CASE REPORT

Manfred N. Hochmeister,[1] M.D.; Bruce Budowle,[2] Ph.D.; Arthur Eisenberg,[3] Ph.D.; Urs V. Borer,[1] and Richard Dirnhofer,[4] M.D.

Using Multiplex PCR Amplification and Typing Kits for the Analysis of DNA Evidence in a Serial Killer Case

REFERENCE: Hochmeister, M. N., Budowle, B., Eisenberg, A., Borer, U. V., and Dirnhofer, R., "**Using Multiplex PCR Amplification and Typing Kits for the Analysis of DNA Evidence in a Serial Killer Case,**" *Journal of Forensic Sciences,* JFSCA. Vol. 41, No. 1, January 1996, pp. 155–162.

ABSTRACT: Analysis of DNA evidence in a serial killer case was performed using the AmpliType® HLA-DQ alpha-, AmpliType® PM-, and the GenePrint™ STR Multiplex System PCR Amplification Kits. In addition, a sex typing procedure using the X-Y homologous gene amelogenin was carried out. DNA profiles from a single hair with attached sheath material, recovered from underneath the seat cover of the suspect's car seat were compared with DNA profiles derived from reference head hairs from a homicide victim. From the evidentiary sample only 9 ng of human DNA could be recovered. In a sample, where the quantity of DNA becomes a critical issue a powerful route is the simultaneous amplification of several loci (multiplex PCR). This is the first report where commercially available multiplex PCR amplification and typing kits have been introduced for the analysis of DNA evidence in a serial killer case and the analysis has been admitted in court.

KEYWORDS: forensic science; criminalistics; human identification, deoxyribonucleic acid (DNA), polymerase chain reaction (PCR), genetic typing, HLA DQA1, LDLR, GYPA, HBGG, D7S8, GC, short tandem repeat (STR) loci, TH01, TPOX, CSF1PO, amelogenin, sexing, reverse paternity, hair

Case History

On September 15, 1990 near the city of Prague (Czechoslovakia) the body of a woman was discovered in a trench, partially submerged in water. The body was left in sexual disarray, naked except for a pair of socks, the legs spread apart, and the genitals exposed. A few thick branches were placed over the body (Fig. 1).

[1]Research Scientists, DNA Identity Laboratory, Institute of Legal Medicine, University of Berne, Switzerland

[2]Research Chemist, Forensic Science Research and Training Center, Laboratory Division, Federal Bureau of Investigation Academy, Quantico, VA.

[3]Director, DNA Identity Laboratory, Department of Pathology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX.

[4]Director, Institute of Legal Medicine, University of Berne, Switzerland.

Names of commercial manufacturers are provided for identification only, and conclusion does not imply endorsement by the authors.

Received for publication 2 March 1995; revised manuscript received 15 May 1995; accepted for publication 16 May 1995.

The identity of the victim was established as the 29-year-old B.B. She was last seen at midnight on September 14, 1990 at the Wenzel-place in Prague and her body was discovered the next morning. The cause of death was determined to be the result of ligature strangulation. The ligature itself was missing, as well as all clothing except for the socks and all personal belongings except for a finger ring. No relevant biological evidence could be recovered from the victim or the crime scene. Until spring 1992 the case remained open.

From January 1991 to April 1992 the bodies of seven prostitutes were discovered in wooded areas in different parts of Austria (one victim near Bregenz, two victims near Graz and four victims near Vienna). The victims were reported missing between 16 days and 353 days prior to the discovery of their bodies. Thus, in four cases the remains were either badly decomposed or skeletonized. All cases shared certain characteristics in regard to the cause and manner of death, the pattern of disposal and positioning of the bodies, the state of dress, and the clothing and personal belongings left behind at the disposal site and on the bodies. In those cases, where the cause of death could be determined, it was found to be ligature strangulation using a piece of the victims clothing (panty hose or body shirt). The bodies were disposed of either naked in sexual disarray or the clothing was partially removed and the genitals, breasts, or buttocks were exposed. In all cases the jewelery was left on the body while personal belongings or clothing were

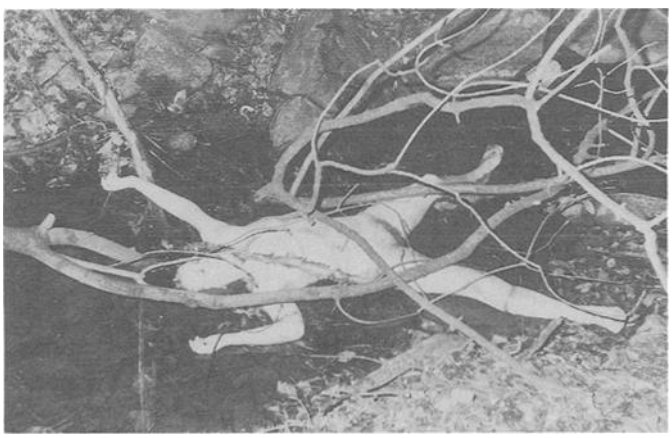

FIG. 1—*Body of the Prague homicide victim.*

missing in part or completely. Fiber evidence was found on one victim and submitted to a crime laboratory for microscopical analysis. However, no relevant biological evidence suitable for DNA analysis could be recovered from any case.

On June 20 and 30, and on July 11, 1991 the bodies of three prostitutes were discovered in Los Angeles. Two victims were found in down town L.A. and one near Malibu, all strangled with their own brassieres. The L.A. Sherrif's Department and the L.A. Police Department established a linkage among the three murders, based predominately on the special characteristics of cuttings applied to all brassieres and the knots found in the ligatures. From one victim (S.E.) vaginal and anal swabs containing sperm cells were obtained. Otherwise, no relevant biological evidence suitable for DNA analysis could be recovered. Those cases were unknown to the authorities in Austria and Czech until September 1991.

