Genotype and Allele Frequency of a 32-Base Pair Deletion Mutation in the CCR5 Gene in Various Ethnic Groups: Absence of Mutation among Asians and Pacific Islanders

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ABSTRACT

Background: A 32-base pair (bp) deletion mutation in the beta-chemokine receptor CCR5 gene has been associated with resistance against human immunodeficiency virus type 1 (HIV-1) infection and disease. Large-scale studies conducted among Caucasians indicate that individuals who are homozygous for this deletion mutation (A32/A32) are protected against HIV-1 infection despite multiple high-risk exposures, whereas CCR5/A32 heterozygotes have a slower progression to acquired immunodeficiency syndrome (AIDS).

Objective: To determine the genotype and allele frequencies of the CCR5 gene 32-bp deletion mutation among ethnically diverse non-Caucasian populations.

Methods: DNA, extracted from blood collected between 1980 and 1997 from 1912 individuals belonging to various ethnic groups, including 363 Caucasians, 303 Puerto Rican Hispanics, 150 Africans, 606 Asians, and 490 Pacific Islanders, were analyzed for the CCR5 gene 32-bp deletion mutation by a polymerase chain reaction (PCR)-based assay, using an oligonucleotide primer pair designed to discriminate CCR5 alleles without restriction endonuclease analysis.

Results: The comparative frequency of CCR5/A32 heterozygosity was 61 of 363 (16.8%) in Caucasians, 17 of 303 (5.6%) in Puerto Rican Hispanics, 9 of 490 (1.8%) in Pacific Islanders, 0 of 606 (0%) in Asians, and 0 of 150 (0%) in Africans.

Conclusions: The data confirm the high frequency of CCR5/A32 heterozygosity among Caucasians, intermediate and low-level A32 allele frequencies among Puerto Rican Hispanics and Hawaiians could be attributed to recent European Caucasian gene flow. By contrast, the inability to detect the A32 allele among Asians and other Pacific Islander groups suggests that other mechanisms are responsible for resistance to HIV-1 infection in these populations.

Key Words: chemokine receptor, human immunodeficiency virus, Melanesia, Micronesia, polymerase chain reaction, Polynesia, population genetics


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Entry of human immunodeficiency virus type 1 (HIV-1) into target cells requires the binding of the external envelope glycoprotein gp120 to both the CD4 molecule and one of several chemokine receptors, recently discovered to function as coreceptors. T-cell line-tropic HIV-1 strains utilize the α-chemokine receptor CXCR4, whereas the β-chemokine receptor 5 (CCR5), which is expressed on monocytes/macrophages, T cells, and granulocyte precursors, is the key cofactor for macrophage-tropic HIV-1 strains, which predominate during the asymptomatic phase of infection.

A 32-base pair (bp) deletion mutation (Δ32) within the second extracellular loop–encoding region of the CCR5 gene, which results in a truncated, nonfunctional protein, has been associated with relative resistance to HIV-1 infection and slower progression to acquired immunodeficiency syndrome (AIDS). Specifically, Δ32/Δ32 homozygotes are protected against acquisition of HIV-1 by the mucosal route despite high-risk exposure, whereas disease progression among CCR5/Δ32 heterozygotes occurs more slowly.

Among Caucasians, the genotype frequencies of CCR5/Δ32 heterozygosity and Δ32/Δ32 homozygosity are 16 to 35% and 1.0 to 3.6%, respectively. Limited information is available on the Δ32 allele frequency among non-Caucasian populations. To address this issue, the authors developed a polymerase chain reaction–based assay to determine the genotype and allele frequencies of this CCR5 polymorphism in nearly 2000 blood samples collected from various populations, particularly those in Asia and the circum-Pacific region.

**Materials and Methods**

**Study Population**

Blood samples, collected from 1980 to 1997, from 1912 individuals (1077 HIV-1-infected and 835 uninfected) belonging to various ethnic groups were studied: 187 HIV-1-seropositive (48 male and 139 female) and 176 HIV-1-seronegative (2 male and 174 female) Caucasians from Hawaii; 303 HIV-1-infected Puerto Rican Hispanics; 150 Africans from Uganda; 606 Asians (279 Vietnamese, 128 Chinese, 109 Japanese, 42 Indian, 59 Filipino, 9 Korean); and 490 Pacific Islanders (253 Polynesians/part-Polynesians from Hawaii, 216 Melanesians from New Britain, and 21 Micronesians from Guam) with or without HIV-1 infection. Samples were collected in conjunction with participation in clinical trials or other studies, as in the HIV-1 sentinel surveillance program in Vietnam and the longitudinal registry for cervical and ovarian cancer among women in Hawaii. Demographic data, retrieved by coded identifiers, were provided by the respective study coordinators.

