DNA profiling in peripheral blood, buccal swabs, hair follicles and semen from a patient following allogeneic hematopoietic stem cells transplantation

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Abstract. Allogeneic peripheral blood stem cells transplantation (allo-PBSCT) or allogeneic bone marrow transplantation (allo-BMT) have been widely used to treat patients exhibiting certain severe illnesses. However, previous studies have shown that the biological materials of allo-PBSCT or allo-BMT recipients may not constitute credible materials for personal identification. In the present study, four types of commonly used samples were collected from a male individual following gender-matched allo-BMT. Autosomal short tandem repeat (STR) and Y-STR markers analysis, based on polymerase chain reaction, were used to evaluate the chimerism status. The results showed that the blood sample were all donor type, the buccal swab sample were mixed chimerism, and the sperm and hair follicle samples maintained a recipient origin of 100%. In conclusion, identical results were obtained by the two methods and it was confirmed that DNA extracted from hair follicles and sperm can be used as a reference for the pre-transplant genotype DNA profile of the recipient in the gender-match allo-BMT or -PBSCT.

Introduction

Allogeneic bone marrow transplantation (allo-BMT) or allogeneic peripheral blood stem cells transplantation (allo-PBSCT) are used commonly as an effective treatment for patients with various hematological malignancies and non-malignant diseases involving bone marrow elements, including severe aplastic anemia, severe combined immunodeficiency states, lymphoma, leukemia and solid tumors (1). A number of studies (2-5) have indicated that the bone marrow stem cells have a significant differentiation capacity as they can differentiate into neural, bone, muscular, cartilage, liver, gut, alveolar, buccal, epidermal or endothelial cells. The study by Tran *et al* (6) confirmed that cells derived from bone marrow migrate to the cheek and differentiate into epithelial cells.

As aforementioned, biological samples from patients who have undergone successful allo-BMT or -PBSCT may not constitute appropriate materials for individual identification or paternity testing.

The aim of the present study was to exploit the sequence-specific primer-based polymerase chain reaction (PCR)-amplified short tandem repeat (STR) analysis method to verify whether blood, hair follicles, seminal stains and buccal swabs are appropriate for personal identification or paternity testing for patients who have undergone a successful allo-PBSCT or -BMT.

Case report

Patient. A 34-year-old male that underwent allo-BMT for chronic myelogenous leukemia requested a paternity test in the Department of Forensic Genetics at the Sichuan University (Sichuan, China). Due to the unique nature of the case, the patient was invited to participate in the chimerism study and informed consent was obtained. As to the current analysis phase, it was 13 years since the patient underwent a successful allo-BMT from an unrelated male donor. There was no evidence of chronic graft-versus-host disease involving the oral mucosa when sampling from the patient.

The post-transplant peripheral blood was collected in EDTA-coated tubes and stored at -20˚C. Buccal swabs with oral epithelial cells were collected from both sides of the oral cavity. Prior to obtaining the buccal swabs, adequate mouthwash was required. The hair follicles were collected from different areas of the scalp. A seminal stain with sperm cells was collected in sterilized gauze and preserved in a dry environment. The oral and hair samples were maintained at room temperature until DNA extraction was performed. The patient provided the pre-transplant blood-stain sample.

DNA extraction. Genomic DNA from all the investigated materials was extracted using Chelex-100 (7). To optimize

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the extraction procedure, one 0.5-cm^2 portion was cut from the buccal swabs, the gauze containing the sperm and the blood stain, respectively. For extraction of the hair samples, a 0.5-cm hair fragment, including the root, was cut. Blood (10 μl), processed buccal swab, seminal stain, blood stain and hair follicle samples were transferred into a sterilized 200-μl eppendorf tube containing 70 μl 5% Chelex-100 and 5 μl proteinase K (10 mg/ml). The samples were incubated for 90 min at 56˚C, and subsequently heated at 98˚C for 10 min and centrifuged at 13,000 x g for 5 min. The DNA concentration of each sample was evaluated with the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

DNA profiling. The 22 autosomal unlinked loci and an additional gender-determining marker, amelogenin, were amplified with the reagents contained in the Expressmarker 22 STR loci direct PCR amplification kit (AGCU Biotechnology Co., Wuxi, China) and analyzed in the GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR products were detected on an ABI PRISM® 3130 Genetic Anaylzer (Applied Biosystems) according to the manufacturer's instructions (8). The electrophoresis data were analyzed using GeneMapper IDX software (Applied Biosystems).

