Distribution of Four HIV Type 1-Resistance Polymorphisms
(CCR5-Δ32, CCR5-m303, CCR2-64I, and SDF1-3′A) in the Bahraini Population

Abdel Halim Salem,1 Eman Farid,2 Raouf Fadel,1 Marwan Abu-Hijleh,1 Wassim Almawi,3 Kyudong Han,4 and Mark A. Batzer4

Abstract

Allelic differences of chemokine (C-C motif) receptor 5 (CCR5) and CCR2, as well as the ligand for the chemokine receptor CXCR4, stromal-derived factor (SDF-1), are known to suppress HIV-1 transmission and to be involved in delay in HIV-1 disease progression. The aim of our study was to investigate the frequencies of four mutations that confer resistance to HIV-1: CCR5-Δ32, CCR5-m303, CCR2-64I, and SDF1-3′A among Bahrainis. We have studied the DNA polymorphisms in 304 unrelated healthy Bahraini individuals without any known history of HIV-1 infection or AIDS symptoms. The CCR5-Δ32 mutation was detected by PCR analysis, while the CCR5-m303, CCR2-64I, and SDF1-3′A mutations were detected by PCR-restriction fragment length polymorphism (PCR-RFLP) tests. Allele frequencies and the fit to the Hardy–Weinberg equilibrium were evaluated using the Arlequin population genetics application. The frequencies of the CCR5-Δ32, CCR2-64I, and SDF1-3′A alleles were 2.8%, 8.9%, and 26.5%, respectively. No mutant alleles were detected for the CCR5-m303 mutation in 304 individuals. We estimated the risk of AIDS onset (relative hazard), computed from the three-locus genotype data. This is the first report of these four mutations conferring resistance to HIV-1 in the Bahraini population. The presence of the CCR5-Δ32 allele among Bahrainis may be attributed to the admixture with people of European descent. The CCR2-64I allele and especially the SDF1-3′A allele are predominant in the Bahraini population and may be associated with resistance to fast HIV-1 infection in Bahrainis, and thus their genotyping can be used for prognosis in HIV-infected individuals.

Introduction

Aquired immunodeficiency syndrome (AIDS) is a disease of the immune system that has been at the center of public health concerns for the past three decades. The chemokine receptors CXCR4, CCR5, and CCR2, G protein-coupled receptors, play a significant role in the fusion of HIV to CD4 target cells. Their identification has led to the discovery of several genetic factors that might control HIV-1 infection.1–5 CCR5-Δ32 is a naturally occurring knockout deletion (32 bp) variant.6 Individuals homozygous for the CCR5-Δ32 allele are highly resistant to HIV-1 infection, whereas the heterozygotes can remarkably delay the progression to AIDS.7–10 A point mutation (CCR5-m303) in the coding region of the CCR-5 gene that confers in vitro and in vivo resistance to the R5 virus has been identified.11 This mutation, when found in the compound CCR5-Δ32 heterozygotes, was associated with enhanced protection. A valine-to-isoleucine substitution at position 64 of the CCR2 protein (CCR2-64I) has been associated with a slower disease progression to AIDS, of about 2–4 years.8 A G-to-A transition at nucleotide 801 in the 3′ untranslated region of the SDF1 gene (SDF1-3′A) has also been reported to slow disease progression,12,13 although this finding is still controversial.14 The protective effects of CCR5-Δ32 and CCR2-64I were observed to be dominant, while that of SDF1-3′A was recessive.8,12

The mutant CCR5-Δ32 is widely observed among individuals of European descent, but it appears to be relatively rare in the Middle East, Asia, and nearly absent in Africa.15–19 However, the CCR5-m303 allele is very rare with an allelic frequency of less than 1%,20 whereas CCR2-64I and SDF1-3′A are distributed in all population groups worldwide.21–23

