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Evidence for recent selection of the CCR5-Δ32 deletion from differences in its frequency between Ashkenazi and Sephardi Jews

S Maayan[1], L Zhang[2], E Shinar[3], J Ho[2], T He[2], N Manni[1], LG Kostrikis[2] and AU Neumann[4]
[1]*Hadassah Hospital, Jerusalem 91000, Israel;* [2]*The Aaron Diamond AIDS Research Center, New York, NY 10016, USA;* [3]*MDA – Israel National Blood Services, Ramat-Gan, Israel;* [4]*Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel*

Recent studies have shown higher frequencies of the CCR5-Δ32 allele and the CCR5-Δ32/Δ32 genotype, which confers protection against HIV infection, in northern Europe as compared to Mediterranean countries. Here, we analyse the prevalence of CCR5-Δ32 in 922 HIV seronegative blood donors in Israel to verify its frequency in Jews of Ashkenazi and Sephardi origin. A significant difference ($P < 0.001$) was found between the CCR5-Δ32 allele frequency in Ashkenazi (13.8%) vs (4.9%) Jews. In contrast, no significant difference was observed in the frequency of the CCR2–64I mutation between Ashkenazi (9.2%) and Sephardi (13.4%) Jews. Using the Island model we calculate that a minimal genetic migration rate of 3% per generation would have been necessary if the higher CCR5-Δ32 prevalence in Ashkenazi is to be fully explained by mixing with the indigenous north-European populations. This putative migration rate is 20-fold higher than that currently estimated from other genes, and would correspond to a non-realistic minimal current admixture of 80%. Thus, our results suggest that a positive selection process for CCR5-Δ32 should have occurred in northern Europe at most a 1000 years ago, after the Ashkenazi Jews separated from their Sephardi kin and moved to north Europe. Genes and Immunity (2000) **1**, 358–361.

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Introduction

Recently, a homozygous mutation of 32 base-pairs deletion (Δ32) in the CC-chemokine receptor 5 (CCR5) locus has been described as conferring *in vitro* resistance of CD4 lymphocytes to infection by HIV-1 of the macrophage-tropic R5 strains.[1–3] In retrospective analyses, the homozygous Δ32/Δ32 genotype has been associated with protection against HIV-R5 infection,[4–6] while the heterozygous wt/Δ32 genotype was associated with a slower rate of progression to AIDS.[5,6] Recent data[7–11] suggests an allelic frequency of 9–18% for the CCR5-Δ32 deletion among Caucasians in northern Europe and North America compared to only 3–6% among Mediterranean populations and 0% among Africans.[6] The highest allelic frequency (20.9%) in this survey was observed[7] among a small number ($n = 43$) of Ashkenazi Jews. We studied Israeli Jewish blood donors of known ancestry in order to expand on that initial observation and look for differences in the distribution of the CCR5-Δ32 allele and genotypes between Ashkenazi and Sephardi Jews. As a control we study the frequency of the CCR2–64I mutation,[12–14] which was also associated with slower HIV disease progression, as function of the two origin groups. In addition, we discuss a number of possible mechanisms to explain the differences in genetic frequencies between Ashkenazi and Sephardi, considering that both groups originated from the same ancestry about a 1000 years ago.

Results

The overall allelic frequency of CCR5 in this Israeli cohort was 9.8% (see Table 1). Of the 922 blood donors, 363 were of Ashkenazi origin and 257 of Sephardi origin (or 443 donors of Ashkenazi descent and 304 of Sephardi descent using more flexible criteria, see methods). The CCR5-Δ32 allele frequency among Israeli Jews of Ashkenazi descent was 13.8%, which is significantly different ($P < 0.001$) in comparison to that of 4.9% for Israelis of Sephardi descent. The mixed group and second generation Israelis had intermediate frequencies. In addition, a significant difference ($P < 0.001$) was noted in the prevalence of the homozygous CCR5 Δ32/Δ32 genotype between Israeli Jews of Ashkenazi (3%) and Sephardi (0.7%) origins. The genotype distributions of the various Israeli subpopulations were in equilibrium as predicted by the Hardy-Weinberg theory, although the total population was out of equilibrium.

The CCR5-Δ32 allele frequency (13.8%) among Israeli donors of Ashkenazi descent was comparable to that measured (10–16%) in recent large surveys of low-HIV-

Correspondence: Avidan U Neumann, Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel. E-mail: neumann@mail.biu.ac.il
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Table 1 CCR5-Δ32 allele and genotype frequencies in Israel according to various Jewish origin groups

Parents' origin	*Number of samples*	*CCR5 wt/wt (%)*	*CCR5 wt/Δ32 (%)*	*CCR5 Δ32/Δ32 (%)*[b]	*CCR5-Δ32 Allele (%)*[b]	*HWE P-value*
Ashkenazi	363[a]	75	22	3[b]	13.8[b]	0.17
Sephardi	257[a]	90	9	0.7[b]	4.9[b]	0.12
Israeli – 2nd generation	148	83	17	0	8.4	0.53
Mixed origin	27	85	11	4	9.3	0.08
Total	992[a]	82	16.0	2	9.8	0.01

