

Mixture Patterned Short Tandem Repeat Profile in a Perimortem Transfused Patient

Hee Jin Seo¹, Sohee Cho¹,
Ji Hyun Lee¹, Sung Hee Lyoo²,
Sohyung Park³, Soong Deok Lee^{1,2}

¹Department of Forensic Medicine,
Seoul National University College
of Medicine, Seoul, Korea, ²Institute
of Forensic Science, Seoul National
University College of Medicine,
Seoul, Korea, ³Medical Examiner's
Office, National Forensic Service,
Wonju, Korea

Received: January 21, 2016
Revised: February 5, 2016
Accepted: February 15, 2016

Correspondence to

Soong Deok Lee
Department of Forensic Medicine,
Seoul National University College of
Medicine, 103 Daehak-ro, Jongno-gu,
Seoul 03080, Korea
Tel: +82-2-740-8359
Fax: +82-2-764-8340
E-mail: sdlee@snu.ac.kr

Recently, it has been reported that transfused patients can generate admixture-like genetic profiles. As genetic material of the donor can survive for a reasonable time after transfusion, the recipient's genomic DNA is likely to have a mixture pattern. An autopsy case of a man transfused perimortem generated a mixture patterned short tandem repeat profile. Notably, the patient was transfused mostly with nuclear-deficient cells, limiting the donor genetic material available for the recipient. As a result, mixture-like patterns were observed consistently, regardless of change in input DNA content; the sample DNA content, which was serially diluted, ranged from 1 ng to 0.0625 ng. The distributions of foreign peaks appeared to be irreproducible, showing stochastic behaviors throughout the genotyped results. This study suggests that a cautious approach is required when genotyping of a patient who has undergone recent transfusion. One must consider the possibility of obtaining a mixture patterned profile in such patients, and therefore, choose parenchymal organs or tissues for reliable results.

Key Words: Perimortem; Blood transfusion; DNA profiling;
Microsatellite repeats

Introduction

Throughout past several decades, various authors have reported that transfusion not only can interfere with but also can alter the recipient's genotype temporarily. Despite the fact that there is no definite worldwide protocol and claims by several authors that transfusion does not affect individual genotype [1], certain countries, such as the United Kingdom, strongly suggest avoiding the use of blood samples from a recently transfused patient for genotyping [2]. While the precise mechanism for such an event remains unknown,

numerous studies have depicted that donor genetic material is capable of surviving in the recipient's system for a reasonable amount of time, and therefore, one must take a cautious approach in samples of recently transfused patients [3,4]. In this study, an autopsy was performed for a patient who was transfused perimortem. In order to examine whether the patient's genomic DNA would generate a mixture-like short tandem repeat (STR) profile, his blood sample was collected during the autopsy, and a sample of a muscle tissue was collected as a reference.

Case Report

An autopsy was conducted for a middle-aged man who had died due to excessive bleeding caused by multiple self-inflicted stab wounds. He had received 3 units of packed red blood cells (pRBC) at the facility where he was treated initially. The patient was then transferred to another facility where he was transfused with 20 units of red blood cells (RBCs), 30 units of platelets, and 17 units of fresh frozen plasma (FFP) at perimortem status. It was not possible to collect the patient's full medical records; therefore, no precise time-dependent diagnostic test results were available for further investigation. During the autopsy, cardiac blood was sampled for genotyping and a part of the iliopsoas muscle tissue was also retrieved.

For genotyping of the blood and muscle sample, DNA was extracted using QIAamp DNA Investigator Kit (Qiagen, Valencia, CA, USA). In order to examine the mixture ratio and peak distribution, extracted DNA from the blood sample was serially diluted to standard 1 ng, then down to 0.5 ng, 0.25 ng, 0.125 ng, and 0.0625 ng. Polymerase chain reaction was performed using PowerPlex Fusion Systems (Promega, Madison, WI, USA) and the products were analyzed with ABI Genetic Analyzer 3130 (Thermo Fisher Scientific, Waltham, MA, USA) and GeneMapper ID Software v3.2.1. (Thermo Fisher Scientific). The experiments were performed in duplicates to evaluate and confirm reproducibility.

The extracted DNA from the muscle tissue provided a consensus profile of the patient (Fig. 1), and the foreign peaks found in the blood samples were inferred based on this profile. Over the range of serial dilutions,

all samples exhibited consistent mixture patterned profiles, regardless of the change in DNA content (Fig. 2). The most prominent mixture patterns developed in the 0.5 ng and 0.25 ng blood DNA samples. Though the detected foreign peaks varied among the samples, several loci showed considerably higher peak-height ratios between the original and donor-suspected alleles than the others did. For instance, for 0.5 ng of blood DNA sample, the height of a foreign peak detected at D18S51 locus was about 35% of the original peak, and for 0.25 ng of blood DNA sample, a foreign peak height measured 46% of the original allele peak height (data not shown).