1Department of Anatomy, 2Department of Microbiology, and 3Department of Biochemistry, College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain.
4Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803.
An accurate estimate of the HIV/AIDS epidemiological situation in Bahrain is impossible because of the lack of updated, consistent data. However, according to a surveillance report by the Ministry of Health in Bahrain, dated June 2002, 122 HIV/AIDS cases were observed. The 2002 Joint United Nations Program on HIV/AIDS report, on the other hand, estimated that the total number of HIV/AIDS cases in Bahrain at the end of 2001 was ~1000 (http://www.undp.org.bh/Files/MDGO3/Goal6.pdf). In spite of the importance of chemokine receptors in AIDS pathogenesis, no information is accessible on the frequency of chemokine receptor gene polymorphisms conferring resistance to HIV-1 infection in the Bahraini population. In this study, we investigated the frequency of CCR5-Δ32, CCR5-m303, CCR2-64I, and SDF1-3’A alleles in the Bahraini population. The relative hazard (RH) index was estimated to evaluate the potential for natural resistance against HIV-1 infection and progression of AIDS.

Materials and Methods

DNA samples

DNA was extracted from 304 blood samples taken from unrelated Bahraini individuals of Arab ancestry (for at least three generations) from the Salmaniya Medical Hospital without any known history of HIV-1 infection or AIDS symptoms. Expatriates were excluded from our study. A written informed consent from all participants included in this study was done under institutionally approved internal review board protocols.

Genotyping

A PCR-based assay was used for genotyping CCR5-Δ32 mutation. A PCR-RFLP assay was used for genotyping CCR5-m303, CCR2-64I, and SDF1-3’A mutations. For genotyping the four loci, CCR5-Δ32, CCR5-m303, CCR2-64I, and SDF1-3’A, the published primer sequences and PCR conditions were adapted.5,12,24,25 The digested PCR products were genotyped by agarose gel electrophoresis. For CCR5, the amplified products can be scored as wild type (225 bp) or mutant (193 bp). For the detection of the CCR5-m303 mutation, the amplified fragment was digested by HincII restriction enzyme at 37°C for 4 h; wild-type (419 bp and 165 bp), heterozygote (584 bp, 419 bp, and 165 bp), and mutant homozygote (584 bp, no restriction site) products were expected. For CCR2, polymorphisms were detected by digesting the PCR products with BsaI at 60°C for 4 h; wild-type product shows up as a single 128-bp band (undigested), heterozygote as 128-bp and 110-bp bands, and mutant homozygote as a single 110-bp band. For SDF1, PCR products were digested with MspI at 37°C for 4 h and genotypes are scored as wild type (202 bp and 100 bp), heterozygote (302 bp, 202 bp, and 100 bp), and mutant homozygote (302 bp, no restriction site).

Statistical analysis

Allele frequencies and the fit to the Hardy–Weinberg equilibrium (HWE) were evaluated by the Arlequin population genetic software.26 Comparison of CCR5-Δ32, CCR2-64I, and SDF1-3’A frequencies between Bahrainis and other populations was determined using Chi-square tests. The level of statistical significance was set at 0.05. To evaluate AIDS onset risk for the Bahraini population, the RH value was calculated based on three-locus genotypes of CCR5-Δ32, CCR2-64I, and SDF1-3’A alleles, not including the CCR5-m303 allele. There are 27 possible three-locus genotypes adapted from the Winkler et al.12 cohort studies that can be grouped into four distinct values. The RH of a population can be estimated as RH = Σ Wp, where W and P are the genotype-specific RH and frequencies summed over all four groups of genotypes. The standard errors (SEs) of population-specific RH estimates are computed assuming that the frequency distributions of genotypes are from multinomial samples.27 Three AIDS definitions, AIDS-1993, AIDS-1987, and Death, following the classification of Dean et al.,9 were considered in the RH evaluations.

Results

The genotype distributions for CCR5-Δ32, CCR2-64I, and SDF1-3’A loci in the Bahraini population are shown in Table 1.

