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Analysis of single nucleotide polymorphisms and its application in a disputed paternity case

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Abstract. We investigated the distribution of allele frequencies for 16 single nucleotide polymorphism (SNP) loci (rs3761455, rs3788319, rs3747173, rs3827413, rs3788306, rs3788359, rs2020943, rs3788545, rs20386, rs20362, rs363780, rs20464, rs492539, rs363754, rs363759, and rs363765) in 120 unrelated healthy Japanese individuals on the basis of multiplexed single nucleotide primer extension using ABI PRISM SNaPshot™ Multiplex Kit and ABI PRISM™ 310 Genetic Analyzer. Simultaneously, we applied these databases to analyze a case of special paternity testing when the putative father and the child's mother were deceased. The DNA of the deceased putative father was extracted only from a formalin-fixed liver tissue.

Keywords: SNPs; Allele frequency; Power of discrimination; Paternity testing; Paternal probability

1. Introduction

There has been recent progress in the areas of research and applied development in the genetic analysis of the single nucleotide polymorphisms (SNPs) employing fluorescent dye labelling technology. The most common application of SNPs is in association studies that detect a statistically significant association between SNP alleles and phenotypes, in order to pinpoint candidate causative genes. Therefore, large databases of well-annotated SNPs have been developed, and the information in these databases is accumulating at a rapid rate. Data derived from analysis of SNPs are being applied in various fields ranging from medical studies of disease mechanisms and individual drug response to population genetics for tracking migration and mixing of ancestral groups, and also in forensic science

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for the identification of human remains and using body samples for the identification of individuals [1].

In this study, we investigated the distribution of allele frequencies for 16 SNP loci (rs3761455, rs3788319, rs3747173, rs3827413, rs3788306, rs3788359, rs2020943, rs3788545, rs20386, rs20362, rs363780, rs20464, rs492539, rs363754, rs363759, and rs363765) in 120 unrelated healthy Japanese individuals on the basis of multiplexed single nucleotide primer extension using ABI PRISM SNaPshot™ Multiplex Kit (Applied Biosystems) and ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems). Simultaneously, we applied these databases to analyze a case of special paternity testing. In this case, the putative father and the child's mother were deceased. The DNA of the deceased putative father was extracted only from a formalin-fixed liver tissue.

2. Materials and methods

The Japanese databases study group of 120 unrelated healthy Japanese individuals comprised 52 males and 68 females. The DNA was extracted from blood by the QIAmp blood kit. For a case of special paternity testing, the DNA of the deceased putative father was extracted by Blin's method from a liver tissue that was formalin-fixed 10 years prior to the experiment [2].

SNP loci were selected from the SNP information supplied by the National Center for Biotechnology Information (NCBI). The allele frequency and genotyping database were used as the first level of study in the Japanese population. SNP scoring was carried out by the single-base extension method [3] using SNaPshot™ Multiplex Kit in accordance with the manufacturer's instructions.

The 16 SNPs were detected from amplified multiplex PCR product using capillary electrophoresis performed on ABI PRISM™ 310 Genetic Analyzer. The analysis of SNPs

Table 1
Allele frequencies and statistical parameters of for the 16 SNPs in 120 unrelated Japanese individuals

Chromosome/Locus	Alleles	Frequencies	Obs. H	Exp. H	PIC	DP	EP
22/rs3761455	A/G	0.1750/0.8250	0.2888	0.2912	0.2471	0.4550	0.1444
22/rs3788319	A/G	0.8083/0.1917	0.3099	0.3125	0.2619	0.4746	0.1309
22/rs3747173	A/G	0.6417/0.3583	0.4598	0.4637	0.3541	0.5999	0.1771
22/rs3827413	A/G	0.7833/0.2167	0.3395	0.3423	0.2819	0.5044	0.1409
22/rs3788306	C/T	0.2917/0.7083	0.4132	0.4167	0.3279	0.5649	0.1639
22/rs3788359	A/G	0.4833/0.5167	0.4994	0.5036	0.3747	0.6457	0.1874
21/rs2020943	C/T	0.8458/0.1542	0.2609	0.2631	0.2268	0.4224	0.1134
22/rs3788545	A/G	0.6875/0.3125	0.4297	0.4333	0.3374	0.5788	0.1687
X/rs20386	A/T	0.5875/0.4125	0.4847	0.4888	0.3672	0.6513	0.1836
X/rs20362	C/T	0.7917/0.2083	0.3299	0.3326	0.2755	0.3899	0.1377
X/rs363780	C/G	0.0667/0.9333	0.1244	0.1255	0.1167	0.1707	0.0584
X/rs20364	C/T	0.7625/0.2375	0.3622	0.3652	0.2966	0.5054	0.1483
X/rs492539	C/T	0.9505/0.0495	0.0941	0.0948	0.0897	0.0958	0.0448
X/rs363754	C/G	0.9208/0.0792	0.1458	0.1470	0.1352	0.1849	0.0676
X/rs363759	G/T	0.9901/0.0099	0.0196	0.0198	0.0194	0.0286	0.0096
X/rs363765	A/G	0.0842/0.9158	0.1542	0.1555	0.1423	0.1848	0.0712

Obs. H: observed heterozygosity; Exp. H: expected heterozygosity; PIC: polymorphic information content; DP: power of discrimination; EP: power of exclusion.

was used with filter set E5 and GeneScan™-120 LIZ size standard by GeneScan Analysis software version 3.1.2 (Applied Biosystems).

For the population data, the gene count method was used to calculate the observed allele frequencies and the observed genotypes at each of the 16 SNP loci. For analysis of population genetics data, the exact tests were performed to evaluate compliance with the Hardy–Weinberg equilibrium [4]. The Essen–Möller formula was used to calculate the paternal probability.

3. Results and discussion

The alleles, genotype frequencies, and statistical parameters of forensic importance for the 16 STR loci are shown in Table 1. For the combined 16 SNP loci, a total of 32 alleles and 48 genotypes were observed in 120 unrelated Japanese individuals, the combined power of discrimination and the combined power of exclusion were 0.999892 and 0.878242, respectively. The utility of the combined set of 16 SNP loci was evaluated to estimate their potential for human identification and kinship analysis.

The analysis result of 16 SNPs in a case of special paternity testing, the DNA of the deceased putative father was extracted from a formalin-fixed liver tissue, and the extracted DNA from the formalin-fixed tissue can be detected accurately using multiplexed single nucleotide primer extension performed on the ABI PRISM™ 310 Genetic Analyzer. By using the Essen–Möller formula, the paternal probability between the deceased putative father and the child was calculated to be 0.993207, and the possibility of existing related to the deceased putative father and the child was not denied in 16 SNP loci. From this value, it was concluded that the paternity of the deceased putative father was "very likely" based on the Essen–Möller's interpretation for the child's father.

The result demonstrated that analysis of 16 SNPs is an extremely effective method for the diagnosis of paternity by using a formalin-fixed liver tissue in the special paternity testing of a deceased parent. This study also indicated that if the length of DNA fragment is longer than 100 bp, would be sufficient for the analysis of SNPs from the liver tissue, even if the liver had been fixed in formalin solution for a prolonged time period. The multiplex SNP typing format presented here appears to be useful for forensic personal identification and anthropological investigations because small amounts of DNA and degraded DNA templates can be reliably typed.

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