

Polymorphisms of Host Genes in Exposed Seronegative Individuals: Impact on HIV-1 Transmission

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Abstract

Polymorphisms in host genes have been shown to affect HIV-1 transmission and disease progression. Our objective was to determine the overall effect of host genes on HIV-1 transmission by characterizing the genetic polymorphisms in CCR1, CCR2, CCR5 and RANTES in exposed seronegative (ES) individuals. The cohorts include 57 ES who have had repeated, unprotected sexual contacts with HIV-1 infected partners, 183 HIV-1 negatives, and 182 HIV-1 positives including 17 long term non-progressors (LTNP). All of the participants are Caucasians. Direct sequencing was used for CCR1, CCR5 and RANTES coding region. PCR or RFLP were used for genotyping CCR5-Δ32, CCR2-64I, RANTES promoter -403A and -28G. Fisher's exact test was used for statistic analysis. No polymorphism except CCR5-Δ32 was found in the coding regions of CCR1, CCR5 and RANTES. The frequency of CCR5-Δ32 homozygote in ES is 3.4%, significantly higher than the non-ES HIV-1 negative and HIV-1 positive groups (P = 0.02). The frequency of CCR5-Δ32 heterozygote in LTNP is 47.1%, significantly higher than in HIV-1 progressors (P = 0.03). These results confirm that CCR5-Δ32 homozygosity is associated with the resistance to HIV-1 infection and that heterozygosity delays disease progression. The frequencies of CCR2-64I homozygote in ES, HIV-1 negative, HIV-1 positive and LTNP are 0.0%, 1.6%, 0.0% and 5.9% respectively, while for heterozygote are 12.1%, 12.6%, 16.1% and 0.0%. There was no significant difference in the frequencies of CCR2-64I genotype and allele among these groups. However, three CCR2-64I homozygotes were found in HIV-1 negative cohort (1.6%) and one in HIV-1 positive cohort (0.6%), suggesting a possible association between CCR2-64I homozygosity and the resistance to HIV-1 infection. The genotype frequencies of RANTES promoter in ES are 67.2% for G1; 1.7% for G2, G5; 0.0% for G3, G6; and 29.4% for G4. Compared with the published data, we found neither G1 nor G4 to be associated with the altered risk of HIV-1 infection in Caucasians either with CCR5-Δ32 (P = 0.55) or without CCR5-Δ32 (P = 0.86). The mechanisms underlying the resistance to HIV-1 infection in ES are likely to be multifarious.

Introduction

Extensive epidemiological data show that some individuals remain uninfected despite multiple sexual contacts with HIV-1 infected partners^{1,2} and the infected individuals varied widely in clinical course. Attempts to elucidate the different HIV-1 exposure outcomes have demonstrated that some genetic polymorphisms in chemokines and chemokine receptors affect HIV-1 transmission and disease progression. Individuals homozygous for CCR5-Δ32, a 32 base pair deletion in the coding region of CCR5, show resistance to HIV-1 infection³⁻⁶. Heterozygosity of this mutation delays disease progression^{5,6}. CCR2-64I, a point mutation in the first transmembrane region of CCR2⁷, and SDF-1 3'A, a point mutation in the untranslated region of SDF-1⁸, has significant impacts on disease progression, but not in all studies. CCR5 promoter mutation was also shown to affect rates of disease progression⁹. Furthermore, RANTES promoter polymorphisms have been shown not only to affect disease progression but also to affect the risk of HIV-1 infection¹⁰⁻¹². The identified genetic factors can only account for a small proportion of the exposed-seronegative individuals (ES) and long term non-progressors (LTNP)^{3,6}, and the inconsistencies of their effects on HIV-1 transmission and disease progression underscore the need for more research. The purposes of this study were to gain a better understanding of the frequency and amount of CCR5-Δ32, CCR2-64I, RANTES promoter and CCR5 promoter sequence variation in diverse cohorts of ES, HIV-1 negative, HIV-1 infected cases, and LTNP.

