TECHNICAL NOTE

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The Effects of Blood Transfusions on PCR DNA Typing at the CSF1P0, TP0X, TH01, D1S80, HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and GC Loci

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ABSTRACT: Pre-transfusion and post-transfusion blood samples from eight individuals were typed at 10 PCR amplified loci. In no case did the PCR DNA profile of the post-transfusion blood sample differ from that of the pre-transfusion profile.

KEYWORDS: forensic science DNA typing, polymerase chain reaction, LDLR, GYPA, HBGG, D7S8, GC, short tandem repeats; PM; D1S80, HLA-DQA1, blood transfusions, CSF1P0, TP0X, TH01

Forensic biology is based on comparison assays carried out between biological material from a crime scene and suspect/victim samples (references). In order for a meaningful comparison to take place, the characteristics found in the crime scene and reference samples must remain unaltered. Validation studies (1–5) have been undertaken to assess the effects of simulated crime scene conditions on PCR DNA typing. Of equal importance in DNA typing are factors which may affect the integrity of reference samples. These effects may similarly impinge on the reliability of the comparison between crime scene material and reference samples.

Blood transfusions, sometimes given to the suspects or victims of a crime, constitute such an alteration. Transfused blood is known to change red blood cell polymorphic enzyme patterns (6). Consequently, if the polymorphic enzymes of the donor and recipient differ, additional donor bands may be detected in the reference pattern. At worst, this could result in an erroneous match between the polymorphic enzymes of a post-transfused blood sample from a suspect and the results found in the crime scene material.

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Today, most forensic laboratories have abandoned polymorphic enzyme assays in favor of the more discriminating DNA typing. Yet we are unaware of any work relating to possible transfusion effects on these assays. In order to determine whether blood transfusions affect PCR typing, a study was carried out on hospital patients who had received varying amounts of whole blood and packed red blood cells, both of which contain leukocytes. Pre- and post-transfusion blood samples were PCR amplified at three STR loci, the five PM loci, D1S80 and HLA-DQA1. The results of this study, together with those of two criminal cases, are presented.

Materials and Methods

Blood Samples

Eight patients were transfused with whole blood and packed red blood cells as indicated in Table 1. Blood was drawn prior to and following the transfusions. In the case of patients 6–8, a blood sample was also taken following a second transfusion.

DNA Extraction

Blood stains were prepared on sterile gauze pads and air dried. DNA was then organically extracted from the stains.

DNA Typing Methods

Simultaneous polymerase chain reaction (PCR) amplification of the three short tandem repeats (STR)—CSF1P0, TP0X, and TH01—was carried out according to the Promega manual (7), except that 16 μg BSA was added to the amplification mixture. The D1S80 loci was amplified as described by Sajantila, et al. (8), except that 3.2 μg BSA was added to the reduced reaction volume of 20 μL and the PCR products were separated on a 0.4 mm thick, 6% continuous polyacrylamide gel. The five PolyMarkers (PM) and HLA-DQA1 were amplified and characterized according to Hayes, et al. (9).

Results

Blood samples from eight patients, who received various quantities of blood components containing leukocytes, were profiled at the PCR STR triplex and the D1S80 loci both before and after

TABLE 1—*Details of the blood transfusions given to eight patients. Patients 1–5 received one transfusion each; patients 6–8 received two transfusions each.*

Patient Number/(Sex)	Number of Transfusions	Blood Product Transfused	Number of Units Transfused	Time Blood Drawn Following Transfusion	Total Number of Units Transfused
1(M)	1	Whole blood	4		
		Packed cells	9	1 day	13
2(M)	1	Whole blood	5	12–20 hours	5
3(M)	1	Packed cells	2	1 day	2
4(M)	1	Packed cells	1	2 days	1
5(F)	1	Packed cells	6		
		Thrombocytes	6	12 hours	12
6(M)*	1	Packed cells	3	2 days	
	2	Packed cells	1	1 day	4
7(F)	1	Whole blood	4		
		Packed cells	10	Immediately	
	2	Whole blood	1		
		Packed cells	1	2 days	16
8(M)	1	Whole blood	8		
		Thrombocytes	6	Immediately	
	2	Whole blood	2		
		Packed cells	2	2 days	18

*Child.

transfusions (Table 2). Samples from five of the eight patients were further profiled at the PM and HLA-DQA1 loci (Table 3).

Case Reports

A man was admitted to hospital after being severely beaten about the head; a week later, he died. During the hospitalization, he received seven units of packed cells. The triplex results obtained on his postmortem blood sample were as follows: CSF1P0 11,11; TPOX 11,8; TH01 9,9. A pre-transfusion blood sample was not available for profiling.