Table 1. Allele and Genotype Frequencies of the CCR5, CCR2, and SDF1 Loci in 304 Bahraini Individuals

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype frequency</th>
<th>Allele frequency</th>
<th>Expected HWE p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5Δ32</td>
<td>wt/wt 288 (94.7%)</td>
<td>Δ32/Δ32 1.0000</td>
<td>0.2053</td>
</tr>
<tr>
<td></td>
<td>Δ32/Δ32 15 (4.9%)</td>
<td>Δ32/Δ32 0.028</td>
<td></td>
</tr>
<tr>
<td>CCR2-64I</td>
<td>wt/wt 252 (82.9%)</td>
<td>64I/64I 0.089</td>
<td>1.0000</td>
</tr>
<tr>
<td></td>
<td>wt/64I 50 (16.4%)</td>
<td>64I/64I 0.089</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wt/64I 2 (0.7%)</td>
<td>64I/64I 0.089</td>
<td></td>
</tr>
<tr>
<td>SDF1-3’A</td>
<td>wt/wt 162 (53.3%)</td>
<td>3’A/3’A 0.5590</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wt/3’A 123 (40.5%)</td>
<td>3’A/3’A 0.5590</td>
<td></td>
</tr>
<tr>
<td></td>
<td>wt/3’A 19 (6.3%)</td>
<td>3’A/3’A 0.265</td>
<td></td>
</tr>
</tbody>
</table>

*Exact probability test26 for significant departure from HWE, implemented in Arlequin (ver. 3.0),26 with 100,000 step Markov chain and 1000 dememorization steps.
6.3% for SDF1

FREQUENCY OF HIV-1 RESISTANCE POLYMORPHISMS

Table 2. Allele Frequencies of CCR5-Δ32, CCR2-64I, and SDF1-3’A in Different Populations

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>CCR5Δ32</th>
<th>CCR2-64I</th>
<th>SDF1-3’A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benin</td>
<td>22</td>
<td>26</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Sudan</td>
<td>22</td>
<td>25</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Nigeria</td>
<td>22</td>
<td>23</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Tunisia</td>
<td>37</td>
<td>145</td>
<td>0</td>
<td>19.3</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>38</td>
<td>250</td>
<td>9.6</td>
<td>—</td>
</tr>
<tr>
<td>Poland</td>
<td>39</td>
<td>1063</td>
<td>—</td>
<td>11.3</td>
</tr>
<tr>
<td>Italy</td>
<td>40</td>
<td>275</td>
<td>4.54</td>
<td>6.55</td>
</tr>
<tr>
<td>Russia</td>
<td>41</td>
<td>171</td>
<td>9.06</td>
<td>10.61</td>
</tr>
<tr>
<td>Greece</td>
<td>42</td>
<td>200</td>
<td>3.25</td>
<td>11.75</td>
</tr>
<tr>
<td>South East Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>43</td>
<td>500</td>
<td>1.5</td>
<td>9</td>
</tr>
<tr>
<td>Japan</td>
<td>44</td>
<td>393</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>China</td>
<td>38</td>
<td>1036</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Middle East</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahrain (present</td>
<td>304</td>
<td>2.8</td>
<td>8.9</td>
<td>26.5</td>
</tr>
<tr>
<td>study)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>45</td>
<td>200</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>17</td>
<td>241</td>
<td>2.1</td>
<td>—</td>
</tr>
<tr>
<td>Kuwait</td>
<td>18</td>
<td>393</td>
<td>1.02</td>
<td>—</td>
</tr>
<tr>
<td>Kuwait</td>
<td>25</td>
<td>113</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>UAE</td>
<td>22</td>
<td>26</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Iran</td>
<td>16</td>
<td>395</td>
<td>1.46</td>
<td>12.21</td>
</tr>
</tbody>
</table>

(16.4%) and 64I/64I (0.7%). In this Bahraini cohort, the frequencies of the wild-type allele CCR2 and the 64I variant were 0.911 and 0.089, respectively. The distribution of genotypes was in agreement with HWE expectations ($p > 0.05$). Table 2 shows the frequency of the CCR2-64I allele in the Bahraini population as well as selected populations from previous studies.

