocytes is relatively high, although exact quantifications are difficult due to the diversity of dandruff in size, structure, and composition. It has been estimated that a dandruff particle of about 0.5 mm in diameter could contain between 500 and 1000 horny cells (25) with a parakeratotic level of about 25% (26). Theoretically, this would correspond to a range of 0.8 to 1.5 ng DNA per dandruff particle.

For DNA quantitation the slot blot technique and hybridization with an alkaline phosphatase labeled probe (D17Z1) followed by chemiluminiscent detection was used. The hybridization method combines several advantages such as high sensitivity and human specificity. In addition, single-stranded, non-purified and even not fully intact DNA over a wide range of average sizes can be detected (27).

The average DNA quantity of 4.7 ng in a single dandruff particle is much higher than expected by theoretical considerations. Nevertheless, the amount of DNA per dandruff particle is highly variable within a wide range of 0.8 to 16.6 ng DNA. These data give a more realistic account of the large variety of dandruff morphology and level of parakeratosis. It was also shown that the DNA quantity for samples from different subjects has much greater variability than from samples of the same subject collected at the same time. Based on the quantity results, it is obvious that the DNA yield for a specific dandruff particle is not predictable and samples have to be analyzed at any rate.

Despite the variety of its appearance we have shown that dandruff of both Type I and Type II are good candidates for successful

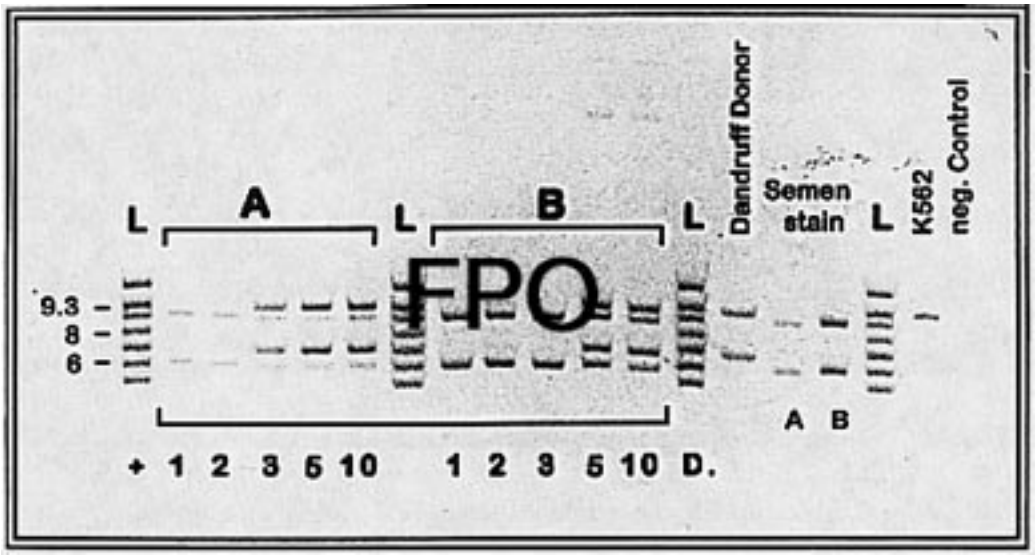

FIG. 9—*Genotyping of mixed stains. Two (A) or ten microliters (B) of whole semen were coextracted with 1, 2, 3, 5, and 10 dandruff particles from a second subject and analyzed for the HUMTH01 locus. For comparison with the mixed samples, DNA of semen stain and dandruff donor (saliva) were genotyped separately resulting in type 6/9 for semen stain and 7/9.3 for dandruff DNA. The allelic ladders (L) contain the alleles 5, 6, 7, 8, 9, 9.3, and 11. Positive and negative controls are indicated.*

DNA typing. DNA extracted from dandruff particles is suitable at least for STR analysis as validated by several STR systems located on different chromosomes [HUMTH01: 11p15.5 (28); HUMVWA: 12p12-12pter (18); HUMFES: 15q25-qter (19); HUMFGA: 4q28 (20); AMELOGENIN: Xp22.1-p2.3 and Yp11.2 (29)]. Although degradation of genomic DNA in dandruff might be expected as a consequence of the ongoing nuclear decomposition during epidermal differentiation, this does not interfere with STR DNA genotyping. Successful PCR analysis of degraded DNA from human remains or aged biological material has been demonstrated by others (30–33). The presence of an intact genome in parakeratotic cells has not been evaluated but could be approximately proved by testing additional STRs and VNTRs. This will be addressed in further investigations.