**Genomic DNA Extraction**

Extraction of genomic DNA from peripheral blood mononuclear cells (PBMC), buffy coat samples, or whole blood blotted onto filter paper was performed in a biosafety laminar-flow cabinet. For the former, PBMC pellets were resuspended in 50 to 250 μL of cell lysis buffer containing 1 × PCR buffer, 2.5 mM MgCl₂, 0.5% NP-40, 0.5% Tween 20, and 120 μg/mL proteinase K. Mixtures were then incubated at 56°C for 60 minutes, heated at 100°C for 10 minutes, and stored at 4°C for later use. DNA extraction from buffy coat samples was accomplished using a commercially available kit (Promega, Madison, WI), and extraction from filter paper-blotted blood samples, collected from HIV-1-infected individuals in the People's Republic of China and Vietnam, was performed according to a previously reported technique. Briefly, filter paper-blotted blood samples were minced with sterile scissors and transferred to a 1.5 mL Eppendorf centrifuge tube (Brinkmann Instruments Inc., Westbury, NY) containing 300 μL of 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 75 μg proteinase K, 0.5% sodium dodecyl sulfate, and 1 mM ethylenediaminetetraacetic acid. Following incubation with occasional gentle inversion at 56°C for 1.5 hour, genomic DNA was isolated using the phenol-chloroform method with ethanol precipitation.

**CCR5 Genotyping by PCR**

A targeted region of the CCR5 gene flanking the 32-bp deletion was amplified by PCR, using an oligonucleotide primer pair designed to discriminate between CCR5 alleles without restriction endonuclease digestion: forward, 5'-GTCTCTCCCAGGAATCATCTTTACCAGATCTC-3'; reverse, 5'-TAGATTTCCGGAGATGAGCATGACCATGACA-3'. Each PCR was conducted in a final volume of 50 μL containing 0.2 to 0.5 μg template DNA, 1 × PCR buffer, 200 μM each dNTP, 1 mM MgCl₂, 1.25 U Thermoherus aquaticus DNA polymerase (Perkin-Elmer Corporation, Norwalk, CT), and 50 nM each primer. Thermocycling conditions consisted of 45 cycles with an initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute. The mutated and unmutated alleles appeared as 147-bp and 179-bp amplicons, respectively, which were easily distinguishable by ethidium bromide-stained gel electrophoresis using 3% NuSieve agarose (FMC BioProducts, Rockland, ME).

To minimize sample carryover, all specimens and reagents were handled in a BSL-2 cabinet or UV-mounted PCR Workstation (C.B.S. Scientific Co., Del Mar, CA). Reagents were dispensed using aerosol-resistant pipette tips. Pre-PCR, thermocycling and post-PCR manipulations were performed in physically separated cubicles. Additionally, two negative controls were used in each PCR run.
Validation of CCR5 Genotyping Assay

To verify that the 147-bp amplified segment was truncated because of the 32-bp deletion, randomly selected PCR amplicons were analyzed by restriction endonuclease digestion, using two enzymes, ApoI and PstI, which made single cuts within and upstream of the 32-bp deletion, respectively (Figure 1, A and B). Each digestion was performed in a 30-μL volume containing the appropriate digestion buffer, 10 μL PCR amplicon, and 5 units of either ApoI or PstI (New England Biolabs, Inc., Beverly, MA), incubated for at least 2 hours at 37°C for PstI and at 50°C for ApoI. Since the single recognition site of ApoI was within the 32-bp deletion, the presence of the deletion mutation resulted in ApoI resistance and PstI sensitivity.

To further confirm the specificity of the PCR-based CCR5 assay, representative PCR products were cloned using the TA cloning kit (Invitrogen, San Diego, CA), and clones were sequenced in both directions on an automated sequencer (model 373A, Applied Biosystems Inc., Foster City, CA) using the same oligonucleotides.

Figure 1. PCR amplification and restriction endonuclease analysis of CCR5 gene. A, Schematic representation (top) and electrophoretic analysis (bottom) of cleavage products of the CCR5 gene by ApoI (cleavage site indicated by closed arrow). Amplified fragments (CCR5/CCR5; lanes 1 and 5; CCR5/Δ32: lanes 2 and 6; Δ32/Δ32: lanes 3 and 7) were size fractionated on 3% NuSieve agarose gel before (lanes 1–3) and after (lanes 5–7) ApoI digestion. Lanes 4 and 8 are 50-bp DNA ladders. B, Schematic representation (top) and agarose gel (bottom) showing the cleavage products of CCR5 gene before and after PstI digestion. Lanes 1 and 2 represent uncut CCR5/CCR5 and CCR5/Δ32, and lanes 4 to 6 are digested CCR5/Δ32, CCR5/CCR5, and Δ32/Δ32. Lane 3 is the 50-bp DNA ladder. C, 3% NuSieve agarose gel showing wild-type CCR5/CCR5 genotype (lanes 1–15) amplified from HIV-1-negative Melanesians from New Britain. Lanes 16 and 17 are the CCR5/Δ32 and CCR5/CCR5 controls, respectively. D, PCR amplification of the CCR5/Δ32 heterozygous genotype in Puerto Rican Hispanics (lanes 1–5) and Caucasians (lanes 9–15). Lanes 6 and 16 are the 50-bp DNA ladder, and lane 7 is the CCR5/CCR5 control.
Table 1. Genotype Frequency of CCR5 Gene 32-bp Deletion Mutation in Various Ethnic Groups

