African Journal of Biotechnology Vol. 10(22), pp. 4544-4549, 30 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2241
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Analysis of three polymorphisms in Bidayuh ethnic of Sarawak population: A report from Malaysia

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Accepted 4 April, 2011

Insertion/deletion polymorphism of YAP (DYS287), M96 and M120 polymorphisms in Bidayuh ethnic populations of Sarawak, Malaysia were analyzed in this study. Genomic DNA was extracted from 180 buccal samples and amplified by Hot-Start PCR method. The amplified PCR products were separated by using 2% agarose gel electrophoresis. 26 out of 180 samples (14.44%) have shown insertion (YAP+) polymorphism, while the rest of the samples (85.56%) have shown deletion (YAP-) polymorphism. M96 derived and ancestral allele reveal 102 (56.67%) and 78 (43.33%), respectively and 96 (53.33%), while for M120 derived allele and 84 (46.67%) for M120 ancestral allele. This study has provided the information about the distribution of YAP, M96 and M120 polymorphisms in Bidayuh ethnic population of Sarawak. This study has to be continued with more samples to determine the YAP polymorphism and also the biallelic markers in Bidayuh population.

Keywords: Y-chromosome, YAP, Sarawak.

INTRODUCTION

Sarawak is a northern state of Malaysia located on the island of Borneo. It is also called Bumi Kenyalang or the Land of Hornbills and is the largest state in Malaysia. The indigenous people make up about 50% of Sarawak population. Among the known groups and sub-groups are Iban, Bidayuh, Kenyah, Kelabit and Penan. Each of them has their own language, culture, religion and lifestyle. Iban is the largest indigenous group in Sarawak, making up almost 38% of the state while Bidayuh make up about 10% of the state. Both Iban and Bidayuh are further divided into smaller sub-groups or branches depending on the geographical area where they reside. The 6 related sub-groups of Iban are Kantu, Mualang, Semberuang, Bugau and Sebaru. The 11 related sub-groups of Bidayuh are Bekati, Binyadu, Jongkang, Ribun, Salako, Lara, Sanggau, Sara, Tringgus, Semandang and Ahe.

Linguistically, Iban and Bidayuh people speak different languages. The Iban people regardless of the sub-groups or branches they are from speak Iban language in a mutually intelligible dialect. Bidayuh on the other hand, have several dialects which are not mutually intelligible. The 6 dialects are spoken by different branches of Bidayuh people depending on their geographical area. There are Bukar-Sadong, Singai-Jagoi, Biatah (Siburan, Padawan and Bia), Braang-Pinyawa, Sepug-Emperoh and Gumbang. Both Iban and Bidayuh language are part of Southeast Asian branch of Austronesian family.

The Bidayuh people are generally known as Land Dayak. They can be found mainly in Lundu and Bau area. Unlike the Iban people, most Bidayuh people live in individual houses rather than longhouses. Most of the Bidayuh people are Christians and animists. Economically, like the Iban people, Bidayuh people also practice rotational agriculture and hunting in the tribal forests.

Y-chromosome markers have been used widely in genetic studies including human origin (or migration) and population studies (Charlesworth, 2003). Y-chromosome markers for example, microsatellites, also known as simple sequence repeat (SSR) or short tandem repeat (STR), biallelic polymorphisms and *Alu* short interspersed elements (SINEs) are among the popular and highly informative tools in studying human population genetics. This is due to the fact that microsatellites are co-dominant and multi-allelic. They are highly reproducible, have high-resolution and are based on PCR (Oliveira et al., 2006).

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Microsatellites are short segment of DNA of 1 to 6 base pairs, composed of repeated sequence of nucleotides usually found in non-coding region of the genome. They are both in eukaryotic and prokaryotic genome. These genetic markers are used as the tools of choice in population genetics due to their unique characteristics such as higher mutation rates compared with the other genes in the genome, patrilineal inheritance and lack of recombination during meiosis.

Alu SINEs (also known as YAP) are transposable DNA sequence of approximately 300 base pairs in length that reproduce by copying itself and inserting into new chromosome locations, a process known as retro-transposition. *Alu* elements are widespread in human genomes reaching about millions of copies. After inserting itself into the genome, the *Alu* element undergoes random mutations and specific base pairs changes. There are approximately 2000 *Alu*s specific to the human genome, which are not found in chimpanzee and gorilla. YAP is thus a useful marker because the ancestral state, which in the absence of the YAP, is known (Hammer, 1994) and it is inherited from parents to children.