Suspect

In May 1991 a potential suspect for the murders in Austria was provided to the Austrian authorities by a retired homicide detective. He recalled the murder of an 18-year-old girl (M.S.) strangled with her own brassiere in Germany in 1974. The suspect pointed out was the 44-year-old J.U. Between 1966 and 1975 he was convicted for 15 different crimes, including attempted rape, and for the murder of M.S. he had been sentenced to life imprisonment in 1976. During his imprisonment, J.U. began a career as a book writer and was able to establish himself as an example of successful rehabilitation. Eventually, he was granted parole and was released from jail on May 23, 1990. In the following two years J.U. was cruising by car through Austria, Germany, Italy and eastern Europe, directing performances of his theater plays, and working as a reporter for newspapers writing articles mainly dealing with prostitution.

Because of the unusually high rate of prostitute murders in Austria between January 1991 and April 1992 and similarities among some of the murders there was strong evidence for the activity of a serial killer. In connection with Interpol open cases in other European countries were investigated. The Czech authorities provided an unsolved case from Prague (B.B., Fig. 1). Checking J.U.'s alibi the police established, that he was in Prague on the same day when B.B. was murdered. Also, for the Austrian cases an association between his traveling activities and the disappearance of the victims could be established. Furthermore, J.U. had flown to Los Angeles on June 10, 1991 and returned to Vienna on July 16, 1991. During his visit in L.A. he managed to get a tour with police officers to prostitute areas under the premise that he worked as a reporter for newspapers. It was during his visit in L.A. that three prostitutes, which had been working the streets near the hotels where he was lodging, were murdered. After his return to Vienna he was under observation during the ensuing few months. He flew to Miami, FL on February 15, 1992, where he was arrested two weeks later and extradited to Austria for his trial.

Circumstantial Evidence

Circumstantial evidence against J.U. was based mainly on the facts that the police was able to establish an association between J.U.'s traveling activities and the murder of the 11 victims. Certain similarities among all cases could be pointed out and J.U. could not provide alibis for any of the cases. Furthermore, there were some strong similarities to the murder of the German girl M.S. in 1974, for which he had been convicted.

Fiber Evidence

Fiber evidence recovered from one of the Austrian victims was analyzed at the crime laboratory of the Scientific Police Institute in Zürich, Switzerland. Two different types of fibers found on that body were reported as being indistinguishable from fibers from J.U. clothing.

Biological Evidence

Vaginal and anal swabs were obtained from one of the L.A. prostitutes (S.E.). The sperm cell fraction from a vaginal swab was typed by restriction fragment length polymorphism (RFLP) analysis and at the HLA-DQA1 locus, and the sperm cell fraction from a rectal swab was typed at the HLA-DQA1 locus by two different U.S. laboratories in 1991. The sperm cell DNA profile in the vaginal vault did not match J.U.'s DNA profile. The DQA1 type from the sperm cells in the rectum was different from that in the vagina vault, but consistent with J.U.'s DQA1 type (3,4). However, since at least two sperm cell donors were present, the possibility could not be ruled out that the semen sample from the rectum might be composed of multiple donors. Depending on the intensity of the dots a HLA-DQA1 typing result 3,4 could be composed of different combinations of the types 3,3; 3,4; and 4,4. If their respective frequencies were added, the frequency of occurrence of the profile was approximately 20% of the population.

Strong evidence that could link the suspect to at least one of the victims was still missing. In June 1992 investigators recovered several head hairs with attached sheath material from underneath the seat cover from the front passenger seat of a BMW. J.U. drove this car when the Prague murder in September 1990 took place. He subsequently sold it and it was finally scrapped. However, the car seats had been removed and had been sitting in a garage for almost 18 months. The recovered hairs were submitted for microscopic analysis to the crime laboratory of the Scientific Police Institute in Zürich, Switzerland in July 1992. Three head hairs found underneath the seat cover of the car seat were microscopically indistinguishable from reference head hairs of the Prague vicitm B.B.

In April 1993 the Institute of Legal Medicine in Berne, Switzerland was requested to determine by DNA analysis whether or not the three head hairs recovered from J.U.'s car seat could belong to the Prague victim B.B. This report describes the analysis and typing strategy of an evidentiary sample where the quantity of DNA became a critical issue.

Material and Methods

Reference Head Hair Samples

Plucked head hairs from different regions of the scalp were obtained during the autopsy of the Prague victim B.B. in September 1990 and stored in paper envelopes at ambient temperature.

Evidentiary Samples

Three head hairs with attached sheath material were submitted for DNA analysis. Microscopic comparison was carried out without mounting them in an embedding medium. The evidentiary hairs were stored in plastic envelopes at ambient temperature.

Reference Blood Samples from J.U.

Reference blood samples from the suspect J.U. were collected in EDTA-tubes. In order to compare the DNA typing results the same genetic markers were analyzed as in the evidentiary sample.

