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Distribution of the HIV resistance CCR5-Δ32 allele among Egyptians and Syrians

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Abstract

A mutant allele of the β-chemokine receptor gene CCR5 bearing a 32-basepair (bp) deletion that prevents cell invasion by the primary transmitting strain of HIV-1 has recently been characterized. Individuals homozygous for the mutation are resistant to infection, even after repeated high-risk exposure, but this resistance appears not absolute, as isolated cases of HIV-positive deletion homozygotes are emerging. The consequence of the heterozygous state is not clear, but it may delay the progression to AIDS in infected individuals. In order to evaluate the frequency distribution of CCR5-Δ32 polymorphism among Egyptians, a total of 200 individuals (154 from Ismailia and 46 from Sinai) were tested. Only two heterozygous individuals from Ismailia carried the CCR5-Δ32 allele (0.6%), and no homozygous (Δ32/Δ32) individuals were detected among the tested samples. The presence of the CCR5-Δ32 allele among Egyptians may be attributed to the admixture with people of European descent. Thus we conclude that the protective deletion CCR5-Δ32 is largely absent in the Egyptian population.

Keywords: Human immunodeficiency virus type 1; Chemokine receptor; Polymorphisms

1. Introduction

A mutant allele of the β-chemokine receptor gene CCR5 bearing a 32-basepair (bp) deletion that prevents cell invasion by the primary transmitting strain of HIV-1 has recently been characterized [1–3]. Individuals homozygous for the mutation are resistant to infection, even after repeated high-risk exposure [1,4], but this resistance appears not absolute, as isolated cases of HIV-positive deletion homozygotes are emerging [5]. There is little information regarding human immunodeficiency virus (HIV) in Egypt. Among IV drug users, a high risk group, the prevalence of HIV infection has already reached 8% in Cairo as of 1994 [6]. HIV infection levels declined between 1987 and 1993 in Cairo among male sexually transmitted disease (STD) patients. In 1987–1988, 0.7% were HIV positive; in 1993, 0.3% were infected. In 1994, there was no evidence of infection among STD patients attending STD clinics in Alexandria and Assiut [6–9]. The estimated percentage of adults living with HIV/AIDS in Egypt at the end of 1999 was 0.02% [8].

Chemokine receptors are cell surface proteins that bind small peptides called chemokines [10]. Chemokines can be classified into three groups based on the number and location of conserved cysteines: C, CC, and CXC. Chemokine receptors are grouped into families on the basis of the chemokine ligands they bind: CC, CXC, or both. The chemokine receptor CCR5 is encoded by

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the CMKBR gene located on the p21.3 region of human chromosome 3 [11]. The chemokine receptor gene CCR5 has become the object of intense interest since the discovery of its role in the entry of human immunodeficiency virus type 1 (HIV-1) into human CD4-positive cells [12,13]. The CCR5 gene product is a member of the seven-transmembrane, G-protein-coupled receptor family [14,15] which, in response to their normal β-chemokine ligands, is involved in the chemotaxis of leucocytes towards sites of inflammation [16]. CCR5 also mediates the entry into cells of the M-tropic strain of HIV-1 that is the primary transmitting form of the virus [17–21].

Almost simultaneously with the findings of the HIV coreceptors, a mutation in the gene that encodes CCR5 was reported that confers a high degree of resistance to HIV infection *in vitro* and *in vivo* [1,4]. This mutation, termed Δ32, consists in a 32 bp deletion that occurs at a site of a repeat motif in the CCR5 gene and results in a frameshift in the coding sequence that produces a non-functional protein, and as a result it is not expressed in the cell membrane [22]. It was believed that individuals with homozygous mutant genotype (Δ32/Δ32) were 100% resistant to HIV infection, at least with M-tropic strains, that use the CCR5 molecule as co-receptors. However, there have been reports of HIV infection in two individuals who are homozygous for the mutant allele (Δ32/Δ32) [5]. These infections could have occurred with T-tropic strains that use the CXCR4 molecule as coreceptors to enter the target cells. It has been difficult to establish if the CCR5/Δ32 genotype confers any degree of resistance to infection. HIV-infected individuals heterozygous for CCR5-Δ32 have about a 2-year delay in progression to AIDS compared with HIV infected individuals who do not carry the Δ32 polymorphism [23–25]. Although the studies on these aspects of disease progression are contradictory [2,3,23,26].

