Int J Legal Med (2012) 126:179–183
DOI 10.1007/s00414-011-0579-0

SHORT COMMUNICATION

Good shedder or bad shedder—the influence of skin diseases on forensic DNA analysis from epithelial abrasions

Thomas Kamphausen · Dirk Schadendorf · Nicole von Wurmb-Schwark · Thomas Bajanowski · Micaela Poetsch

Received: 1 February 2011 / Accepted: 29 April 2011 / Published online: 15 May 2011

Abstract The successful analysis of weak biological stains by means of highly sensitive short tandem repeat (STR) amplification has been increased significantly over the recent years. Nevertheless, the percentage of reliably analysable samples varies considerably between different crime scene investigations even if the nature of the stains appears to be the same. It has been proposed that the amount and quality of DNA left at a crime scene may be due to individual skin conditions (among other factors). Therefore, we investigated DNA from handprints from 30 patients acutely suffering from skin diseases like atopic dermatitis, psoriasis or skin ulcer before and after therapy by STR amplification using the new and highly sensitive Powerplex® ESX17 kit in comparison to 22 healthy controls. Handprints from atopic dermatitis patients showed a correct and reliable DNA profile in 90% and 40% of patients before and after therapy, respectively. Regarding psoriasis patients, we detected full DNA profiles in only 64% and 55% of handprints before and after therapy. In contrast, in ulcus patients and controls, full DNA profiles were obtained in much lower numbers. We conclude that active skin diseases like atopic dermatitis or psoriasis have a considerable impact on the amplificable DNA left by skin contact with surfaces. Since up to 7% of adults in European countries suffer from one of these diseases, this could explain at least partially the varying quality of DNA from weak stains.

Keywords STR · Low copy number DNA · Dermatopathy · Crime scene investigation

T. Kamphausen · T. Bajanowski · M. Poetsch (✉)
Institute of Legal Medicine, University Hospital Essen,
Hufelandstr. 55,
45122 Essen, Germany
e-mail: micaela.poetsch@uk-essen.de

D. Schadendorf
Department of Dermatology, University Hospital Essen,
Essen, Germany

N. von Wurmb-Schwark
Institute of Legal Medicine, University of Schleswig-Holstein,
Campus Kiel,
Kiel, Germany

Introduction

Due to a constant improvement in forensic genetic analysis, e. g. the development of so-called mini-short tandem repeat (STRs) [1–5], the DNA detection threshold becomes lower and lower. Meanwhile, DNA amounts down to 25 pg can be successfully investigated, allowing the analysis of samples formerly known as not suited for DNA investigation. This is especially true for minimal stains such as epidermal abrasions or handprints on different surfaces. Nevertheless, there are samples, especially epidermal abrasions, which sometimes are easy to analyse and sometimes still show poor results in DNA analysis [1, 6, 7]. Here, it might be difficult to explain to the police or at court why there are sometimes significant differences regarding genetic typing results from apparently similar traces. Some authors propose that due to their individual skin conditions, some individuals seem to be "good shedders", who constantly leave detectable amounts of DNA on a surface contacted, while others do not shed so much DNA during close contact [8–10]. Other studies investigated predominantly the influence of surface conditions and the manner of DNA application [11, 12].

If there are "good shedders" and "bad shedders", this leads to the question: Which factors determine quantity and

quality of DNA left on different surfaces? Here, even a recent review [13] emphasised the importance for more studies regarding such variables. Beside possible extrinsic factors like temperature or air moisture, an important intrinsic factor could be the proliferation rate of the skin, which is directly associated to the number of scaled epithelial cells. This proliferation rate depends on a variety of individual factors like age, gender, differences in skin structure or specific skin diseases. Therefore, we systematically investigated the influence of atopic dermatitis, psoriasis and dermal ulcers known to be accompanied by an increased proliferation rate of the skin on the transfer of epithelial cells on surfaces by means of forensic DNA analysis.