DNA Extraction and Quantification

DNA was isolated from one centimeter of the root portion from each of the hairs. The extraction of the reference head hairs and the evidentiary samples was carried out independently at different times. Each hair was incubated overnight at 56°C in 400 μL stain extraction buffer (10 mM Tris, 10 mM EDTA, 100 mM NaCl, 39 mM DTT, 2% SDS) and 10 μL Proteinase K (20 mg/mL). On the following day an additional 10 μL Proteinase K (20 mg/mL) were added, and the samples were incubated for two more hours at 56°C. The solution was extracted with 500 μL phenol-chloroform-isoamylalcohol (25:24:1) and subsequently extracted in 1 mL water saturated n-butanol to remove traces of phenol. The aqueous phase was then transferred to a Centricon™ 100 microconcentrator tube (1) containing one mL sterile water. The volume was brought up to a total volume of two mL with sterile water, the sample reservoir was sealed with parafilm, and the tubes were subjected to centrifugation at 1000 g for 30 min. Then two mL of sterile water were added to the sample reservoir, and the reservoir was sealed with new parafilm. Again the tubes were centrifuged at 1000 g for 30 min. The DNA was recovered by back centrifugation at 1000 g for five min. The final sample volume was approximately 25–40 μL. Ten percent of each retentate was used to determine the quantity of human DNA as described previously (2). Samples containing a head hair from a known donor and a sample containing no hair served as reagent positive and negative control samples, respectively.

Amplification and Typing of the Extracted DNA Using the AmpliType™ HLA-DQ Alpha Amplification and Typing Kit

Nine nanograms of human DNA could be extracted from one of the three evidentiary hair samples from the suspect's car. From the other two samples no DNA could be extracted. In June 1993 two ng of DNA from this evidentiary sample and two ng of DNA from a reference head hair sample were amplified and typed for HLA-DQA1 according to the protocol contained in the AmpliType™ HLA-DQ alpha Amplification and Typing Kit (3), except that each PCR contained 16 μg BSA (Sigma Cat. No. A 3350)/ 100 μL PCR mix.

Purification and Recovery of Genomic DNA from the HLA-DQA1 Amplification Product and Subsequent Amplification and Typing with the AmpliType® PM PCR Amplification and Typing Kit

From the evidentiary sample and a reference head hair sample the HLA-DQA1 results were obtained (type 1.1,4). In January, 1994 in order to conserve as much original evidentiary sample DNA as possible for further analysis, the genomic DNA from the HLA-DQA1 amplification product was purified, recovered and subsequently amplified as described previously by Lorrente et al. (4) and Hochmeister et al. (5). Briefly, 35 μL of the HLA-DQA1 products were used for typing. The remaining portion of each sample has been stored at −20°C. Subsequently each solution was transferred to a Centricon™ 100 microconcentrator tube containing two mL of sterile water and purified by removing the primers, dNTPs and salts. The purified retentate containing the genomic DNA as well as the DQA1 amplification product was recovered by back centrifugation. The final sample volume was approximately 20–30 μL. Twenty μL of each retentate were amplified in a 100 μL PCR in a Perkin Elmer Thermal Cyler 480 using the AmpliType® PM PCR Amplification and Typing Kit (Roche Molecular Systems, Inc., Branchburg, NJ) (6) and typed for five loci according to the manufacturers protocols, except that each PCR contained 16 μg BSA/100 μL PCR mix. The PM Kit includes reagents that direct the simultaneous amplification of specific regions of six genetic loci: HLA DQA1 (previously referred to as HLA DQ alpha; PCR product size 242/239 bp) (7). Low Density Lipoprotein Receptor (LDLR; PCR product size 214 bp) (8), Glycophorin A (GYPA; PCR product size 190 bp) (9), Hemoglobin G Gammaglobin (HBGG; PCR product size 172 bp) (10) D7S8 (PCR product size 151 bp) (11), and Group Specific Component (GC; PCR product size 138 bp) (12).

Purification and recovery and amplification and typing procedures of the evidentiary sample were carried out independently from the reference head hair samples. Positive and negative controls were processed at all times.

Amplification and Typing of the Extracted DNA Using the GenePrint™ STR Multiplex System

In April 1994, two ng of DNA from each sample were amplified and typed simultaneously at three polymorphic STR loci (TH01, TPOX and CSF1P0) using the GenePrint STR Multiplex System (Promega Corporation, Madison, WI, USA) according to the specifications of the GenePrint™ STR Systems Technical Manual (13) and Huang et al. (14), except that each PCR contained 16 μg BSA/100 μL PCR mix. This multiplex PCR amplification kit includes reagents that direct the simultaneous amplification of specific regions of three tetrameric STR loci: HUMTH01 (PCR product size 179–203 base pairs) (15,16,17), TPOX (PCR product size 232–248 base pairs) (18), and CSF1PO (PCR product size 295–327 base pairs) (19).

Briefly, the PCR was carried out in 50 μL reaction volumes in a Perkin Elmer 480 thermal cycler. Five μL of PCR product were mixed with 2.5 μL 3 X STR loading dye and 2.5 μL of this mix were loaded onto a denaturing polyacrylamide gel (4%T, 5%C, 31 cm long and 0.4 mm thick) containing 7 M urea and 0.5 × Tris-Borate-EDTA buffer. Electrophoresis was carried out on an SA 32 Electrophoresis Apparatus (GIBCO BRL, Gaithersburg, MD, USA). The conditions for electrophoresis were set at a constant power of 40 watts and electrophoresis was carried out at ambient temperature. Electrophoresis was stopped when the xylene cyanol dye migrated 6 cm from the anode (approximately 1 h 15 min).

Allele designations were determined by comparison of the sample fragments with those of the allelic ladders supplied in the kit. Allele designations were made according to recommendations of the DNA commission of the International Society of Forensic Haemogenetics (20).

Sex-Determination

Finally in June 1994, one ng of DNA from each sample was amplified at the X-Y homologous amelogenin gene, as described previously (21). The amplification products were visualized after ethidium bromide staining on a 3% NuSieve/1% SeaKem agarose gel. Male and female control DNA was processed in the same manner.

