Genetic Polymorphism in \text{CX}_3\text{CR}1 and Risk of HIV Disease

Faure et al. (1) reported that allele M280 of the chemokine receptor/HIV coreceptor \text{CX}_3\text{CR}1 is associated with both increased risk of HIV infection and accelerated HIV disease progression in studies of Caucasian HIV cohorts from France. In the seroconverters (SEROCO) cohort, the relative risk (RR) for AIDS was 2.13 for those homozygous for the M280 allele \((n = 16)\) compared with those homozygous for the reference allele T280 \((n = 306; P = 0.039)\). In the SEROCO, standard progressor (IMMUNOCO), and long-term asymptomatic (ALT) cohorts, association of homozygous M280 \((2)\) with HIV infection was significant at \(P < 0.045\). Here we report that we were unable to confirm these associations in three North American (NA) cohorts of HIV-1 seroconverters: the D.C. Gay cohort (DCG), the Multicenter AIDS Cohort Study of homosexual men (MACS), and the Multicenter Hemophilia Cohort Study (MHCS).

Allele M280 has two nonsynonymous single nucleotide polymorphisms (SNPs), causing substitution of isoleucine (I) for valine (V) at codon 249 and methionine (M) for threonine (T) at codon 280. Three other possible alleles are formed by these SNPs: V249 M280, V249 T280, and I249 T280. V249 M280 has not been observed, an indication of complete linkage disequilibrium; for that reason, and to conform with the nomenclature previously used by Faure et al. (1), we refer to this allele as M280. V249 T280 was the most common allele in all racial groups (data not shown) and was similar in frequency between Caucasian random blood donors from North America, at 72.2\% \((3, 4)\), and those from France, at 74.3\% \((1)\). Allele M280 was present in 20.2\% of NA Caucasian random blood donors, compared with 13.5\% of those in France, and 7.7\% of NA Caucasian random blood donors possessed I249 T280, compared with 12.2\% from France. Genotypes were in Hardy-Weinberg equilibrium within each racial group, which suggests a lack of selective pressure on these alleles.

No significant difference was observed between exposed but uninfected \((n = 109)\) and HIV-1-infected \((n = 573)\) Caucasian MACS participants in the distribution of any compound \text{CX}_3\text{CR}1 genotype \((P = 0.72)\) or allele \((P = 0.82)\), a finding that fails to support a role for \text{CX}_3\text{CR}1 in HIV transmission among homosexual men. Using a Cox proportional hazards model (PROC PHREG, SAS Institute, Cary, NC), progression rates to AIDS and all-cause mortality were not significantly different in individual or combined NA cohorts for M280 homozygotes relative to T280 homozygotes (Table 1). Our power to detect this effect, given the previously reported relative risk of 2.13 in the SEROCO cohort \((1)\), is 0.65. M280 heterozygosity was weakly associated with a 1.5-year delay in median time to both AIDS \((RR = 0.77, P = 0.05)\) and all-cause death \((RR = 0.77, P = 0.07)\) in the combined NA cohorts (Table 1 and Fig. 1A). This result was attributable primarily to the MACS cohort \((RR = 0.73; P = 0.06)\), although a trend toward reduced progression that was not statistically significant was also noted in MHCS and DCG. The RR of 0.77 is similar to that reported previously for the MACS for the CCR532 and CCR2-64I HIV coreceptor variants \((4–6)\). This delay in progression was not seen in the French SEROCO cohort; however, the power in that study to detect this association was only 0.33.

Consistent with delayed disease progression, the receptor encoded by allele M280 had 15 to 50\% activity, compared with the reference receptor encoded by allele V249 T280, for all three informative HIV-1 envelope glycoproteins tested in a standard HIV fusion assay, despite equivalent receptor expression on the cell surface (Fig. 1B).

On statistical grounds, the discrepancy between these results and those reported by Faure et al. is not necessarily surprising. With respect to M/M280, both studies have limited power, because homozygosity for this allele is uncommon \((n = 19\) in this study, \(n = 16\) in the study of Faure et al.). Moreover, the confidence intervals from the two studies overlap for both the M/M280 and T/M280 data. Taken together, the two studies suggest at best a modest protective effect of the T/M280 genotype and a modest adverse effect of the M/M280 genotype on HIV progression rate. Whether one or both of these associations occurs by chance alone, or whether, paradoxically, both are true will require a larger consortium or meta-analysis study that will have sufficient power.