Fig. 1 Standardized creation of handprints

Material and methods

Patients and controls

The study comprised 30 patients (12 women and 18 men) acutely suffering either from atopic dermatitis (10 patients, age 1–65 years), psoriasis (11 patients, age 18–83 years) or dermal ulcer (10 patients, age 63–90 years). One patient was suffering from a skin ulcer and psoriasis. Samples were collected in 2009 and 2010 in the Department of Dermatology, University Hospital Essen. Atopic dermatitis is a chronic or chronically relapsing inflammatory dermopathy which may be a hereditary immunological disorder linked to an increased IgE level in blood, a disproportion between TH1 leucocytes and TH2 leucocytes, antibodies against bacterial antigens or even autoantibodies against epidermal proteins. Atopic eczema with itching as a main symptom is caused by transepithelial loss of water and an altered composition of dermal lipids with a damaged barrier layer and a dry, scaling skin. Psoriasis is a chronic, autoimmune dermatopathy which causes red, scaly psoriatic plaques of inflammation caused by excessive keratinocyte production. Skin ulcers are caused by dysfunctional venous valves leading to edema, local inflammation, eczema, skin thickening, fibrosis and subsequently to ulcer predominantly located in the malleolus areas.

Handprints

The patients and controls created a handprint by pushing their right hand for 10 s on a melamine-coated board (Fig. 1) previously cleaned/treated with DNA *AWAY*® (Molecular BioProducts, San Diego, USA) and rinsed with ETOH 70% to avoid contamination with foreign DNA. Handprints were taken before and after a dermatological therapy of 7 to 14 days on average to compare the amount of transferred DNA and the subsequent DNA typing success. Cells were collected from the melamine-coated board by wiping it with DNA-free swabs, which were moistened with a lysis buffer. For comparison purposes, a buccal swab from every patient was taken.

Additionally, samples from 22 healthy test persons (11 women and 11 men, age 23–55 years) were taken and treated in the same way. All samples were obtained after informed consent and with approval of the Medical Ethics Committee at the University of Duisburg-Essen in accordance with the declaration of Helsinki and national laws.

DNA extraction

DNA extraction from buccal swabs was done using innuPREP® DNA Mini Kit (Analytikjena®, Jena). DNA extraction from artificially created stains (handprints) was performed using a slightly modified phenol/chloroform method as published by DeSalle and Bonwich [14].

DNA quantification

DNA content of the artificial stains produced before the beginning and after the conclusion of a dermatological therapy was measured in a real-time PCR using the Quantifiler® Human DNA quantification kit (Applied Biosystems) according to the manufacturer's instructions. Every sample was analysed in triplets using 2 µl of DNA-containing solutions each. This assay had a reliable and reproducible detection threshold down to 25 pg; less DNA amounts were also detectable but not always with 100% correctness.

DNA amplification and electrophoresis

The amplification protocol for the multiplex PCR Kit Powerplex® ESX 17 followed the manufacturer's instructions (Promega, Mannheim), with a reduced PCR volume of

12.5 µl in the GeneAmp® PCR system 9700 (Applied Biosystems). The employment of this nonstandard reaction was done to save money for this study and the following routine investigations. This reduced volume assay has been thoroughly and independently tested according to the existing quality managements. In each amplification, a positive control (100 pg 9947A) and a no template control negative control (sterile water) were analysed. Amplification products were separated and detected on the ABI310 Genetic Analyzer (Applied Biosystems) in comparison to the allelic ladder, which is a component of the kit. Electrophoresis results were analysed using the GeneMapper® ID Software v3.2. Allele peaks were interpreted when greater than or equal to 50 RFUs.

Results and discussion

DNA yield from handprints

Real-time PCR results showed total DNA amounts from 0 to 160 ng (up to 3.7 µg/µl) for the handprints. Maximum yield was 160 ng, 53 ng, 44 ng and 38 ng for patients with atopic dermatitis, psoriasis patients, ulcer patients and controls, respectively (Fig. 2).

Detection of DNA profiles from the handprints

The results obtained from the handprints were classified as follows:

- Full profile—in all investigated STRs (n=16), the full profile of the patient/control person could be obtained, and the number of STRs with allelic drop-in was three or below
- Partial profile—in more than half of the investigated STRs, the full profile of the patient/control person could be obtained, and the number of STRs with allelic drop-in was five or below
- No profile—in three or less STRs, the alleles of the patient/control person were found.