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>HIV-1-Infected</th>
<th>HIV-1-Uninfected</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CCR5/CCR5</td>
<td>CCR5/Δ32</td>
</tr>
<tr>
<td>Asian</td>
<td>408</td>
<td>408 (1.000)</td>
</tr>
<tr>
<td>Chinese</td>
<td>113</td>
<td>113 (1.000)</td>
</tr>
<tr>
<td>Japanese</td>
<td>20</td>
<td>20 (1.000)</td>
</tr>
<tr>
<td>Vietnamese</td>
<td>279</td>
<td>279 (1.000)</td>
</tr>
<tr>
<td>Filipino</td>
<td>6</td>
<td>6 (1.000)</td>
</tr>
<tr>
<td>Indian</td>
<td>0</td>
<td>0 (0.000)</td>
</tr>
<tr>
<td>Korean</td>
<td>0</td>
<td>0 (0.000)</td>
</tr>
<tr>
<td>Puerto Rican</td>
<td>303</td>
<td>286 (0.944)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>187</td>
<td>155 (0.829)</td>
</tr>
<tr>
<td>African</td>
<td>150</td>
<td>150 (1.000)</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>29</td>
<td>28 (0.966)</td>
</tr>
<tr>
<td>Polynesian</td>
<td>28</td>
<td>27 (0.964)</td>
</tr>
<tr>
<td>Micronesian</td>
<td>1</td>
<td>1 (1.000)</td>
</tr>
</tbody>
</table>

Frequencies are shown in parentheses.
The approximately 17% CCR5/Δ32 heterozygosity rate detected among HIV-1 infected and uninfected Caucasians and the Δ32/Δ32 homozygosity rate of 1.7% in HIV-1-seronegative Caucasians in this study fell within the range reported previously for Caucasian populations.7-9,11 Despite the apparent resistance afforded by the CCR5 gene 32-bp deletion mutation, protection against HIV-1 infection is not absolute, as evidenced by reports of HIV-1 infection among Δ32/Δ32 homozygotes.17-19 Thus far, however, acquisition of HIV-1 infection in such individuals has been by the parenteral, rather than mucosal route, suggesting utilization of the CXCR4 coreceptor by dual-tropic strains of HIV-1 among infected Δ32/Δ32 homozygotes.

A CCR5/Δ32 heterozygous genotype frequency of 6.7% has been reported previously for Hispanics living in the United States who trace their heritage to Mexico as well as to Central and South America.12 In the present study, a similar overall low-level CCR5/Δ32 heterozygosity of 5.6% was found among Puerto Ricans. Although Puerto Ricans are generally classified as Hispanics, their genetic composition is rather more complex and diverse, with admixture of Caucasian, Taino (and possibly Caribe) Indian, and African traits to varying degrees. In this regard, albeit to a lesser extent, the low Δ32 allele frequency among Hawaiians can be accounted for by recent Caucasian gene flow or admixture.

Not unexpectedly, CCR5/Δ32 heterozygosity was found among several long-term survivors. However, many more individuals infected with HIV-1 for more than 13 years and having relatively high CD4 counts and low plasma viral loads did not possess the Δ32 allele, indicating that polymorphisms other than the CCR5 gene 32-bp deletion mutation may account for slower progression to AIDS in such individuals. Specifically, a G to A nucleotide substitution at position 190 in the CCR2 protein (CCR2-64l), and this point mutation appears to be operative in delayed progression to AIDS.20-22 In addition, a CCR5 gene mutation at position 303 (M303) from T to A has recently been identified to be an accomplice in conferring resistance to HIV-1 infection.23 The M303 mutation introduced a premature stop codon in the CCR5 gene, which results in the loss of the expression of a functional coreceptor. On the other hand, individuals with overproduction of beta-chemokines appear to resist HIV-1 infection, even in the absence of such polymorphisms in the CCR5 or CCR2 gene.24

Inability to detect the Δ32 allele in HIV-1-infected and -uninfected Asian populations in the present study is consistent with previously published reports based on smaller numbers of individuals.7,9,14,15 However, the absence of the CCR5 gene 32-bp deletion mutation among Asians, as well as in Polynesian, Micronesian, and Melanesian populations, does not preclude, in such ethnic groups, the existence of other polymorphisms of chemokine receptor genes associated with slower progression to AIDS. For example, the extraordinarily high frequencies of the SDF-1-3’A allele among non-Austronesian highlanders of Papua New Guinea and aboriginal populations of Australia warrants careful clinical correlation.25

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REFERENCES


