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Research paper

Genetic analysis of 15 mtDNA SNP loci in Chinese Yi ethnic group using SNaPshot minisequencing

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ABSTRACT

SNaPshot minisequencing is a rapid and robust methodology based on a single base extension with a labeled ddNTP. The present study detected 15 selected SNPs in the mitochondrial DNA (mtDNA) control and coding regions by minisequencing methodology using SNaPshot for forensic purpose. The samples were collected from 99 unrelated individuals of the Yi ethnic minority group in Yunnan Province. We have predominantly found high-frequency transitions (91.7%) and a significantly lower frequency of transversions (8.3%). The nt152, 489, 8701, 10,398, 16,183, and 16,362 loci were highly polymorphic, while the nt231, 473 and 581 loci were not polymorphic in the studied population. Based on these 15 SNPs, a total of 28 mtDNA haplotypes were defined in 99 individuals with the haplotype diversity of 0.9136. Also, we compared the mtDNA sequences of Yi group and other 9 populations worldwide and drew a Neighbor-Joining tree based on the shared 12 mtDNA SNP loci, which demonstrated a close relationship between Yi and Bai groups. In conclusion, the analysis of the 15 selected SNPs increases considerably the discrimination power of mtDNA. Moreover, the SNaPshot minisequencing method could quickly detect mtDNA SNPs, and is economical and sensitive. The set of selected 15 SNPs is highly informative and is capable for anthropology genetic analysis.

1. Introduction

Human mitochondrial DNA (mtDNA) present outside of the nucleus is a small, closed circular double-stranded DNA molecule (Bogenhagen and Clayton, 1974). The complete nucleotide sequence of the 16,569 bp human mtDNA was first determined in 1981 (Anderson et al., 1981) and then revised in 1999 (Andrews et al., 1999). The revised mtDNA sequence was named revised Cambridge reference sequence (rCRS). The copy number of mtDNA per mitochondrion ranges from 1 to 15 with the average around 4–6, and the copy number of mtDNA per cell is estimated to be about 500 (Satoh and Kuroiwa, 1991; Chen et al., 2015). MtDNA is maternally inherited as haplotype-block and thus has a low recombination rate (Giles et al., 1980). Moreover, the mtDNA sequence evolution rate is much higher than that of nuclear gene (Wallace et al., 1987; Chen et al., 2009; Chen et al., 2008a). Along the history and with the human migration, substantial variations arose and accumulated sequentially in the mtDNA, resulting in the divergence of maternal lineages from different geographical regions. Thus, the analyses of these mtDNA mutations enable us to infer the history of human evolution and patterns of migration. In addition, it is an available genetic marker for the studies of anthropology.

In the field of forensic genetics, the above mentioned characteristics have made the mtDNA useful for human identification testing and especially for the analysis of highly degraded materials or samples containing infinitesimal nuclear DNA such as skeletal remains and hair shafts (Coutinho et al., 2014; Kohnemann and Pfeiffer, 2011; Budowle and van Daal, 2008; Kohnemann et al., 2010; van Oven et al., 2011; Haak et al., 2010). In the previous studies of restriction fragment length polymorphism (RFLP), many stable loci were obtained and could be used to identify different mtDNA haplogroups. Most of the mutations observed in both the control and the coding regions of mtDNA in modern human populations have occurred on these preexisting haplogroups (Graven et al., 1995). The bottleneck of using RFLP in identification testing is the

Abbreviations: mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; rCRS, revised Cambridge reference sequence.

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limited discrimination power, and it can only provide less than sufficient information in evolutionary studies. Recently, in order to improve the discrimination power of mtDNA, the interest in point mutation also known as single nucleotide polymorphism (SNP) has increased. The mtDNA SNPs can be analyzed in amplicons shorter than 150 bp (Kohnemann et al., 2008), therefore they became available for the analysis in degraded samples which could not be genotyped by STR typing.

Yi group is one of the 56 ethnic groups officially recognized by the People's Republic of China. In the 6th Chinese population census in 2010, the population of Yi was 8,714,393 and ranked the 7th among all the ethnic groups in China. The Yis are mainly distributed over the provinces of Yunnan, Sichuan, Guizhou, and the Guangxi Zhuang Autonomous Region. There are more than five million Yis residing in Yunnan province (http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/indexch.htm).

Previously, SNaPshot minisequencing has been utilized to subtype mtDNA and resolve mitochondrial macro-haplogroups by many researchers (Schlebusch et al., 2009; Grignani et al., 2006; Quintans et al., 2004; Alvarez-Iglesias et al., 2007). Here is the first study of the selection of 15 SNPs from both the control and the coding regions of mtDNA in the Yi ethnic group using SNaPshot minisequencing method. We found that the SNaPshot minisequencing methodology was a convenient, robust, and efficient method for mtDNA SNP typing. Furthermore, the relationships between Yi and other 9 populations were analyzed based on the pair-wise comparisons at the 12 shared SNPs (nt16,183/16,234/16,327/16,362/150/152/189/195/199/231/473/489).

