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Validating the consistency of cSNPs analysis results between DNA and RNA using SNaPshot method

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ABSTRACT

The use of coding region single nucleotide polymorphisms (cSNPs) was recently proposed as a potential method for individual identification because it allows mRNA profiling and DNA typing to be performed concurrently. Nevertheless, availability of this approach still needs some further validation in different aspect. In this study, we have initially selected several SNP loci located in mRNA molecules that were confirmed to be highly expressed in blood. Both coding regions (CDRs) and untranslated regions (UTRs) were taken into consideration during the screening. Genomic DNA (gDNA) and total RNA from venous blood samples were isolated, followed by the synthesis of first-strand complementary DNA (cDNA) using purified RNA samples. Subsequently, the genotypes of these SNPs were respectively determined with gDNA and cDNA by using SNaPshot method. The PCR primers for cDNA were designed to span an intron in order to ensure that the amplification products were not due to the presence of potential DNA contamination. In summary, our study revealed a high consistency of cSNP analysis results between DNA and RNA on capillary electrophoresis platform, which highlighted the potential use of cSNP in forensic investigation.

1. Introduction

Single nucleotide polymorphism (SNP) is a kind of DNA sequence polymorphism extensively existed in human genes. Due to its relatively large amount, low mutation rate and short amplicon required for detection, it has aroused an increasing attention in marker selection related forensic research [1]. Through the progress of human genomics related research, a large number of SNPs have been identified on coding genes [2]. Haas et al. [3] proposed that coding region SNPs (cSNPs) have the potential to be applied in origin identification of body fluids or tissues, for it can establish a unique association between a specific DNA profile and a specific body fluid type. However, the consistency of cSNP analysis results between DNA and RNA was not critically discussed. The purpose of this study was to investigate the consistency of cSNP analysis results between DNA and RNA on capillary electrophoresis platform. Several SNP loci located in mRNA molecules that were confirmed to be highly expressed in blood were selected and then the genotypes of these SNPs were respectively determined with genomic DNA (gDNA) and first-strand complementary DNA (cDNA) by using SNaPshot method [4].

2. Materials and methods

2.1. Marker selection

Our selected cSNPs are located on mRNA molecules that specifically expressed in blood [5,6]. Both coding regions (CDRs) and untranslated regions (UTRs) were taken into consideration during the screening. PCR primers for gDNA and cDNA were respectively designed. The cDNA primers were designed to span at least an intron to prevent the interference of potential DNA contamination. Primers used in single-base extension (SBE) were the same for gDNA and cDNA amplicons.

2.2. Sample preparation

20 venous blood samples were collected from healthy volunteers with informed consent. Genomic DNA were extracted using the PureLink Genomic DNA Kits (Thermo Fisher Scientific). Total RNA isolation and purification were performed as previously described [7]. First-strand cDNA was then synthesized using purified RNA samples and SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific).

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Table 1
Basic information and genotyping results about selected cSNP markers.

SNP	Gene	Region (Consequence)	SBE strand	Alleles (FWD)	Number of Missing Peaks	Amplicon Length (cDNA)
rs3746731	CD93	CDR (missense)	FWD	AG	2	379bp
rs504574	ANK1	CDR (synonymous)	FWD	CG	0	200bp
rs229592	SPTB	CDR (synonymous)	FWD	AG	0	164bp
rs2072081	SLC4A1	3' UTR	REV	GT	4	464bp
rs17121881	AMICA1	CDR (missense)	FWD	AT	1	241bp
rs3753058	CD3G	CDR (missense)	REV	GT	0	123bp

2.3. PCR and SNaPshot

The PCR reactions were performed using the Multiplex PCR Master Mix (QIAGEN). Specificity of the amplification product was then verified by polyacrylamide gel electrophoresis. SBE reactions were performed using the SNaPshot Multiplex kit (Thermo Fisher Scientific). The fluorescence-labeled extension products were separated by capillary electrophoresis on a 3130 Genetic Analyzer (Thermo Fisher Scientific).