Determination of the Frequency of the Derived Profile

Allele and genotype frequencies for the nine PCR-based genetic markers were determined previously in a Swiss Caucasian population sample (22,23). The frequency of the multiple locus DNA

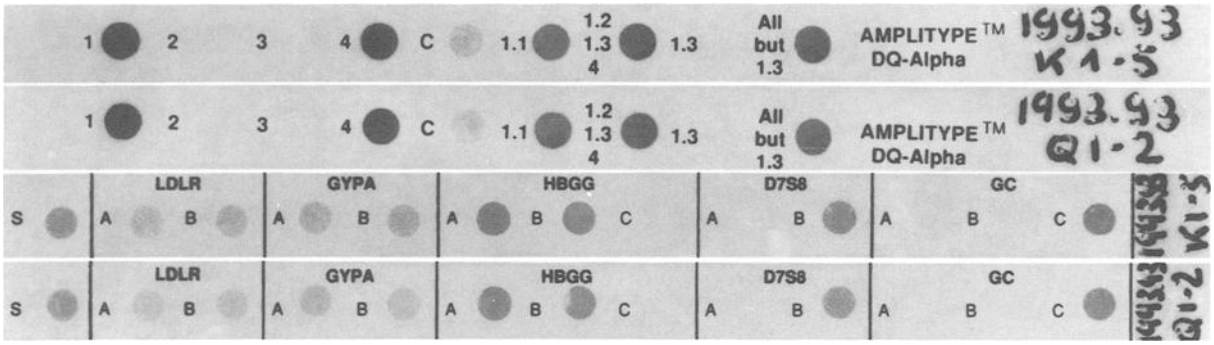

FIG. 2—*HLA DQA1 and polymarker probe strips with the typing results from a reference head hair from the victim (1993.93 K1-5) and from the recovered hair from the car seat (1993.93 Q1-2). The genomic DNA was recovered from the DQA1 amplification product and subsequently amplified at the PM loci. Due to this approach the "S" dot on the PM strips is more intense and the intensity of the PM alleles is either lighter than or equivalent to the "S" dot. Both samples show the same typing results.*

profile was estimated using the observed allele frequencies and the product rule under the assumption of independence (23).

Reverse Paternity Analysis

In order to confirm the identity of the individual contributing the hair sample recovered from the suspect's car by different means, a reverse paternity analysis was performed. A reference blood sample was collected by venipuncture in EDTA-tubes from the mother (E.H.) and the father (W.S.) of the Prague homicide victim B.B. DNA was extracted (24), quantified by slot blot analysis (2), and amplified and typed at nine genetic loci (HLA-DQA1, LDLR, GYPA, HBGG, D7S8, GC, HUMTH01, TPOX, CSF1PO). The typing results were compared with the evidentiary sample recovered from the suspects car and those from B.B.'s reference hair samples. The likelihood ratio was calculated by the equation: Probability (given the DNA typing results—evidence is from their child)/Probability (given the DNA typing results—evidence is not from their child). In general, if an individual is heterozygous at a given locus, the frequency of passing on the obligate allele to the child is 0.5; and if she or he is homozygous it is 1. Therefore if both parents are heterozygous at a given locus the numerator equals 0.5×0.5; if one is homozygous it equals 0.5×1; and if both are homozygous it equals 1×1. The denominator is equal to the frequency of the genotype of the evidentiary sample.

Results

From one head hair from J.U.'s car 9 ng of human DNA were isolated. No DNA could be extracted from the other two samples. The DNA from the evidentiary sample (1993.93 Q1–2) and the DNA from a reference head hair (1993.93 K1–5) were amplified successfully and typed at nine genetic loci: HLA DQA1, LDLR, GYPA, HBGG, D7S8, GC (Fig. 2); and HUMTH01, TPOX, CSF1P0 (Fig. 3). Sex typing at the X-Y homologous gene amelogenin revealed one 106 basepair band consistent with a female type (data not shown). All control samples typed correctly. The DNA profiles obtained from B.B.'s reference head hairs matched the DNA profiles obtained from the evidentiary head hair from the suspect's car (Table 1). Based on Swiss Caucasian population data and the assumption of independence this multiple locus DNA profile occurs in approximately 1 in 2.1 million women.

The results of the reverse paternity analysis are shown in Fig. 4 and Table 2. The donor of the evidentiary sample could not be excluded as the child of B.B.'s mother (E.H.) and father (W.S.).