Biallelic polymorphisms, including single-nucleotide polymorphism (SNP) are another useful Y-specific marker used to study the human evolutionary history from paternal lineages (Gayden et al., 2007). A SNP is a variation of DNA sequence when a single nucleotide in the genome is substituted or differs between paired chromosomes or two individuals belonging to the same species.

The method used in this study was based on polymerase chain reaction (PCR). PCR is an effective and inexpensive technique of amplifying DNA fragments. However, prior to the thermal cycling, PCR reactions are susceptible to non-specific binding of primer to DNA template, contaminant DNA and even to other primers which eventually leads to generation of non-specific products and primer artifacts. Therefore, Hot-Start PCR was used to reduce the generation of non-specific products and primer artifacts.

An increasing number of human Y chromosome-specific polymorphic markers are used to examine the evolution of human populations from different geographic areas. Characteristics such as patrilineal inheritance, lack of recombination and differential mutation rates represent attractive and powerful tools for the study of population migration patterns, admixture, selection and relatedness (Jobling and Tyler 1995; Yuet et al., 2005, 2008). The objective of present study was to analyze the Y-STR polymorphism (DYS287, M96 and M120) in Bidayuh ethnics of Sarawak populations.

MATERIALS AND METHODS

A total number of 180 samples were collected from Bidayuh ethnic from Sarawak, Malaysia. Each respondent has to be interviewed to make sure (the pure and unmixed respondent) their parents come from the same ethnics. Mixed respondents were excluded from this study. Genomic DNA samples were obtained from buccal cells using sterile cytology brushes by brushing the buccal mucosa. To avoid any growth of microorganisms that destroy the DNA, the brushes were then dried at room temperature for 15 to 20 min. After drying the brushes, DNA samples were stored in a cool box at 4°C during transportation. Any unwanted cells that were trapped in the brushes were washed with distilled water before further analysis.

Genomic DNA extraction

Genomic DNA was extracted from the buccal cell samples by using Qiagen DNA extraction kit. The steps to extract the genomic DNA from the blood were carried out by using manufacturer's protocol. The extracted genomic DNA was used as the templates for DNA amplification by polymerase chain reaction. The extracted genomic DNA was stored in -20°C freezer for further analysis.

Quantification of genomic DNA

The extracted genomic DNA concentration was determined using Biophotometer (Eppendorf, Germany). The reading of A_{260} and A_{280} of the genomic DNA against sterile distilled water as blank was obtained. The genomic DNA concentration in µg/ml was determined by multiplying the absorbance with 50, because 1.0 absorbance unit was equivalent to 50 µg/ml.

Polymerase chain reaction (PCR)

In this study, Hot-Start PCR technique was used to amplify the target products of the respective markers. Hot-Start PCR is a modified technique of conventional PCR, where the DNA polymerase is blocked or isolated from the PCR cocktail until an optimum temperature is reached. This prevents the DNA polymerase from binding unspecifically to the primers, which could lead to unspecific extension of primers.

To determine the insertion/deletion polymorphism of the YAP and the ancestral/derived state of M96 and M120, flanking primer pairs (Table 1) synthesized by Research Biolabs, Malaysia was used. PCR amplification was performed with a 25 µl reaction mixture that contained 0.2 µmol of each primer, 0.4 mmol/l each dNTP, 2.0 mmol/l $MgCl_2$, 1 x Taq buffer and 1 unit of Fermentas *Taq* DNA polymerase (New England Biolabs, Beverly, MA, USA). The PCR cycling conditions were carried out on Biorad machine (BioRad Laboratories, Hercules, California, USA). Table 2 shows the PCR cycling conditions and the PCR product sizes for the respective genes.

