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Occurrence of CCR5 Heterozygous 32 bp Deletion in Nepali Ethnic Groups

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ABSTRACT

The Chemokine (C-C) receptor 5 (CCR5) as one of the small signaling proteins, is a HIV-1 resistant gene. The major boosting to the study of CCR5 gene among ethnic groups in the world is the detection of 32 bp deletion in its heterozygous and homozygous condition which is responsible for relative or absolute resistance to HIV-1 infection. A total of 456 samples belonging to six Nepalese ethnic groups were subjected to genotyping by the use of PCR-RFLP for detecting 32 bp deletion on exon 3 of CCR5 gene. Finally, allele frequencies of 32 bp deletion among Nepalese ethnic groups were calculated by the use of Hardy-Weinberg formula for analysis and interpretation. Chidimar ethnic group, for the first time, showed heterozygous 32 bp deletion at the population level in Asia-pacific region is an excitement in which Chidimar might have conferred resistance against HIV-1 infection in Nepal.

Keywords: CCR5 gene, 32 bp deletion, HIV-1 infection, genotypes, ethnic groups.

INTRODUCTION

Chemokine (C-C) receptor 5 (CCR5), one of small signaling proteins, belongs to CC type subfamily of chemokines (Samson *et al.* 1996). CCR5 gene is also alternatively known by CCCKR5, CMKBR5, CKR5 or Chem13 (Samson *et al.* 1996; Dean *et al.* 1996). Major attraction towards the CCR5 gene began, when it was identified as a co-receptor for the human immunodeficiency virus–1 (HIV-1) (Deng *et al.* 1996; Dragic *et al.* 1996). CCR5 and fusin are two co-receptor promoting the fusion of HIV-1 with the plasma membrane of CD4+ cells. More specifically, CCR5 was responsible for facilitating the entry of macrophage-tropic strains of HIV-1 (Deng *et al.* 1996). CCR5 was localized to chromosome 3p21 (Liu *et al.* 1996).

The researches from different perspectives on the CCR5 gene were initiated after identifying its molecular basis (Liu *et al.* 1996; Samson *et al.* 1996; Mummidi *et al.* 1997) for HIV-1 resistance. The genomic structure of CCR5 is rather interesting. It contains 4 exons with only 2 introns. The exons 2 and 3 are joined together without intron and the exon 4 contains the open reading frame (Mummidi *et al.* 1997; Fig 2a & 2b).

After detecting the thirty-two base pair deletion (<32 or 32 bp deletion) in two independent studies in relation to HIV-1 patients, it was hypothesized that the 32 bp deletions in its heterozygous and homozygous condition may be responsible for relative or absolute resistance to HIV-1 infection (Samson *et al.* 1996; Liu *et al.* 1996). This kind of 32 bp deletion provides a protective mechanism for those individuals who are homozygous confer resistance to HIV-1 infection despite high–risk exposure, whereas those who are heterozygous appear to have slower progression to AIDS following infection. It provided a major boosting to the study of 32 bp deletion among many ethnic groups. A rapid PCR based assay was devised to detect the 32 bp deletion among individuals of major global populations (Martinson *et al.* 1997). The genotype survey of CCR5-del32 allele across Eurasia gave a clear picture of its frequency cline north-to-south downhill gradient and ethnic specificity (Stephens *et al.* 1998). It is also reported that the individuals having 32 bp deletions are at reduced risk of developing asthma (Hall *et al.* 1999). There are no reports on the prevalence of the CCR5 gene among a vast body of Nepalese ethnic groups till now. Therefore, genotyping was performed for the detection of 32 bp deletion among six Nepalese ethnic groups to evaluate its allele frequency.

MATERIALS AND METHODS

Altogether 456 samples belonging to six Nepalese ethnic groups were subjected to genotyping for detecting 32 bp deletion within the exon 3 of CCR5 gene (Table 1). Different methods were implemented to extract DNA from blood as well as nails samples.