The duplicates of blood DNA sample of 0.5 ng produced typical mixture-like electropherograms, though the called foreign alleles varied in each sample (Fig. 3B, C). The mixture patterns were irreproducible, presuming that the foreign alleles found in each sample were stochastic.

Discussion

The patient who received perimortem transfusion developed a mixed patterned profile, as donor genomic DNA can co-exist within the recipient's blood system [2,5]. A remarkable point was that the patient's profiles had multiple foreign peaks in every diluted DNA sample, although he was transfused with nuclear-deficient blood components: mostly RBCs, platelets, and plasma, which limit the presence of donor DNA that could enter into the subject's system. Considering the ratio between his original DNA in blood and newly transfused foreign genetic material, if any, the peak height ratios found

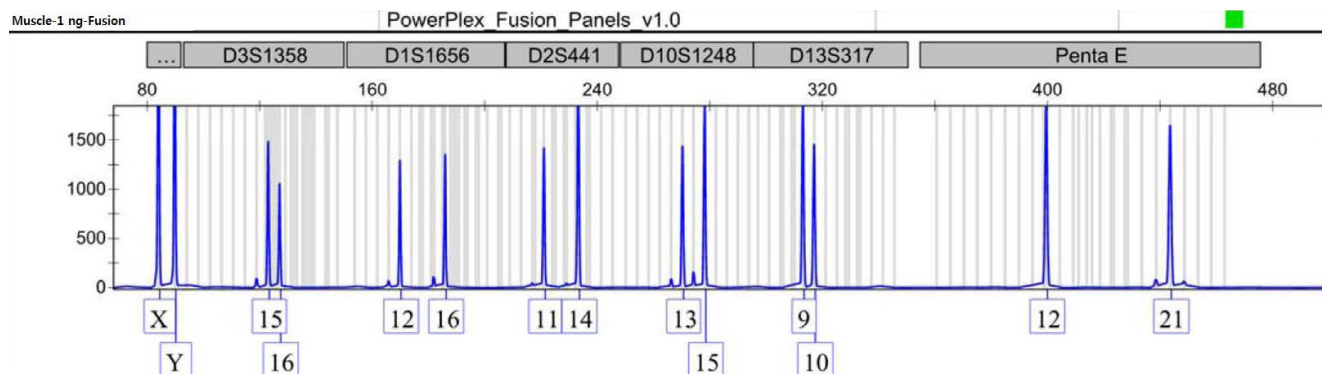


Fig. 1. An example of the patient's consensus profile from a muscle tissue sample.

