LETTER TO THE EDITOR

32 bp DELETION IN CCR-5 GENE AND HUMAN IMMUNODEFICIENCY VIRUS EPIDEMIC IN THE CZECH REPUBLIC

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CCR-5 is a receptor for chemotactic cytokines which functions also as a co-receptor for macrophage-tropic strains of human immunodeficiency virus type 1 (HIV-1). A 32 bp deletion in CCR-5 gene (CCR-5Δ32) is present in approximately 20% of Caucasians but is almost absent in Africans and Asians (*1,2*). People homozygous for Δ32 are resistant to HIV-1 infection (*1,3*), though protection is not absolute (*4*). While it may postpone the disease progression (*2,3,5*), heterozygosity in CCR-5 is not a barrier to HIV-1 infection (*3*).

The Czech Republic is a post-communist country in the middle of Europe with a remarkably low incidence of HIV infection. By the end of 1997, 361 HIV-positive individuals were reported in the 10 million Czech population, however, the estimated number was much higher, about 2,000 (0.02%) in comparison with 510,000 (0.22%) in Western Europe. To test a hypothesis that this low incidence may be connected with increased frequency of CCR-5Δ32, we typed 386 healthy unrelated Czech individuals for wild type and mutant CCR-5 alleles. Though Czechs are of Caucasoid origin, due to history of migration of other ethnic groups through the country, a higher frequency of the mutant allele was not *a priori* excluded.

Genomic DNA was extracted from peripheral blood by a salting out method (*6*) and amplified by polymerase chain reaction (PCR) with primers 5'-CTTCATTACACCT GCAGCTCT-3' (sense) and 5'-CACAGCCCTGTGCCTCT TCTTC-3' (antisense). The reaction mixture (10 μl) contained 2.5 μg/ml DNA, 67 mmol/l Tris.HCl pH 8.8, 16.6 mmol/l $(NH_4)_2SO_4$, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 0.1% Tween-20, 1 nmol/ml primers, 17 U/ml Taq polymerase. After initial denaturation (94°C for 2 mins), the PCR was run using a touchdown principle (*7*): the annealing temperature was lowered after each cycle of the first 10 cycles by 1°C. The cycling conditions were as follows: 94°C for 10 secs, 67°C – 1°C per cycle for 50 secs, 72°C for 30 secs. The next 20 cycles were run at constant annealing temperature of 58°C as follows: 94°C for 10 secs, 58 °C for 50 secs, 72°C for 30 secs. The PCR was finished by incubation at 72°C for 2 mins followed by 25°C for 1 sec. The PCR products were separated by electrophoresis on 2% agarose gel in a Tris-borate buffer at 5.6 V/cm for 50 mins.

The specificity of the method used is illustrated on the figure which shows wild type homozygote CCR-5 (182 bp, lane 2), mutant homozygote CCR-5 (150 bp, lane 3), heterozygote CCR-5 (lane 4) and size markers (Promega, lanes 1 and 5, size in bp).

We found the frequency of homozygous wild type individuals of 78.8% (304/386), that of homozygous mutant individuals of 0.3% (1/386), and that of heterozygous individuals of 21% (81/386) in accord with the reported findings on other Caucasian populations (*1*). Though mutations, unrecognized by the method used in this study (e.g. nucleotide substitutions in the tested region and mutations outside the tested region) may exist, the CCR-5 deletion mutation does not seem to be the cause of the low incidence of HIV infection in the Czech Republic. Therefore, other factors should be considered. E.g. the existence of the „iron

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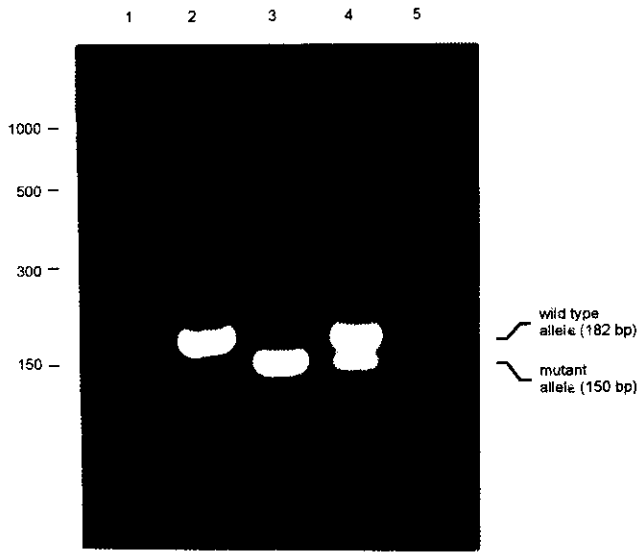
Abbreviations: HIV = human immunodeficiency virus; PCR = polymerase chain reaction

curtain" on the western border of the Czech Republic may have delayed the penetration of HIV into our population. In addition, effective preventive epidemiological measures (e.g. free syringe supply to drug abusers, blood donors screening) may also have contributed to the low frequency of HIV infection. Anyway, a more intensive research into molecular epidemiology of HIV infection is needed to reveal the cause of low HIV incidence in our country. In conclusion, our data suggest that Czechs should not rely on the CCR-5 deletion as a factor conferring natural resistance to HIV infection.



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