Multi-SNP ANALYSIS OF CCR5-CCR2 GENES IN ETHIOPIAN JEWS: MICRO-EVOLUTION AND HIV-RESISTANCE IMPLICATIONS

Korostishevsky M.1, Bonne'-Tamir B.1, Bentwich Z.2, Tsimanis A.2

1Department of Human Molecular Genetics and Biochemistry, Tel Aviv University, Israel; 2Institute of Clinical Immunology and AIDS, Kaplan Medical Center, Rehovot, Israel

*Corresponding author: e-mail: korost@post.tau.ac.il

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SUMMARY

Motivation: This is the first report concerning CR5 SNPs in the Ethiopian Jewish population. CCR5 and CCR2 genes have been implicated in HIV disease progression, resistance or non-progressive infection. To determine the influence of host genetics on HIV infection, we examined 29 HIV-seronegative individuals of Ethiopian descent for polymorphisms in the CCR5-CCR2 gene region and compared the results with those of 13 exposed but uninfected individuals. Multi-SNP analysis was used for sample comparisons and for population relationship estimates.

Results: Using multi-SNP analysis, no significant differences in the genotypes frequencies between the studied groups was found ($\chi^2_{3df} = 4.662, p = 0.198$). The pattern of CCR5-CCR2 genetic variations in Ethiopian Jews resembles the one found in Asian populations and is distinguished from the one found in Africans.


INTRODUCTION

The chemokine receptor CCR5 is an essential co-receptor for the cellular entry of R5 strains of HIV-1, which predominate in the early stages of infection (Moore et al., 1997). Following infection with HIV-1, the majority of patients develop AIDS within 10 years, a small subset of infected individuals rapidly progress to AIDS, and less than 5% remain asymptomatic without antiretroviral therapy. There is evident that, even after being repeatedly exposed to HIV, some individuals remain seronegative.

It has been proposed that HIV infection and disease progression might be genetically controlled. Functionally important polymorphisms in the regulatory region of CCR5 gene, a 32-base pair deletion in the coding part of CCR5 (CCR5-Δ32) and a single conservative valine-to-isoleucine (V64I) mutation in CCR2 coding region (CCR2-G190A) have been identified (Carrington et al., 1999). These polymorphisms are distributed through human populations with differing frequencies depending on the ethnic groups or on particular population groups that contain distinctive set of haplotype pair combinations (Gonzalez et al., 1999). The finding that polymorphisms in the promoter region of CCR5 are associated with differential HIV-1 disease progression and susceptibility of cells to HIV-1 infection suggests that these haplotypes are not functionally similar.

The best genetic feature characterized is the CCR5-Δ32 deletion that results in synthesis of a short, nonfunctional CCR5 protein and the absence of cell surface CCR5
expression. Thus, the mechanism of protection most likely involves a reduction in the number of CCR5-positive target cells. The CCR2-V64I mutation confers resistance to AIDS progression, probably due to the heterodimerization and sequestration of the CCR5 receptor (Mellado et al., 1999). Mutations in the CCR5 promoter region determine the level of CCR5 gene transcription and production of the corresponding mRNA. It has also been hypothesized that polymorphisms in the CCR5 promoter region may influence cell surface expression and consequently could influence individual susceptibility to HIV. However, these data must be used with caution: firstly, a recent cohort study of Ugandan population shows no association between CCR5 polymorphisms and the rate of disease progression (Ramaley et al., 2002). Secondly, similar expression level of CCR5 was found in HIV-exposed uninfected female prostitutes and in unexposed control individuals from Kenya and Ethiopia (Fowke et al., 1998; Messele et al., 2001). Thirdly, in vitro infection study revealed that PBMC isolated from HIV-highly exposed uninfected and unexposed Thai women carrying different CCR5 haplogroups, had no differences in susceptibility to HIV-1 infection (Kulkarni et al., 2003).

Present-day Ethiopian Jews lived in the north of Lake Tana in Gondar. During Ethiopian civil war (1984–1985) and then in 1989, several thousands of Ethiopian Jews were airlifted to Israel. Some of them arrived in Israel with dramatic health problems including a variety of immune-mediated and infectious diseases. There is abundant evidence that chemokines and cytokines production, which is under genetic control, may exert certain disease occurrences and outcomes.

Using evolutionary-based CCR5 haplotype classification (Gonzalez et al., 1999), we have characterized the DNA polymorphisms at the loci that encode CCR5 and CCR2 receptors, in two groups of Ethiopian Jews: healthy individuals without any history of HIV infection and individuals who were exposed but uninfected. We verified the presence of CCR5-A32 deletion and genotyped sequence variations for the single-nucleotide polymorphism (SNP) G208T, T627C, A676G and C927T in the CCR5 promoter region as well as G190A mutation in the coding part of CCR2 gene.

We estimated the magnitude of LD between the SNPs and performed multi-SNP analysis between the samples. Using the haplotype distribution data for different ethnic groups, we investigated the phylogenetic relationships for Ethiopian Jews.

SAMPLES AND METHODS

A total of 29 HIV-1-negative (control) and 13 exposed but uninfected seronegative (ESN) Ethiopian Jews were sampled for this study. The evaluation of the clinical status of individuals including the presence of antibodies to HIV-1 and HIV-1 viral particles has been performed at the Kaplan Medical Center, Rehovot, Israel. All samples were coded and blind-tested. Informed consent was obtained for the collected samples.