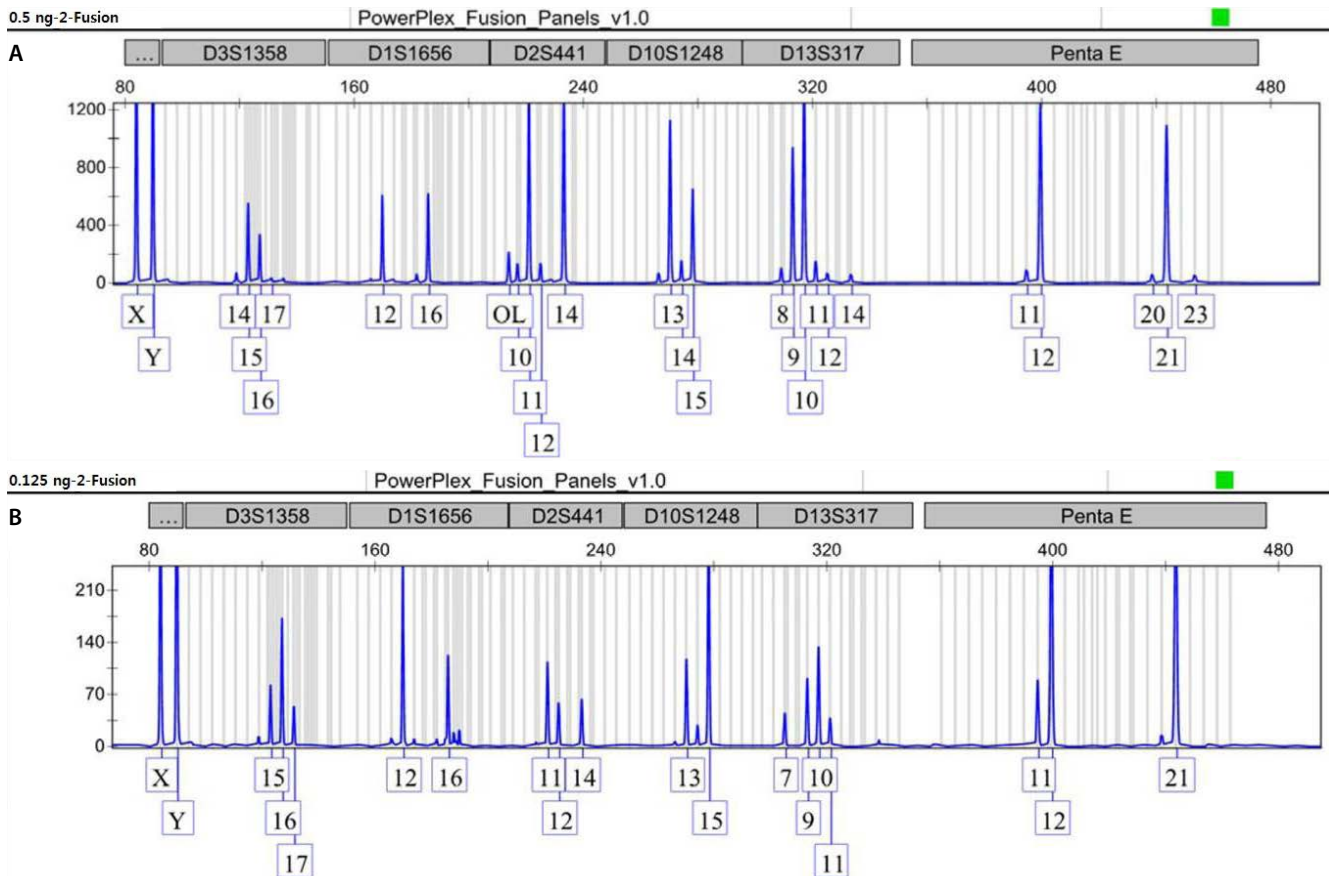


Fig. 2. Overview of foreign peaks detected in blood DNA sample diluted to 0.5 ng (A) and 0.125 ng (B).

on data analysis seemed to be remarkably higher than artifacts, the threshold of which is usually set at about 15% of the original allele's height in forensics field. The mechanism for such phenomenon is unknown yet, but one of the possible reasons could be that transfused blood components may have contained cell-free donor genomic DNA, abundant enough to enter his system and survive. Studies have shown that when normal blood is donated, nuclear fragments can be released owing to lysis of white blood cells, which tends to increase significantly over time [6]. If the blood is donated and stored for several days before transfusion, it is likely that the amount of cell-free genomic DNA increases substantially [5,7]. Moreover, if such stored pRBC units containing donor leukocytes are separated into different compartments and these are then transfused into this patient, it is likely that extensively larger amounts of cell-free DNA could have been introduced into the recipient's body [6], and may have proliferated within the system for a reasonable amount of time after his

death [8].

Another possible idea could be that clotting factors contained in the plasma and platelets may have increased the donor's cell-free DNA in the recipient's system. According to Lee et al. [6], most cell-free genomic DNA in the serum or plasma is generated during the process of clotting. Cell-free DNA can be accumulated during clotting as a result of cell destruction [6]. As massive amount of platelets and FFP are transfused (30 units and 17 units, respectively), a bulk of clotting factors may have also been transfused, releasing donor cell-free DNA into the recipient's body [6]. Nonetheless, these are only speculations, and the exact cause and mechanism for such an event is unknown.

This study proposes that when genotyping patients with recent blood transfusion, portions of the profile generated may be derived from unknown contributors and that those could interfere with the recipient's genuine profile [2,9]. As there are many aspects of

