FALSE RESULTS IN THE HLA-DQα TYPING: TWO CASES REPORTED

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INTRODUCTION

DNA analysis by PCR-based polymorphisms is routinely carried out in the forensic laboratory. The most relevant advantage of the *in vitro* amplification is the high sensitivity of this technique that permits successful characterisations from nanograms of (also degraded) DNA samples (Bloch, 1991; Reynolds and Sensabaugh, 1991).

The HLA-DQA1 locus (DQα according to the old nomenclature) is one of the systems most used and the genetic polymorphisms of this locus can be shown by several methods: sequence analysis (Gyllensten and Herlich, 1988), revers dot blot hybridisation with Allele Specific Oligonucleotides (ASO) (Saiki, 1989) and digestion with restriction enzymes.

We present here two cases where the characterisation of the HLA-DQA1 locus by ASO gave unreliable results.

MATERIALS AND METHODS

Case1

A 55-year-old woman was murdered, 13 years previously, by multiple head injures (laceration of the scalp and fissured fractures of the skull); her clothes, however, showed 2 large bloodstains (ref. n. 418 and 419) and 4 other bloodstains (ref. n. 414-417) that were used, at the time of DNA analysis, as reference samples of the victim. The reference sample of the suspect (who is the child of the victim) was the Na2EDTA blood collected at the time of the DNA analysis (ref. n. 423).

Case 2

A paternity test was carried out on Na2EDTA blood samples of a mother-son couple (ref. n. 487 and 486) and on tissue samples collected at autopsy from the body of the alleged father who has died 6 years before. The tissue samples were the following: cartilage of the sternum (497/5), cartilage of the ear (497/1) and ligamentus of the acetabulum (497/17).

DNA extraction

The DNA was extracted from all the samples by SDS/Proteinase K buffer, phenol purification and ethanol precipitation. Chelex procedure was also employed for DNA purification from the bloodstains for PCR amplification. The presence of human DNA was demonstrated in all the samples by slot blot hybridisation with a P^{32} alphoid sequence.

PCR-based polymorphisms analysis

The following systems were amplified: Coll2AI, D1S80, HumTH01, HumVWA, MBP-B and SE33. The PCR products of STR systems were separated by electrophoresis in polyacrylamide gels (5-8%) in 1xTBE buffer. TBE-agarose gel electrophoresis (3.3 %) was carried out to type D1S80 and Coll2AI loci. The HLA-DQA1 system was amplified and typed by the Amplitype Kit (Perkin-Elmer) according to the manufacturer's instruction.

The DQA1 locus was furthermore amplified by published protocols (Saiki, 1989) in 25 µl reaction tubes and the amplification products direct sequenced by PCR (Gyllensten, 1989). A

373A Sequencer (Applied Biosystem) was employed to analyse the sequence reactions. Negative and positive amplification controls were always included.

HPLC (High Performance Liquid Chromatography) analysis

About 10 μg of the DNA extracted from bloodstains 418 and 419 and from 2 postmortem tissue samples (ref. n. 497/5 and 497/17) were hydrolysed in 90 % formic acid at 170 C for 30' (Pääbo, 1989). After lyophilization, the samples were redissolved in 1 % HCl and analysed by reverse-phase HPLC using a Res Elut C18 5 μ column (Varian) in acetonitrile/Na-acetate buffer. Hydrolysed human DNA was used as reference standard; the base-specific peaks were identified by comparison wiht hydrolysed dNTPs (Boëhringer).

RESULTS

Case 1

The first characterisations at the HLA-DQA1 locus showed a 1.1/4 genotype in the samples 416T and 418 (Fig. 1). As these results excluded the parental relationship between the victim and her child (sample 423; genotype: 2/3), further characterisations were carried out: new amplification solutions were used and others bloodstains typed. The results are shown in Fig. 1. New extractions were then carried out from the bloodstains and all typed at locus HumTH01 as genotypes 6/9; the son of the victim was typed as 9/9.3. The bloodstains 418 and 419 were further typed in 3 other STR systems and they have been shown to share the same genotypes (7/11 at locus MBP-B; 14/18 at locus HumVWA, 14/21 at locus SE33); however there was always a "match" with the sample of the suspect.