Materials and Methods

Clinical samples. A total of 422 Caucasian individuals enrolled at the University of Washington Primary HIV Infection Clinic and Fred Hutchinson Cancer Research Center from 1993 to 2001 were evaluated. The subdivision was: exposed-seronegative (ES, n=57) who were multiply exposed through unprotected sexual activity with known HIV-1 positive partners², HIV-1 seronegative individuals (n=183); HIV-1 infected individuals (n=165); and LTNP (n=17) who show no sign of immunodeficiency for more than 10 years. Only Caucasian individuals were included from all ES, HIV-1 negative and positive cohorts.

Genotyping. Peripheral blood mononuclear cells (PBMC) were obtained from blood by Ficoll-Histopaque method. DNA extracted from these samples were used for single PCR or multiplex PCR¹³. Direct sequencing was used for CCR1, CCR5, RANTES coding region and CCR5 promoter region. PCR or RFLP were used for genotyping CCR5-Δ32, CCR2-64I, CCR5 promoter 59029, RANTES promoter -403A and -28G.

Statistical analysis. The association between possession of a genotype or haplotype and risk of acquiring HIV-1 was evaluated using Fisher's exact test. All analyses were performed using PRISM version 3.0 and InStat Version 3.0 (GraphPad Software, INC, San Diego, CA).

