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Case report

Experience with the PCR-based HLA-DQα DNA typing system in routine forensic casework

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Summary. The results of HLA-DQα typing from 42 routine forensic cases using the polymerase chain reaction (PCR) were analyzed regarding the reliability, discrimination efficiency and informative value of this system in a given case. The cases included stain typing from a variety of different substrates, i.e. blood and semen stains, mixed body fluids, single hairs, cigarette butts, material from fingernail scratches, as well as identification and paternity cases on postmortem and fixed tissue. A total of 125 individual stain and tissue samples were included. PCR amplification was achieved in 70% of these samples. In cases with mixed body fluids, e.g. sperm and vaginal cells from rape cases, DQα typing was always carried out successfully. However, only approx. 42% of all samples that could be typed were relevant regarding the inclusion or exclusion of a suspect. This was mostly due to the limited number of alleles that can be typed at the HLA-DQα locus or to the fact that the stain or hair samples did not originate from the perpetrator, but from the victim or from other persons not related to the crime.

Key words: Polymerase chain reaction (PCR) – HLA-DQα typing – Forensic stain analysis – Deoxyribonucleic acid (DNA)

Zusammenfassung. Die Ergebnisse der HLA-DQα-Typisierung unter Anwendung der Polymerase-Kettenreaktion (engl. Abk. PCR) wurden anhand von 42 Routine-Spurenfällen in Hinsicht auf die Zuverlässigkeit, die Unterscheidungskraft und den Beweiswert ausgewertet. Es wurden dabei eine Reihe verschiedener Körperflüssigkeiten und -gewebe untersucht, so z.B. Blut- und Spermaflecken, Mischsekrete, Einzelhaare, Zigarettenkippen sowie Fingernagelschmutz von Kratzspuren. Außerdem wurden postmortal asservierte sowie fixierte bzw. Paraffin-eingebettete Gewebe im Zusammenhang mit Abstammungs- und Identitätsgutachten typisiert. Dabei konnten insgesamt 125 Einzelspuren untersucht werden. Die PCR-Typisierung war in 70% der Proben erfolgreich. Mischsekrete, z.B. aus Sperma- und vaginalen Epithelzellen von Sexualdelikten, konnten in allen Fällen typisiert werden. Allerdings war das Ergebnis nur in ca. 42% der typisierten Spuren informativ in Hinsicht auf Einschluß oder Ausschluß von Tatverdächtigen. In den meisten Fällen lag dies daran, daß entweder kein Merkmalsunterschied zwischen Spur und Opfer vorlag oder daß die Blut- oder Haarspuren nicht vom Täter, sondern vom Opfer bzw. von unbeteiligten Zeugen stammten und somit für die Tat nicht relevant waren.

Schlüsselwörter: Polymerase Kettenreaktion (PCR) – HLA-DQα Typisierung – forensische Spurenkunde – Desoxyribonukleinsäure (DNA)

Introduction

The PCR-based HLA-DQα DNA typing system (Saiki et al. 1986, 1989) has been evaluated in a number of studies regarding its population genetics as well as its reliability in forensic stain typing (Helmuth et al. 1990; Westwood and Werrett 1990; Comey and Budowle 1991; Reynolds et al. 1991; Schneider et al. 1991a, 1991b). In these studies the general robustness of this system for forensic stain analysis could be demonstrated and subsequently HLA-DQα was the first PCR typing system to be introduced in routine casework.

The AmpliType HLA-DQα system (Cetus, Emeryville, CA, USA) uses a reversed dot blot method, in which the amplified DNA is labelled during PCR by incorporation of biotinylated primers. The allele-specific oligonucleotide (ASO) probes for the 6 DQA specificities are immobilized side-by-side on a probe strip. The amplified DNA is hybridized under stringent conditions to the ASO probe strip and the results are visualized in a color reaction catalyzed by a streptavidin-alkaline phos-

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phatase complex bound to the biotinylated PCR products. The limitations of this system lie in the relatively high frequencies of the 21 DQA genotypes (between 0.5 and 12.5% in the German population; Schneider et al. 1991b). Thus the system can be used to screen large numbers of samples for the purpose of including or excluding suspects, but it is usually not sufficient to provide strong evidence for positive identification of a stain donor.