SDF1-3’A

Genotyping of the Bahraini subjects for the SDF1-3’A mutation revealed a genotype distribution of 53.3%, 40.5%, and 6.3% for SDF1/SDF1, SDF1/3’A, and 3’A/3’A, respectively. The frequencies of the wild-type allele SDF1 and the 3’A variant were found to be 0.735 and 0.265, respectively. No significant deviations from the HWE were observed ($p > 0.05$). Table 2 shows the frequency of the SDF1-3’A allele in the Bahraini population as well as selected populations from previous studies.

CCR5-m303

The CCR5-m303 genotype was determined by PCR-RFLP from 304 Bahraini individuals. No individual was found to be homozygous or heterozygous for the CCR5-m303 mutation. According to the data analysis, the frequency of CCR5-m303 allele is less than 0.3% (less than 1 in 304). It is of interest that the test used for detection of the CCR5-m303 mutation allows simultaneous detection of the CCR5-Δ32 mutation.

Relative hazard (RH) evaluation

Estimates of the RH values for the Bahraini population are presented in Table 3. RH values are relatively high for all three AIDS-defining criteria (AIDS-1993, AIDS-1987, and Death).

Table 3. The RH Values in the Bahraini Population

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>RH1 (SE)</th>
<th>RH2 (SE)</th>
<th>RH3 (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahrain</td>
<td>304</td>
<td>0.89 (0.03)</td>
<td>0.88 (0.03)</td>
<td>0.85 (0.03)</td>
</tr>
</tbody>
</table>

*The RH values were calculated based on three AIDS definitions, AIDS-1993 (RH1), AIDS-1987 (RH2), and Death (RH3).*

Discussion

Recently, Hutcheson et al. detected 20 AIDS restriction genes from the genome-wide screening of common genetic polymorphisms among candidate genes by using the combination of high-throughput genotyping techniques and HapMap annotation of human SNP variants. The AIDS restriction genes might affect susceptibility to HIV infection or to AIDS progression. Among them, mutations of the CCR5, CCR2, and SDF1 genes, associated with suppressing HIV-1 infection or delay in disease progression, have been well-characterized and studied in different populations. Therefore, the assessment of mutation frequencies offering genetic resistance to HIV/AIDS in different populations might be helpful in predicting the dynamics of HIV/AIDS epidemics as well as gaining insight about their origin.

The frequency of the CCR5-Δ32 allele in the Bahraini population studied is slightly higher than that reported for other Arabian populations but it is consistent with data reported from other populations with non-European ancestors. Within the Middle Eastern populations, the frequency of the mutant CCR5-Δ32 allele reached its highest among Bahrainis, 2.8%; Iranians, 2.4%; Saudi, 2.1%; and its lowest among Kuwaitis, 1%; and Egyptians, 0.5%; and is completely absent in individuals from the United Arab Emirates. Our results suggest that the CCR5-Δ32 allele is detected at very low frequency in Arabian populations. However, the CCR5-Δ32 allele is observed mostly in European populations. The marginal presence of the allele seen in the Bahraini population could be due to gene flow from people of European descent.

The CCR5-m303 mutation is characterized by an open reading frame single T-to-A transversion at nucleotide 303, which indicates a nonsense mutation in the first extracellular loop of the chemokine receptor protein at amino acid 101. This mechanism of protective action of the CCR5-64I substitution is indirect. CCR2-64I protein can preferentially dimerize with CCR5 and CXCR4. In addition, the CCR2-64I substitution is located in a transmembrane domain of the CCR-2 protein, which is not a part of the HIV-binding site. Thus, the mechanism of protective action of the CCR2-64I mutation might be indirect. CCR2-64I protein can preferentially dimerize with CXCR4 polypeptides, whereas wild-type CCR2 protein does not.