Results

FIG.1. Map of CCR2, CCR5 Promoter, CCR5, and Nucleotide Variants

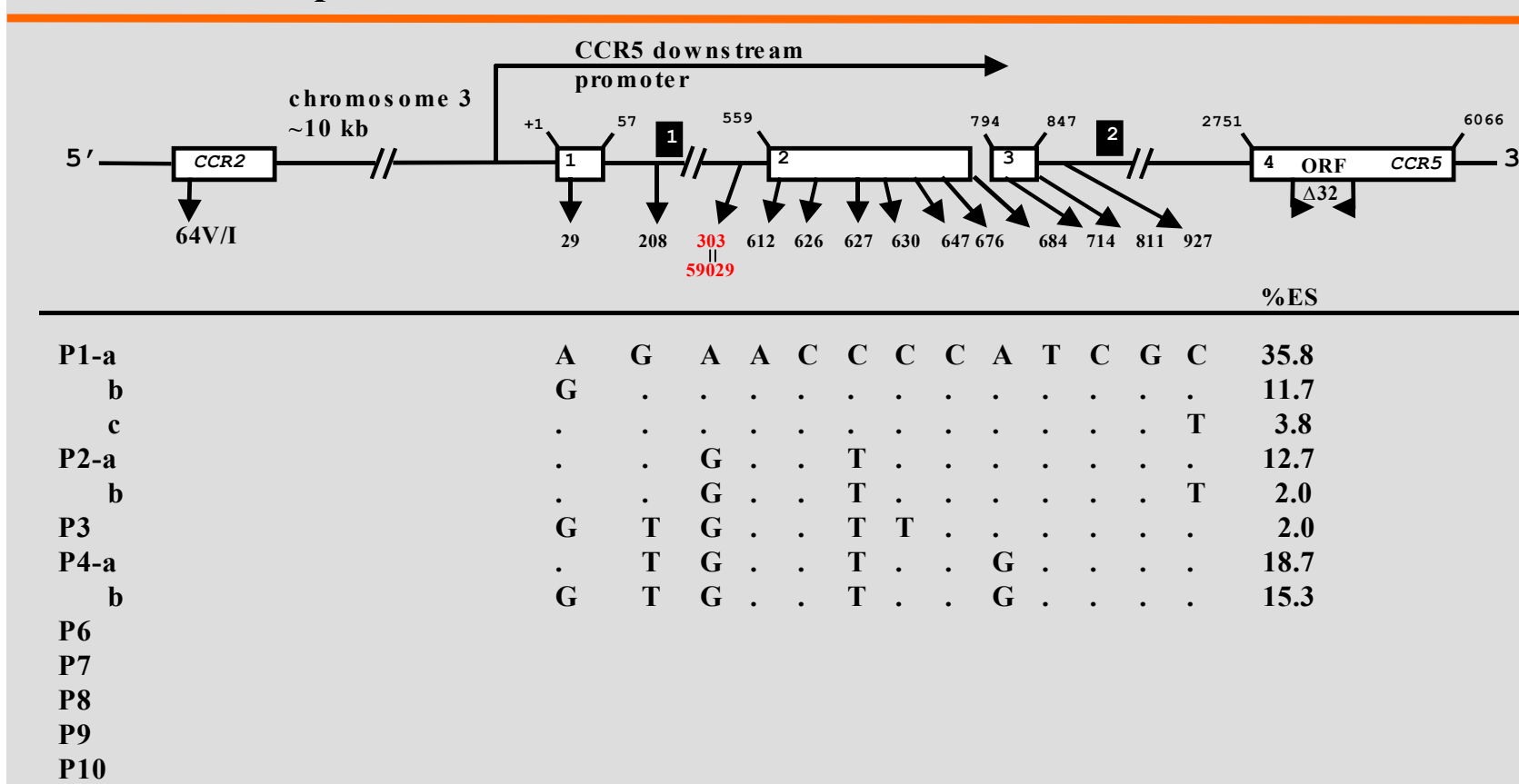


FIG.2. CCR5-Δ32 Genotype in ES and Local Caucasian Populations

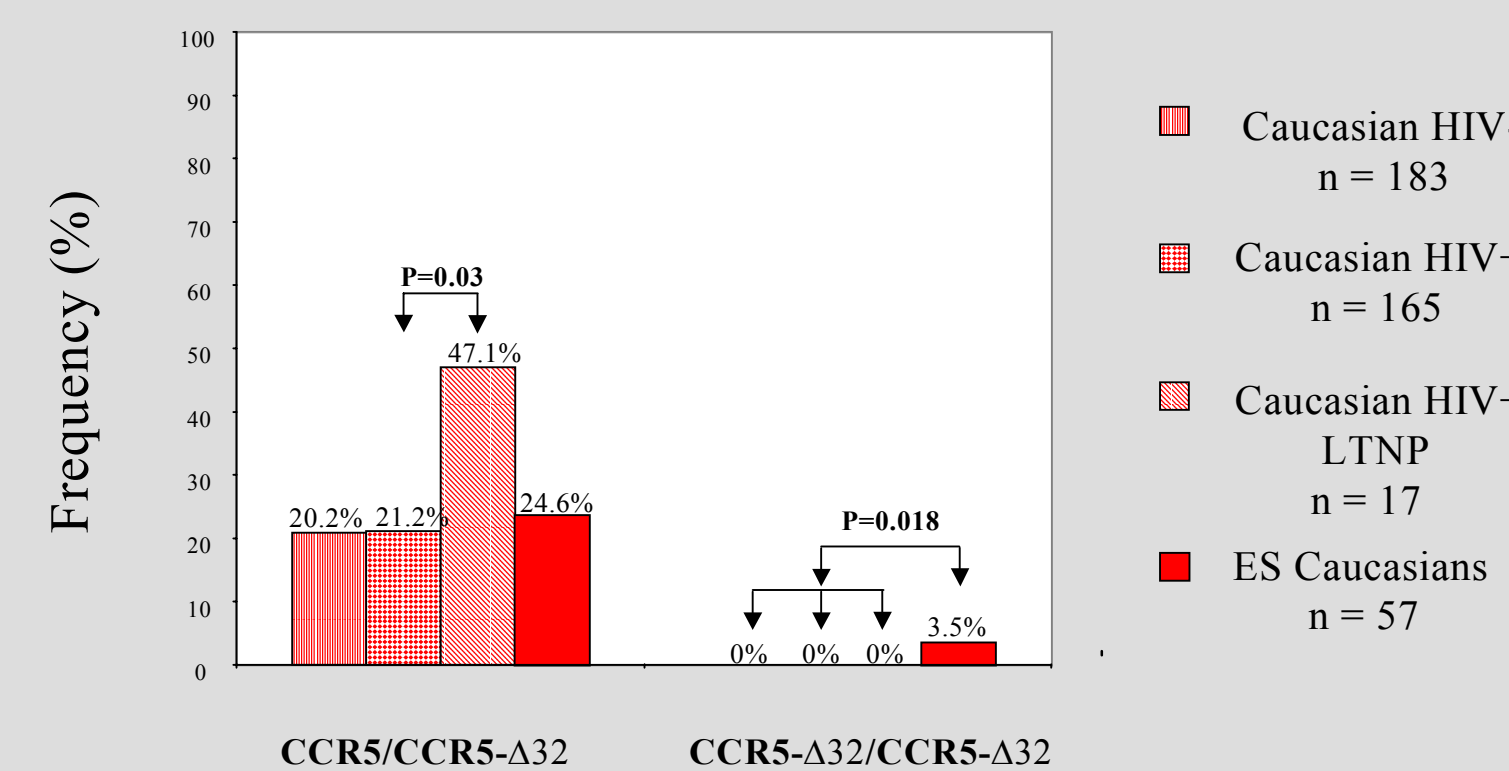


