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Distribution of Alcohol Related ALDH2 Genotypes among Six Ethnic Groups of Nepal

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ABSTRACT

This paper attempts to detect alcohol related ALDH2 genotypes among six ethnic groups of Nepal. The ALDH2*2 allele is rarely found in Caucasians but readily detect in some 50 percent of the Orientals (Mongolians). Altogether 456 blood cum nail samples were collected from six ethnic groups and DNA extraction was carried out by NaI and Phenol-Chloroform methods and finally the samples were subjected to detect the distribution of ALDH2 genotype frequencies by the use of PCR-RFLP method. Allele frequencies were also calculated by the use of Hardy-Weinberg formula for analysis and interpretation. In the case of Nepalese ethnic populations, the ALDH2*2 allele frequency was found to range from the lowest (1%) in the Raute to the highest (8%) in the Gurung through the middle values found in the Chepang (2%) and the Thakali (2.7%), respectively. The prevalence of ALDH2*2 allele in the Dravidian Munda does not rule out its possible genetic admixture with either the Caucasoid or other ethnic groups. ALDH2*2 allele was totally absent in the Caucasoid Chidimar. Therefore, the ALDH2 gene allele frequency demonstrated ethnic distinction. The Chepang showed significant deviation from Hardy-Weinberg expectation.

Key words: ALDH2*2 allele frequency, alcoholism, Mongolians, Caucasians, genotypes, ethnic groups.

INTRODUCTION

Scientists have been working to unravel the complex relationship between genes and behavior to understand the excessive alcohol addiction and hope to find out better treatment. One of such genes is the enzyme acetaldehyde dehydrogenase (ALDH). Nearly all Caucasians have 2 major ALDH isoforms in the liver: a cytosolic ALDH1 and a mitochondrial ALDH2 (EC1.2.1.3). ALDH2, one of the two major isoforms, is of considerable interest because it is found in two polymorphic forms, the wild type of gene (ALDH2*1) encodes the active enzyme whereas the mutant type gene (ALDH2.*2) encodes the inactive enzyme. The ALDH2*2 allele is rarely found in Caucasians but readily detected in some 50% of the Orientals (Agarwal *et al.* 1981; Impraim *et al.* 1982; Oota *et al.* 2004; Chai *et al.* 2005) and is inherited as autosomal dominant. ALDH2 is involved in alcohol (ethanol) metabolism. During metabolism, alcohol is converted into acetaldehyde by a group of enzymes, which, in turns, is converted into the acetate. The acetate so produced by acetaldehyde oxidation is rapidly metabolized into carbon dioxide and water.

If the ALDH2 gene is a mutant one (ALDH2*2), the acetaldehyde will directly go to the blood as the toxic product will not be converted into carbon dioxide and water via acetate. It will be responsible for acute alcohol intoxication due to accumulation of acetaldehyde. Facial flushing, nausea, dizziness and tachycardia characterize this alcohol intoxication (Yokoyama *et al.* 2005). This kind of alcohol intolerance is due to the absence of the enzyme coded by ALDH2, frequently found in Mongoloid persons (Goedde *et al.* 1979 & 1992). ALDH2 is another burning example of human co-adaptation with another enzyme alcohol dehydrogenase (ADH) to their exposed environment in relation to the particular diet (Ikuta *et al.* 1986; Luo *et al.* 2006). ALDH2 acts in detoxifying a wide variety of organic compounds, toxins and pollutants. Defects in ALDH leads to very rare Sjogren-Larsson syndrome in the ratio of 1:200,000 in humans (DeLaurenzi *et al.* 1996).

One of the most studied polymorphism is a single base-pair mutation (G1510A) in exon 12 of ALDH2 gene that causes an E487K substitution (ALDH2*2 allele) resulting in the inactivation of the enzyme. The ALDH2 locus was assigned to the distal part of the long arm of the chromosome 12 by means of a cDNA probe and Southern blot analysis (Hsu *et al.* 1985). Therefore, the most interesting polymorphism at nucleotide G1510A

for ALDH2*2 allele was taken as a genetic marker to characterize its prevalent rate among Nepalese ethnic groups through the PCR-RFLP approach. The aim of the present study is to detect the distribution pattern of ALDH2 genotype frequencies among six ethnic groups of Nepal.

MATERIALS AND METHODS

Altogether 456 samples belonging to six Nepalese ethnic groups; namely Raute, Chidimar, Thakali, Gurung, Chepang and Munda were collected from different six places in Nepal (Fig.1) which were later subjected to genotyping for detecting the frequency of the ALDH2 gene by the use of PCR-RFLP approach (Table 1 & Fig. 1). NaI and Phenol-Chloroform methods were implemented to extract DNA from blood as well as nails samples.