DNA genotyping of dandruff resulted in an overall yield of 90%. Only two of the samples failed, possibly due to a minute amount of extracted DNA or the presence of inhibitors of PCR.

In fact, DNA content of one of those two samples is very low (0.2 ng/10 μL) and the average DNA quantity per dandruff particle is the lowest in the range of all samples tested (0.8 ng). This sample derived from a ten year old child. Dandruff is relatively unusual and mild in children and uncommon below the age of 5 (34). Dandruff from children are much smaller in size than from adults resulting in a lower yield of DNA which is a disadvantage for genotyping.

DNA quantity of the second sample which failed in DNA profiling is 0.5 ng/10 μL. Eight other samples from different subjects contain about the same amount of DNA but STR analysis was successful. Therefore, the failure of the second sample is possibly due to the presence of PCR inhibitory substances. The sample derived from a female subject who had freshly dyed red hair. Staining was very intense so that stain was visible on dandruff particles as a reddish shimmer. In those cases, PCR efficiency could be possibly improved by purification of the extracts on QIAamp® columns, by phenol-chloroform extraction or other appropriate methods. Additional purification of samples has not been tested yet.

Inhibitory effects to some extent may also play a role in case of the three samples for which PCR amplification was less efficient although DNA quantity is relatively high (0.5 to 1.5 ng/10 μL). The nature of those presumed inhibitors is not known. Chemical compounds from a variety of different shampoos or other haircare products used by the 35 subjects might be candidates but in 90% of the samples had no obvious effects. In addition, DNA coextracted from the normal scalp microflora or from elevated levels of specific microorganisms which were supposed to play a role in dandruff etiology did not interfere with STR analysis.

Furthermore, it could be demonstrated that DNA typing is also possible using only a single dandruff particle if extraction volume is reduced to 100 μL of Chelex (or less). These results were independent from the type of dandruff morphology. In contrast, data from DNA quantitation revealed that the DNA yield per dandruff particle varies within a wide range. Therefore, the efficiency of STR analysis from a single dandruff particle may vary correspondingly.

Dandruff particles are usually 0.5 to 1 mm in diameter in size and therefore visible by the naked eye. Nevertheless, it can sometimes be confused with other artificial particles. An appropriate dandruff stain detection assay, as known for bloodstains for example, is not available apart from microscopic examination which is not recommended if only few dandruff particles have to be analyzed. A more indirect proof of biological material of human sources is given by direct STR PCR analysis because it has been shown for a variety of STR polymorphisms to be specific for humans (33). Van Oorshot et al. 1994 (35) have tested numerous different species for the HUMTH01 PCR system. Cross amplification was only observed with two relatively closely related primate species (gorilla and chimpanzee). Other primate species, e.g. orangutan and pig tail macaque, and all the other species tested failed. More recently, a comprehensive species study including bacterial strains, several non-primate and 10 primate species has been performed using seven STR loci as multiplex (36). Although several signals had been observed mainly for the closely related species to humans (great apes), they were designated as atypical alleles,

present both within and outside the human loci regions and profiles appeared to be unbalanced.

Concerning the prevalence of dandruff among populations, very few references are given in the literature. Differences in human races or influences of geography and climate are not known. At least seasonal variations have been determined that dandruff declines in the summer months and is more common in fall (37). Those seasonal variations were shown to exist also on the level of DNA quantity per dandruff particle. Samples collected from the same subject but at large intervals of time clearly show a decrease in the DNA amount from winter/fall to summer/spring.