FIG.3. CCR2-64I Genotype in ES and Local Caucasian Populations

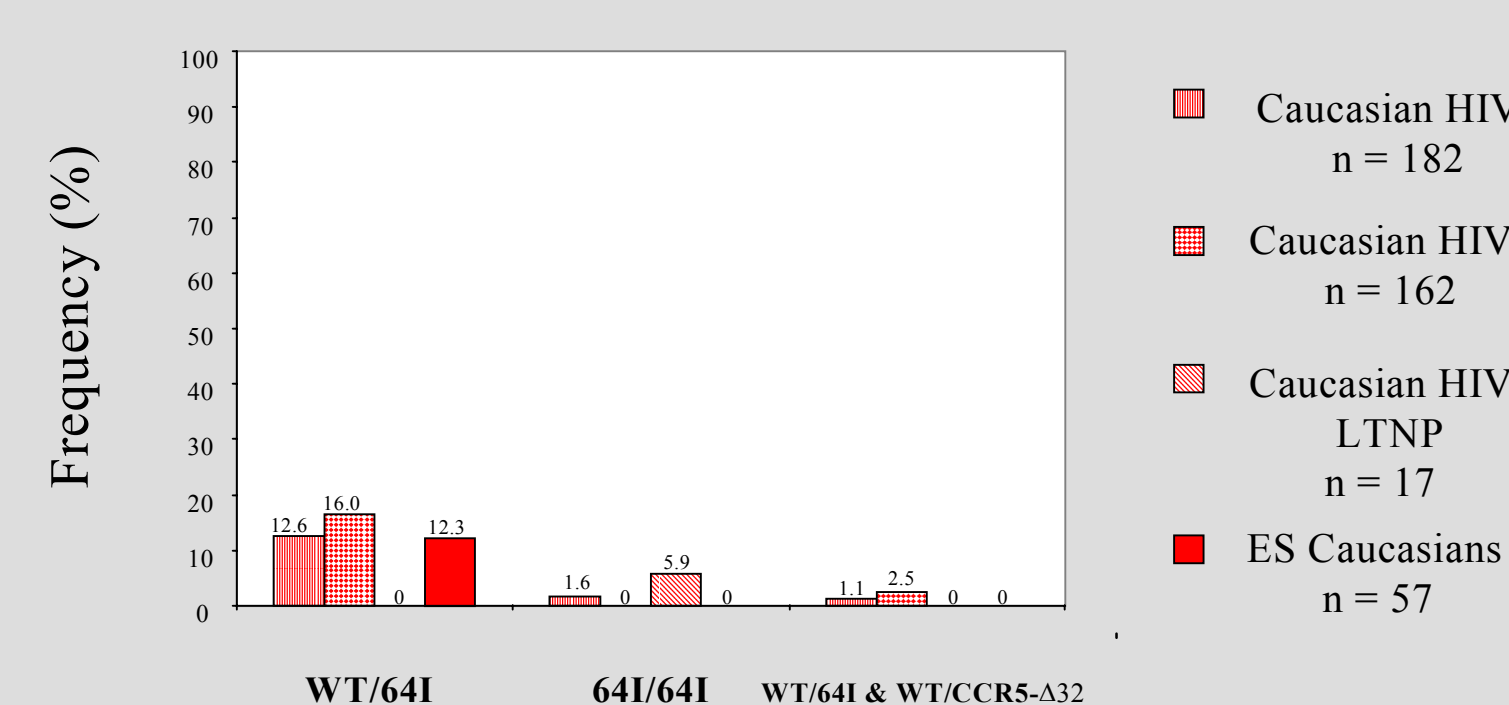


FIG.4. Frequency of CCR5 Promoter Haplotypes

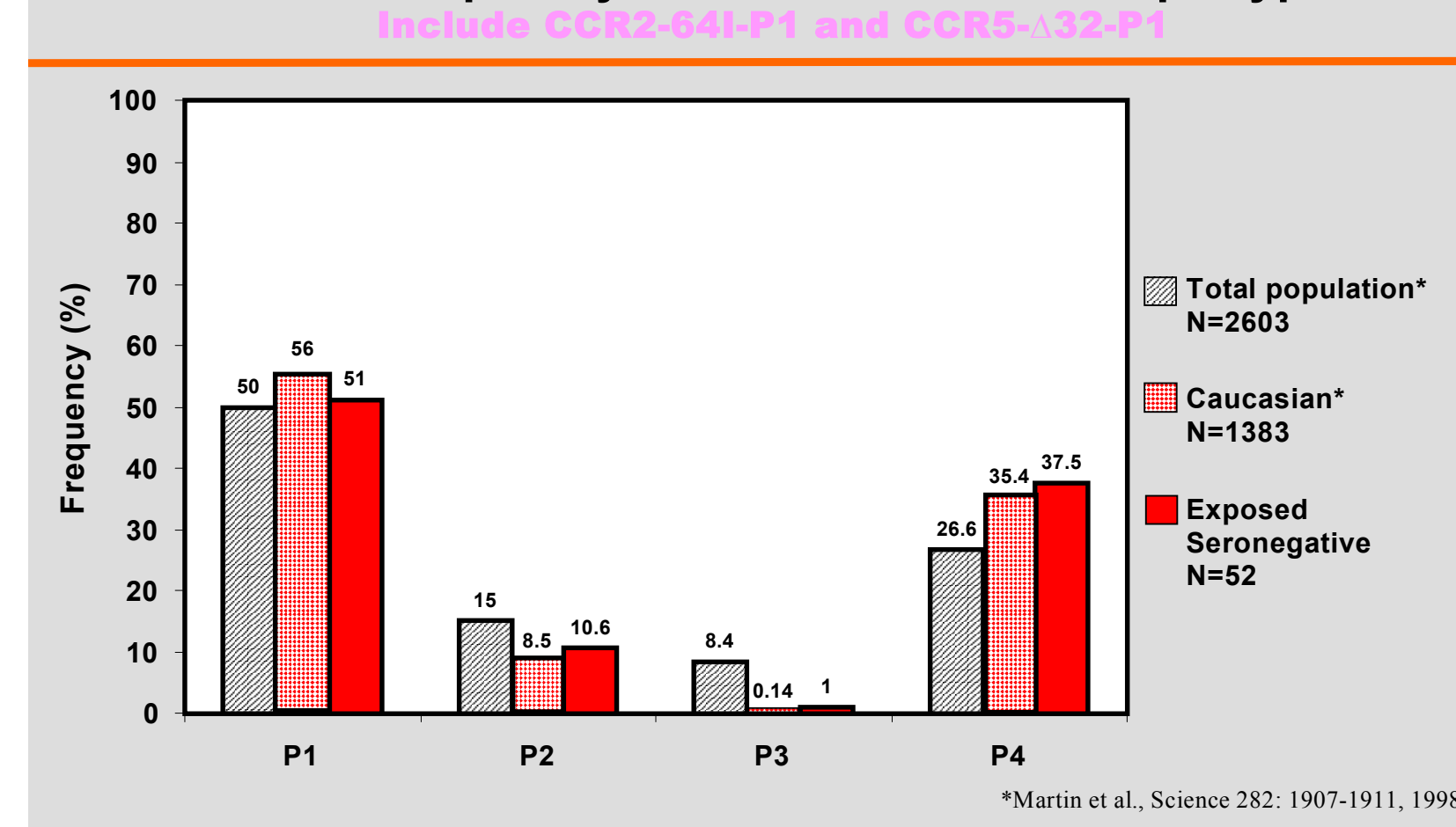