Alternatively, the discrepant results could be due to differences in cohort composition. Known differences include gender (the NA

Table 1. Association of \text{CX}_3\text{CR}1 allele M280 with HIV disease outcomes in NA cohorts. The relative risk adjusted for age at seroconversion for AIDS and all-cause death was calculated using homogenous T280 as the referent group and a Cox proportional hazards model. Allele T280 refers to the combination of alleles V249 T280 and I249 T280. Descriptions of the cohorts and anonymous blood donors can be found in previous publications \((3, 4, 11–14)\). We analyzed the DNA samples of all participants whose seroconversion date was known or could be accurately estimated. Outcomes were right-censored as of 31 December 1995, to eliminate effects of highly active antiretroviral therapy (HAART). The final analytic sample included 685 HIV+ Caucasian men representing a total of 323 (47\%) cases of AIDS and 276 (40\%) all-cause deaths. All participants had both \text{CX}_3\text{CR}1 alleles defined. Approximately 99\% of the participants were successfully traced through the end of the follow-up period. The mean \pm SD survival times for the combined cohorts were 7.63 \pm 3.42 and 8.28 \pm 3.38 years for AIDS and all-cause death, respectively. Cox proportional hazards models (PROC PHREG, SAS Institute, Cary, NC) were used to assess association between these genotypic groups (separately and with T/M280 and M/M280 combined) and AIDS and all-cause mortality. Each model was adjusted for age at seroconversion and was performed separately for each cohort and the cohorts combined. Age of seroconversion for the seroconvertent cases was determined as the midpoint between the date of the last seronegative and date of the first seropositive HIV quantitative test result, whereas for the seroreconvertent cases in the DCG cohort it was determined by the geographic area of recruitment of the study participants—1 June 1980, for subjects in NYC, and 1 June 1981, for subjects in Washington, D.C., on the basis of available information regarding the HIV-1 epidemic in those cities \((15, 16)\).

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Genotype</th>
<th>(n)</th>
<th>AIDS 1987 Risk ratio (95% CI)</th>
<th>(P)</th>
<th>All-cause death Risk ratio (95% CI)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACS</td>
<td>T/T280</td>
<td>300</td>
<td>1.0</td>
<td>0.73</td>
<td>0.53–1.01</td>
<td>1.0</td>
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<td></td>
<td>T/M280</td>
<td>127</td>
<td>0.91 (0.34–2.47)</td>
<td>0.86</td>
<td>0.63 (0.16–2.6)</td>
<td>0.52</td>
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<tr>
<td></td>
<td>M/M280</td>
<td>12</td>
<td>0.9 (0.36–3.86)</td>
<td>0.78</td>
<td>0.49–1.54</td>
<td>0.63</td>
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<tr>
<td>DCG</td>
<td>T/T280</td>
<td>67</td>
<td>1.0</td>
<td>0.90</td>
<td>0.51–1.58</td>
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<td></td>
<td>T/M280</td>
<td>24</td>
<td>1.18 (0.36–3.86)</td>
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<td>0.49–1.54</td>
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<tr>
<td></td>
<td>M/M280</td>
<td>114</td>
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<td>0.49–1.54</td>
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<tr>
<td>MHCS</td>
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<td>1.0</td>
<td>0.85</td>
<td>0.45–1.61</td>
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<td></td>
<td>T/M280</td>
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<td>1.36 (0.33–5.62)</td>
<td>0.67</td>
<td>N/A</td>
<td>0.48</td>
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<td>M/M280</td>
<td>8</td>
<td>1.10 (0.57–2.15)</td>
<td>0.77</td>
<td>0.53 (0.31–1.84)</td>
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<td>Combined</td>
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<td>1.0</td>
<td>0.77</td>
<td>0.58–1.02</td>
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<td>0.77</td>
<td>0.53 (0.31–1.84)</td>
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<tr>
<td></td>
<td>M/M280</td>
<td>19</td>
<td>1.10 (0.57–2.15)</td>
<td>0.77</td>
<td>0.53 (0.31–1.84)</td>
<td>0.53</td>
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</table>
Fig. 1. Cx3CR1 allele M280 is associated with delayed HIV disease progression in NA cohorts and has impaired HIV coreceptor activity. (A) HIV disease association. Kaplan-Meier tests for determining probability of survival without AIDS-1987 were performed using groupings of T/T280 (the referent group) and T/M280 + M/M280 genotypes for the combined cohorts. When only T/M280 data were considered, the curve was not significantly changed. (B) Impaired HIV coreceptor activity. Materials and methods for the cell-cell fusion assay are as previously performed (17). Shown is the mean ± SEM of three experiments performed in triplicate. Statistical significance was assessed using a two-tailed t test. Inset: FACS analysis performed on NIH 3T3 cells used in the fusion assay. Cells transfected with plasmids encoding Cx3CR1 variants M280 or V249 T280 or vector alone (pSG59) were stained with rabbit polyclonal antisera specific for Cx3CR1 as previously described (18).

cohorts were entirely male, whereas the SEROCO cohort included 22% females), HIV risk category (26% heterosexual and 7% intravenous drug abuse in SEROCO, versus none in the NA cohorts; 22% hemophiliacs in the NA cohorts, versus none in SEROCO), and median length of patient follow-up (73 months for SEROCO versus 89 months for NA cohorts). Cohort differences have also been observed for other HIV disease-associated chemokine and chemokine receptor variants (4–10). Nevertheless, at present, the results from this study and from that of Faure et al. (1), taken together, do not support a clear and consistent role for Cx3CR1 in HIV pathogenesis.

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References and Notes
2. In [1], M280 was incorrectly printed as T280 in this context.
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