Agarose gel electrophoresis

The PCR products were detected using 2% agarose gel electrophoresis. To prepare 2% agarose gel, 0.8 g of agarose (Promega, Madison, USA) powder was weighed and the agarose was heated in a microwave oven by adding 40 ml of 1 × TBE buffer to dissolve completely. The agarose solution was then swirled gently before pouring into the gel mould. 8 µl of amplified PCR products was mixed with 1.5 µl loading dye and the products were separated in origins electrophoresis tank (Elchrom Scientific AG, Switzerland) at 80 to 90 volts in 1 × TBE buffer for 40 to 50 min and stained with 0.5 µg/ml of ethidium bromide for 20 min. The size of the PCR products was determined by comparing it with a 100 bp/1kb. DNA ladder (Fermentas, Hanover, USA) was used as a DNA marker.

Staining and visualization

The agarose gels were stained with ethidium bromide (Sigma St.

Table 1. List of primers used for hot-start PCR.

Marker	Forward and reverse primer	Control primer
YAP	5'-CAGGGGAAGATAAAGAAATA-3' 5'-ACTGCTAAAAGGGGATGGAT-3'	-
M96	5'-GAAAACAGGTCTCTCATAATAG-3' 5'-GAAAACAGGTCTCTCATAATAC-3'	5'-AAGGTCACTGGAAGGATTGC-3'
M120	5'-TCCGTTTTTTGATGTGGAAATAT-3' 5'-CCGTTTTTTGATGTGGAAATAC-3'	5'-AACTTTAAGGCACTTCTGGC-3'

Table 2. PCR conditions used for hot-start PCR and the PCR products sizes.

Marker	Condition	Temperature/time	Fragment size (bp)
YAP	Initial denaturation	94°C /2 min	Insertion - 450, Deletion - 150
	Denaturation	94°C /1 min	
	Annealing	51°C for 2 min	
	Extension	72°C for 1 min	
	Final extension	72°C for 5 min	
	Number of cycles	30	
M96 and M120	Initial denaturation	94°C /4 min	Ancestral (M96)-392 Ancestral (M120)-294
	Denaturation	94°C /30 s	
	Annealing	63°C for 30 s	
	Extension	72°C for 1 min	
	Final extension	72°C for 10 min	
	Number of cycles	40	

viewing. The genomic DNA and PCR amplified products were visualized under UV light and images were captured with Alpha Imager (Alpha Innotech, San Leandro, CA).

RESULTS

The YAP+ was successfully amplified as a 450 bp amplicon (Figure 1). The frequency of YAP+ and YAP- polymorphism in 180 Bidayuh ethnics are summarized in Table 3. Out of the 180 subjects, 14.44% of subjects show YAP+ while 85.56% of subjects are having YAP- polymorphism. The ancestral allele of M96 was successfully amplified at 392 bp, while the derived allele of M96 showed very faint or absence of the band at 392 bp (Figure 2). 56.67% of subjects have ancestral allele, while 43.33% of subjects have the derived allele of M96 polymorphism (Table 3). The ancestral allele of M120 was amplified as a 294 bp amplicon (Figure 3). The frequencies of the ancestral and derived alleles were 53.33 and 46.67%, respectively.

DISCUSSION

This study has shown that the Bidayuh population lacks the YAP insertion polymorphism. From the result obtained, only 14.44% of the subjects have YAP+ polymorphism. On the other hand, 85.56% of the subjects have YAP- polymorphism. This percentage indicates that there is lack of YAP insertion polymorphism in Bidayuh ethnic of Sarawak population. An extensive study of YAP insertion/deletion polymorphism in European, African, Asian and Oceanian populations was carried out by Hammer (1994). He found that the frequency of YAP insertion polymorphism (YAP+) was significantly high in the African populations, followed by the Europeans, Ocea-nians and lastly, Asians with exception to the Japanese population. Another study done by Hammer and Horai (1995) observed that in Japanese population, 43% of Japanese subjects have YAP+ polymorphism, the highest frequency ever discovered in Asian population among Ahmadiyya Muslim males from Qadian, district Gurdaspur of Punjab (Badaruddoza et al., 2008) YAP+ insertion was not found. In all Middle Eastern Muslim populations the frequency of YAP insertion is found to be in the range of 11 to 25% but none of the north Indian and South Indian populations had this insertion (Deka et al., 1996; Al-Zahery et al., 2003). The result obtained from the study on Bidayuh subjects is consistent with the