FIG.5. CCR5 Promoter P1/P1 Homozygotes Distribution in Caucasian Populations

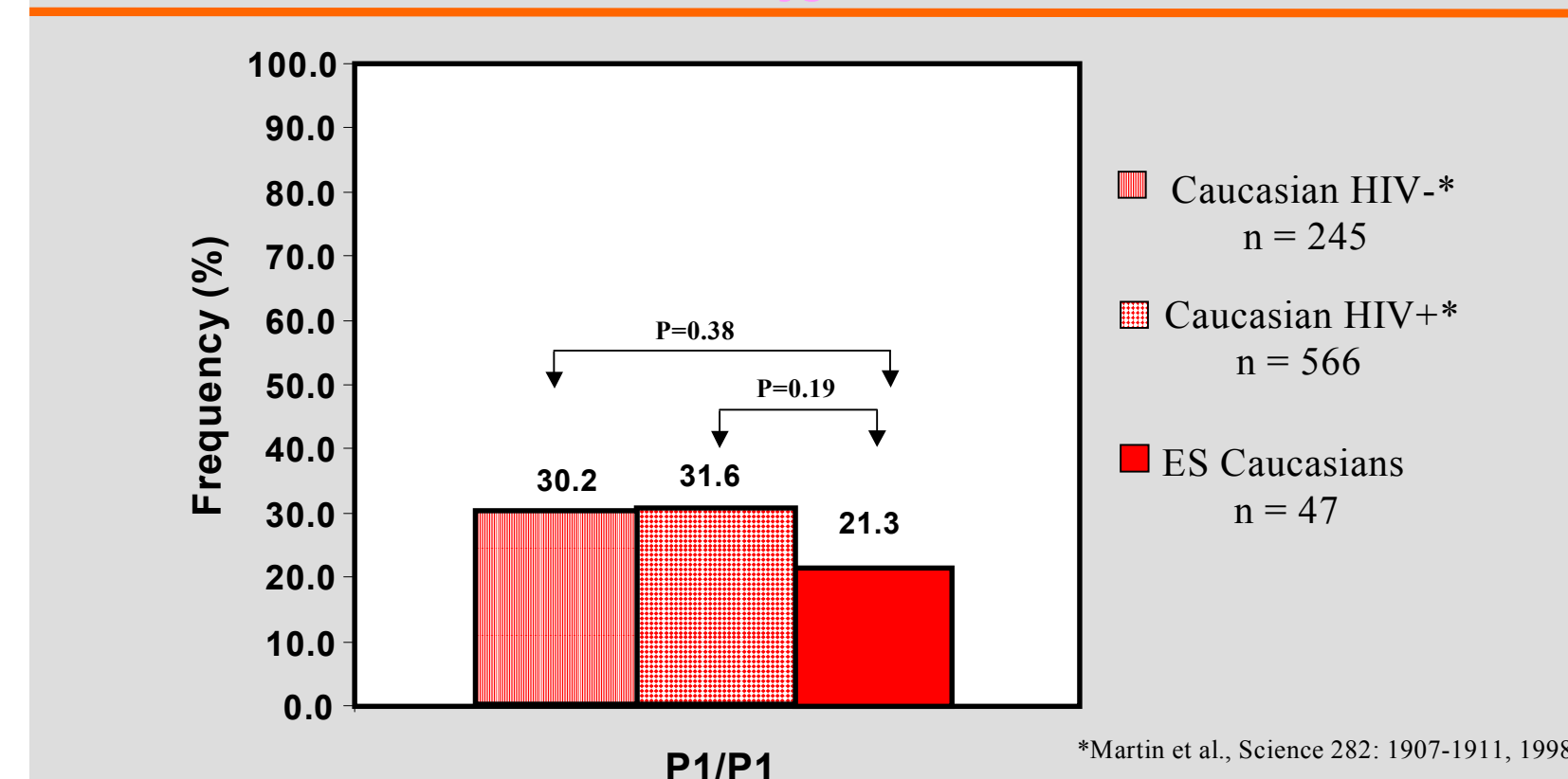


FIG.6. CCR5 Promoter P1/P1 Genotype Distribution

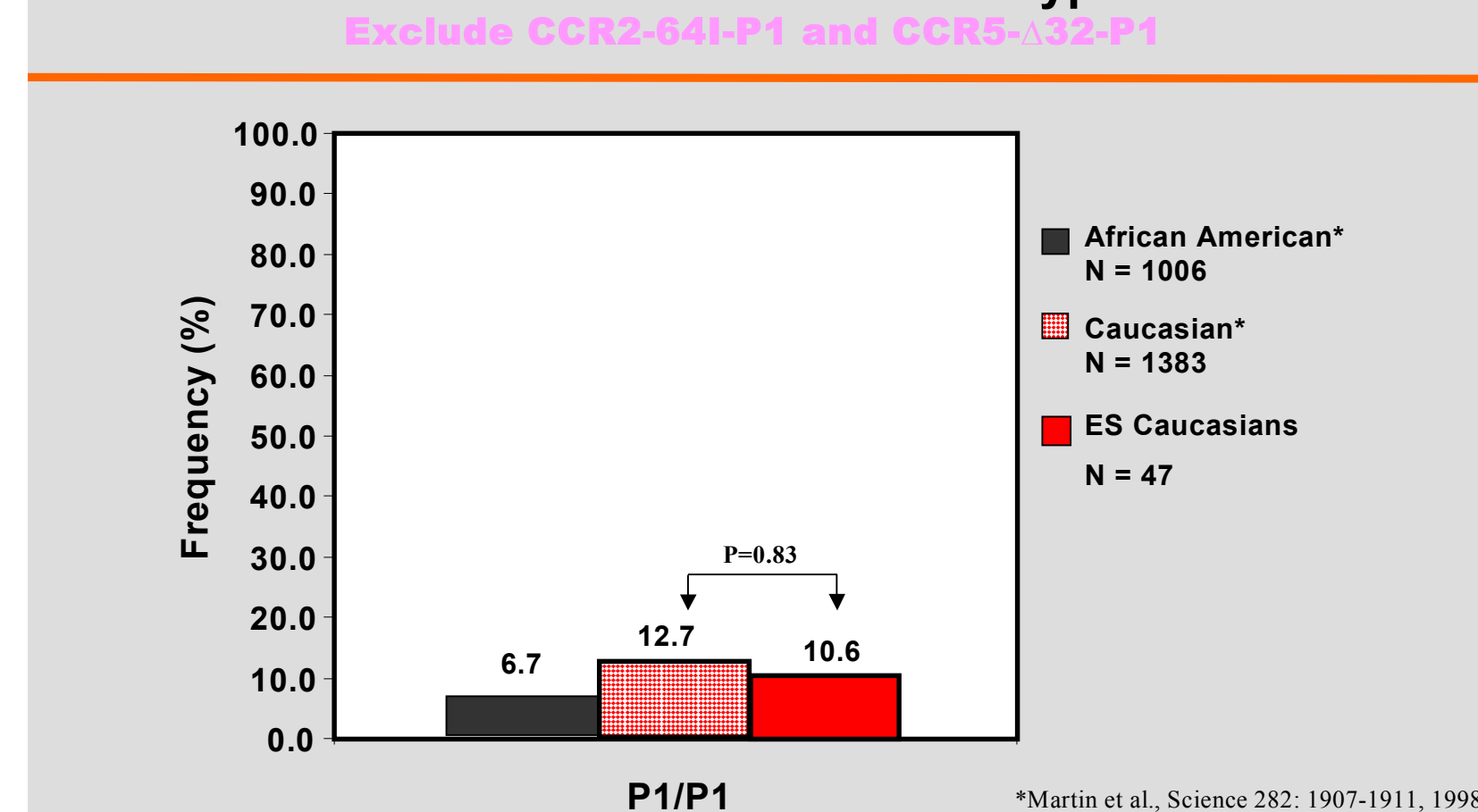