Fig. 1. Map and location of the indigenous populations studied in Nepal

PCR amplification was performed using an allele specific forward and reverse primer set: ALDH2U (5'-CAAATTACAGGGTCAACTTGCT-3') and ALDH2D (5'- CCACACTCACAGTTTTCTCTT-3'), respectively. The underline base near the 3' end of the primer was mutant–specific substitution. 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 (ApplieBiosystems).

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. The PCR products were 135 bp single fragments for mutant allele and the subsequent *Ksp632I* digestion at 37^0C for overnight cleaved the mutant type allele into 112 bp and 23 bp fragments. These fragments were visualized by 5% agarose gel electrophoresis followed by staining with ethidium bromide and photos were taken by the printgraph (Bioinstrument Atto, Japan). Finally, allele frequencies (ALDH2*1 & ALDH2*2 alleles) of the ALDH2 gene among Raute, Chidimar, Thakali, Gurung, Chepang, and Munda were calculated by the use of Hardy-Weinberg formula for analysis and interpretation (Table 1).

RESULTS

Fig. 2 reveals the genomic structure and Fig. 3 shows the band pattern of the PCR product after digesting with restriction enzyme, *Ksp632I*. Eliminating the *Ksp632I* restriction site from 112 bp and 23 bp fragments lengths, respectively, produced a single 135 bp fragment. ALDH2 gene analysis shows genomic structure of the ALDH2 gene with 13 exons modified from Peterson *et al.* (1999) and designing an allele specific mutant primer set flanking point mutation at nucleotide 1510 changing from G to A in the exon 12.

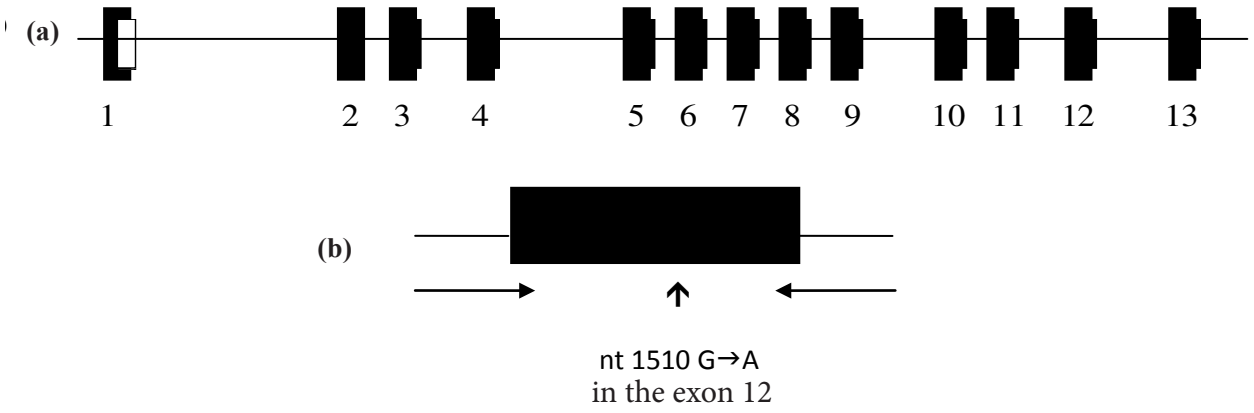

Fig. 2. ALDH2 gene analysis: (a) Genomic structure of the ALDH2 gene with 13 exons modified from Peterson et al. (1999). (b) Designing an allele specific mutant primer set flanking point mutation at nucleotide 1510 changing from G to A in the exon 12.

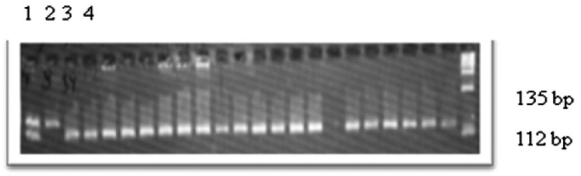

Fig. 3. PCR-RFLP products of the ALDH2 gene: PCR Products of ALDH2 exon 12 generated by PCR-RFLP digested with *Ksp632I*. A 135 bp mutant fragment was cleaved into two wild fragments of 112 and 23 bp. The fragments were visualized on 5% agarose gel electrophoresis followed by staining with ethidium bromide. Lanes 1&2: heterozygous and mutant homozygous; lanes 3 & 4: wild types.

Table 1 shows the allele frequencies of the ALDH2 gene in 6 Nepalese ethnic groups genotyped. The ALDH2 gene system is controlled by two alleles, ALDH2*1 & ALDH2*2, respectively. In the mutant type (inactive form), ALDH2*1 was replaced with ALDH2*2. The ALDH2 gene allele frequency demonstrated ethnic distinction. ALDH2*2 allele was totally absent in the Caucasoid Chidimar, but it was found in all Mongoloid ethnic groups, even in small percentage. This kind of genotype detections were not yet carried out among other Nepali ethnic groups, which is not possible to explore and to compare with other indigenous ethnic groups.

