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Ethno-genetics of AIM1 gene relating to Human Skin Pigmentation among Six Indigenous Nationalities in Nepal

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ABSTRACT

This paper attempts to find out allele frequencies of AIM1 gene among six ethnic groups in Nepal by detecting a total number of 456 blood and nail samples through an allele specific PCR amplification. The research findings revealed that the AIM1 codon L374F polymorphism distinctively showed conspicuous ethnic distribution of the two alleles; namely allele "F" was found mostly high (11.4%) in the Caucasoid Chidimar and rarely low (1.1%) in the Dravidian Munda whereas Mongoloid Chepang, Gurung, Raute and Thakali entirely lacked the "F" allele and showed the monomorphic type for the "L" allele. The codon L374F could be the best genetic marker for distinguishing Caucasian populations from Mongoloid ones and would explain the ethno-genomics of human skin coloration among many ethnic groups in the world.

Keywords: Human skin pigmentation, melanoma, polymorphism

INTRODUCTION

Human antigen in melanoma (AIM1) differs from the Ser/Thr kinase, also called "AIM-1 located on the human chromosome 17 (Katayama *et al.* 1998) and that of the mouse absent in melanoma gene (aim1) found on the mouse chromosome 10 (Teichmann *et al.* 1998). The medaka-fish homologue, also known as AIM-1, was found to be 55 percent identical to Human AIM1 (Fukamachi *et al.* 2001). The mouse underwhite locus (uw-locus) gene was renamed as membrane-associated transporter protein (Matp) and its human homologue as MATP (Xu *et al.* 2012). The human AIM1 gene known alternatively as MATP was mapped to chromosome 5p (Newton *et al.* 2001). AIM1 protein identified as an antigen in human melanoma encodes a protein of 530 amino acids (Harada *et al.* 2001). The main function of AIM1 as a transporter in melanin synthesis has been suggested by its 12-domain membrane pass sharing similarity with the structure of the sucrose transporter in plants (Fukamachi *et al.* 2001).

There is not known sucrose transporter in mammals, it is feasible that MATP co-transports a sugar molecule with a proton. MATP protein plays a critical role in vertebrate pigmentation, presumably mediating the transport of a critical substance across the melanosome membrane (Newton *et al.* 2001, Graf *et al.* 2007, Guedj *et al.* 2008). The most common AIM1 codon L374F polymorphism was identified in the MATP coding sequencing corresponding to the exon 5 in the normally pigmented individuals (Newton *et al.* 2001, Yuasa *et al.* 2006, Bin *et al.* 2015). For the first time, this polymorphism was used in our laboratory to investigate the genetic variation among the representatives of major human populations with varying degrees of skin pigmentations (Nakayama *et al.* 2002, Stokowski *et al.* 2007, Graf *et al.* 2007). This prompted me to use AIM1 codon L374F polymorphism as a newly identified human genetic marker for skin color to investigate among six Nepalese ethnic groups with different skin pigmentations. In this context, this study attempts to find out allele frequencies of AIM1 gene among six ethnic groups in Nepal.

MATERIALS AND METHODS

Altogether 456 samples belonging to six Nepalese ethnic groups were subjected to genotyping for detecting the frequency of the AIM1 gene L374F polymorphism (Table 1 & Fig. 1**).** NaI method was implemented to extract DNA from the whole blood, Phenol-Chloroform method was used to extract DNA from the cultured cells and modified method was used to extract DNA from nail samples.

Fig. 1. Map and location of Nepalese indigenous nationalities.

One time PCR amplification was performed using allele specific two forward primers and a universal reverse primer, namely L374 & F374 (5'-TTGGATGTTGGGGCTTG-3' & 5'-TTGGATGTTGGGGCTTC-3') and (5'-TCCCTTTCATTTTCCAGAGA-3'); respectively (Nakayama *et al.* 2002). The underline base near the 3' end of forward primer was mutant–specific substitution. This allele specific PCR was carried out to identify L374F genotypes (Fig. 2b).

Components of PCR reaction were PCR buffer I (Applied Biosystems, Japan), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 pM/ul reverse and forward primer, and 0.03 unit/ul AmpliTaq Gold (Applied Biosystems).

The conditions for the PCRs were an initial denaturation at 95^0C for 9 min, 40 cycles of 94^0C for 30 sec, 60^0C for 30 sec and 72^0C for 1min with additional extension at 72^0C for 5 min in the last cycle.

There were two PCR reaction mixtures for each sample for wild and mutant type forward primers with a universal reverse primer. After allele specific PCR, the PCR products were 317 bp single fragments were visualized by 2.5 percent agarose gel electrophoresis followed by staining with ethidium bromide and photos were taken by the printgraph (Bioinstrument Atto, Japan). Lanes 1 and 2 were genotyped in two reaction mixtures as wild type. But lanes 3 and 4 were genotyped as heterozygous and lanes 5 and 6 as the mutant homozygous; respectively (Fig. 2). Finally, allele frequencies of the AIM1 codon L374F polymorphism among Nepalese ethnic groups were calculated by the use of Hardy-Weinberg formula for analysis and interpretation.

RESULTS

Fig. 2 revealed the genomic structure of the AIM1 gene with total 7 exons constituted from Newton *et al.* (2001).