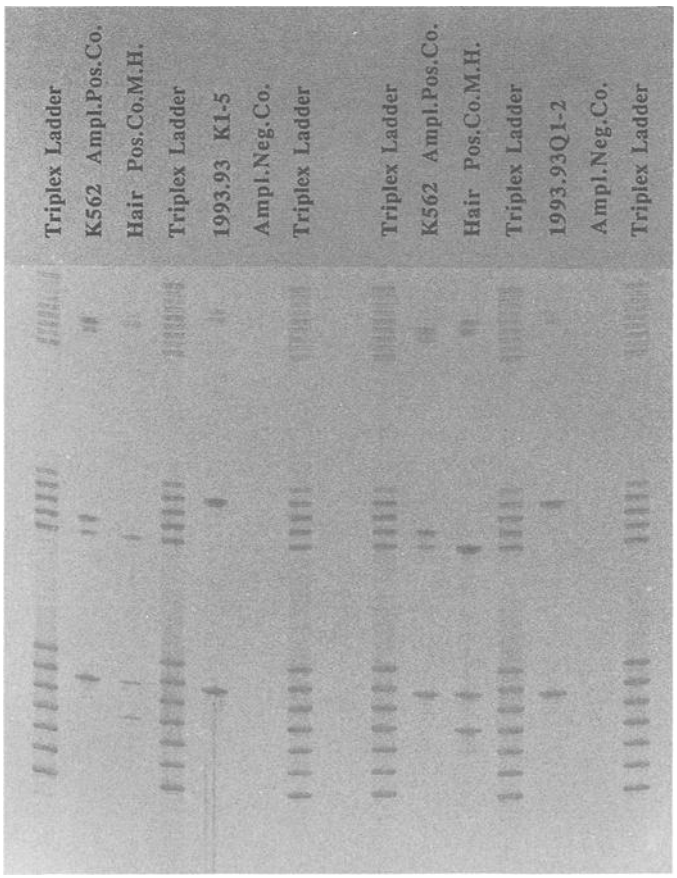

FIG. 3—*Silver-stained DNA profiles of the three simultaneously amplified STR loci TH01, TPOX, and CSF1P0. Thus, each lane displays genetic information for three loci. The DNA typing results from a reference head hair from the victim (1993.93 K1-5) are the same as those from the recovered head hair from the car seat (1993.93 Q1-2). The STR ladder alleles from bottom to top are: TH01: 5,6,7,8,9,10,11; TPOX: 8,9,10,11,12; CSF1P0: 7,8,9,10,11,12,13,14,15.*

Given the DNA typing results it is 102 times more likely that the evidentiary hair sample was from the offspring of E.H. and S.H. as compared to an unrelated random individual. If for the reverse paternity calculation a neutral prior probability of 0.5 is used than there is a 99.0% probability that the evidentiary hair sample was that of E.H.'s and W.S.'s child B.B.

TABLE 1—*Typing results and genotype frequencies from the evidentiary and reference hair samples.*

	DQA1	LDLR	GYPA	HBGG	D7S8	GC	TH01	TPOX	CSF1PO	SEX
Evidentiary Sample	1.1.4	AB	AB	AB	BB	CC	9.3.9.3	11.11	11.12	female
Reference Sample	1.1.4	AB	AB	AB	BB	CC	9.3.9.3	11.11	11.12	female
Genotype Frequency	0.075	0.45	0.57	0.47	0.16	0.29	0.078	0.068	0.206	0.5
Frequence of occurence	1 in 13					1 in 2300			1 in 2.1 million	female

TABLE 2—*Typing results and likelihood ratio from the reverse paternity analysis. Obligate paternal and maternal alleles are marked bold.*

	DQA1	LDLR	GYPA	HBGG	D7S8	GC	TH01	TPOX	CSF1PO
Mother of the Homicide Victim	2,**4**	**AB**	**AA**	**AB**	**AB**	**CC**	9,**9,3**	10,**11**	10,**12**
Hair recovered from Suspects Car	**1.4,4**	**AB**	**AB**	**AB**	**BB**	**CC**	**9.3,9.3**	**11,11**	**11,12**
Father of the Homicide Victim	**1.1**,4	**AA**	**AB**	**AA**	**AB**	**AC**	**8,9.3**	8,**11**	**11**,12
Likelihood ratio	3.13	1.02	1.00	1.00	1.41	1.68	3.01	3.70	1.21
Combined LR = 101.94									

In this special case the remaining portions of each HLA-DQA1 amplification were purified in Centricon 100 microconcentrator tubes and the genomic template DNA was recovered for subsequent multiplex PCR. The purpose was to obtain more information from the previous analyzed sample and to save the remaining 7 ng of original sample DNA for additional future tests. When using special precautions, the reliability of the HLA-DQA1-Centricon 100-PM procedure or the so called sequential multiplex amplification (SMA) could be demonstrated by Lorente et al. (4) and Hochmeister et al. (5). The genomic template DNA was successfully recovered from the HLA DQA1 amplification products and reutilized for the amplification and typing of the Polymarker loci. When subjected to agarose electrophoresis, all six amplification products were clearly visible with the DQA1 product in excess (data not shown). Since by Centricon-100 microfiltration the genomic DNA and the DQA1 product are recovered, the latter also serves as a template in the subsequent PM amplifications. Therefore, there will be more DQA1 product after the PM amplification than would be expected when only genomic DNA is used as a template and it should be expected that the "S" dot will be more prominent than the PM alleles in such samples (Fig. 2). However, the reliability of reading and interpreting the types from such PM probe strips has been demonstrated when certain practices are maintained (5).[5]

Discussion

DNA typing has been shown to be a powerful tool for identify purposes. The detection of variable number of tandem repeat (VNTR) sequences by restriction fragment length (RFLP) analysis can produce very high levels of discrimination, but requires a minimum of 25 to 50 ng of relatively undegraded DNA. Genetic information from samples with degraded DNA or from samples which do not contain as much DNA may be obtained by amplification of the DNA using the PCR. PCR-based tests, including the amplification of the HLA DQA1 locus (25), the PM loci (26,27,28) various VNTR loci (29,30), microsatellite typing (31,32), and mitochondrial DNA sequence analysis (33,34) have been successfully applied to the analysis of DNA extracted from various sources, including hairs (35,36).

However, by analyzing only one or a few PCR-based loci, for instance the HLA DQA1 locus, a main disadvantage is that the results provide limited information for forensic casework when samples cannot be excluded. Analyzing additional markers via individual amplification is relatively easy in PCR based testing and usually only 1–2 ng of DNA are required per test. However, in samples where the quantity of DNA becomes a critical issue (that is, in a range below 10 ng) each PCR-based test usually consumes 1–2 ng of sample DNA. In those samples a powerful alternative route is the simultaneous amplification of several loci using several sets of primers in one tube under the same set of PCR conditions (multiplexing). Currently, two multiplex PCR amplification and typing kits are commercially available. The AmpliType® PM PCR Amplification and Typing Kit uses the same reverse dot blot format as the HLA-DQA1 test (3). With the GenePrint™ STR Multiplex System typing of the individual loci is performed by separation on denaturing polyacrylamide gels and silver staining (13). These two multiplex PCR amplification and typing kits enable the amplification and typing of specific regions of nine genetic markers by consuming at most four nanogram of sample DNA. Therefore they are powerful tools when sample size and the power of discrimination of the applied tests become important issues. Additionally, these PCR-based tests are relatively simple and can be implemented into most application orientated laboratories.