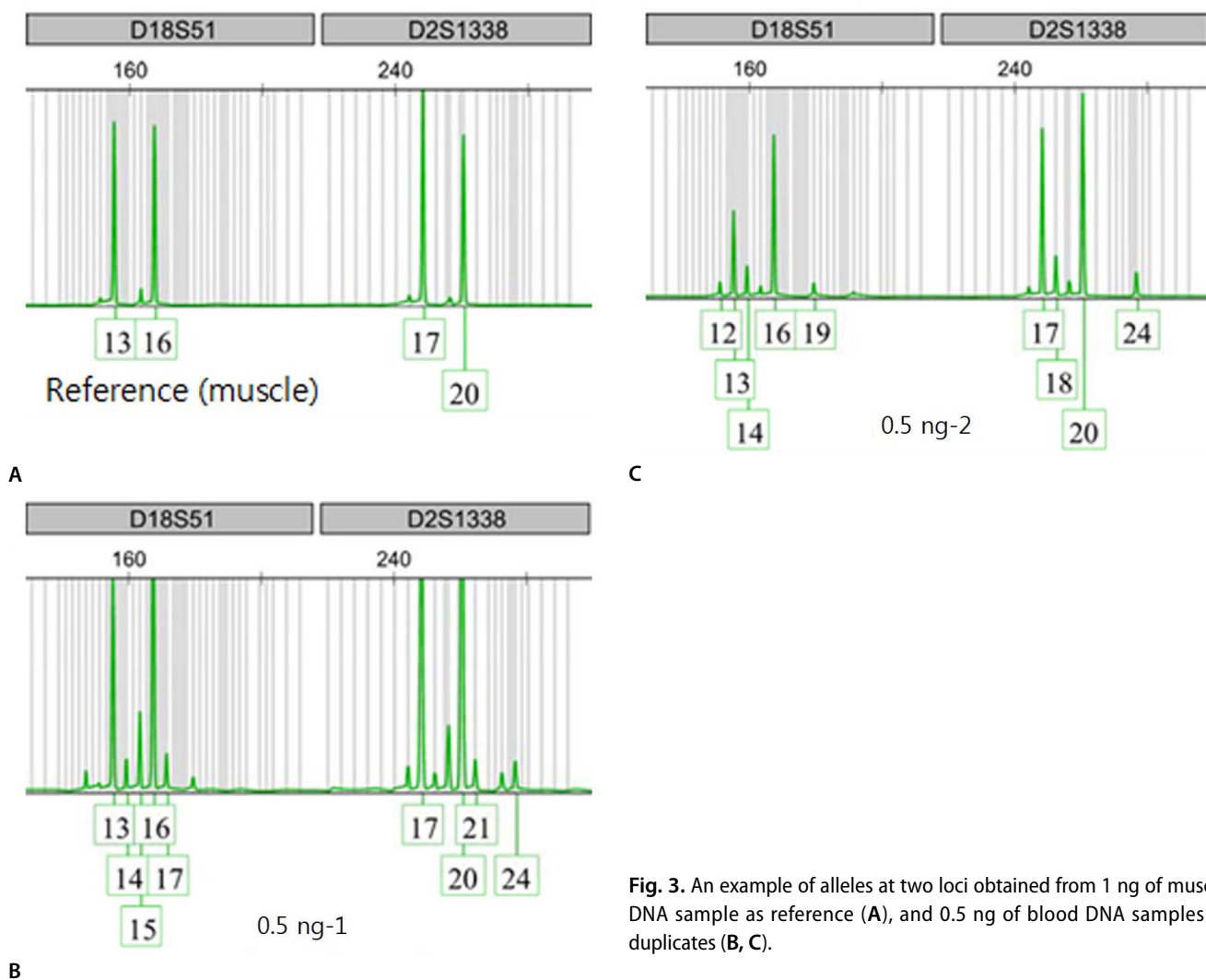


Fig. 3. An example of alleles at two loci obtained from 1 ng of muscle DNA sample as reference (A), and 0.5 ng of blood DNA samples in duplicates (B, C).

post-transfusion that can influence STR profiling, for instance, the amount and types of blood components transfused, the time period between the last transfusion and sample collection, it is recommended that one should be careful when collecting reference samples. Because studies have shown that a recipient may show mixed profiles other than blood, additional hair sampling may be necessary for confirmation in certain cases [3]. This study emphasizes that the reference site must be chosen carefully, and a site from parenchymal organs or tissues may be ideal for reference purposes.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2014M3A9E1069989).

References

- Wenk RE, Chiafari PA. DNA typing of recipient blood after massive transfusion. *Transfusion* 1997;37:1108-10.
- Graham EA, Tsokos M, Rutty GN. Can post-mortem blood be used for DNA profiling after peri-mortem blood transfusion? *Int J Legal Med* 2007;121:18-23.
- Dauber EM, Dorner G, Mitterbauer M, et al. Discrepant results of samples taken from different tissues of a single individual. *Int Congr Ser* 2004;1261:48-9.

4. Vietor HE, Hallensleben E, van Bree SP, et al. Survival of donor cells 25 years after intrauterine transfusion. *Blood* 2000;95:2709-14.
5. Gong MN, Sai Y, Zhou W, et al. Genotyping patients with recent blood transfusions. *Epidemiology* 2003;14:744-7.
6. Lee TH, Montalvo L, Chrebtow V, et al. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. *Transfusion* 2001;41:276-82.
7. Schechter GP, Whang-Peng J, McFarland W. Circulation of donor lymphocytes after blood transfusion in man. *Blood* 1977;49:651-6.
8. Utter GH, Reed WF, Lee TH, et al. Transfusion-associated microchimerism. *Vox Sang* 2007;93:188-95.
9. Jung JY, Park SH, Park SW, et al. Mixed DNA profiles in transfused deceased case. *Korean J Sci Crim Invest* 2015;9:206-12.