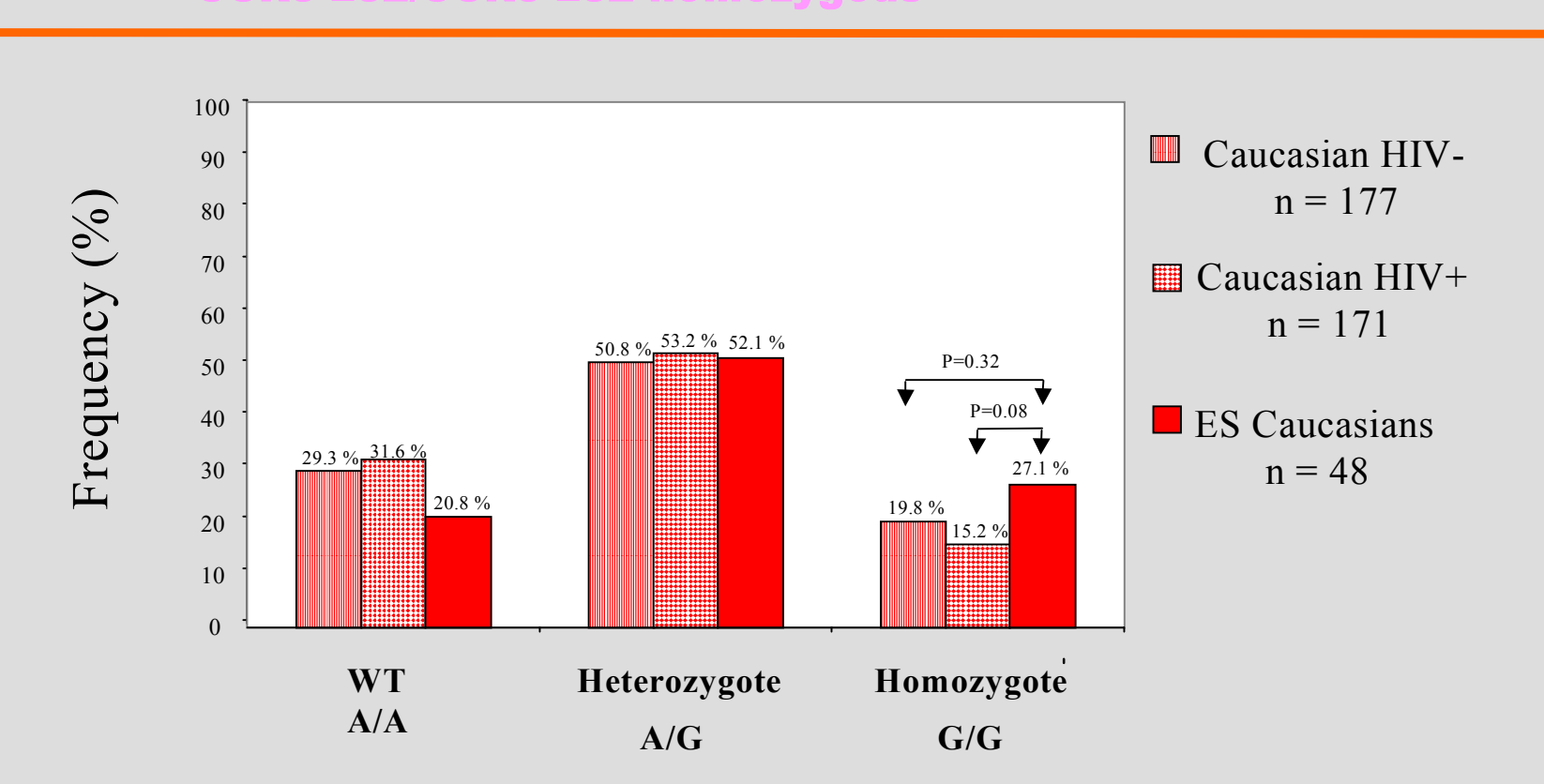


FIG.7. CCR5P-59029 Genotype in ES and Local Caucasian Population



Conclusions

- Higher frequencies of CCR5-Δ32 homozygous mutations in ES cohort (P=0.056 vs HIV-1 positive; P=0.018 vs both HIV-1 positive and negative individuals).
- The frequency of CCR5-Δ32 heterozygotes in LTNP is 47.1%, significantly higher than HIV-1 positive groups (P = 0.03).
- No polymorphisms was found in other coreceptor coding region in ES cohort.
- No significant difference in the frequencies of CCR2-64I genotype/allele was seen among ES, HIV-1 negative and positive individuals.

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