[a]According to the more relaxed criteria, in which one parent could be born in Israel, there are 443 Ashkenazi and 304 Sephardi (thus total $n = 922$) with no significant changes in the allele and genotype frequencies in these groups. [b]Difference between Ashkenazi and Sephardi is statistically significant ($P < 0.001$).

risk populations in north-eastern Europe,[8,9] from where the Ashkenazi Jews originate. The Ashkenazi CCR5-Δ32 allele and Δ32/Δ32 genotype frequencies are, however, higher ($P < 0.001$) than those reported (8–9% and 1–1.5% respectively) for North America and central/western Europe Caucasians.[6–9] No significant differences ($P > 0.1$) were found when comparing Israeli donors of Sephardi origin to large Greek-Cypriot[10] or Italian[11] cohorts. We have checked and ruled out the possibilities that extensive family ties among the blood donors, or that individuals with high risk for HIV, may have influenced the mutation prevalence in the different sub-groups. In addition, our allele frequency data among individuals of Ashkenazi descent are similar to those obtained in recent smaller surveys of CCR5-Δ32 based on samples of the National Centers for Genetic Diseases in Israel[15] and in France,[16] in which high risk of HIV is not expected.

In order to verify if differences between Ashkenazi and Sephardi Jews exist also in other CC-chemokine receptor genes we have analysed in our cohort the frequency of the CCR2–64I mutation (see Table 2), which is associated with slower progression to AIDS in a number of retrospective analyses.[12–13] We have found no significant difference in the frequency of the CCR2–64I allele between the Ashkenazi (9.2%, $n = 142$) and the Sephardi (13.4%, $n = 67$, $P > 0.2$). Although these results are only for a subset of our cohort, note that the difference in CCR5-Δ32 is already significant in that subset.

Analysis and discussion

In this report we summarize findings from the largest survey of the frequency of CCR5-Δ32 mutation among Israeli Jews. Our main finding is the significant difference in allele frequency, and homozygous Δ32/Δ32 genotype, between Israelis of Ashkenazi descent and of Sephardi descent. In contrast, we have found no difference in the frequencies of the CCR2–64I mutation between Ashkenazi and Sephardi Jews in Israel. This result was expected since there are no reported differences in the CCR2–64I mutation between individuals of northern European or Mediterranean origin.[14] The difference in the CCR5-Δ32 frequency between Ashkenazi and Sephardi Jews corresponds to the difference in CCR5-Δ32 prevalence between north European and Mediterranean populations in general.[7–11] Thus, the high frequency of the CCR5-Δ32 allele in Ashkenazi, as compared to Sephardi Jews, could be explained by mixing of indigenous north European genes into the Ashkenazi population.[17–18] According to that scenario, Ashkenazi and Sephardi Jews had the same CCR5-Δ32 frequency until the Ashkenazi separated and moved to north-eastern Europe, where they mixed with a local population rich in CCR5-Δ32 and acquired it through the ensuing genetic migration from indigenous north Europeans.

Here, we use the next assumptions in order to obtain a *minimal* estimate of the necessary migration rate that will give the current high frequency of CCR5-Δ32 in Ashkenazi Jews: (a) the gene migration took *at most* G=50 generations, starting at the time when Ashkenazi Jews first moved to north-eastern Europe (about 1000 AD). (b) The CCR5-Δ32 allele frequency of the Ashkenazi population before admixture with the indigenous population was *maximally* equal to that of the Sephardi Jews today, $P_0 = 0.049$. (c) The CCR5-Δ32 allele frequency of the non-Jewish north-eastern European population was *always equal to its maximal* present frequency, Pm=0.16. Using these parameters in a calculation based on the Island model[19] (Eq. 1 in Methods) gives a *minimal* migration rate of M=0.03 per generation. Thus (using Eq. 2 in Methods), the present genetic admixture of north-European origin in Ashkenazi Jews should have been *minimally* of the order of A=80% *if* the high allele frequency of CCR5-Δ32 in Ashkenazi Jews is fully due to migration.

Table 2 CCR2-64I allele and genotype frequencies in Israel according to various Jewish origin groups

Parents' origin	*Number of samples*	*CCR2 wt/wt (%)*	*CCR2 wt/64I (%)*	*CCR2 64I/64I (%)*	*CCR2-64I Allele (%)*	*HWE P-value*
Ashkenazi	142	82.4	16.9	0.7[a]	9.2[a]	0.84
Sephardi	67	74.6	23.9	1.5[a]	13.4[a]	0.82
Israel – 2nd generation	37	78.4	21.6	0	10.8	0.46
Total	246	80.1	19.1	0.8	10.4	0.64

[a]No significant difference between Ashkenazi and Sephardi ($P < 0.2$) in CCR2-64I, although for same set of 246 samples there is a significant difference ($P < 0.01$) in CCR5-Δ32.