We obtained very different results from the three patient groups (Fig. 3). A full profile could be found from almost all atopic patients before therapy (90%) and for nearly half of them after therapy (40%; Fig. 4). Here, a clear effect of the therapy could be seen. Regarding psoriasis patients, in 64% and 55% of handprints, a full profile could be shown before and after therapy, respectively. In only one patient was no profile found after therapy in contrast to a full profile before therapy. In the others, the impact of therapy measured by forensic DNA analysis was only minor or nonexistent. The lowest number of patients with a full profile before and after therapy was observed in ulcer patients (20% and 30%, respectively). Here, the changes after therapy were only minimal. Nevertheless, even in ulcer patients, we detected more people with a full profile than in our controls (9%).

Lowe et al. considered that individuals can be categorised as “good shedders” and “bad shedders” depending on their ability of depositing DNA traces on handled objects [8]. They detected 18 “good shedders” out of 30 volunteers, who left a full profile on sterile plastic tubes held in the closed fist 15 min after washing. This result could be confirmed by Djuric et al. [9]. In contrast, Phipps and Petricevic did not observe any “good shedders” in a group of 60 volunteers, following similar test conditions [10]. Beside differences in sensitivity due to individually used examination and amplification methods, Phipps and Petricevic considered that the approach to classify individuals as “good” and “bad” shedders could be too simplifying and takes no account on further variables influencing the amount of deposited DNA. Despite using a much more

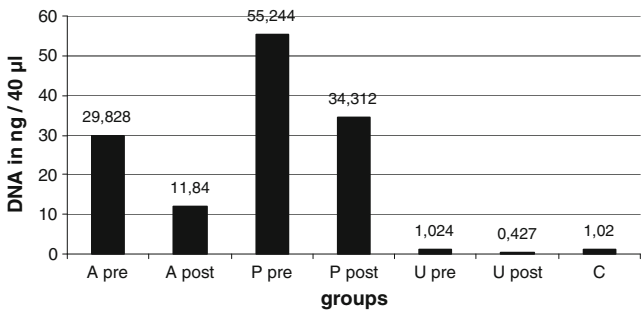

Fig. 2 DNA yield from whole handprints of persons with different skin conditions. Shown are the mean values of nuclear DNA in the whole DNA extract after real-time PCR quantification. From all patient groups (*A* atopic dermatitis, *P* psoriasis, *U* ulcer), samples were taken before and after specific skin treatment. For comparison, a control group from people not suffering any pathological skin condition was additionally investigated

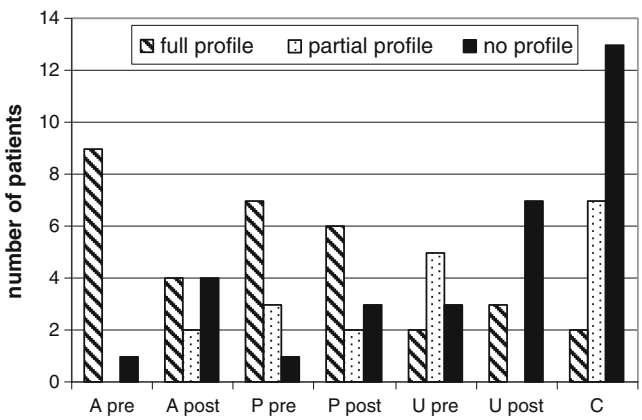

Fig. 3 A schematic presentation of results after STR analysis; *A* atopic dermatitis, *P* psoriasis, *U* ulcer, *pre* before therapy, *post* after therapy, *C* controls