The global distribution of the CCR5-Δ32 alleles among diverse human populations is widely variable. The CCR5-Δ32 allele is more prevalent in European populations and almost absent from Asian groups [2]. Within European populations a wide variation of 10–20% in CCR5-Δ32 frequency was observed with a gradient, uppermost at the north around the Baltic Sea down to the Mediterranean coast. It was inferred that most, if not all CCR5-Δ32 alleles originate from a single mutation event, and that this mutation event probably took place a few thousand years ago in Northeastern Europe [11]. The frequency of the mutation is 20.93% in Ashkenazi Jews [27,28]. In European-derived populations from the United States and Southern Europe, CCR5-Δ32 was observed at a frequency of 5–10%, but decreased to 2–5% in Hispanic populations, throughout the Middle East or in the Indian continent. However, this allele is almost absent among African-Americans in whom admixture with people of European descent has been considerable [27]. In Asian populations, the CCR5-Δ32 alleles were absent among Japanese, Filipino, Korean, Chinese and Indian populations studied [29]. In Latin America, the CCR5-Δ32 was not detected among 32 individuals from Venezuela or in Amerindian groups [1,27]. The frequency of the allele reaches 5.3% in Columbia [30].

The age of the CCR5-Δ32 allele has been estimated to be between 700 and 3500 years old [31,32] with ancient DNA evidence suggesting an age of 2900 years old [33]. The high frequency of the CCR5-Δ32 allele in some populations is presumably the consequence of natural selection possibly driven by an increased resistance to an infectious agent [34–36]. The geographic spread of the CCR5-Δ32 resistance allele has recently been studied to determine how selection and dispersal have interacted during the history of the allele [37]. In the present study, the distribution of the CCR5-Δ32 allele among Egyptians and several other diverse human populations was analyzed.

2. Materials and methods

The human population samples used for this study have been described previously and were available from previous studies [38,39]. The samples studied were collected from unrelated individuals from six populations: Arabic countries (Egypt and Syria), European (German, Greek and Turkish Cypriots), sub-Saharan Africa (African American) under institutionally approved internal review board protocols with informed consent. DNA was prepared from blood leukocytes by standard methods [40]. The genotype determination was carried out as previously described [24]. The specific segment of CCR5 gene was amplified by polymerase chain reaction (PCR) using the following primers [30]: CCR5-F (5′-ACCAGATCTCAAAAAGAAGGTCT-3′) and CCR5-R (5′-CATGATGGTGAAGATAAGCCTCACA-3′). PCR amplification was carried out in 25 μl reactions containing 20–100 ng of template DNA, 40 pM of each oligonucleotide primers, 200 μM dNTPs, in 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.4) and Taq DNA polymerase (1.25 Units). The reaction were subjected to 32 cycles: an initial denaturation of 150 s at 94 °C, 1 min denaturation at 94 °C, 1 min at the annealing temperature 55 °C, extension at 72 °C for 1 min. Following the amplification cycles a final extension was performed at 72 °C for 10 min. For analysis, 20 μl of each sample was fractionated on a 3% agarose gel with 0.05 μg/ml ethidium bromide. PCR products were directly visualized using UV fluorescence. For the wild type genotype (CCR5/CCR5), the PCR product was 225 bp while a product of 193 bp indi-