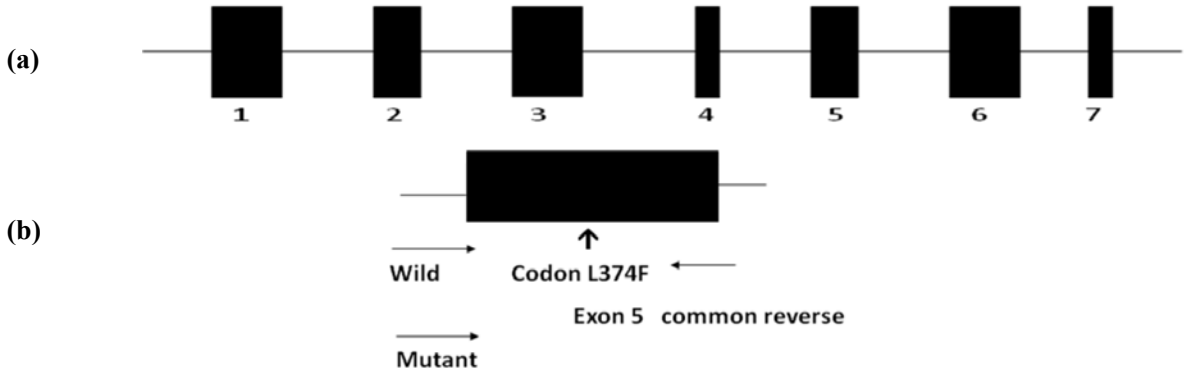

Fig. 2. AIM1 gene analysis: (a) Genomic structure of the AIM1 gene with 7 exons constituted from Newton *et al.*(2001). (b) Designing the allele specific primer set flanking AIM1 codon L374F polymorphism in the exon 5.

Fig. 3 depicted the designing the allele specific primer set flanking AIM1 codon L374F polymorphism in the exon 5. It was the product of the different lane patterns with a single band (317 bp) after performing allele specific PCR. Lane 1 was only amplified but not the lane 2 for the wild type. Both the lanes 3 and 4 were amplified for the wild as well as mutant type for heterozygous. Lane 5 was not amplified for wild type but the lane 6 for the mutant type. Therefore, without using restriction enzyme, genotyping of different ethnic groups could be done by allele specific PCR amplification.

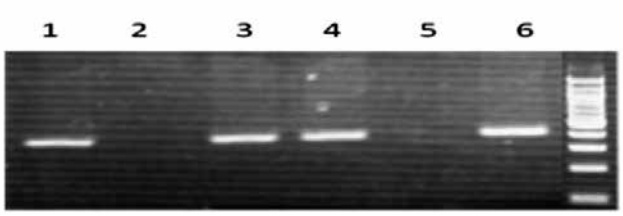

Fig. 3. PCR-RFLP products of the AIM1 gene: PCR Products of AIM1 codon L374F polymorphism in the exon 5 generated by allele specific PCR. A 317 bp single fragment was visualized on 2.5 percent agarose gel electrophoresis followed by staining with ethidium bromide. Genotypes were recognized by allele specific PCR. Each sample contained double lanes. Lanes 1 and 2: wild; lanes 3 and 4: heterozygous; lanes 5 and 6: homozygous types.

Table 1 shows the allele frequencies of the AIM1 codon L374F polymorphism genotyped among six Nepalese ethnic groups. This polymorphism is controlled by two alleles, leucine (L) and phenylalanine (F); respectively.

Table 1. Allele frequencies of AIM1 Codon L373F among 6 different ethnic groups in Nepal

Ethnic groups	Locality (District)	Population	Allele frequency of	
			Leu	Phe
Chepang	Chitwan	72	1.000	0.000
Chidimar	Banke	35	0.886	0.114
Gurung	Kaski and Shyangja	68	1.000	0.000
Munda	Jhapa and Morang	88	0.989	0.011
Raute	Dadeldhura	102	1.000	0.000
Thakali	Mustang and Myagdi	91	1.000	0.000

The AIM1 codon L374F polymorphism distinctively showed conspicuous ethnic distribution of the two alleles. The allele "F" was found mostly in the Caucasoid Chidimar and rarely in the Dravidian Munda. Its frequency ranged from the highest 11.4 percent in the Chidimar to 1.1 percent in the Munda whereas Mongoloid Chepang, Gurung, Raute and Thakali entirely lacked the "F" allele and showed the monomorphic type for the "L" allele. The result segregated the Caucasoid from the Mongoloid ethnic groups with a little chance of genetic admixture of the Munda with the Caucasians.

DISCUSSION AND CONCLUSION

Graf *et al.* (2005) found a significant association between 2 polymorphisms of the MATP gene in Caucasians, an 814G-A transition resulting in a glu272-to-lys substitution (G272K) and an 1122C-G trans-version resulting in a phe374-to-leu substitution (F374L). The systematic ethnic study on the distinctive distribution of AIM1 codon L374F polymorphism was firstly carried out among White South African, Ghanaian, Japanese and New Guinea Islanders with a contrast of skin pigmentation (Newton *et al.* 2001, Yuasa *et al.* 2006, Bin *et al.* 2015). The "F" allele was only detected exclusively in White South Africans (89%) but not at all in all ethnic groups studied (Nakayama *et al.* 2002). The White South Africans were extremely found to show predominant in the "F" allele. In this aspect, the present study is consistent with the only previous researches on different populations. The Caucasoid oriented blackish Chidimar showed the prevalence of the "F" allele but less in amount (11.4%) in comparison to White South Africans. Small percentage of the prevalence of the "F" allele in the Munda provided the evidence of its possible genetic admixture with the Caucasians. The codon L374F could be the best genetic marker for distinguishing Caucasian populations from Mongoloid ones and would explain the ethno-genomics of human skin coloration among many ethnic groups. The genetic variation for skin color background will be fascinating, if ethnic populations are closely watched from Nigeria to Norway and Bali to Italy.

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