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Validation of SNPs as markers for individual identification

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Abstract

Nowadays, the DNA markers used for individual identification in forensic science are on the one hand based on repeat sequences (STR and VNTR) on genomic DNA, and on the other hand based on the mitochondrial hypervariable regions 1 and 2. An alternative to these classical markers could be single-nucleotide polymorphisms (SNPs), as they present advantages over them. Even if most SNPs are biallelic and, therefore, of limited discriminatory value, a vast number of those loci exist and are being catalogued at a rapid rate. Furthermore, those markers have a sex-unspecific transmission, are less subjects to mutations as they can be found in coding regions of DNA and their detection and analysis are relatively easy to achieve. Finally, the analysed DNA sequence is much shorter than the one used for STR research, and SNP profiles could have an application in anthropology and legal medicine where samples are old and often degraded. The aim of this study is the selection of 11 informative SNPs, i.e., conserved, phenotypically not expressed and represented at high frequencies in populations, their analysis by sequencing and the confirmation of their mendelian transmission. The principle of this method is compatible with different SNP analysis platforms (i.e., pyrosequencing, MALDI-TOF MS, SnaPShot, etc.) and could allow the achievement of a genetic profile specific for an individual in an extremely short time and at low costs. The number of SNPs present on human genome being vast, the list of the selected ones could be even longer.

Keywords: Single-nucleotide polymorphism (SNP); Forensic; Individual specific genetic profile

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1. Introduction

The polymorphisms of the human genome provide excellent means for discrimination between individuals. Single-nucleotide variations (SNPs) have the potential to revolutionize human genetic studies with utility as genetic markers in linkage and association studies of complex genetic traits, population and evolutionary genetics, as well as positional cloning and physical mapping.

Despite the fact they are biallelic and, therefore, of limited discriminatory value, SNPs offer several attractive features as genetic markers, including the following.

Autosomal SNPs are the most frequently found DNA sequence variations in the human genome, and have a low mutation rate, as they can be found in coding regions of DNA. They have a highly stable inheritance unlike repeat polymorphisms, which are detrimental to inheritance analysis and a sex-unspecific transmission unlike mtDNA or the Y chromosome. Most SNPs must be neutral, even though a proportion contributes to disease susceptibility and resistance. Finally, their detection and analysis of SNPs are relatively easy to achieve, as they do not require comparative allele sizing.

In the case of forensic and anthropology applications, the particular advantage of SNPs over other genetic markers is in the length of the studied DNA region. Indeed, the DNA sequence needed being much shorter, SNP profiles could have an application in the identification of individuals for forensic medicine and anthropology studies where often only small or degraded samples are available for DNA analysis. Furthermore, it has been demonstrated that relatively small arrays of approximatively 50 loci are comparable to existing STR multiplexes provided that they are chosen so that the alleles range in proportion between 0.2 and 0.8 [1].

The aim of the present study is the development of a new method for forensic DNA typing. Therefore, a panel of informative biallelic markers, i.e., phenotypically not expressed but conserved and represented at high frequencies, is analysed by sequencing in order to establish individual specific profiles.

2. Materials and methods

For this study, most of the SNPs are located in coding regions where they are least subject to variations. However, it has been checked they were reported as not, or at least by now not reported as, linked to a genetically caused pathology and that they were not used as markers for linkage disequilibri studies.

The markers allelic frequencies were also taken in consideration in order for the most represented allele of each SNP to have a maximum frequency of 65%.

Nine individuals belonging to three unrelated families (father, mother and son) were genotyped for all the 11 selected markers. DNA was extracted from blood samples.

The sequencing reaction was accomplished thanks to the ABI Prism® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems). Raw sequencing data are obtained thanks to the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) and interpreted by the DNA Sequencing Analysis Software (Applied Biosystems). After

Table 1
Results obtained from the analysis of 11 biallelic markers in nine individuals belonging to three unrelated caucasian families

	Gene or sequence containing the target SNP										
	C8α	WI 867	WI 1126	WI 1888	WI 1732	WI 681	WI 1349	WI 2054	WI 2032	PCI	C1r
Father A	C/C	G/A	T/T	G/G	T/T	G/G	C/A	C/C	C/G	C/T	C/C
Mother A	C/C	G/A	T/C	A/A	C/C	A/A	C/C	C/T	C/G	C/T	C/T
Son A	C/C	G/A	T/T	A/G	T/C	A/G	C/C	C/C	C/C	C/T	C/T
Father B	A/A	G/G	T/C	A/A	T/C	A/G	A/A	T/T	C/G	C/C	C/T
Mother B	C/C	A/A	C/C	A/A	C/C	A/G	C/A	T/T	C/C	C/C	C/T
Son B	A/C	G/A	C/C	A/A	T/C	A/G	C/A	T/T	C/G	C/C	C/T
Father C	A/C	G/G	T/T	A/G	T/C	G/G	C/C	T/T	C/G	C/T	C/C
Mother C	A/A	A/A	T/C	A/G	T/C	A/G	C/A	C/C	C/G	C/C	C/T
Son C	A/C	G/A	T/C	A/G	T/C	G/G	C/C	C/T	C/C	C/C	C/T
Ref.	[2]	[3,4]	[3,4]	[3,4]	[3,4]	[3,4]	[3,4]	[3,4]	[3,4]	[5]	[6]

conversion of the raw data the sequences were analysed using the Sequence Navigator Software (Applied Biosystems).

3. Results

As can be seen in Table 1, the obtained results allow the conclusion that the chosen SNPs are polymorphic, they obey the rules of mendelian transmission and provide an individual specific genetic profile. The obtained electropherograms allowed a perfectly unambiguous allele determination.

4. Discussion

The results obtained up to now show the project is to be continued as the next steps are the most important ones. In order to reach the informativity of existing STR multiplexes, the study should be extended to about 50 biallelic loci. Calculation of the heterozygosity and reliability of the profiles require the genotyping of a large number of families. Finally, testing the method on degraded samples should assess its utility in forensic cases.

This method could allow the achievement of a genetic profile specific of an individual in extremely short time (e.g., within custody delays or even within 24 h) and at low costs.

The number of SNPs present on human genome being vast, the list of the selected ones could be even longer.

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