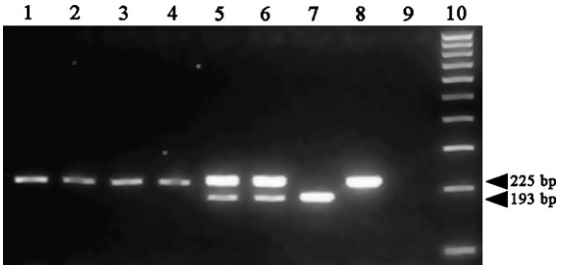

Fig. 1. Determination of CCR5 genotypes by agarose gel electrophoresis. DNA fragment patterns of persons with homozygous wild type (CCR5/CCR5) (lanes 1–4 and 8), homozygous 32 bp deletion (Δ32/Δ32) (lane 7) or heterozygous genotypes (CCR5/Δ32) (lanes 5 and 6), are shown. Lane 9 is a negative control and lane 10 is 100 bp marker. The black arrows refer to the fragment size of 225 bp for wild type and 193 bp for mutant alleles.

cated a homozygous mutant (Δ32/Δ32). The presence of both fragments indicated a heterozygous genotype (CCR5/Δ32) (Fig. 1). The allelic and genotypic frequencies were compared and differences evaluated using the chi square test for goodness of fit (χ^2). The significant deviation from Hardy–Weinberg equilibrium (HWE) was evaluated by the same test.

DNA sequencing was performed on gel purified PCR products that had been cloned using the TOPO-TA cloning vector (Invitrogen) using chain termination sequencing [41] on an Applied Biosystems 3100 automated DNA sequencer. The automated DNA sequencing was employed to confirm the authenticity of the amplified PCR products.

3. Results

The CCR5 genotype was determined by polymerase chain reaction (PCR) from six diverse population samples. As shown in Table 1, we found that 152 individuals from Egypt living in Ismailia were homozygous for the wild-type CCR5 allele and two were heterozygous for the CCR5-Δ32 allele, giving a CCR5-Δ32 allelic frequency of 0.006. None of the individuals tested was homozygous for the mutation (Δ32/Δ32). No mutant alleles were identified in 46 Egyptian individuals from the Sinai. Based on the allele frequencies it is possible to predict the genotype frequency considering that they follow the Hardy–Weinberg equilibrium. This means that the frequencies have a binomial distribution according to the following equation: $p^2 + 2pq + q^2 = 1$, where p and q are the allelic frequencies of CCR5 and Δ32, respectively, and p^2, $2pq$ and q^2 are the genotype frequencies of CCR5/CCR5, CCR5/Δ32, and Δ32/Δ32, respectively. No significant deviations from the Hardy–Weinberg equilibrium were observed. Table 1 shows the frequency of the CCR5-Δ32 allele in the populations analyzed here as well as selected populations from previous studies. The frequency of the CCR5-Δ32 allele was high among German Europeans (12.8%), moderate in Turkish individuals from Turkey (6.3%) and from Cyprus (3.2%). It is found at a very low frequency in Cyprus (Greek)

Table 1
CCR5 genotypes in diverse human populations

Population	No. of studied	CCR5/CCR5	CCR5/Δ32	Δ32/Δ32
Egypt-Ismailia	154	152	2	0
Egypt-Sinai	46	46	0	0
Syria	69	68	1	0
Lebanon [31]	51	51	0	0
Saudi Arabia [27]	241	231	10	0
Saudi Arabia [31]	100	100	0	0
United Arab Emirates [56]	26	26	0	0
Yemen [27]	34	34	0	0
Sudan [56]	25	25	0	0
Kenya [27]	80	80	0	0
Nigeria [27]	111	110	1	0
African-American	87	85	2	0
German European	74	58	13	3
Turkey [11]	104	91	13	0
Cyprus (Turkish)	47	44	3	0
Cyprus (Greek)	56	55	1	0
Kuwait [42]	393	385	8	0
Syria [42]	106	103	3	0
Jordan [42]	52	52	0	0
Iraq [42]	13	13	0	0
Iran [42]	84	80	4	0
Russia [42]	176	133	43	0

Data from previous studies is denoted by the citations.

