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Brief Communication

Applicability of the parentally imprinted allele (PIA) typing of a VNTR upstream the *H19* gene to forensic samples of different tissues

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Abstract

The parentally imprinted allele (PIA) typing that we have recently developed determines parental alleles at a VNTR locus in the differentially methylated region upstream of the human *H19* gene. The usefulness of this typing was demonstrated by its application to blood samples in paternity cases. However, its applicability to other tissue DNA remains to be tested. DNA samples from fifteen different postmortem tissues such as cerebrum, skeletal muscle and skin were examined, all of which were obtained from three autopsy cases 2–11 h after death. DNA was digested with a methylation-sensitive *Hha*I enzyme and diluted solutions of the digests were subjected to the first PCR amplification, providing amplification of only the paternal *H19* methylated allele. Subsequent VNTR typing was carried out for the amplified products to determine which allele was of paternal origin. No tissue-dependent difference was observed and all the samples examined, though degraded, were successfully used for determining the paternal allele. These results substantiate the usefulness of PIA typing in forensic examinations. Its application to two identity cases, a burned male body and a male body with adipocere formation, was also shown.

Keywords: Parentally imprinted allele (PIA) typing; Human *H19* gene; DNA methylation; VNTR; Forensic tissue samples

1. Introduction

VNTR and STR have been widely employed as useful markers for forensic examinations, such as in the case of personal identification and paternity disputes. Conventional DNA typing with those markers is, however, unable to distinguish between two alleles transmitted from one's parents. We have recently developed a novel method consisting of conventional genotyping and parentally imprinted allele (PIA) typing of a VNTR locus 5′ to the *H19* imprinted gene [1]. PIA typing can determine the paternal allele by distinguishing allelic differences in the DNA methylation status, and the usefulness of this typing was demonstrated by its applicability to blood samples in paternity cases. However, blood samples are rarely provided in forensic examinations, especially in the identification of charred fire victims, body parts and putrefied corpses. Therefore, this typing's applicability to other tissue DNA should also be tested. One drawback of PIA typing may be in distinguishing allelic differences in the DNA methylation. It is known that some imprinted genes, including the Wilms' tumor gene and the insulin-like growth factor type 2 gene, show differential methylation confined to only certain tissues or to certain stages of development [2–6]. Therefore, there may be tissues showing no difference in methylation within the probe region of the PIA typing.

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In this study we tested the applicability of this protocol to DNA from fifteen different postmortem tissues from autopsy cases. Personal identity cases were also examined. The results demonstrate that all of the DNA samples, though degraded, could be used for determining the paternal allele using PIA typing.

2. Materials and methods

2.1. DNA samples

Fifteen kinds of postmortem tissue specimens were obtained from three autopsy cases (C_1, C_2 and C_3). In the C_1 (approximately four hours after death), 12 samples were obtained from blood, cerebrum, cerebellum, liver, kidney, spleen, skeletal muscle, heart (myocardial apex, atrioventricular muscle), uterus, ovary and skin. In the C_2 (approximately eleven hours after death), 8 samples were taken from blood, cerebrum, cerebellum, heart (myocardial apex, atrioventricular muscle), prostate, testis and aorta. In the C_3 (approximately two hours after death), 8 samples were collected from blood, liver, kidney, spleen, thymus, skeletal muscle, testis and skin. In two identity cases (X_1 and X_2), DNA sample was obtained from thigh muscle in the X_1 and bone marrow in the X_2. DNAs of the putative families were isolated from their blood with written consent. Genomic DNA was extracted by proteinase K digestion and phenol/ chloroform extraction from these tissues.

2.2. Genotyping and PIA typing of the VNTR

The genotyping was carried out using a set of primers (5′-CCATTACTTATATCTGGGTAGGTC-3′ (A) and 5′-GTCATCTAGATAGACACATGAGC-3′ (B)), which produces about a 400–600 bp fragment, as described in our previous paper [1].

The PIA typing was also performed according to the method presented previously. Approximately 500 ng of *Hha*I-digested DNAs were diluted to several concentrations. One μl of each dilution was amplified in a 10 μl reaction using Expand Long Template PCR System™ (Roche, Basel, Switzerland) and 10 pmol of each primer according to the recommendation of the manufacturer. The primers used were 5′-GGGTCATTATAGACGCAATCG-3′ (C) and 5′-AGAACCTGTTGGGCGGTTAGA-3′ (D). The cycling profile was 95 °C for 2 min, 12 cycles of 98 °C for 20 s, 63 °C for 30 s, 68 °C for 90 s and 27 cycles of 98 °C for 20 s, 63 °C for 30 s, 68 °C for 90 s extending the elongation of 20 s for each cycle, and then extension of 68 °C for 7 min. The amplified products were separated by polyacrylamide gel electrophoresis in order to check whether they were generated. One μl of the PCR mixture from the most diluted sample providing a 1.7 kb-fragment was subjected to the VNTR typing as a template without purification procedure.

3. Results and discussion

3.1. Application of the PIA typing to tissue DNA samples

In this study, we carried out the PIA typing avoiding the gel purification of the 1.7 kb fragment amplified with the C and D primers as described in Section 2. The modified method gave the same result as the PIA typing protocol reported previously [1]. This made the PIA typing more simple and less time-consuming.

Human DNA was extracted from fifteen different postmortem tissues: cerebrum, cerebellum, liver, kidney, spleen, skeletal muscle, heart (myocardial apex, atrioventricular muscle), uterus, ovary, skin, prostate, testis, aorta, thymus, and blood. Those tissues were obtained from three autopsy cases (C_1, C_2 and C_3) 2–11 h after death. The lower panel of Fig. 1 shows the size of their DNA. In medico-legal death investigations, genomic DNA recovered from biological materials is often degraded extensively. As predicted, DNA samples, except for blood DNA shown in lanes 1 and 21, were partially degraded. The DNA sample (lane 14) obtained from the cerebrum in C_2 was extremely degraded. The others contained high-molecular-weight DNA of more than 20 kb pairs in length. Those DNA samples were examined to test whether or not the present protocol can provide DNA typing of the parentally imprinted allele.

The upper panels in Fig. 1 show the PIA typing results. In C_1, its genotype consisted of alleles 6/9 as shown in lane G. The allele detected by PIA typing using blood DNA (lane 1) was allele 6, indicating that the band was of paternal allele. All other tissue samples (lanes 2–12) of this case gave the same allele 6. A faint band of the maternally-derived allele due to incomplete digestion with *Hha*I was observed in this typing result. Other kinds of postmortem tissues such as prostate, testis, aorta and thymus were examined in C_2 and C_3. The alleles detected with PIA typing for these tissues were ones derived from the paternal alleles; in C_2 the allele was 7 (lane 13) and in C_3 the allele was 9 (lane 21). These results indicated that the methylation status of the *H19* region in those tissue DNA samples was the same as that of blood DNA obtained.

Jinno et al. identified sites in the *H19* upstream repeat region characteristic of a paternal-specific methylation imprinting [7,8]. This region is different from the region used in this study and located −2.0 to −3.3 kb upstream from the transcription start site. Frevel et al. found an altered methylation pattern in Wilms tumors, however, that was biallelically methylated [9]. In the −6.5 to −9.0 kb region examined in this study, no such tissue-specific or tumor-specific difference was observed in the fifteen different tissue