This putative minimal migration rate (3% per generation), which is necessary to explain the high CCR5-Δ32 frequency in Ashkenazi, is 20 times larger than that currently estimated from other genes.[20] Moreover, it would imply a non-realistic genetic admixture of minimally 80% north-European origin in Ashkenazi Jews, instead of 3–30% as currently estimated.[17,18,20] Therefore, the higher prevalence of the CCR5-Δ32 mutation in Ashkenazi cannot be fully explained by mixing with the north-European population. Bottlenecks or cofounder effects, which are not considered by the Island model used here to calculate the migration rate, could contribute to the elevated allele frequency in Ashkenazi.[21,22] However, bottlenecks could not alone explain the observed high frequency in Ashkenazi Jews from many different countries of origin.

Thus, our results suggest that alternatively, or additionally, the CCR5-Δ32 frequency increased in the Ashkenazi Jews in north-eastern Europe due to a selective advantage, in parallel to such selection in indigenous populations as recently proposed.[8,9] Such a selection event or continuous selection process, eg due to some disease as in the case of malaria and sickle cells,[23,24] should have occurred at most 1000 years ago since that is the earliest time Ashkenazi Jews separated from their Sephardi kin. Our data cannot, however, determine if the origin of the CCR5-Δ32 deletion occurred much earlier than 1000 years ago or not.[8,9] Also, it cannot be ruled out that the difference in CCR5-Δ32 frequency is possibly the result of a piggyback effect due to selection of another gene that is linked to CCR5-Δ32. Additional microsatellite frequency and linkage disequilibrium analysis in Ashkenazi and Sephardi could shed more light on these questions. Further understanding of non-HIV based selection of CCR5-Δ32 is important for the development of anti-HIV therapy.

Patients and methods

Subjects

A total of 923 unselected consecutive blood donors were tested at two major blood banks in Israel. With consent, information was obtained on the place of birth of the donors and their parents. All blood samples in the study were seronegative for HIV, Hepatitis B and Hepatitis C. All participants were screened by an interview before donating blood in order to exclude the possibility that individuals of high-risk behavior for HIV infection participate in the study. In addition, most individuals in this study were 'pedigree' blood donors, who frequently donate blood and are highly motivated and well informed about the practice of self-exclusion.

The study individuals were divided into the following origin groups:

1. Ashkenazi origin: Both parents born in a northern/eastern European country. This category included the following countries of origin: England, France, Germany, Poland, Russia, the Baltic states, and other eastern European countries with the exception of Bulgaria.

2. Sephardi/Oriental origin: Both parents born in Mediterranean or Middle-Eastern countries. This category included the following countries: Spain, Italy, Greece, Bulgaria, Turkey, Morocco, Algeria, Tunis, Libya, Egypt, Syria, Iraq, Iran, and Yemen.

3. Israelis, 2nd generation: Both parents born in Israel.

4. Mixed origin: One parent born in a northern-European country and the other in a Middle-Eastern country.

We have also used a more relaxed criterion by which the origin group was defined by the origin of only one of the parents if the other parent was born in Israel. We have verified that the allele and genotype frequencies did not significantly change with the more relaxed criteria, although the number of individuals in the Middle-Eastern and European groups were larger then. Our study is focused on comparing results obtained from individuals of Ashkenazi versus Sephardi origin.

Genotyping

CCR5-Δ32 genotypes were determined by PCR and gel electrophoresis. A portion of the CCR5 gene was amplified by PCR from genomic DNA and analysed on a 4% Metaphore agarose gel (FMC BioProducts). Primers CCR5c, 5′-CAAAAAGAAGGTCTTCATTACACC-3, and CCR5d, 5′-CCTGTGCCTCTTCTTCTCATTTCG-3, that flank the 32 bp deletion were used to generate wild-type and deleted fragments of 189 bp and 157 bp, respectively. The PCR reaction mixture contained 0.25 mM of dNTPs, 20 pmol of each primer and 0.5 unit of Taq polymerase in 1x Reaction Buffer (Boehringer Mannheim, Germany). Each PCR amplification consisted of 40 cycles with the first five cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec. Genotyping of the CCR2 gene was done by a spectral genotyping assay, using molecular beacons in combination with real-time PCR as previously described.[13]

Statistical analysis

Chi-square and Fisher exact tests were used to determine statistical significance of differences in genotype and allele distributions among different sub-groups. The statistical significance of the Hardi-Weinberg Equilibrium (HWE) was tested by a Chi-square test with 1 degree of freedom. Significance was established at $P = 0.03$.

Calculation of migration rates and admixture

The genetic migration rate per generation, M, from one population to another can be calculated with the solution of the Island model iteration process,[19] by

$$M = 1 - [Pt\text{-}Pm)/(P_0\text{-}Pm)] \wedge (1/G) \quad (1)$$

where P_0 and Pt are the initial and present allele frequencies in the population studied, Pm is a constant allele frequency in the population where migration came from and G is the number of generations that the migration took place in. The total genetic admixture, A, is then expressed[20] by

$$A = 1 - (1\text{-}M) \wedge G \quad (2)$$

after G generations of continuous genetic migration.

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