In this survey, we summarize our experience with the application of the HLA-DQα system in routine forensic casework over the last 2 years. The cases include stain typing of a variety of different substrates, i.e. blood and semen stains, mixed body fluids, hairs, cigarette butts, material from fingernail scratches, as well as identification and paternity cases.

Material and methods

The PCR typing results described below are compiled from 42 separate cases. The stain material was usually referred to our institute by the police or by courts in the process of routine investigations or courtroom hearings in the context of capital crime cases. The age of the stain material was up to 11 years old. Twelve of these cases have already been discussed in more detail in a previous publication (Rittner et al. 1991), but are included in the compiled data of this survey for completeness.

The extraction of DNA from the various substrates and tissues was carried out according to previously published methods (Gill et al. 1985; Giusti et al. 1986; Higuchi et al. 1988; Ogata et al. 1990; Comey and Budowle 1991; Schneider et al. 1991b). In all cases presented, the amount of DNA extracted was not sufficient for conventional DNA typing using VNTR single locus probes as indicated by fluorometric measurement of the DNA concentration. All DNA samples extracted from stain material were subjected to microdialysis on filter discs (VSWP 002500, Millipore GmbH, Eschborn, FRG) against 0.1 × TE (1 m*M* Tris, 0.1 m*M* EDTA, pH 7.5) for 3 h. AmpliType HLA-DQα typing was carried out according to the manufacturer's instructions using a Perkin-Elmer thermocycler (TC-1, Perkin-Elmer GmbH, Überlingen, FRG). Routinely, 32 cycles of amplification were carried out for all samples, except hair, where 40 cycles were used. If no amplification was achieved after 32 cycles, another aliquot of the stain DNA was dialzyed again and amplified for 40 cycles.

Results and discussion

The 42 cases can be divided into 3 categories (numbers given in brackets): i) forensic stain analyses from rape and/or murder cases ($n = 30$), ii) identification cases involving fire victims, body parts, or blood samples for alcohol testing ($n = 5$), and iii) parentage testing in cases with deceased putative fathers on very old blood samples, paraffin embedded tissues or post-mortem tissues, sometimes obtained after exhumation of the corpse ($n = 7$). Most of the cases involving stain analyses usually required the typing of several forensic stains, sometimes from different origins or from different stain carriers. The various stains and tissues subjected to PCR analysis are listed in Table 1 according to the nature of the biological sample. The success rate of PCR amplification and the informative value regarding inclusion or exclusion of a suspect (or putative father in paternity cases) are also given. In a significant number of cases, a clear result was obtained confirming the victim's or the suspect's genotype, but which was not useful for the investigation. In some cases, the stain donor's genotype was revealed but no suspect was available for comparison.

Finally, a number of typing results were inconclusive due to the fact that the sample was contaminated or had a mixed genotype pattern which was uninterpretable. The typing results from fresh reference blood samples obtained from victims or suspects are not included in this table, but are taken into account for the evaluation of the stain typing results in the context of each case.

PCR analysis of blood and semen stains was successful in 60% of cases. Contamination of bloodstains was observed in only 2 samples. In 2 cases involving analysis of numerous bloodstains, the observed genotypes were identical either with those of the 2 victims, where evidence from the perpetrator was expected, or with the suspect's genotype in a case where the police looked for the victim's blood.