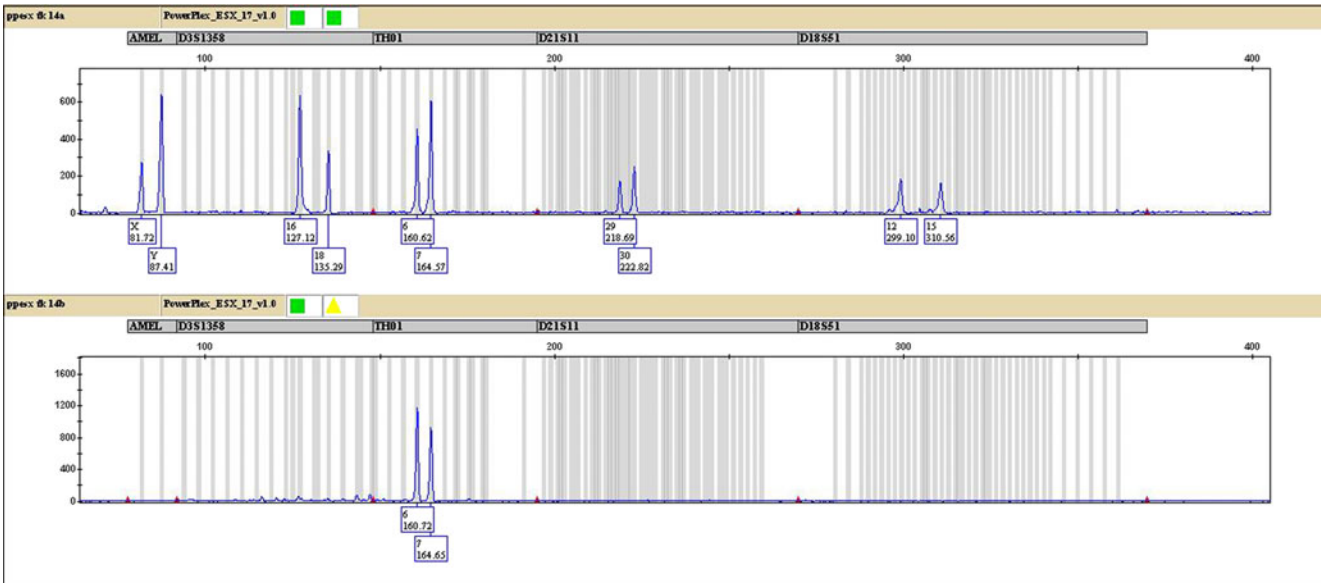

Fig. 4 Representative electropherogram of PPESX17® analysis of a handprint from an atopic dermatitis patient before (*above*) and after therapy (*below*)

sensitive amplification kit in this study (Powerplex ESX17 versus SGMplus in the studies of Lowe et al. and Phipps and Petricevic [8, 9], Identifiler for [7]), we found mostly "bad shedders" among healthy test persons. Our results indicate that under our standardized experimental conditions, healthy skin does not leave enough cellular material on a plain surface to create a full DNA profile regularly. Similar results were seen in the cohort of patients suffering from skin ulcer solely. The lack of impact of the ulcer on the skin of the hand is plausible since the ulcer is localized at the lower leg.

In contrast, dermatopathies localized on the hands seem to sufficiently increase the amount of cellular material left on a surface to produce adequate DNA profiles, especially if they are not treated.

If—as in this study—very low levels of DNA template are amplified, allele drop-in and drop-out artefacts are known to occur [15]. In the literature, PCR reactions involving DNA template levels below approximately 100 pg of DNA have early been shown to display such stochastic fluctuation phenomena [16, 17]. In a recent study, Cowen et al. [18] showed that allele drop-in predominantly occurs in stutter position. In our study, we observed 193 allele drop-in artefacts in different STRs, 171 of these were in stutter position (89%), supporting the results of Cowen et al. In our atopic patients, drop-in and drop-out occurred mostly in less than three STRs, rendering an easy to analyse (full) profile. All psoriasis patients exhibiting only drop-in artefacts had a full profile (according to our above-mentioned classification), whereas drop-out artefacts or both kinds of artefacts in a psoriasis patient profile occurred always in at least four STRs (partial profile). In contrast, in ulcus patients and controls, mostly drop-in and drop-out artefacts were found in more than seven STRs in one person, thus resulting in a negative profile. Regarding the occurrence of drop-in and drop-out artefacts, we can summarize that 0.5 ng and more DNA always led to a full profile without any discrepancies. When employing very low DNA amounts (100 pg and less), the number of drop-in artefacts is considerably increased, while drop-out artefacts occur already sporadically when having less than 500 pg. There was no strong correlation between a full or partial profile and the amount of DNA template, implying that not only the simple number of template molecules but also possibly the status of degradation influences PCR success.