We used genomic DNA obtained from peripheral blood lymphocytes. The DNA samples were subjected to a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. PCR amplification was performed to amplify CCR5 promoter and CCR5 and CCR2 genes fragments covering the polymorphic sites.

Possible differences in the frequency of each of the SNP genotypes or alleles between the samples were estimated using the $\chi^2$ test, as described elsewhere. The Arlequin software package, http://Lgb.unige.ch/arlequin, was used to evaluate genetic distances between different populations, and to calculate the maximum likelihood (ML) of haplotype frequencies. Based on the ML haplotype frequency estimates, the likelihood ratio test (LRT) for sample differentiation was performed as we previously described (Korostishevsky et al., 2006). PHYLIP (http://evolution.gs.washington.edu/phylip.html) package was used for phylogeny inferences based on the CCR2-CCR5 region genetic distances.
RESULTS AND DISCUSSION

Two groups of Ethiopian Jews were genotyped for the CCR5-Δ32, CCR2-G190A and CCR5 promoter alleles constituting the CCR5 human haplotypes. We performed DNA PCR by use of primers that amplified the region encoding the 32-bp deletion. No CCR5-Δ32 deletions were detected in both groups. CCR5-Δ32 allele is very common in Caucasians, but no such allele was reported in people of African and Asian descent including Ethiopian Jews (Kantor, Gershoni, 1999).

A discrepancy between the control and ESN individuals was observed for the T627C SNP only ($\chi^2_{1df} = 4.140$, $p = 0.042$). This deviation in allele frequency of one from 5 SNPs was not preserved after the Bonferroni correction (data not shown). The haplotype distributions in the samples are presented Table 1. The multi-SNP likelihood ratio test (Korostishevsky et al., 2006) did not elicit significant differences between the studied groups ($\chi^2_{3df} = 4.66$, $p = 0.198$).

Table 1. ML estimates of haplotype frequencies in ESN and Control samples

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>HH-code</th>
<th>ESN</th>
<th>Control</th>
<th>OR</th>
<th>LRT* (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-G-C-A-C</td>
<td>HHE</td>
<td>0.385</td>
<td>0.207</td>
<td>1.859</td>
<td></td>
</tr>
<tr>
<td>G-T-G-C</td>
<td>HHIC</td>
<td>0.231</td>
<td>0.431</td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td>A-G-C-A-T</td>
<td>HHF*2</td>
<td>0.269</td>
<td>0.207</td>
<td>1.301</td>
<td></td>
</tr>
<tr>
<td>G-G-T-A-C</td>
<td>HHA</td>
<td>0.115</td>
<td>0.155</td>
<td>0.744</td>
<td></td>
</tr>
</tbody>
</table>

Our results indicate that the HHF*2 frequency is slightly higher in the ESN individuals than in the group of HIV-1-negative Ethiopian Jews (26.9 % vs. 20.7 %), although the difference did not attain statistical significance. In the control group, the most common CCR5 haplotype was HHC (43.1 %), but in the group of ESN individuals the most common was HHE (38.6 %). These two haplotypes have significantly higher frequencies in Caucasians (Gonzalez et al., 2000). The minor haplotype in both studied groups was HHA (15.5 % and 11.5 %, respectively), which was more frequent in Africans. Genotyping of 29 HIV-negative and 13 ESN Ethiopian Jews failed to detect presence of HHD and/or HHB haplotypes, which were reported as specific to African population.

Recently, a comparative analysis of CCR5 polymorphisms in HIV-exposed uninfected individuals from two ethnic groups, Caucasian and Asian, was undertaken (Gonzalez et al., 1999; Mangano et al., 2000). Nevertheless, in vitro infection experiments showed that PBMC isolated from the HIV-1-exposed and unexposed seronegative women carrying different CCR5 haplogroup had no differences in susceptibility to HIV-1 infection (Yang et al., 2003).

The genetic affinities of the Ethiopian Jews were investigated using classical autosomal markers, as well as DNA, mtDNA and Y-chromosome markers. It was demonstrated that the Ethiopian Jews are a mixture of African and Caucasian (Asian) population and are significantly different from other Jewish communities. Several authors argued that Ethiopian Jews were derived mostly from Africans. However, both cultural and historic evidence suggest close affinity between Ethiopians and Asian populations (Near East and southern Arabia) (Ritte et al., 1993; Hammer et al., 2000).

We used a tree reconstruction to investigate population relationships according to CCR5-CCR2 polymorphism. The dendrogram shows two well-defined groups. The first one contains two populations: Non-Pygmy and American-Africans. The second group contains the remaining six populations which are further divided. Within this group, Ethiopian Jews are “sisters” to Asian populations (non-Indians and Indians), while the Thai population is found to be the most distant (Fig. 1).

In conclusion, our results indicate that there is no significant difference in the distribution of CCR5 haplotypes among ESN and control individuals. We also did not observe preponderance of AIDS-“protective” 303-G, 627-T and 676-G alleles in ESN.
individuals. On the contrary, frequencies of these alleles are higher in the control group. The pattern of CCR5-CCR2 genetic variations in Ethiopian Jews resembles the one found in Asian populations and is distinguished from that found in Africans.

Figure 1. UPGMA tree based on CCR5-CCR2 haplotype frequencies.

REFERENCES