Amplification of genomic DNA was carried out by using two different sets of primers on exon 3 (Figs. 2a & 2b). The 1st specific set of primers was used only for PCR

for detecting 32 bp deletion directly without using the restriction enzyme (Fig. 2b). But the 2nd set of primers was used to amplify the fragment length and then to digest with the restriction enzyme in order to confirm the results obtained by the 1st with that of the 2nd set of primers.

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

For the 1st specific set of primers, a 182 bp fragment length flanking the deletion site of the CCR5 gene was amplified by PCR using the previously applied (Liu *et al.* 1996) CCR5-specific forward and reverse primers: SP4.760 (5'-CCTCATTACACCTGCAGCTCT-3') and PM6.942 (5'-CACAGCCCTGTGCTTCTTCTT-3').

For the 2nd set of primers, a 735 bp fragment length of the CCR5 gene was amplified by PCR using the previously applied (Samson *et al.* 1996) forward and reverse primers: CCR5F (5'-CCTGGCTGTCGTCCATGCTG-3') and CCR5R (5'-CTGATCTAGAGCCATGTGCACAACTC T-3'). PCR was performed in a PCR machine (Program Temp. Control System PC-800, Japan) with the same PCR conditions for both sets of primers. Components of PCR reaction were PCR buffer I (Applied Biosystems, Japan), 1.65mM $MgCl_2$, 0.22 mM each dNTP, 0.2pM/ul reverse and forward primer, and 0.05U/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 30sec, 62^0 for 30sec and 72^0 for 1min with additional extension at 72^0C for 5 min in the last cycle. The expected PCR products for the 1st specific set of primers were 182 bp and 150 bp fragments in wild and deletion types, respectively (Fig. 3). Similarly, the expected PCR products for the 2nd set of primers were 735 bp and 703 bp, respectively. But it was difficult to distinguish the big sized (703 bp) mutant band from the wild one (735bp). Therefore, subsequent EcoRI digestion at 37^0C for overnight cleaved the wild 735 bp into a common band of 332 bp for both alleles and into 403 and 371 bp bands for the wild type and mutant alleles, respectively (Figs. 2a & 2b). After PCR, the fragments were visualized in 3 % agarose gel followed by staining with ethidium bromide and photos were taken by the printgraph (Bioinstrument Atto, Japan). Results from both the primer sets were matched each other. Finally, frequencies of the CCR5 32 bp deletion among Nepalese ethnic groups were calculated by the use of Hardy-Weinberg formula (Table 1).

RESULTS

Fig. 3 shows the band pattern in two different primer sets. For the 1st set of primers, a 182 bp and 150 bp fragments could easily be distinguished for wild and 32 bp mutant alleles, respectively (Figs. 2a & 2b & Fig. 3). But for the 2nd set of primers, only the 735 bp fragment could be cleaved into a common band of 332 bp and into 403 bp and 371 bp wild and mutant type alleles, respectively (Fig. 2b). Both results were compatible each other.

Table 1. Distribution of genotypes and frequency of <32 allele of the CCR5 gene among six Nepalese indigenous populations

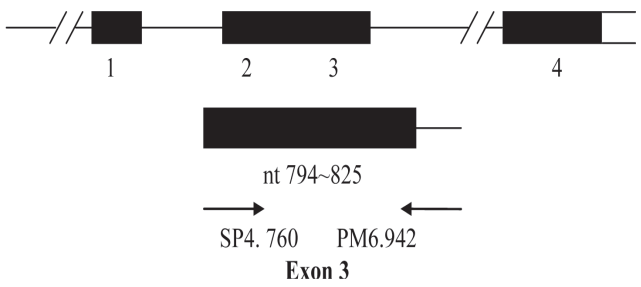
		Genotype				<32 allele		
Population	n	CCR5/CCR5	CCR5/<32	<32/<32	P value	frequency	±	SE
Chepang	72	72	0	0	-	0.000	±	0.000
Chidimar	35	29	6	0	>0.05	0.086	±	0.033
Gurung	68	68	0	0	-	0.000	±	0.000
Munda	88	88	0	0	-	0.000	±	0.000
Raute	102	102	0	0	-	0.000	±	0.000
Thakali	91	88	3	0	>0.05	0.016	±	0.009