Profiling of the 22 STR loci along the Y-chromosome was carried out using the Prototype PowerPlex Y23 System kit (Promega Corp., Madison, WI, USA) following the manufacturer's instructions (9). Subsequently, allele detection was performed with GeneMapper ID v3.2 software (Applied Biosystems).

Results and Discussion

DNA profiling in all the samples are shown in Table I. In the study, the autosomal and Y-STR markers analyses of blood were 100% donor type. The DNA profiling obtained from the hair follicle and sperm samples were 100% recipient type. However, in the buccal swab samples, the result showed a manifest mixture of two sources of DNA (58% donor). There was no evidence of contamination in any sample.

In forensic science, DNA material recovered from a crime scene has become standard forensic evidence for investigating and solving a wide spectrum of crimes, including murder and rape (10). Forensic scientists use DNA from various biological samples found at crime scenes to identify the criminal suspect by DNA profiling. Different types of DNA material have the ability to undergo a DNA match based on a hypothesis that all the cells in the human body have a consistent DNA profile (11).

However, in certain unique cases, this hypothesis is invalid. For any subject who has undergone a successful allo-BMT or -PBSCT for various severe diseases, blood is not suitable for personal identification due to a conversion to complete donor type (12). When blood cells become the donor genotype, it was previously believed that the other cells remain the same as the recipient origin. However, previous studies (2-5) indicate that stem cells can differentiate into a number of cell types *in vivo*. A previous study identified that male bone marrow-derived cells differentiate into epithelial cells and migrate into the cheek (6). Coincidentally, certain studies have detected donor-derived cells in buccal swabs (5,12,13). In a study of the most frequently used

Table I. Results of the STR profiling.

STR loci	Buccal swab	Blood	Hair	Sperm	Pre-transplant
Autosomal					
D3S1358	15,16,17	15	16,17	16,17	16,17
D13S317	11,12	11,12	11,12	11,12	11,12
D7S820	10,11	11	10,11	10,11	10,11
D16S539	9,11,12	11,12	9	9	9
Penta E	12,17,20	17,20	12,20	12,20	12,20
D2S441	11,11.3	11	11.3	11.3	11.3
TPOX	8,9	8,9	8,9	8,9	8,9
TH01	7,9.3	7	7,9.3	7,9.3	7,9.3
D2S1338	17,19,20	17,19	19,20	19,20	19,20
CSF1PO	11,12	11,12	12	12	12
Penta D	9,12	9,12	9	9	9
D10S1248	14,15	14,15	15	15	15
D19S433	13,14,15.2	14	13,15.2	13,15.2	13,15.2
Vwa	14,19	14	19	19	19
D21S11	32,32.2,33	32,33	32.2	32.2	32.2
D18S51	14,15,16	15,16	14,15	14,15	14,15
D6S1043	12,13,17,19	12,19	13,17	13,17	13,17
D8S1179	11,13,17	13,17	11,13	11,13	11,13
D5S818	9,10,11,13	10,11	9,13	9,13	9,13
D12S391	17,18,19,24	18,24	17,19	17,19	17,19
FGA	22,23,25	23,25	22	22	22
Amelogenin	X,Y	X,Y	X,Y	X,Y	X,Y
Y					
DYS576	17,23	17	23	23	23
DYS389I	13,14	14	13	13	13
DYS448	17,20	17	20	20	20
DYS389 II	29	29	29	29	29
DYS19	14,15	15	14	14	14
DYS391	10	10	10	10	10
DYS481	23,25	25	23	23	23
DYS549	12	12	12	12	12
DYS533	11	11	11	11	11
DYS438	10,11	10	11	11	11
DYS437	14,15	14	15	15	15
DYS570	18	18	18	18	18
DYS635	20,21	21	20	20	20
DYS390	24	24	24	24	24
DYS439	12,13	12	13	13	13
DYS392	13,14	13	14	14	14
DYS643	10,11	11	10	10	10
DYS393	12,14	14	12	12	12
DYS458	15,17	15	17	17	17
DYS385	12,13,18	12,18	13,18	13,18	13,18
DYS456	15,17	17	15	15	15
YGATAH4	12	12	12	12	12

STR, short tandem repeat.