2. Materials and methods

2.1. MtDNA-SNP selection

A set of 15 mtDNA-SNPs (nt150, 152,189, 195, 199, 231, 473, 489, 581, 8701, 10,398, 16,183, 16,234, 16,327 and 16,362), which are termed by position in the mitochondrial genome according to the rCRS, was chosen from previously published works for their relatively high polymorphism in Chinese groups (Gao, 2006; J H, 2005; Tang, 2003).

2.2. Samples

Blood samples were taken from 99 unrelated individuals of the Yi ethnic minority in Yunnan Province, China. DNA was extracted from 10 μl EDTA blood samples using the phenol–chloroform method. Informed consentwas obtained from all the individuals in accordance with the Humane and Ethical Research Principles of Xi'an Jiaotong University Health Science Center, China. And the study was approved by the ethical committee of Xi'an Jiaotong University Health Science Center, China.

2.3. PCR-multiplex

The selected 15 SNPs were separated into two multiplex reactions. Multiplex 1 included a selection of 8 SNPs (150, 152, 199, 489, 8701, 10,398, 16,183 and 16,234). Multiplex 2 contained a set of 7 SNPs (189, 195, 231, 473, 581, 16,327 and 16,362). Primers were designed and selected using Primer Premier 5.0 software (PREMIER Biosoft, California, US), which permitted analysis in multiplex reactions with applicable annealing temperatures and amplicon lengths.

We performed both multiplexes in a 20 μl reaction volume comprising 1 ng of DNA template, 2 μl 10×buffer, 1.5 μl $MgCl_2$ (25 mM), 1 unit of Gold™ DNA polymerase (Applied Biosystems, Foster City, California, USA), 2 μl dNTP (2 mM) and 1 μl multiplex primers (10 μM). Amplification was carried out with a first denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min with a final extension at 72 °C for 7 min.

2.4. SNaPshot reaction

After amplification, a purification process was performed in order to remove primers and unexhausted dNTPs. According to the instruction of Exo-sapIT, 5 μl of PCR product was incubated with 2 μl of Exo-sapIT for 15 min at 37 °C followed by 15 min at 85 °C to inactivate the enzyme, and then at 4 °C until further use. The two minisequencing reactions were performed in a GeneAmp 9700 PCR thermocycler (Applied Biosystems, Foster City, California, USA) and both in 7 μl volume. The first reaction consisted of 2 μl SNaPshot Multiplex, 2.1 μl SNaPshot primer and 2.9 μl purified PCR products. The second reaction contained 1.5 μl SNaPshot Multiplex, 2.1 μl SNaPshot primer and 3.4 μl purified PCR products. The single base extension was performed in 25 cycles under the following conditions: 96 °C denaturation for 10 s, annealing at 50 °C for 5 s and extension at 60 °C for 30 s. Here the SNaPshot Multiplex contained the enzyme, the buffer and the 4 ddNTPs labeled with 4 different fluorescent dyes: ddATP with dR6G™, ddTTP with dROX™, ddCTP with dTAMRA™ and ddGTP with dR110™. Using this strategy, the single combined ddNTPs can be easily detected. And we could run together an assay with an A/G polymorphism and a C/T polymorphism even if the primers were of the same length.

Primer Premier 5.0 software was used to design the minisequencing primers which were modified by the addition of non-homologous tails at the 5′-end so that, by the size of the primer we can distinguish the different SNP sites. All minisequencing primers were purified by HPLC to remove incomplete primer synthesis products. After minisequencing reactions, a purification treatment to get rid of the unincorporated ddNTPs can help prevent high background signal and non-specific signal. The 7 μl products were mixed with 0.7 μl of SAP. Incubation was carried out at 37 °C for 60 min, followed by 15 min at 85 °C for enzyme inactivation and at 4 °C until further use.

The minisequencing products (1.2 μl) were mixed with 10 μl formamide and 0.5 μl of GeneScan LIZ-120 (Applied Biosystems, Foster City, California, USA). Sample analysis was performed on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, California, USA). Electrophoresis parameters were used as follows: sample introduction for 10 s at 15 kV, electrophoresed for 20 min at 15 kV and 9 μA. GeneScan ™3.7 Software (Applied Biosystems, Foster City, California, USA) was used to analyze the resulting data.

2.5. Genetic distance analysis.

The nucleotides at the selected mtDNA SNP loci were aligned with rCRS for statistical analysis. Software Arlequin 3.0 (Excoffier et al., 2005) and Mega 4.0 (Tamura et al., 2007) were employed to analyze the pair-wise genetic distances among Yi, Egyptian (Saunier et al., 2009), Tunisian (Turchi et al., 2009), Bai (Chen et al., 2015), Guangdong Han (Chen et al., 2008b) and other five Han populations (Liaoning Han,

Table 1
The allele frequencies of 15 mtDNA SNPs loci in Chinese Yi group (n = 99).

Loci (nt)	Allele	Frequency
150	C/T	0.86/0.14
152	T/C	0.76/0.24
189	A/G	0.98/0.02
195	T/C	0.96/0.04
199	T/C	0.97/0.03
231	C	1
473	C	1
489	T/C	0.57/0.43
581	A	1
8701	A/G	0.54/0.46
10,398	G/A	0.53/0.47
16,183	A/C	0.77/0.23
16,234	C/T	0.92/0.08
16,327	C/T	0.93/0.07
16,362	T/C	0.55/0.45