Nevertheless, these cases demonstrate the reliability of the system, since the genotypes of the 3 individuals were obtained consistently from up to 8 different blood-

Table 1. Success rate and informative value of different types of evidence material

Evidence sample	Total number	Amplification		Inclusion	Exclusion	Genotype confirmed[a]	No suspect	Inconclusive[b]
		Positive	Negative					
Blood stains	40	24	16	4		18		2
Semen stains	1	1		1				
Single hairs	42	29	13	6	5	5	8	5
Finger nail material	19	14	5	4		6		6
Post mortem blood/tissue	6	6		5	1			
Fixed (embedded) tissue	4	2	2	1			1	
Cigarette butts	3	2	1	2				
Mixed body fluids	10	10		6	2		2	
Total numbers	125	88	37	29	8	29	11	13

[a] Genotype confirmed: The victim's or suspect's genotype was confirmed, but not relevant for the investigation
[b] Inconclusive: contaminated sample or uninterpretable genotype

Table 2. Analysis of mixed body fluids using differential lysis

Cases (stain material)	Differential lysis	Total extraction	Complete separation	Genotype(s) observed	
				in female fraction[a]	in male fraction[a]
1. Vaginal swab on slide		+		Mixed, female > male	
2. Anal swab	+		+	f	m
3. Vaginal swab	+			f	f/m
4. Vaginal fluid stain	+			f/m	m
5. Vaginal fluid stain	+			f/m	m
6. Vaginal swab	+		+	f	m
7. Vaginal fluid stain	+			f/m	m
8. Mouth swab		+		Mixed	
9. Vaginal swab	+			–	f/m
10. Vaginal swab	+		+	f	m
Total	8	3	2		

[a] f = female, m = male, f/m = mixed genotypes, – = no result

stains. In some instances with unsuccessful amplification, the amount of stain material was insufficient to determine its nature prior to PCR typing. It was therefore decided to use the specificity of HLA-DQα typing as a tool to test whether the material was of human origin.

Single hair typing was mostly done on hairs without sheath material. Usually, the root portion of the hair (1–2 cm) was cut off and completely extracted for DNA. About two-thirds of the hairs could be typed successfully. As described previously (Schneider et al. 1991a), the typing results from single hairs were often not useful in a given case, since either the hair's genotype was identical to that of the victim or, in cases where the hairs were collected from the scene of crime (e.g. a stolen car or a piece of equipment or clothing), various genotypes were found originating either from the owner of the item, or from other unknown individuals probably not connected to the crime. If PCR amplification was carried out successfully on hair DNA, genotypes could be interpreted without problem and contamination was only observed in a few cases. The oldest hair that could still reliably by typed was pubic hair from a murder victim who had been killed 11 years earlier.

Skin tissue samples scratched from the perpetrator during the crime and collected from the fingernails of the victim (or vice versa) have been studied in 5 cases. Usually, the fingernail ends of the victim were cut off during autopsy and were inspected visually for adhering tissue using a stereo microscope. In 2 cases the suspect's genotype matched the scratched tissue from the fingernail. The other samples which could be amplified only carried the victim's genotype or had mixed genotypes, which could not be further interpreted, since no suspects were available for comparison.

Postmortem blood and tissue samples, as well as fixed or paraffin-embedded tissues, were mostly typed for the purpose of identification and in cases of controversial paternity. Postmortem samples could all be typed successfully, even after exhumation of the body. In contrast, formalin-fixed or paraffin-embedded tissue samples could not always be typed. In one case, DQα typing of formalin-fixed brain tissue from a murder victim revealed the genotype DQA 1.2, whereas from the victim's mouth swab stored frozen, as well as from the results of vaginal swab sample typing after differential lysis it was observed that the victim's genotype was DQA 1.2,4. Thus allelic dropout had occurred during PCR of DNA from the fixed tissue sample. It was found in other studies that the fixative as well as the fixation period both have a strong impact on the success rate of PCR amplification (Pötsch et al. 1992). DNA can always be extracted in sufficient amounts, but it might not be suitable for subsequent PCR amplification, possibly due to cross-linking of the DNA strands induced by the fixative formaldehyde.

Both successfully typed cigarette butts were from filter cigarettes. The paper cover and the inner filter material were extracted separately, but only the paper cover yielded sufficient DNA for DQα typing. The butts had been typed previously in a different laboratory for ABO blood groups by absorption elution, and the genotype obtained from both systems matched the suspect's genotype.