In the case of Nepalese Mongoloid ethnic populations, the ALDH2*2 allele frequency was found to range from the lowest (1%) in the Raute to the highest (8%) in the Gurung through the middle values found in the Chepang (2%) and the Thakali (2.7%), respectively. The prevalence of ALDH2*2 allele in the Dravidian Munda does not rule out its possible genetic admixture with either the Caucasoid or other ethnic groups. ALDH2*2 allele frequency among Nepalese Mongoloids was found to be consistent with range of Tibetan and Mongolians rather than other Chinese and Japanese populations (Chen *et al.* 1994). The ALDH2*2 allele was totally lacked in the individuals of the Caucasoid Chidimar. After analyzing genotype frequency data, the Chepang showed significant deviation from Hardy-Weinberg expectation. This genetic marker is distinctly found only among the Nepali Mongolians (Raute, Gurung, Thakali, and Chepang), but is totally absent in Nepali Caucasian population (Chidimar) and Dravidian Munda which is consistent to the other findings.

Table 1. Distribution of ALDH2 genotypes and frequency of the ALDH2*2 allele among six Nepalese indigenous populations

		Genotype				ALDH2*2 allele		
Population	**n**	**1/1**	**1/2**	**2/2**	**P value**	**frequency**	**±**	**SE**
Chepang	72	70	1	1	<0.001	0.021	±	0.012
Chidimar	35	35	0	0	-	0.000	±	0.000
Gurung	68	57	11	0	>0.05	0.081	±	0.023
Munda	88	88	0	0	-	0.000	±	0.000
Raute	102	100	2	0	>0.05	0.010	±	0.007
Thakali	91	86	5	0	>0.05	0.027	±	0.012

Note- n is the number individuals subjected to analysis. Genotype distribution was tested for conformity with Hardy-Weinberg equilibrium by means of x^2 test.

DISCUSSION

The present study is the first report on the distribution of genotypes and the allele frequency of the ALDH2 gene in Nepalese indigenous populations. The initial alcohol sensitivity, quite common in individuals of Mongoloid origin, might be due to a delayed oxidation of acetaldehyde rather than it's higher than normal production by atypical alcohol dehydrogenase (Goedde *et al.* 1979). The ALDH2*2 allele is frequent in, but confined to Asian individuals, and it appears to be a determinant against alcoholism. On the other hand, alcohol-drinking individuals having the ALDH2*2 genotype are at substantially high risk of developing esophageal and upper aero-digestive tract cancers, head and neck cancers, stomach cancer, colorectal cancer (nome@ galton.ucl.ac.uk, 2002; Ding *et al.* 2010; Matsuo *et al.* 2013).

The ALDH2*2 allele frequency found in Nepalese Mongoloids is much closer to Tibetans and Mongolians than to other Asian Mongoloids supports their proximal

genealogical relationship, probably with Tibetans. The absence of the inactive form of the ALDH2 gene in the Caucasoid Chidimar is consistent with the previous reports on the Caucasoid and other non-Mongoloid populations (Chen *et al.* 1992; Goedde *et al.* 1992; Kim *et al.* 2008). Rare prevalence of ALDH2*2 allele in the Munda provides a clue of its genetic mixture in the past with other Caucasoid and other populations. In addition to this, the prevalence of the ALDH2*2 allele in Nepalese ethnic groups will protect them from the exposure to various alcohol-related diseases because of the protective nature of the ALDH2*2 allele against alcoholism (Hira *et al.* 2013) This kind of protective mechanism of the ALDH2*2 allele against has already been reported, especially in East Asians (Chen *et al.* 1999; Peng *et al.* 2007). The occurrence of the ALDH2*2 allele in the ethnic groups will lead to the genetic adaptation in relation to feeding system along with environments. The findings of the research are consistent to other researches carried out elsewhere (Hsu *et al.* 1985; Goedde *et al.* 1992; Chen *et al.* 1994; Peng *et al.* 2007; Kim *et al.* 2008; Ding et al. 2010; Matsuo *et al.* 2013).

CONCLUSION

The ALDH2*2 allele frequency found in Nepalese Mongoloids is much closer to Tibetans and Mongolians than to other Asian Mongoloids. The most important dimension of the prevalence of ALDH2*2 allele among Nepalese ethnic groups is to use the genotyping data as the tool to compare the various ethnic groups based on their ethnic differences. The consistent findings are observed in Nepali ALDH2 gene frequencies of six ethnic groups. ALDH2*2 is a genetic marker for Nepali Mongoloid population in order to distinguish it from Caucasians.

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