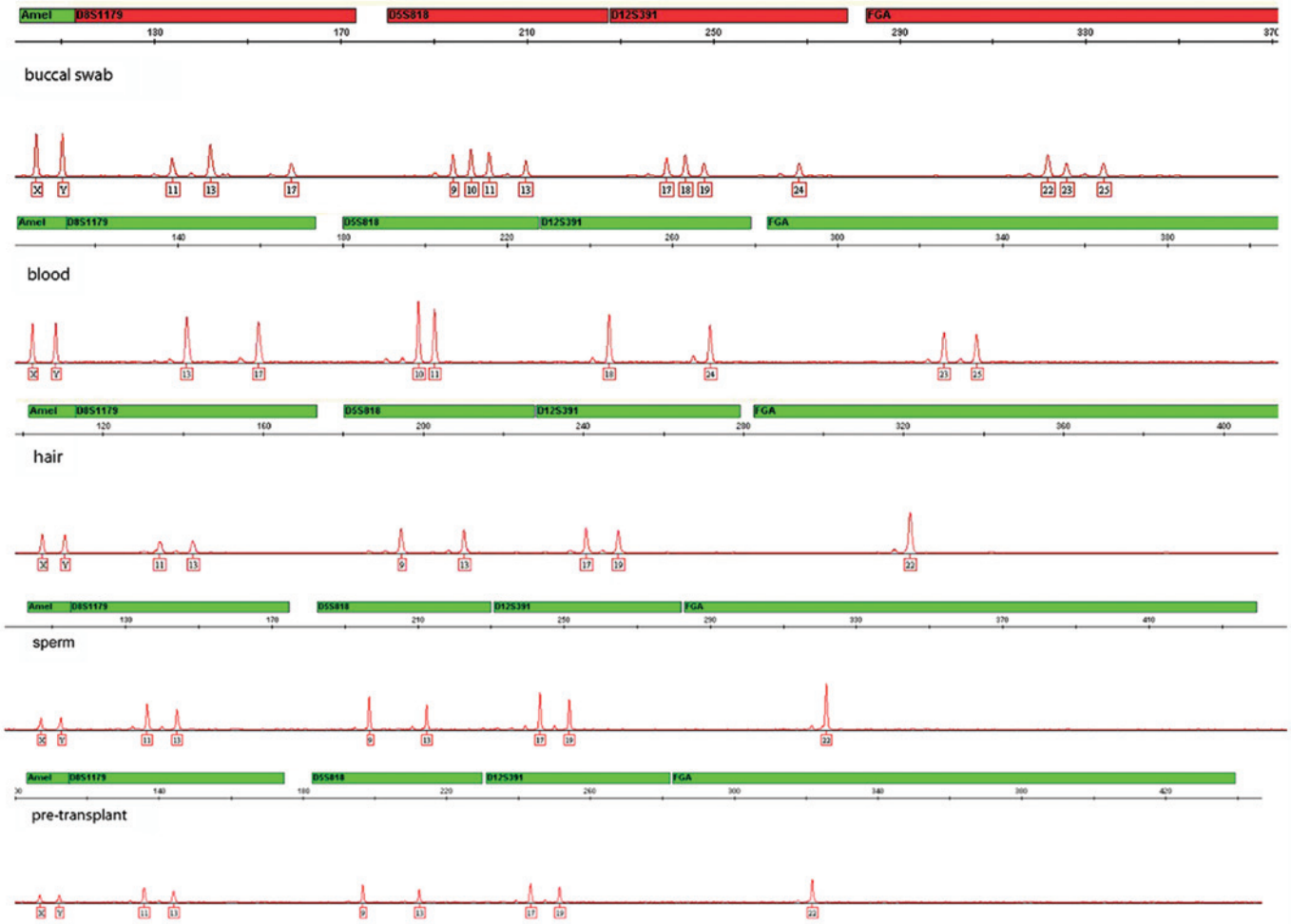

Figure 1. Representative results of autosomal STR analysis from all the samples (ROX dye-labeled markers). STR, short tandem repeat.

materials in forensic investigations, the donor-derived cells were detected not only in blood and buccal swabs, but also in fingernails (14). As with fingernails, the hair has the same ectodermal origin. However, whether the hair follicles are the only reliable source of biological material remains disputable. Semen is often used as critical evidence in forensic cases, and determining whether the genotype of semen is changed is essential. Mammalian spermatogenesis is a complicated progress; a mature spermatozoa is differentiated from the spermatogonial stem cells (15). There is no evidence that bone marrow stem cells are able to differentiate into sperm cells.