These observations imply that CCR2-64I delays AIDS progression by limiting the transition from CCR5 to CXCR4 in infected individuals, a turning point in the collapse of the CD4-T lymphocytes and a prelude to AIDS-defining disease. The frequency of the CCR2-64I allele was common in all ethnic groups with the following allele frequencies: 10% in whites, 15% in African-Americans, and 25% in Asians. The frequency of the CCR2-64I allele in Bahrainis is slightly lower than that reported for other Middle Eastern populations.
The frequency of the mutant CCR2-64I allele reached its highest among Iranians, 12.2%; Kuwaitis, 12%; and Bahrainis, 8.9%, and reached its lowest in individuals from the United Arab Emirates, 6%. The worldwide data show complete absence of this allele in some Southeast Asian populations. Our results suggest that the distribution of the CCR2-64I allele among Bahrainis is comparable to other Arabic populations.

The protective effect of the SDF1-3'A mutation is recessive. In other words, it is observed only in homozygotes and no difference is apparent in this respect between wild-type homozygotes and heterozygotes. The prevalence of the SDF1-3'A is quite wide-ranging and it is extremely high in Oceania and relatively low in African populations. The frequency of SDF1-3'A in other populations ranged from 3% to 43%. Within the Middle Eastern populations, the frequency of the mutant SDF1-3'A allele is higher among individuals from the United Arab Emirates, 35%; Bahrainis, 26.5%; and Kuwaitis, 25.9%, which suggests that the distribution of the SDF1-3'A allele among Bahrainis is comparable to other Arabic populations.

Analysis of the genotypic frequencies did not show any significant deviation from HWE in the Bahraini population, implying the absence of detectable differential selection among individuals with and without the mutations.

We estimated the average RH values based on three-locus genotypes to determine the potential risk of AIDS onset in Bahrainis (Table 3). Geographically close populations tend to share comparable allele frequencies at the three loci, resulting in similar RH values across these populations. The Bahraini population exhibits somewhat similar RH values (0.85–0.89) as compared to those of the United Arab Emirates, which suggests that the distribution of the SDF1-3'A allele among Bahrainis is comparable to other Arabic populations.

In contrast, Oceanic populations have been reported to have the lowest RH value, which implies that they have the greatest protection from HIV-1 infection or AIDS onset. The worldwide data suggest that Southeast Asian populations and African populations have been observed to have the highest RH values, which is consistent with the high prevalence of AIDS epidemics in these two regions. It is likely that the intermediate frequencies of two mutant alleles (SDF1-3'A and CCR2-64I) and the very low frequency of CCR5-D32 resulted in high RH values for Bahraini population.

Most of the data currently available for Middle Eastern populations are related to the CCR5-D32 mutation. Very little is known regarding the frequencies of the other three HIV/AIDS resistance mutations in most Arabic and Middle Eastern countries. The aim of this study was to obtain such information for the Bahraini population.

Conclusions

The frequency of the CCR5-D32 allele among Bahrainis and Arabs in general is very low and probably derived from admixture with people of European descent. The CCR2-64I and especially SDF1-3'A mutations are sufficiently common in the Bahraini population and may be associated with slower HIV-1 progression in Bahrainis. The presence of these mutations can be used in part for prognostic genotyping in HIV-infected individuals. High RH values among Bahrainis suggest that this population is vulnerable to HIV infection. This is the first report of CCR5-D32, CCR5-m303, CCR2-64I, and SDF1-3'A polymorphisms in the Bahraini population, information on host genetic factors in this population that will prove useful for future studies of contributors to the prevention of HIV-1 infection or the delay of disease progression.

Acknowledgments

We thank Thomas J. Meyer for useful comments during the preparation of this manuscript. This work was supported by grants from the Arabian Gulf University, Grant 51 (A.-H.S.), and the State of Louisiana Board of Regents Governor’s Bio-technology Initiative GBI (2002-005) (M.A.B.).

References

8. Smith MW, Dean M, Carrington M, et al.: Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. Science 1997;277:959–965.
FREQUENCY OF HIV-1 RESISTANCE POLYMORPHISMS


Address correspondence to: Mark A. Batzer
Department of Biological Sciences
202 Life Sciences Building
Louisiana State University
Baton Rouge, Louisiana 70803
E-mail: mbatzer@lsu.edu