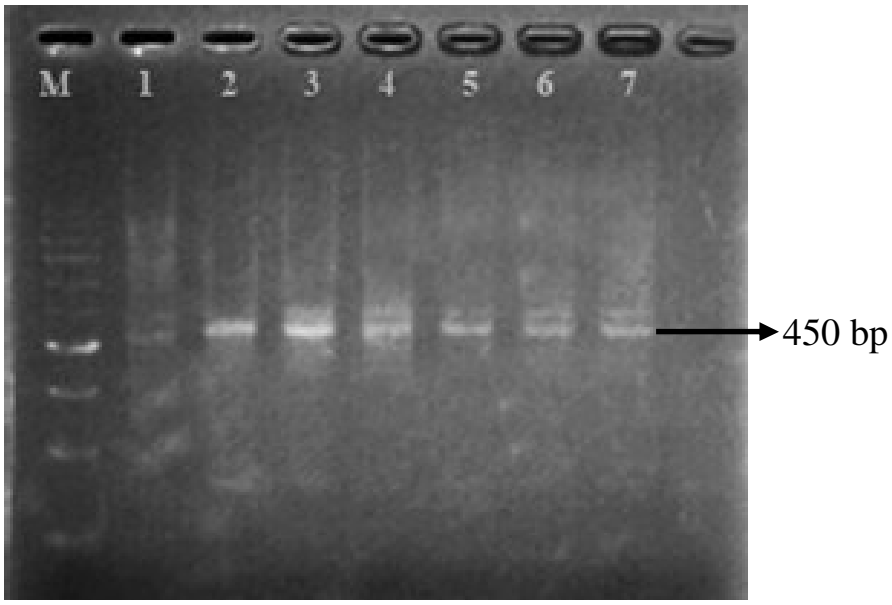

Figure 1. Gel electrophoresis showing amplification of YAP. YAP+ was successfully amplified at 450 bp.

Table 3. Distribution of YAP+ and YAP- in 180 Bidayuh subjects.

Polymorphism	Number (percentage)
YAP(+)	26 (14.44%)
YAP(-)	154 (85.56%)
M96 derived allele	102 (56.67%)
M96 ancestral allele	78 (43.33%)
M120 derived allele	96 (53.33%)
M120 ancestral allele	84 (46.67%)

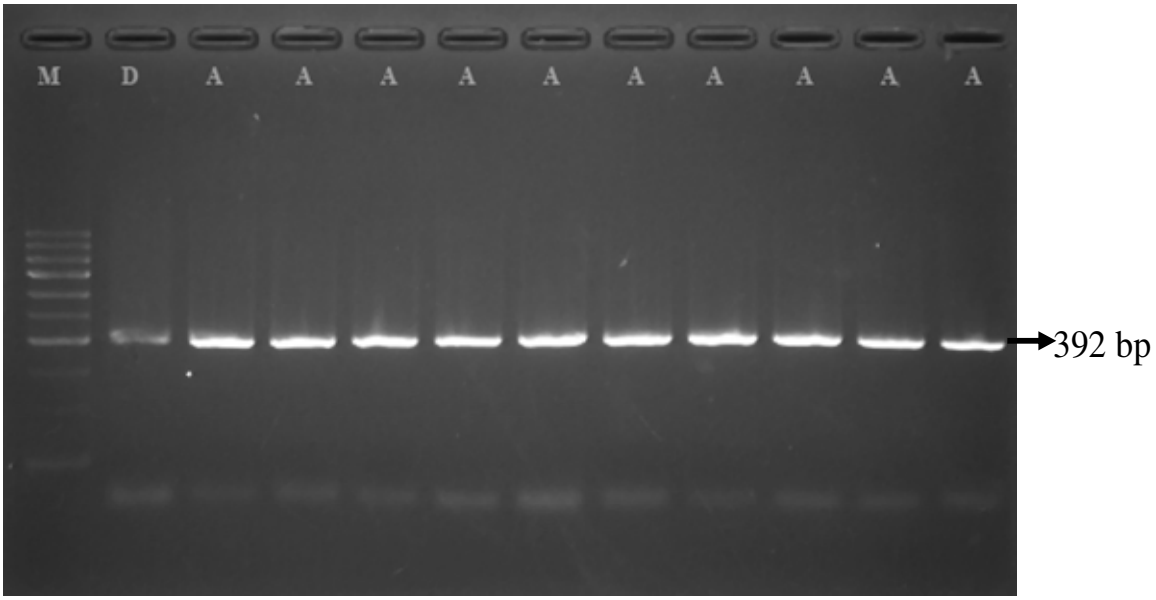

Figure 2. Gel electrophoresis showing amplification products of M96 ancestral and derived allele.