(0.9%) and is completely absent from populations of African origin except Nigerians and African Americans, where it is detected at very low frequency. Within the Arabic populations, the frequency of the mutant Δ32 allele reached its highest among Saudis 2.07% [27] and Iranians 2.38% [42]. In a previous study done by Stephens et al. [31], no mutant alleles were detected among 100 individuals from Saudi Arabia. By combining both studies together, the frequency of CCR5-Δ32 among Saudis drops to 1.5%. This frequency is still higher than any of the other Arabic countries (Table 1). The frequency of the CCR5-Δ32 was very low among Syrians (0.7%), Egyptians from Ismailia (0.6%) and is completely absent from individuals from Lebanon, Sudan, Yemen, the United Arab Emirates and Egyptians from the Sinai (Table 1). Our findings suggest that the CCR5-Δ32 allele is nearly absent from Arabic populations. However, the near absence of the CCR5 gene 32-bp deletion mutation does not preclude the existence of other polymorphisms of chemokine receptor genes associated with slower progression to AIDS within these populations.

4. Discussion

Despite the apparent resistance afforded by the CCR5-Δ32 mutation, protection against HIV-1 infection is not absolute, as evidenced by reports of HIV-1 infection among Δ32/Δ32 homozygotes [5,43,44]. 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 homozygotes. Recently, it was demonstrated that HIV could use other chemokine receptors such as CCR2 and CCR3 as coreceptors [45]. Therefore, mutations in these molecules or in the chemokine receptor ligands might explain the resistance to HIV infection of some individuals. However, the mutation CCR2-64I was not associated with a resistant phenotype but rather with a delayed in disease progression [46]. Similarly, the polymorphism SDF-1-3′ A reported in the α-chemokine SDF-1 was associated with delayed onset of AIDS related symptoms [47].

The general absence of CCR5-Δ32 in sub-Saharan Africa suggests a recent origin of the mutation in agreement with a variety of other age estimates [31–33], but also suggests that it is not a significant factor in the prevalence or transmission of HIV-1 within Africa. The high frequency of CCR5-Δ32 in Europe may indicate that it too has had a long term selective advantage against infectious diseases using CCR5 as entry cofactors, as suggested previously [3,34–36]. Alternatively, this may be the result of a previously-neutral polymorphism whose selective advantage is just becoming apparent.

The high frequency of the CCR5-Δ32 allele in some populations could affect the epidemiology of the disease and the mean progression rate of HIV-positive individuals in these populations. It may also favor the emergence of strains that can bypass the CCR5 defect by using other coreceptors efficiently [18,20,48]. The uneven distribution of the genotype across geographic and racial groups may be related to some previous epidemic, restricted to Europe that led to a survival advantage among persons homozygous or heterozygous for the CCR5/Δ32 genotype and resulted in a concentration of the CCR5/Δ32 genotype in persons of European ancestry. The bubonic plague, which claimed the lives of 25–33% of Europeans during the Black Death from 1346 to 1352 and other large epidemics such as small pox have been used as examples to support this [49–53]. Thus, certain populations appear to have some increase in survival advantage in the current HIV epidemic. Conversely, populations with lower frequencies of the CCR5-Δ32 genotype might be expected to have a higher prevalence of HIV infection or a more rapid course of the epidemic. A greater risk for HIV infection has been found among African Americans than among European Americans from the United States, when known risk factors, including social class, were controlled [54]. The prevalence of the CCR5-Δ32 allele is lower in African Americans than in European Americans. While increasing HIV prevalence in parts of Asia and Africa may be attributed to social and demographic factors, as well as differences in the phenotype of circulating viruses [55], the racial distribution of HIV risk raises the possibility that differences in the distribution of the CCR5-Δ32 allele or other heritable host factors/mutations may influence the rate of transmission or the speed of the epidemic in different racial groups.

In the Arabic countries, the frequency of the mutant allele CCR5-Δ32 is very low and probably derived from admixture with the populations of European descent rather than as a result of parallel independent mutations. Future studies are needed to study the chromosomal haplotypes carrying this mutation and the mutation frequency among the rest of the Arabic populations.

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