

A PRELIMINARY STUDY ON THE POLYMORPHISM OF HYPERVARIABLE D17S5 LOCUS IN UNIMAS CHINESE POPULATION

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ABSTRACT

Variable number of tandem repeats (VNTRs) is one of the most useful tools in the study of polymorphism and have spurred studies in many populations throughout the world. Here we report a preliminary VNTR study of the polymorphism of the hypervariable locus D17S5, in the Chinese community in UNIMAS. Samples were obtained from seventy-five unrelated Chinese individuals. The study showed the presence of six alleles ranging from 170bp to 520bp in sizes. The allele frequencies ranged from 0.02 to 0.44 with heterozygosity value of 0.32. The distributions of the D17S5 alleles in the UNIMAS Chinese population were also determined with allele 2 having the highest frequency and alleles 5 and 6 being the lowest frequencies, respectively.

Keywords: VNTR, D17S5, polymerase chain reaction, forensic identification, allele distribution

INTRODUCTION

Approximately 30% of the human genome is made up of human tandem repeats. Human minisatellites or variable number tandem repeat (VNTR) loci have repeat units of between 6 to more than 100 base pairs long, depending on the locus, and are co-dominance (Tamaki & Jeffreys 2005). The high polymorphism characteristic of VNTRs makes them suitable markers for use in genetic identification, forensic medicine, paternity testing and population genetics (Gasparini *et al.* 1991; Hartl & Clark 1997). The VNTR D17S5 locus is located on chromosome number 17 and comprise of 19 repeat units (Horn *et al.* 1989; Kijas *et al.* 1994; Pinheiro *et al.* 1996). The repeat unit has tandem repeats of 70 base pairs and alleles range from 170bp to 1430bp in length.

Database for hypervariable locus, especially D17S5, in the Malaysia population has not been well established for many of the races especially in Sarawak. Previous similar studies were conducted in Peninsular Malaysia looked at the polymorphism in several markers such as the mitochondrial DNA, CYP2C9 enzyme and the use of cytochrome P450 (Yang *et al.* 2004; Hoong & Lek 2005; Zainuddin *et al.* 2006). The Chinese community in UNIMAS came from all over Malaysia including Sabah and Sarawak. Therefore

this study would eventually enable further population studies such as in the origin; ancient migration and movement of the Chinese community can be studied. Here we report a preliminary analysis of the D17S5 locus from the Chinese population in UNIMAS.

MATERIALS & METHODS

Sample Collection and DNA extraction

A non-invasive method of DNA sample collection was conducted by buccal swab technique and DNA isolation was conducted (Richards *et al.* 1993). Samples were collected from 75 unrelated individuals. Informed consent was obtained from all donors before the DNA samples were collected.

Polymerase Chain Reaction (PCR) and Gel analysis

PCR of hypervariable locus was conducted in 25µl total PCR volume consisted of 7.5 µl sterile water, 2.5 µl 10X buffer with (NH₄)₂SO₄ (Vivantis), 1 µl 10mM dNTP mix (Fermentas), 1 µl D17S5 Forward primer (5'-CACAGTCTTTATTCTTCAGCG-3'), 1 µl D17S5 Reverse primer (5'-CGAAGAGTGAAGTGCACAGG-3'), 0.5 µl of

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5U/ μ l *Taq* DNA polymerase (Vivantis), 10 μ l DNA and 20 μ l mineral oil. The PCR condition was as follows; denaturation at 94°C for 30 seconds, annealing at 65°C for 1 minute, amplification at 72°C for 1 minute, final extension at 72°C for 10 minutes and the PCR was repeated 30 cycles. PCR products were analysed in 1.2% agarose gel in 1X TAE (Tris base, glacial acetic acid, 0.5 M EDTA) and documented using Kodak Gel Documentation System.

Statistical Analysis

Statistical analysis of data were calculated using equation by Carr (2004).

$$f(A) = (2x + y) / 2N,$$

*where f represents the allele frequency, x represents homozygote individuals, y represents heterozygote individuals and N represents the sample size.

RESULTS AND DISCUSSION

A total of six different sizes were generated from the PCR representing the alleles in D17S5 locus ranging from 170bp to 520bp (Figure 1). Table 1 shows the number of homozygotes and heterozygotes and the allelic frequencies for the D17S5 locus calculated using the equation by Carr (2004). Allele 2 was found to be the most frequent allele (0.44) while alleles 5 and 6 exhibited the lowest frequency (0.02

each).