Strategy Used for the PCR Analysis in the Case

A single hair was the only biological evidence which potentially could link the suspect to one of the victims. Since only 9 ng of human DNA could be recovered, sample size and power of discrimination of the applied tests were important issues. In June 1993, two ng of DNA from the evidentiary sample were used to type HLA-DQA1. The HLA-DQA1 types of the victim's reference sample and the evidentiary sample matched. The frequency of

[5]After the trial 2 ng of the original DNA from the remaining portion of the evidentiary sample and 2 ng of DNA from a reference head hair sample were amplified and typed with the AmpliType® PM PCR Amplification and Typing Kit. As expected, the "S" dot was much lighter than for those samples where the template DNA had been recovered from the HLA-DQA1 products. The evidentiary head hair recovered from the car showed the same typing results as the reference head hair from the victim.

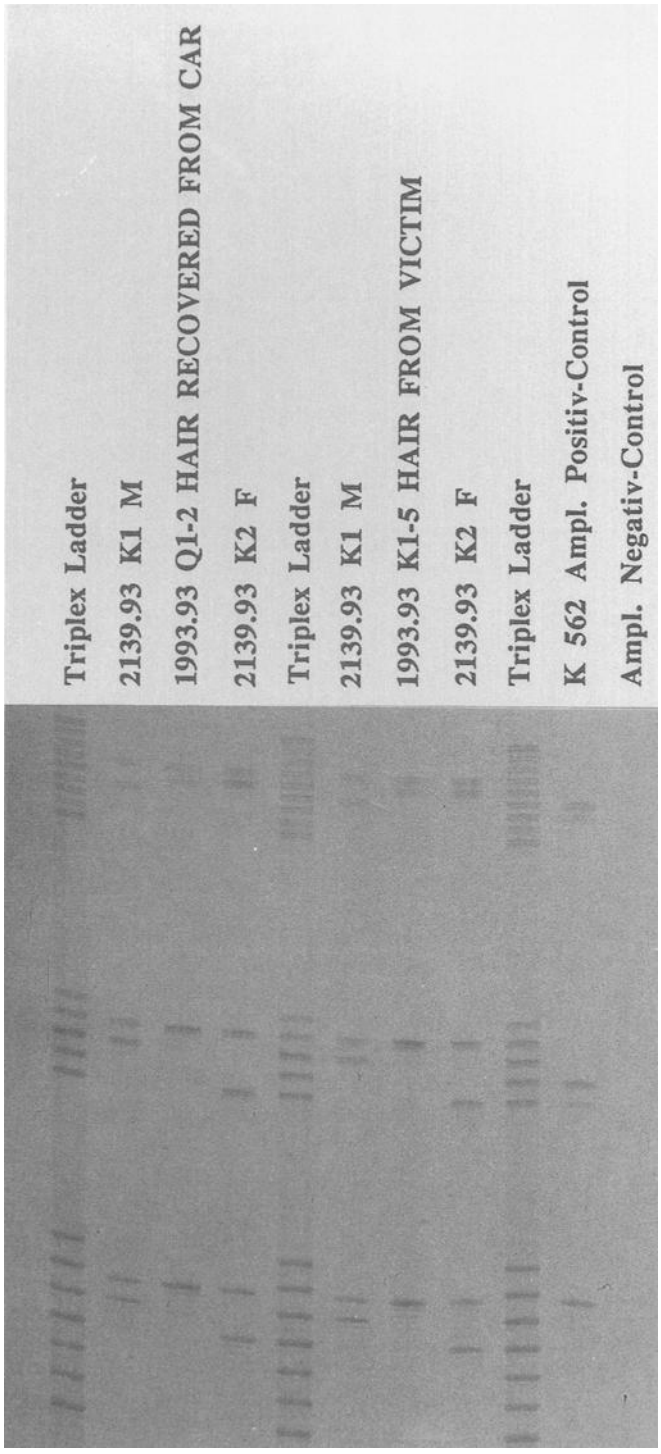

FIG. 4—*Reverse paternity analysis. Silver-stained DNA profiles of the three simultaneously amplified STR loci TH01, TPOX, and CSF1P0. The DNA profiles from a reference head hair from the victim (1993.93 K1-5) and the evidentiary sample recovered from the suspect's car (1993.93 Q1-2) were compared with the DNA profiles of the victim's mother (2139.93 K1 M) and father (2139.93 K2 F). No exclusion was found. The STR ladder alleles from bottom to top are: TH01: 5,6,7,8,9,10,11; TPOX: 8,9,10,11,12; CSF1P0: 7,8,9,10,11,12,13,14,15.*