Conclusions

Despite the low number of patients investigated, our results propose that dermatopathies associated with an increase keratinocyte turnover such as inflammation of the palms may be one cause for the different DNA quality and quantity observed in epithelial abrasions or swabs from any handled material. A high proliferation rate of the skin results not only in more scaled cells but also in a higher number of cells containing only slightly degraded DNA. Since 2% to 4% and 2% to 3% of adults in Germany and other European countries [http://www.gbe-bund.de, 19] suffer from atopic dermatitis and psoriasis, respectively, a considerable number of culprits in crime scene investigations could suffer from such dermatopathies, rendering a very good DNA profile when touching objects. At court, this may be mentioned as one possible reason for major and apparently strange differences between amplification results of epithelial abrasions.

References

1. Poetsch M, Kamphausen T, Bajanowski T, Schwark T, von Wurmb-Schwark N (2011) Powerplex ES versus Powerplex S5—casework testing of the new screening kit. Forensic Sci Int Genet 5(1):57–63
2. Poetsch M, Bayer K, Ergin Z, Milbrath M, Schwark T, von Wurmb-Schwark N (2011) First experiences using the new Powerplex® ESX17 and ESI17 kits in casework analysis and allele frequencies from two different regions in Germany. Int J Legal Med (in press) PMID: 20567841
3. Wiegand P, Kleiber M (2001) Less is more—length reduction of STR amplicons using redesigned primers. Int J Leg Med 114:285–287
4. Grubwieser P, Mühlmann R, Berger B, Niederstätter H, Pavlic M, Parson W (2006) A new "miniSTR-multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA. Int J Leg Med 120:115–120
5. Wiegand P, Klein R, Braunschweiger G, Hohoff C, Brinkmann B (2006) Short amplicon STR multiplex for stain typing. Int J Leg Med 120:160–164
6. Balogh MK, Burger J, Bender K, Schneider PM, Alt KW (2003) Fingerprints from fingerprints. Progr Forensic Gent 9:953–957
7. Wickenheiser RA (2002) Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci 47:442–450
8. Lowe A, Murray C, Whitaker J, Tully G, Gill P (2002) The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Sci Int 129:25–34
9. Djuric M, Varljen T, Stanojevic A, Stojkovic O (2008) DNA typing from handled items. Forensic Sci Int Genet 1:411–412
10. Phipps M, Petricevic S (2007) The tendency of individuals to transfer DNA to handled items. Forensic Sci Int 168:162–168
11. Goray M, Eken E, Mitchell RJ, van Oorschot RAH (2010) Secondary DNA transfer of biological substances under varying test conditions. Forensic Sci Genet 4:62–67
12. Raymond JJ, van Oorschot RAH, Gunn PR, Walsh SJ, Roux C (2009) Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes. Forensic Sci Int Genet 4:26–33
13. Van Oorschot RAH, Ballantyne KN, Mitchell RJ (2010) Forensic trace DNA: a review. Investig Genet 1:14
14. DeSalle R, Bonwich E (1996) DNA isolation, manipulation and characterization from old tissues. Genet Eng 18:13–32
15. Walsh PS, Erlich HA, Higuchi R (1992) Preferential PCR amplification of alleles: mechanisms and solutions. PCR Methods Appl 1:241–250
16. Kimpton CP, Oldroyd NJ, Watson SK, Frazier RR, Johnson PE, Millican ES, Urquhart A, Sparkes BL, Gill P (1996) Validation of highly discriminating multiplex short tandem repeat amplification systems for individual identification. Electrophoresis 17:1283–1293
17. Frégeau CJ, Fourney RM (1993) DNA typing with fluorescently tagged short tandem repeats: a sensitive and accurate approach to human identification. Biotechniques 15:100–119
18. Cowen S, Debenham P, Dixon A, Kutranov S, Thomson J, Way K (2010) An investigation of the robustness of the consensus method of interpreting low-template DNA profiles. Forensic Sci Int Genet. doi:10.1016/j.fsigen.2010.08.010
19. Thyssen JP, Johansen JD, Linneberg A, Menne T (2010) The epidemiology of hand eczema in the general population—prevalence and main findings. Contact Dermat 62:75–87