In another case, a man and a woman suffered multiple stab and axe wounds. Both were transfused before blood samples were taken for comparison with blood found at the scene of the stabbings. Details of the transfusion were unavailable, as were pre-transfusion blood samples. The DNA profile for the man was: CSF1P0 10,10; TPOX 11,9; TH01 9.3,9; D1S80 25,18 and for the woman: CSF1P0 11,10; TPOX 9,8; TH01 9,8; D1S80 24,24.

TABLE 2—*STR and D1S80 typing results on the blood of eight pre- and post-transfusion patients.*

Patient Number	Number of Transfusions*	PCR STR Results			PCR DIS80 Results
		CSF1P0	TPOX	TH01	
1	0	12,10	11,8	6,6	37,18
	1	12,10	11,8	6,6	37,18
2	0	12,11	10,8	9,9	24,24
	1	12,11	10,8	9,9	24,24
3	0	15,12	8,8	9.3,7	24,18
	1	15,12	8,8	9.3,7	24,18
4	0	12,10	8,8	8,7	31,24
	1	12,10	8,8	8,7	31,24
5	0	11,10	10,8	9.3,9	24,24
	1	11,10	10,8	9.3,9	24,24
6	0	12,11	11,8	9,6	29,24
	1	12,11	11,8	9,6	29,24
	2	12,11	11,8	9,6	29,24
7	0	11,11	9,8	9.3,6	>41,24
	1	11,11	9,8	9.3,6	>41,24
	2	11,11	9,8	9.3,6	>41,24
8	0	10.10	8,8	9.3,6	28,18
	1	10,10	8,8	9.3,6	28,18
	2	10,10	8,8	9.3,6	28,18

*Details of the transfusions appear in Table 1.

Discussion

Previously, we reported on a case in which blood recovered from a crime scene and the post-transfusion sample of a suspect's blood matched by four RFLP probes as well as at the D1S80 locus (10). No DNA transfusion effects were apparent in the suspect's post-transfusion blood sample.

In the present study, the ten PCR amplified loci assayed were also unaffected by blood transfusions, irrespective of the quantity of blood transfused or the time interval between the completion of the transfusion and the drawing of blood sample. Furthermore, in the two criminal cases, there was no indication of mixed profiles in the STR triplex or the D1S80 locus.

In neither the study nor the case reports were samples of the donor bloods available for testing. However, approximately fifty other donor blood samples, taken from blood unit segments, have been successfully amplified in our laboratory at the three STR loci used in this study. We have also typed D1S80 from more than ninety donor bloods, six of which were outdated (up to 3.5 months). This demonstrates that donor blood samples contain PCR amplifiable DNA.

We would expect that other DNA laboratories have tested blood samples from persons who have received blood transfusions. However, we are unaware of any reports of transfusion effects on DNA typing, a fact that is consistent with our findings.

Other factors such as carcinomas (11), toxicological substances (12) and bone marrow transplants (13) are known to alter DNA profiles. This may lead to confusion in the interpretation of crime scene results in the absence of reference samples. For example, a female, who receives a male bone marrow transplant will have Y chromosomes in her peripheral blood cells (13). As a result, this female's blood, found anonymously at a crime scene, may be missexed. By contrast, blood transfusions, given either before or after the deposition of biological material at a crime scene, would not appear to cause erroneous results in forensic DNA comparisons.

TABLE 3—*PolyMarker and HLA-DQA1 typing results on five pre- and post-transfused patients.*

Patient Number	Number of Transfusions*	PM Results					HLA-DQA1
		LDLR	GYPA	HBGG	D7S8	GC	
1	0	AB	AB	AA	AB	AB	1.3–4
	1	AB	AB	AA	AB	AB	1.3–4
2	0	BB	AB	AA	AB	AB	1.3–4
	1	BB	AB	AA	AB	AB	1.3–4
5	0	AB	AA	AB	BB	CC	1.1–2
	1	AB	AA	AB	BB	CC	1.1–2
7	0	BB	AA	AB	AB	CC	1.3–4
	1	BB	AA	AB	AB	CC	1.3–4
	2	BB	AA	AB	AB	CC	1.3–4
8	0	AB	AB	AB	AB	AC	3–4
	1	AB	AB	AB	AB	AC	3–4
	2	AB	AB	AB	AB	AC	3–4

*Details of the transfusions appear in Table 1.

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