occurrence of this type in the Swiss Caucasian population is approximately 1 in 13. In January 1994 the genomic DNA was recovered from the HLA-DQA1 amplification product and typed at five more genetic markers (LDLR: AB, GYPA: AB, HBGG: AB; D7S8: BB, and GC: CC). Again the victim's reference hair sample and the evidentiary hair sample matched. The likelihood of occurrence of these five genetic markers in the Swiss Caucasian population (Table 1) is approximately 1 in 180 individuals. Combined with HLA-DQA1 the frequency of the derived DNA profile is approximately 1 in 2300 individuals. In April 1994 from the remaining seven ng DNA of the sample two ng were used for multiplex PCR of specific regions of three tetrameric STR loci using the GenePrint™ STR Multiplex System. The victim's reference hair sample and the evidentiary hair sample again matched at three genetic markers. The likelihood of occurrence of these three genetic markers in the Swiss Caucasian population (Table 1) is approximately 1 in 900 individuals. However, combined with HLA-DQA1 and the PM loci, the power of discrimination increased dramatically to approximately 1 in 2.1 million individuals. Finally, the sex of the evidentiary sample was determined as female, using 1 ng of the remaining 5 ng of original sample DNA for amplification of a specific region of the amelogenin gene. Therefore, the frequency of occurrence of the derived profile in the Swiss Caucasian population was determined to be very rare—approximately 1 in 2.1 million females. Furthermore, the reverse parentage analysis did not exclude the donor of evidentiary sample as E.H.'s and W.S.'s child. The suspect itself was excluded in all genetic markers to be a contributor to the sample.

The Trial

The trial was held in Graz, Austria from April to June 1994. There is no equivalent to the Frye standard in the Austrian court system. However, the DNA methodology including the underlying population genetics statistics applied in this case was considered so well accepted, or noncontroversial, that the judge ruled it admissible without holding a pretrial hearing. The DNA evidence was presented by a qualified expert, who had participated in the analysis, in an extensive one and a half day hearing. This included the education of the jury by addressing the principles of DNA analysis and PCR. Photographs of all typing results (HLA-DQ alpha and PM-strips as well as the polyacrylamide gels) had been mounted on large cardboard-posters for court-room demonstration.

The Defense

The defense chose two ways to attack the analysis. The first argument was that although the DNA tests were carried out properly there were gaps in the chain of custody. This argument was refuted by demonstrating an exact protocol of the chain of custody of the evidence by all participating laboratories and investigators. For the DNA part, it was explained that the known and questioned samples had been received, extracted, amplified and typed independently. The second argument was over the statistical basis of using a small Swiss population sample (n = 100) to calculate the frequency of occurrence of the particular DNA profile. However, despite the size of the population sample, tests for independence had been carried out (22,23) that have sufficient power to detect departures from expectations that would affect estimates of the likelihood of occurrence of a PCR-based DNA profile for forensic identity testing. It was shown that the Swiss population sample used in this case can yield reliable estimates of the frequency of

a multiple PCR-based DNA profile. Furthermore, frequencies for different population samples were provided, demonstrating that the particular nine-marker profile was very rare in all populations (37).

The Verdict

J.U. was sentenced to life imprisonment for the murder of nine women, including the Prague victim B.B., where the DNA evidence had been analyzed. DNA evidence played a central role in the trial and established with a high degree of certainty that this particular victim was in contact with his car. In addition, other circumstantial evidence, eye witnesses and the recovered fiber evidence came into play. The verdict was read to J.U. at 9 p.m.; six hours later he hanged himself in his cell.

Conclusions

In summary, this is the first report where commercially available multiplex PCR amplification and typing kits have been introduced for the analysis of single-hair DNA evidence in a serial killer case and the analysis has been admitted in court. Since the quantity of DNA was the critical issue in this case, a special typing strategy was used, including sequential multiplex amplification (SMA). This approach, as well as the multiplex PCR was accepted by the court as properly generated in the case in question.

Acknowledgment

We would like to thank Dr. J. Schumm and Dr. C. Sprecher at Promega Corporation, Madison, WI, for kindly providing the GenePrint™ STR Multiplex System PCR Amplification Kits.