3. Results and discussion

All 6 SNPs were successfully detected in gDNA samples, while some loci were missing in a few cDNA samples, which might be due to mRNA degradation. In general, loci with longer amplicon lengths were more prone to have missing peaks (Table 1). Although we have tried to minimize the amplicon length, the problem cannot be solved because some cSNPs are far from adjacent introns. Representative capillary electrophoresis genotyping results of one blood sample and the negative control were shown in Fig. 1, in which SNPs and detected alleles are labelled. All loci are successfully detected in this sample, while the negative control showed no valid peak. As for other samples tested, the exist genotyping results in gDNA and cDNA samples were consistent for the 6 SNPs despite those missing peaks. Overall, the peak heights were more balanced in gDNA samples compared to that in cDNA samples. This might be due to the fact that the expression level of different mRNA has an intrinsic difference. Besides, the weak fluorescence signals of thymine might interference the genotyping results in some heterozygotes. Therefore, when dealing with these heterozygotes, the genotyping results need to be carefully inspected.

4. Conclusion

In this study, we have selected several SNPs located in mRNA molecules that were confirmed to be highly expressed in blood. GDNA and total RNA from venous blood samples were isolated, followed by the synthesis of first-strand cDNA. Subsequently, the genotypes of these SNPs were respectively determined with gDNA and cDNA by using SNaPshot method to validate their consistency. Our experiment showed that despite some missing peaks in cDNA samples, the exist genotyping results in gDNA and cDNA samples were consistent for the 6 SNPs. Therefore, cSNPs have the potential to be applied in practical forensic investigation.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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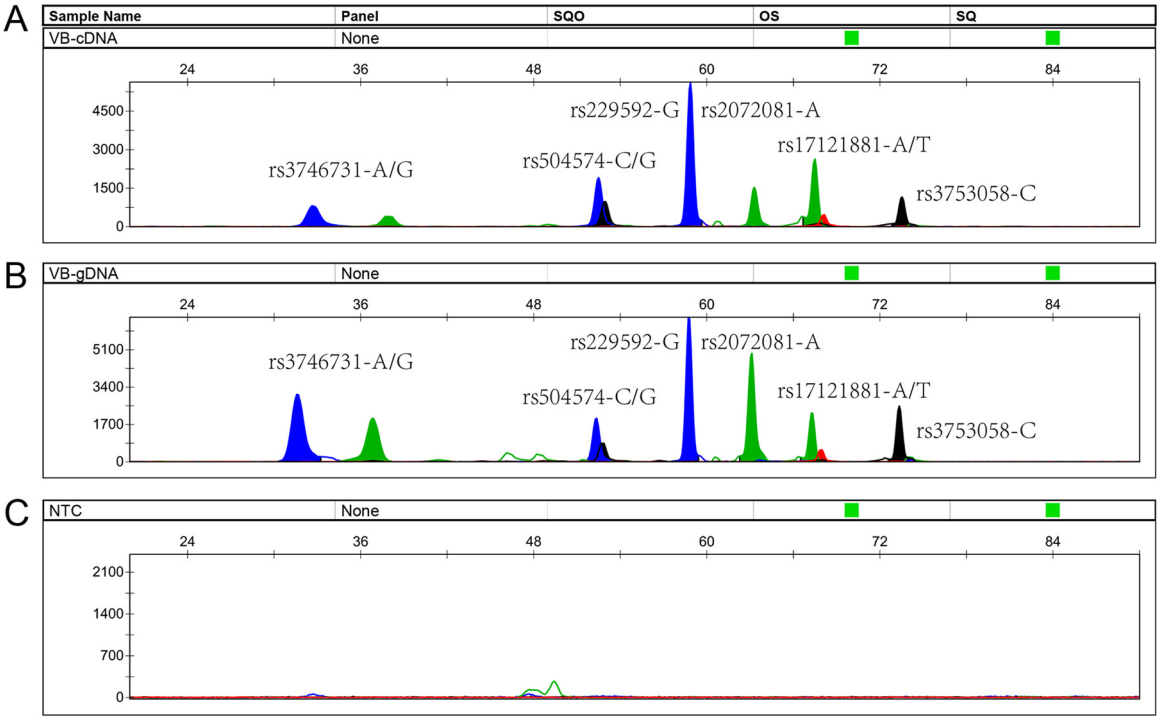

Fig. 1. Capillary electrophoresis genotyping results. (A) Venous blood cDNA. (B) Venous blood gDNA. (C) Negative control.

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