Based on estimations from specialists, dandruff is a widespread problem among about 20% of the population. Therefore, the occurance of dandruff particles can be assumed everywhere. This might be an advantage of additional samples in respect to forensic application but could also be a source of inadvertent contamination. It has been shown, that dandruff particles coextracted with other stains such as bloodstains or semen stains could—to a certain extent—induce mixed stains. Obviously, the extraction procedure of a sample as well as the DNA quantity of dandruff particles are deciding factors. Comey et al. (38) reported that induced contamination of 1 μL or 10 μL of a bloodstain coextracted with ''30 s shed scalp cells'' from a laboratory worker was not observed in HLA DQα genotyping. Negative results were also obtained for control samples of PCR premix solution and extraction buffer, respectively, mixed with shed scalp cells. In the course of their validation studies of the DQα system, the authors have tested four different extraction protocols using organic extraction with phenol/chroroform, organic extraction modified by a water presoak of the stains or followed by centricon purification, and non-organic extraction which included sample treatment with Triton X-100 and proteinase K. It was shown that the modified organic extraction procedures and the non-organic extraction method as well were all more effective in yielding amplifiable DNA than the organic extraction protocol. Unfortunately, it is not clear which extraction method was used especially for the induced contamination experiment with shed scalp skin. Nevertheless, Chelex extraction had not been tested at that time but was supposed to be more efficient as the authors stated themselves. So, perhaps the extraction method might be crucial. In addition, the nature of ''shed scalp cells'' was not described so that it is not known whether there was DNA and if any how much DNA was present in the sample.

In contrast, Kitchin et al. (39) reported that an alarming source of contamination can derive from the skin surface of PCR operators. Again, the authors did not characterize exactly the source of contamination but assume ''dead epidermal cells'' derived from the exposed facial skin surfaces of one of the operators. Contamination material was introduced to PCR premix just by shaking the head of this lab worker over open tubes resulting in false positives. The contamination could be prevented by protecting the head of the operator with a disposable mask and mob cap. As a preliminary proof of the contradictory findings we repeated both experiments adding one or more dandruff particles from two different subjects directly into the PCR premix. First results were consistent with those from Kitchin and coworkers (data not shown) but these findings have to be reexamined and further contamination studies are addressed to the future.

In general, a risk of contamination introduced by dandruff DNA is evident for typical stains such as blood or semen and has to be considered as an additional source of contamination during collection of stains from a crime scene as well as during handling of samples in the laboratory.

Concerning our present experience with masks from crime scenes it is recommended to collect separately a number of dandruff particles both, from the outside and inside of a mask. Avoiding the induction of mixed samples, it is highly recommended to collect additionally some single dandruff particles separately and better extract in 100 μL or less Chelex solution. In addition, as a matter of routine the lab workers wear disposable masks and mob caps during collection of the samples.

In conclusion, the results of our study verify that genotyping of human dandruff is possible and could be useful in a forensic context. Dandruff can be considered as an additional or alternative sample, if present, when others like hair roots or saliva from a mask, for example, failed in DNA analysis. Dandruff particles can occur on several substrates e.g., headwear of all kind including crash helmets and masks, outer clothing and covers of automobile head rests. On the basis of the epidermal origin of dandruff, more attention should be paid in crime cases to all possible sources of epithelial cells e.g., cuticles on adhesive tape.

Acknowledgments

The authors would like to thank Ms. Stephanie Merten for her excellent technical assistance. Special thanks are also addressed to all subjects who supported this study by their generous donation of dandruff particles. We are grateful to Prof. Hevert, Basotherm, Germany, to Dr. Feistkorn, Wella, Germany, to Prof. Runne, University of Frankfurt/Main, Germany, and to Prof. Tronnier, University of Witten-Herdecke, Germany, for their helpful informations about dermatological aspects. We are also grateful to Prof. Bratzke and Richard Zehner, Institute of Legal Medicine, Frankfurt, Germany, for providing the equipment used for DNA quantitation and to Ms. Schöttler as well for her technical support. We thank Catherine Duffek and Claus Morhart for reading the manuscript.

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Additional information and reprint requests:
Dr. Birgit Herber
Hessisches Landeskriminalamt (HLKA)
Department of Biology (HSG 73)
Hɵlderlinstr. 5
D-65187 Wiesbaden, Germany