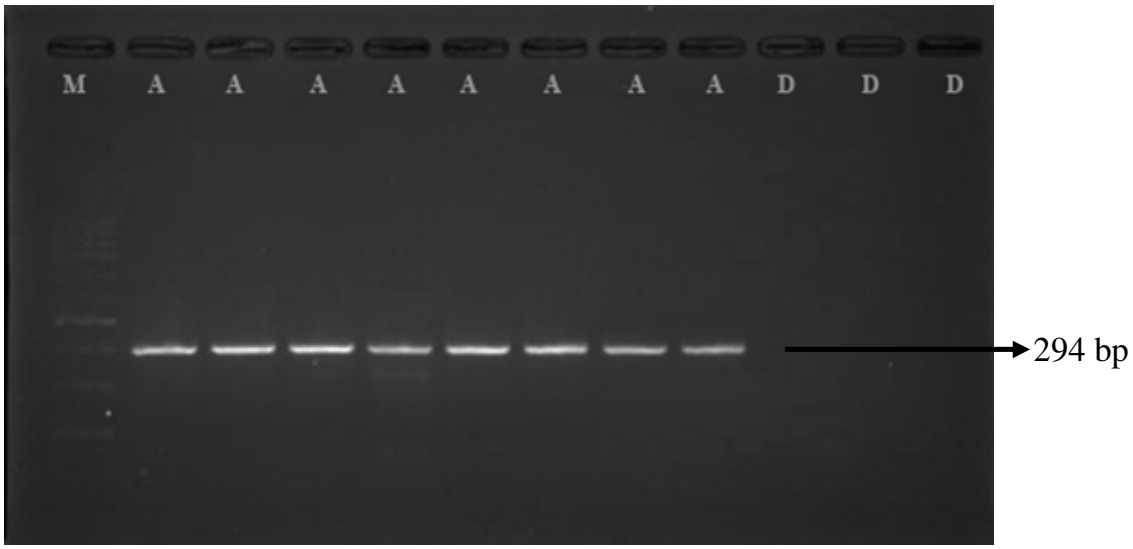

Figure 3. Gel electrophoresis showing amplification of ancestral allele of M120 polymorphism at 294 bp

study done by Hammer (1994) and Badaruddoza et al. (2008) which concluded that most Asian populations lack the YAP+ with the exception of the Japanese population. This may be due to the fact that *Alu* element was originally inserted on the chromosome of the Sub-saharan African populations, which explains the lack of YAP+ in the Asian population. The small percentage of YAP+ found in Bidayuh ethnics suggest that the probability of population admixture have taken place many years ago.

M96 biallelic polymorphism is a single base substitution polymorphism, where the base G has been substituted to C (G > C). 56.67% of Bidayuh subjects possess the ancestral state, while the remaining 43.33% of subjects have the derived state of the allele (Table 3). M96 originated in Africa and later dispersed to Middle East and Europe about 20,000 years ago (Hong et al*.,* 2008). Silva et al. (2007) has studied the Y-chromosome genetic variation in 127 male subjects from Rio de Janeiro of Brazilian populations. This study has utilized 28 Y chromosome-specific biallelic markers including M96. They found that the frequency of M96 in Rio de Janeiro population was at 0.018 (N = 112) after removing all chromosomes of Sub-saharan Africa or Amerindian origin.

Like M96, M120 biallelic polymorphism is a novel Y-specific biallelic marker characterized by single base substitution from T to C (T > C). From the result, 53.33% of Bidayuh subjects show the ancestral allele, while 46.67% have derived allele. M120 biallelic polymorphism originated in Asia and represent recent founder paternal Native American radiation into the Americas. Sharma et al. (2007) has studied the Y-specific biallelic markers in Indian population including the M120. 630 samples belonging to different regions of India were screened for M120 polymorphism, only one individual was found to have M120 polymorphism, representing Q1 lineage. From the comparison of the frequency of M120 mutation found in Bidayuh ethnic of Sarawak population and that of the Indian population, we could conclude that there is no close linkage between these both populations.

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