Regarding this, autosomal STR-PCR analysis is frequently applied to evaluate the origin of cells following allo-BMT or -PBSCT. In the present study, the analysis of autosomal STR markers was performed in the origin materials from the recipient using the Expressmarker 22 STR loci direct PCR amplification kit. The Expressmarker 22 system is a PCR-based amplification kit that targets a panel of 21 autosomal unlinked STR loci, which are D3S1358, D13S317, D7S820, D16S539, Penta E, D2S441 (FAM dye-labeled), TPOX, TH01, D2S1338, CSF1PO, Penta D, D10S1248 (HEX dye-labeled), D19S433, vWA, D21S11, D18S51, D6S1043 (TAMRA dye-labeled), D8S1179, D5S818, D12S391 and FGA (ROX dye-labeled), and an additional gender-determining marker Amelogenin (ROX dye-labeled), which was used to distinguish the X and Y chromosomes. These autosomal STR analysis results are in accordance with earlier study (12), as the hair follicles of the recipient following successful allo-BMT had a completely autologous profile. The genotypes that were detected in the blood samples were derived entirely from the donor, and the genotypes detected in the buccal swab samples were mixed chimerism. The results of autosomal STR analysis from the sperm sample were identical to the results from the hair follicles. These observations indicate that the DNA profiling detected from the hair follicle and sperm samples were 100% recipient type. In the buccal swab samples, the results showed two apparent sources of DNA (the mixture of the recipient and donor patterns). According to the standardized calculation procedure of chimerism values (16), the peak area from the donor accounted for 58% of the total signal. The results are presented in Fig. 1 (ROX dye-labeled markers).

From the autosomal STR markers analysis, the results supported the previous conclusion that considered hair follicle and sperm as the only reliable source of biological material for forensic personal identification. In the present study, the male patient received HLA-matched transplantation from an unrelated male donor 13 years ago. Based on the gender-matched engraftment, Y-STR multiplex analysis was performed using