Mixed body fluids containing male sperm and female (vaginal, anal or oral) epithelial cells were obtained from swabs, cell smears on microscope slides or body fluid stains on the victim's clothings. PCR typing was preferred to VNTR analysis either because only a small number of sperm was found, or the material was aged (up to 2 years storage at room temperature) and the extracted DNA degraded, or most of the stain material was consumed for previous investigations by other laboratories. Ten cases involving the analysis of mixed body fluids are listed in Table 2 together with the method applied for DNA extraction and the results. Differential lysis was carried out in 8 cases. In 3 cases, complete separation of male and female cells was achieved. The other 5 cases resulted in mixed genotypes observed either in the "male" or in the "female" fraction. In all cases, a mixture was found in only one of these fractions, so that an interpretation of the results was possible. Also, the results from stain typing were always consis-

Table 3. HLA-DQα typing in two rape and murder cases

Samples	HLA-DQA
Case A:	
Victim A (spleen)	*1.3,4*
Anal swab victim A:	
1. "Female" fraction	1.3,4
2. "Male" fraction	1.1,3 + 1.3,4
Swab container victim A:	
1. "Female" fraction	1.3,4
2. "Male" fraction	*1.1,3* + 1.3,4
Case B:	
Victim B (hair)	*1.2,2*
Semen stain on pubic hair victim B	*1.1,1.2*
Suspect 1 (deceased): blood	1.2,4

tent with those from the victims' blood providing further evidence for the reliability of the HLA-DQα system. In 2 cases, the sperm count after elution and microscopical analysis was so low that a total extraction without differential lysis was done. Comey and Budowle (1991) studied the efficiency of the DQα system to detect mixed samples and found that the minority genotype was faintly visible, although less intense than the control dot, up to a ratio of 1:100.

The usefulness of the HLA-DQα system is illustrated by 2 rape and murder cases presented in Table 3. The crimes were committed in the same city and the investigating authorities speculated that they might have been carried out by the same individual. A deceased suspect was identified. The typing results revealed that the semen stains from the 2 victims had completely different genotypes. Also, these genotypes did not match the deceased suspect's genotype. Therefore it had to be concluded that the crimes were probably committed by different individuals. Since the first suspect was excluded, 21 additional suspects were subsequently typed. Among these, only 2 individuals matched the perpetrator's genotype in case A (DQA 1.1, 3), which has a frequency of 3.8% in the German population, whereas none of the 21 suspects matched the semen stain genotype in case B (DQA 1.1, 1.2; 5.2% genotype frequency).

The results from bloodstain and hair typing clearly show that the majority of stains collected from the scene of crime very often turn out to be irrelevant for the case. The chance to obtain clues leading to the identification of a stain donor connected to the crime increases with the immediate proximity of a given stain to the victim.

The cases presented in Table 3 illustrate that the HLA-DQα system can be helpful for rapidly screening large groups of suspects before more laborious and time-consuming typing methods, e.g. VNTR probe typing, are applied. It is also obvious, however, that a non-exclusion based only on DQα typing alone is not sufficient for proving that a person is the stain donor. Further PCR typing systems have to be introduced into routine casework to achieve more powerful exclusion probabilities. The amplification of small VNTR loci (amplified fragment length polymorphisms – AMP-FLP's) or of short tandem repeats (STR's; tri- and tetranucleotide repeats) combine the sensitivity of the PCR technology with the high genetic variability of tandemly repeated sequences (Weber and May 1989; Budowle et al. 1992; Edwards et al. 1992; Rand et al. 1992). Regarding AMP-FLP systems with PCR-generated fragment sizes of up to 1 kb, however, it has to be taken into consideration that they are more sensitive to degraded DNA than the HLA-DQα system with a PCR fragment size of 250 bp. Thus the HLA-DQα system is also suitable to test the quality limits of the stain DNA. STR system should also be equally robust in this respect, as they are represented by small amplification products of similar size. In paternity and identification cases as well as in selected stain cases on samples without mixed genotypes according to the HLA-DQα typing results, we have recently started to use the PCR-based 3'-ApoB (Boerwinkle et al. 1989) and D1S80 (MCT 118) systems (Kasai et al. 1990). However, further validation work on AMP-FLP and STR systems is required before they can be fully applied in routine casework.

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