Similar studies on the allele frequency distribution of the D17S5 locus showed that 12 alleles were observed in the North Vietnamese population, 10 alleles were found in the Japanese population, and 10 alleles were found in both the Northern Han and the Hui populations (Katsuyama *et al.*, 1998). In the Vietnamese population, allele B8 was the most common allele (14.7%) with a heterozygosity value of 83.76%. The most frequent alleles found in the Japanese population was allele number 4 while allele number 1 was the most frequent in both the Northern Han and the Hui populations. The observed heterozygosity values in the Japanese, Northern Han and Hui populations were 0.774, 0.0341 and 0.909 respectively. The Chinese community in this study showed a heterozygosity value of 0.32 which was higher than the value of the Northern Han population living in Xining, China but lower than those found in the Japanese and the Hui populations.

On the other hand, additional comparison of the D17S5 allelic frequency in the Chinese community samples with the Melanau population of Sarawak (Sulaiman, 2006, unpublished data) indicated that the Melanau population has 3 additional alleles compared to the Chinese samples, with the highest frequency found was allele number 1 while the lowest frequency were alleles number 7 and 9, but with lower observed heterozygosity value (0.1935).

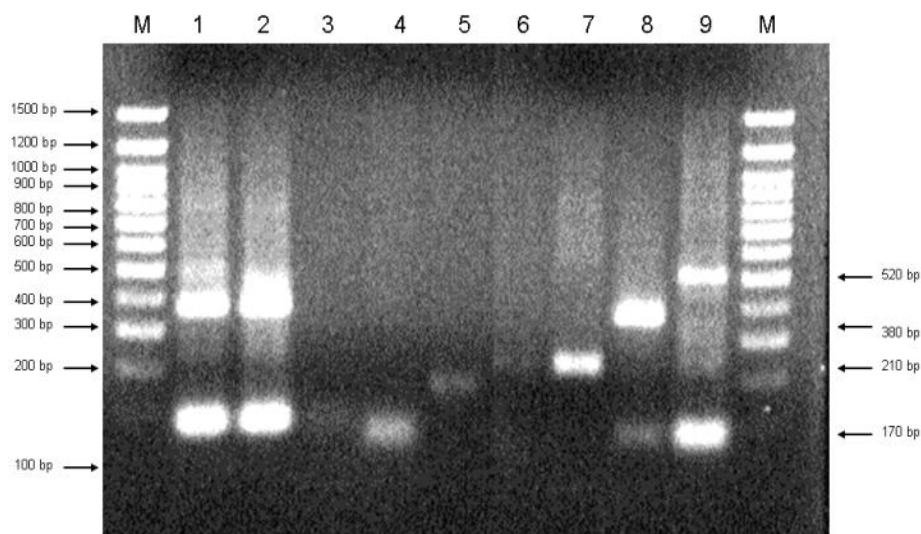


Figure 1. Agarose gel electrophoresis of D17S5 PCR products. Lanes 1-9 are the PCR products representing the 4 alleles present in the population. Lanes M are the Forever 100 base pairs ladders (Seegene).

Table 1. Allele frequencies calculated according to the equation reported by Carr (2004) for the different alleles.

Allele	Size	Number of homozygotes	Number of heterozygotes	Allele frequency
1	170	8	15	0.31
2	240	18	8	0.44
3	310	4	-	0.08
4	380	4	6	0.14
5	450	1	-	0.02
6	520	-	2	0.02

CONCLUSION

This study has successfully amplified and resolved the D17S5 alleles. The various sizes of the alleles were determined and 6 different alleles were found in the Chinese population of UNIMAS that ranged from 170 base pairs to 520 base pairs. The statistical analyses carried out also showed that the highest frequency was allele number 2 while the lowest allelic frequencies were allele number 5 and 6. The distributions of alleles of the Chinese population in UNIMAS were also found not to be evenly spread.

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CONSENT STATEMENT

Prior to the start of the project, this project have been reviewed by UNIMAS Ethical Committee for Research and have been given consent to be performed. The procedures of the protocols are within the guidelines of the 1964 Declaration of Helsinki. The donors for DNA samples have been informed prior to donation of the purpose of the project and that the DNA will be kept confidential. Upon agreeing to the conditions, the donors DNA was then labeled anonymously and only used for the said project. Subsequent DNA samples that are not used are disposed of via autoclave.

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