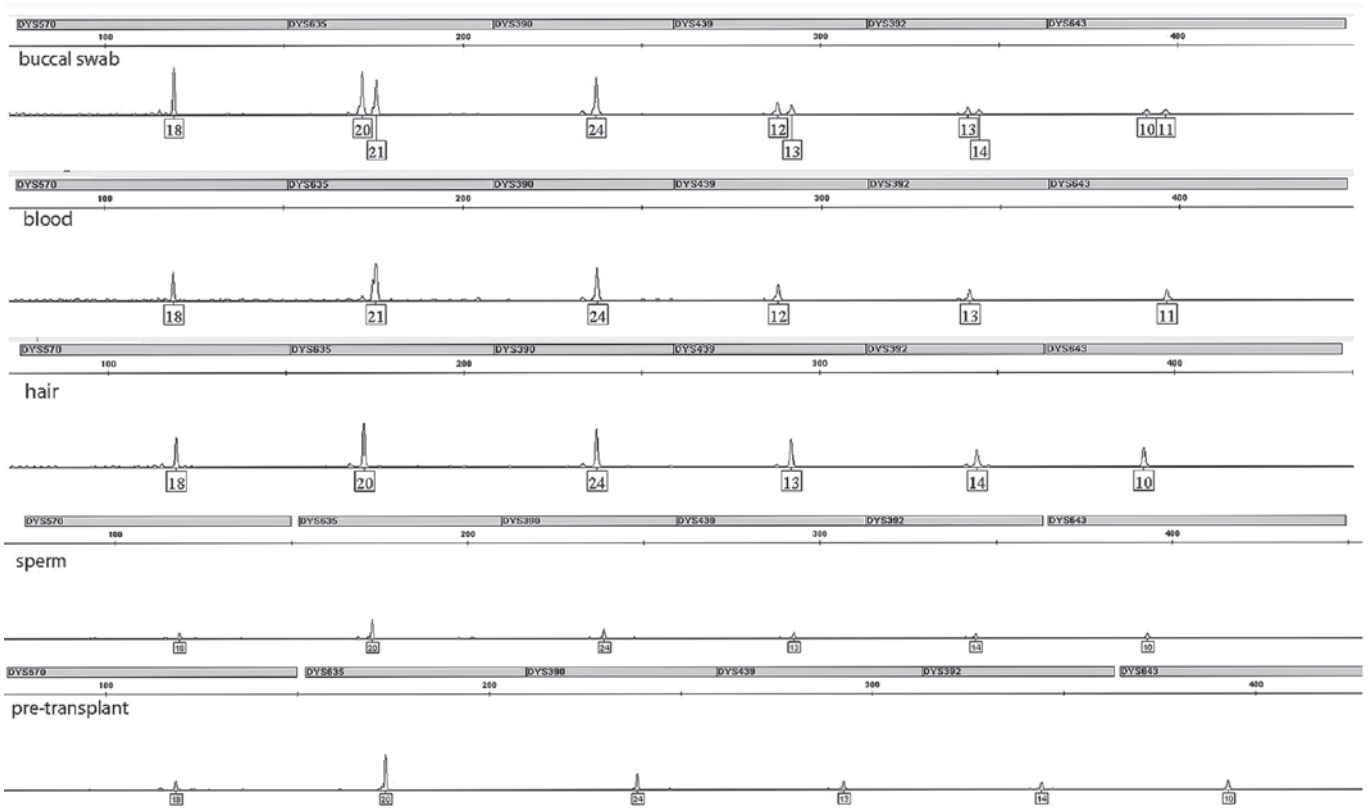

Figure 2. Representative results of Y-STR analysis from all the samples (electrophoretic profiles of DYS570, DYS635, DYS390, DYS439, DYS392 and DYS643 loci). STR, short tandem repeat.

the Prototype PowerPlex Y23 System as the subsequent step. The PowerPlex Y23 System was able to detect the minor alleles in male/male DNA admixtures (17). According to the manufacturer's instructions, all the samples were obtained and demonstrated successful typing. Fig. 2 shows the representative electrophoretic profiles of the DYS570, DYS635, DYS390, DYS439, DYS392 and DYS643 loci. The profiles indicated a mixture of the recipient and donor patterns in the buccal swab, the full donor pattern in the blood sample, and the full recipient profile in the hair follicle and sperm samples. However, previous studies revealed the Y-STR haplotype from the donor in all the post-transplant blood and buccal swab samples, and even in post-transplant hair follicle samples obtained from female patients following gender-mismatched engraftment (11,13). A previous study has already demonstrated that an extremely small quantity of male material in mixed male/female DNA samples can be detected by analysis with the Y-STR system (18). The main reason behind this is due to the lack of Y-STR haplotype in female DNA, which may not affect the specificity of the Y-STR system. Notably however, the presence of the minor contribution of Y-STRs may not be revealed when using the identical Y-STR system in male/male admixture conditions.

In the present study, the results have to be considered following the DNA analysis of samples, which are the most commonly used sources for forensic and clinical genetic investigations. As adult stem cells differentiate into blood and epithelial cells, buccal swab may not serve as an ideal source of pre-transplant DNA profiling of the recipient origin. The genotypes that were detected from blood samples were completely derived from the donor. However, due to the highly-conserved process of spermatogenesis, as shown in the results, the DNA typing analysis from the sperm sample represents the recipient genotype prior to transplantation. Regarding the hair follicle sample, it has been shown that the DNA profiling remains completely the same as the recipient origin.

In conclusion, the analysis based on autosomal and Y-chromosome STR markers, which was performed in a male recipient with gender-matched allo-BMT, showed that identical results were obtained using the two methods. The investigation confirmed that the DNA extracted from hair follicles and sperm can be used as a reference for the pre-transplant genotype DNA profile of the recipient in the gender-match allo-BMT or -PBSCT. However, further studies should be conducted to verify these findings in larger samples.

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