Moreover, sequence analysis of the amplification products of the samples 418 and 419 demonstrated the presence of 239 bp fragments that show a 0201 genotype.

HPLC analysis shows the presence of anomalous peaks.

Case 2

The results of the characterisations at VNTR and STR systems are reported in Table 1.

By ASO, the HLA-DQA1 results were 1.1/4 in the mother and 4/4 in the son while the samples 497/5 and 497/17 were typed as 1.1/1.3 and 1.2/4 respectively. Samples 486 and 487 were sequenced and the results confirmed. However, sequence analysis of amplification products of the samples 497/5 and 497/17 shows the presence in both of 239 bp fragments corresponding to 0501 genotype. These two DNA templates were also analysed by HPLC: this shows the presence of an anomalous chromatogram.

DISCUSSION

Two cases are reported where ambiguous results by HLA-DQA1 typing by ASO were obtained. The case 1 refers to the DNA typing of 13-year-old bloodstains. The reasons of these results are unknown and several mechanism could be involved: contamination by exogenous DNA, inhibitors, etc. Nevertheless, in samples 418 and 419 only the presence of 239 bp long PCR products was identified; these fragments, however, have been sequenced as 0201 genotypes (a 2/2 genotype was also clearly typed by AS0 in sample 414T) (Fig. 1).

More reliable was the PCR analysis of STR loci. Four of them (MBP-B, HumTH01, HumVWA and SE33) were successfully typed and the results obtained were in agreement with those expected: same genotype in the samples 418 and 419 and one allele shared with the sample of the child.

As regards the paternity here presented (Case 2), it has been practically proven by six PCR-based polymorphisms. Moreover, the sequence analysis at HLA-DQA1 locus shows the presence in the alleged father of 239 bp fragments, corresponding to allele 0501 (allele 4 in the

old denomination). These data further confirm the paternity (genotype of the son: 4/4). The reasons of these ambiguous results obtained by ASO typing are being investigated.
As the HPLC analysis shows the presence, in both cases, of anomalous peaks, also the role of highly damaged templates is being considered (Golenberg, 1994).

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ACKNOWLEDGEMENTS

We thank Prof. B. Brinkmann and Dr. S. Rand (University of Münster, Germany) for their helpful suggestions in proceeding of this work.

Table 1. The alleles shared between the son and alleged father are underlined. *: not performed; **: the sequence analysis of others PCR products are being carried out.

System	mother	son	497/1	497/5	497/1
Coll2AI	13/13	13/13	13/13	13/13	13/13
D1S80	18/24	17/18	*	17/18	17/18
WVA-A	17/19	15/19	15/18	15/18	15/18
MBP-B	10/12	7/10	7/7	7/7	7/7
THO1	6/9.3	6/9.3	6/6	6/6	6/6
SE33	20/26	16/20	*	16/19	16/19
HLA-A1	0101/0501	0501/0501	*	01../0501**	01../0501**

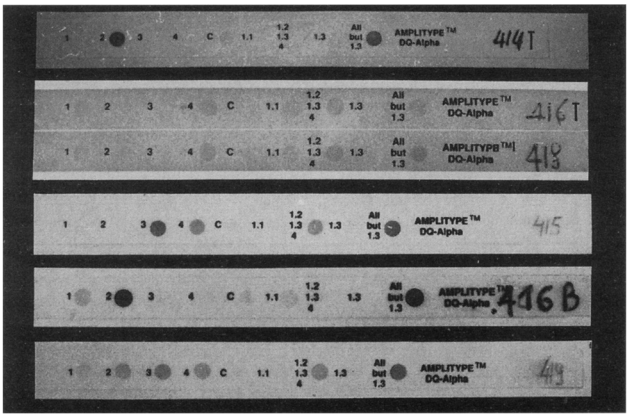

Fig. 1. HLA-DQA1 system. Revers dot blot hybridisations of the PCR products of 13-year-old bloodstains.