References

(1) Amicon, Centricon™ microconcentrators user guide, Amicon Division, W. R. Grace & Co.-Conn., Danvers, MA, 1989.
(2) Waye JS, Presley LA, Budowle B, et al. A simple and sensitive method for quantifying human genomic DNA in forensic specimen extracts. Bio Techniques 1989;7:852–5.
(3) AmpliType™ HLA DQ alpha forensic DNA amplification and typing kit user guide, Roche Molecular Systems, Inc., Branchburg, New Jersey, USA, 1992.
(4) Lorente M, Lorente JA, Wilson M, Budowle B, Villanueva E. Sequential multiplex amplification (SMA) of genetic loci: a method for recovering template DNA for subsequent analyses of additional loci. Int J Legal Med 1994;107:156–8.
(5) Hochmeister M, Budowle B, Borer UV, Dirnhofer R. A method for the purification and recovery of genomic DNA from an HLA DQA1 amplification product and its subsequent amplification and typing with the AmpliType® PM amplification and typing kit. J Forensic Sci 1995;40:(4).
(6) AmpliType™ PM PCR amplification and typing kit user guide, Roche Molecular Systems, Inc., Branchburg, New Jersey, USA, 1994.
(7) Gyllenstein UB, Erlich HA, Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQ alpha locus. Proceed Natl Acad Sci USA 1988;85:7652–56.
(8) Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russel DW. The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA, Cell 1984; 39:27–38.
(9) Siebert PD, Fukuda M. Molecular cloning of a human glycophorin B cDNA: nucleotide sequence and genomic relationship to glycophorin A. Proceed Natl Acad Sci USA 1987;84:6735–9.
(10) Slightom JL, Bechl AE, Smithies O. Human fetal g-gammaglobin and alpha-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicate genes. Cell 1980;21:627–38.
(11) Horn GT, Richards B, Merrill JJ, Klinger KW. Characterization and rapid diagnostic analysis of DNA polymorphisms closely linked to the cystic fibrosis locus. Cell 1980;21:627–38.
(12) Yang F, Brune JL, Naylor S, Apples RL, Naberhaus KH. Human group-specific component (Gc) is a member of the albumin family. Proceed Natl Acad Sci USA 1985;82:7994–8.
(13) Promega, Technical Manual # TMD004, GenePrint™ STR Systems, 1994, Promega Corporation, Madison, WI.
(14) Huang NE, Schumm J, Budowle B. Chinese population data on 3 tetrameric short tandem repeat loci—HUMTH01, TPOX, and CSF1PO—devised using multiplex PCR and manual typing. Forensic Sci Int 1994 (in press).
(15) Edwards A, Civitello A, Hammond HA, Caskey CT. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756.
(16) Edwards A, Hammond H, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric repeat loci in four human population groups. Genomics 1992;12:241–53.
(17) Polymeropoulos MH, Xiao H, Rath DS, Merril CR. Tetranucleotide repeat polymorphism at the human tyrosine hydrolase gene (TH). Nucleic Acids Res 1991;19:3753.
(18) Anker R, Steinbrueck T, Donnis-Keller H. Tetranucleotide repeat polymorphism at the human thyroid peroxidase (hTPO) locus. Hum Mol Genet 1992;1:137.
(19) Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R. Evaluation of thirteen STR loci for use in personal identification applications. Am J Hum Genet 1994 (in press).
(20) Recommendations of the DNA Commission of the International Society for Forensic Haemogenetics, Int J Leg Med 1994 (in press).
(21) Sullivan, KM, Mannucci, A, Kimpzon, CP, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X–Y homologous gene amelogenin. BioTechniques 1993;15(4):-636–41.
(22) Hochmeister M, Budowle B, Borer UV, Dirnhofer R. Swiss population data on the loci HLA-DQ alpha, LDLR, GYPA, HBGG, D7S8, GC, and D1S80. Forensic Sci Int 1994;67:175–84.
(23) Hochmeister, MN, Budowle, B, Schumm, JW, Sprecher CJ, Dirnhofer R. Swiss population data and forensic efficiency values on 3 tetrameric short tandem repeat loci-HUMTH01, TPOX, and CSF1P0—derived using a STR Multiplex System. Int J Legal Med 1995 (in press).
(24) Budowle B, Baechtel FS. Modifications to improve the effectiveness of restriction fragment length polymorphism typing. Appl Theoret Electrophor 1990;1:181–7.
(25) Blake E, Mihalovich J, Higuchi R, Walsh PS, Erlich HA. Polymerase chain reaction (PCR) amplification and human leukocyte antigen (HLA)-DQ alpha oligonucleotide typing on biological evidence samples: casework experience. J Forensic Sci 1992;37(3):700–26.
(26) Budowle B, Lindsey J, DeCou JA, Koons BW, Giusti A, Comey CT. Validation and population studies of the loci LDLR, GYPA, HBGG, D7S8, and Gc (PM loci), and HLA-DQ alpha using a multiplex amplification and typing procedure. J Forensic Sci 1995;40(1):45–54.
(27) Hochmeister M, Budowle B, Borer UV, Rudin O, Bohnert M, Dirnhofer R. Confirmation of the identity of human skeletal remains using multiplex PCR amplification and typing kits. J Forensic Sci 1995;40(4).
(28) Herrin G Jr., Fildes N, Reynolds R. Evaluation of the AmpliType® PM DNA test system on forensic case samples. J Forensic Sci 1994;39(5):1247–53.
(29) Hochmeister MN, Budowle B, Borer UV, et al. Typing of DNA extracted from compact bones from human remains. J Forensic Sci 1991;36(6):1649–61.
(30) Sajantila A, Ström M, Budowle B, Karhunen PJ, Peltonen L. The polymerase chain reaction and post mortem forensic identity testing: application of amplified D1S80 and HLA DQ alpha loci to the identification of fire victims. Forensic Sci Int 1991;(51):23–34.
(31) Hagelberg E, Gray IC, Jeffreys AJ. Identification of the skeletal remains of a murder victim by DNA analysis. Nature 1991; 352:427–9.
(32) Jeffreys AJ, Allen MJ, Hagelberg E, Sonnberg A. Identification of

the skeletal remains of Josef Mengele by DNA analysis. Forensic Sci Int 1992;56:65–76.

(33) Holland MM, Fischer DL, Mitchel LG, Rodriguez, WC, Canik JJ, Merril CR, and Weedn VW. Mitochondrial DNA sequence analysis of human skeletal remains: identification of remains from the Vietnam War. J Forensic Sci 1993;38(1):542–53.

(34) Fisher DL, Holland MM, Mitchell L, Sledzik PS, Webb Wilcox A, Wadhams M, Weedn VW. Extraction, evaluation and amplification of DNA from decalcified and undecalcified United States Civil War bone. J Forensic Sci 1993;38(1):60–8.

(35) Higuchi R, von Beroldingen CH, Sensabaugh GF, Erlich HA. DNA typing from single hairs. Nature 1988;332:543–6.

(36) Uchihi R, Tamaki K, Kojima T, Yamamoto T, Katsumata Y. Deoxyribonucleic acid (DNA) typing of human leukocyte antigen (HLA)-DQA1 from single hairs in Japanese. J Forensic Sci 1992;37:853–9.

(37) Lander ES, Budowle B. DNA fingerprinting dispute laid to rest. Nature 1994;371:735–8.

Address requests for reprint or additional information to
Manfred Hochmeister, M.D.
Institute of Legal Medicine, Berne
Buehlstrasse 20, CH-3012 Switzerland
Fax: ++41 31 631 38 33