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

The most important thing is that the < 32 allele was found among two out of six ethnic groups genotyped. There was a major difference in frequencies between the Caucasoid and Mongoloid populations. The highest frequencies of the mutated allele were found for the first time among the Caucasoid Chidimar (8.6%) living in the low land

Terai close to Indian border. The 2nd highest frequency was found in the Thakali group (1.6%). The Gurung, the Chepang, the Munda and the Raute did not show 32 bp deletions. The detection of the 32 bp deletion in Thakali individuals supported the possible gene admixture with the Caucasoid during their evolution (Table 1).

(a)



(b)

Fig.2. CCR5 gene analysis: (a) Genomic map of the human CCR5 gene with 4 exons modified from Mummidi et al (1997). (b) Designing a specific primer set flanking the 32 bp deletion site for the PCR.

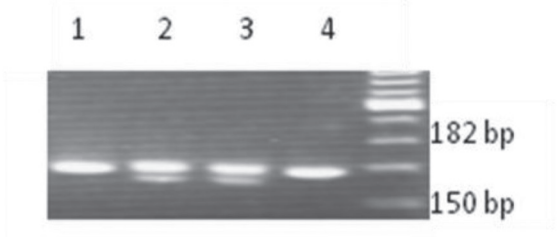


Fig.3. PCR products of the CCR5 gene: CCR5 32 bp deletion in heterozygous state amplified by the onetime PCR with specific primer set. Lanes 2 & 3: 182bp (wild type); lane 1 & 4: 150 bp (32 bp deletion in heterozygous condition).

DISCUSSION

For the first time, the present study detected and documented the prevalence of CCR5 32 bp deletion allele among Nepalese ethnic groups. As in Europe (Lucotte and Mercier, 1998; Lucotte, 2001), a north-to-south cline was not applicable to Nepalese context due to different ethno-geographical accommodation in the natural environment. Various Mongoloid groups inhabit Northern Nepal where the occurrence of the CCR5 32 bp deletion is very rare in comparison to the Caucasoid. Mid-hill Nepal is co-inhabited by both Mongoloid and Caucasoid groups. The low land Terai is dominated by Caucasoid. But the north south internal migration has changed the whole scenario of population composition.

The frequency of CCR5 32 bp deletion among Nepalese ethnic group ranged from 0% in the Gurung, the Chepang, the Munda and the Raute, to 1.6% in the Thakali and to 8.6 % in the Chidimar. The Chidimar ethnic group showed the highest % of CCR5 32 bp deletion allele without any European admixture found in Indian Subcontinent. In India alone, allele frequency of 32 bp deletion was detected less than 1% (Husain *et al.* 1998). A gene frequency of approximately 10% was found for the 32 bp deletion in populations of European descent (Martinson *et al.* 1997). The hypothesis of northern European origin of delta-32 and Viking-mediated dispersal, which was originally proposed by Lucotte and Mercier (1998), was also supported by other similar researches (Novembre *et al.* 2005).The highest and the lowest frequencies were detected in Finnish/Mordvinian populations (16%) and Sardinia (4%) (Libert *et al.* 1998) respectively. Some results in Slovakian populations do not indicate that a relatively low incidence of HIV-1 infection in Slovakia could be caused by the CCR5-Δ32 mutation (Takacova *et al.* 2008). Some findings support previous data showing Delta-32 as a genetic protective factor against HIV-1 infection in Mexican women, as well as in women from other populations (Estrada-Aguirre *et al.* 2013). Recently a positive correlation has been established between CCR5-Δ32 and lupus nephritis risk in the Chinese Han ethnic population by playing a role in lupus nephritis susceptibility (Cheng *et al.* 2014).

This sort of first report of CCR5 32 bp deletion in Nepali Chidimar and Thakali has suggested the further need of carrying out the molecular analysis in order to show whether CCR5 32bp deletion confers the resistance to the HIV-1 infection among many ethnic groups in Nepal or not.

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