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The Δ ccr-5 Mutation in the CCR-5 Chemokine Receptor Gene Conferring Resistance to Human Immunodeficiency Virus Type 1: Frequency in Kuwait

Key Words

HIV-1
CCR-5
 Δ ccr-5
Polymerase chain reaction
Genetic resistance
Allele frequency

Abstract

Objective: The homozygous deletion of 32 nucleotides in the CCR-5 gene (Δ ccr-5) confers resistance to macrophage-tropic HIV-1. The aim of this study was to determine the frequency of Δ ccr-5 in populations residing in Kuwait. **Methods:** A polymerase chain reaction (PCR) test for Δ ccr-5 has been developed. The specificity of the test has been proved by direct sequencing of the PCR products amplified from individuals homozygous for the wild-type allele and from Δ ccr-5/wild-type heterozygotes. **Results:** Among 353 healthy individuals residing in Kuwait (270 Kuwaiti citizens and 83 Bedouins) tested for the Δ ccr-5 mutation, 8 heterozygotes have been identified, 4 in each group. No homozygotes for the Δ ccr-5 deletion have been found. The frequency of the Δ ccr-5 allele in Kuwaiti nationals and Bedouins residing in Kuwait is 0.74 and 2.4%, respectively. **Conclusion:** The Δ ccr-5 mutation is present in populations residing in Kuwait, though its frequency is quite low and can hardly influence the pace of HIV-1 spreading in Kuwait.

Introduction

One of the most challenging and intriguing observations in the HIV/AIDS field is the existence of individuals remaining HIV-free, despite repeated exposure to the virus through

sexual contacts [1, 2]. Such 'exposed-uninfected' individuals are 'naturally resistant' to HIV infection. Such resistance has been considered to be genetic, but until recently proofs have been lacking.

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In 1996, after a decade of unsuccessful attempts, the 'second HIV receptor' (also called 'coreceptor') was identified for both T cell line (T)-tropic and macrophage (M)-tropic strains of HIV-1. Both coreceptors turned out to be members of the seven-transmembrane domain, G-protein-coupled chemokine receptor family. The CXCR-4 chemokine (also known as fusin or LESTR) serves as the coreceptor for T-tropic HIV-1, while the CCR-5 chemokine (also known as C-C CKR-5) does the same for M-tropic HIV-1 [3–8].

The relevance of the HIV coreceptor to the problem of natural resistance to HIV-1 infection and AIDS became apparent when identical homozygous mutations were found in some 'HIV-1 exposed-uninfected' individuals [9]. The mutation (a 32-nucleotide deletion in the CCR-5 gene, called Δ CCR-5) results in truncation of the CCR-5 protein. The truncated CCR-5 fails to mediate chemokine signaling and to facilitate entry of HIV-1 into the macrophagic target cells. As a result, the cells bearing the mutation become resistant to HIV-1. Individuals carrying Δ CCR-5 in the homozygous state are highly resistant to HIV-1 infection, though the resistance is not absolute [10, 11].

Unexpectedly, the Δ CCR-5 mutation has been found to be quite common among individuals of West European descent (mutant allele frequency 0.080–0.092). At the same time, the mutation has not been found in individuals of African, South American and Japanese origin [9, 10]. The frequency of Δ CCR-5 in other ethnic groups was unknown at the beginning of this study.

In this paper, we present the first data on the frequency of the Δ CCR-5 mutation in an Arab population. The preliminary report of our study was presented at the 5th Kuwait International Conference on AIDS, March 17–19, 1997 [12] and at the Kuwait University Faculty of Medicine Poster Day, April 29–30, 1997 [13].

Fig. 1. Typical results of identification of w/w and Δ CCR-5/w genotypes by PCR. M = Markers (50, 100, 200, 300, 400, 500, 700, 1,000, 1,500 and 2,000 bp); lanes 1, 5 = Δ CCR-5/w heterozygotes (wild-type allele band 230 bp, Δ CCR-5 allele band 198 bp, the band above the 230 bp consists of heteroduplexes); lanes 2, 3, 4, 6 = wild-type homozygotes.

Materials and Methods

DNA Samples

Whole blood samples from 353 individuals (270 Kuwaiti citizens and 83 Bedouins residing in Kuwait) were collected and used for extraction of DNA. The DNA was extracted from leukocytes using the Chelex 100 method [14]. The study group included voluntary blood donors and healthy relatives of the individuals referred to the Kuwait Medical Genetics Centre.

Polymerase Chain Reaction for Detection of the Δ CCR-5

The primers AV-66 (5'-CTGTGTTTGCGTCTCTCC-3') and AV-67 (5'-CCTGTGCCTCTTCTCTCTCA-3') were used to amplify the 230-bp fragment of the CCR-5 gene containing the site of mutation. The reaction mixture included standard polymerase chain reaction (PCR) buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM Mg₂Cl), 50 μ M of deoxynucleoside triphosphates, 10 pmol of each primer and 5 μ l of Chelex-100-extracted DNA from leukocytes in a total volume of 25 μ l. Thermocycling (94, 55 and 72°C, 30 s at each temperature, 30 cycles) was performed in a Perkin-Elmer system 9600. Standard precautions to prevent carry-over artifacts were strictly followed [15].

		10	20	30	40	50
CCR5-X91492	1	CAGGAATCAT	CTTTACCAGA	TCTCAAAAAG	AAGGTCTTCA	TTACACCTGC
CCR-5-W/W	1	CAGGAATCAT	CTTTACCAGA	TCTCAAAAAG	AAGGTCTTCA	TTACACCTGC
CCR-5-Δ/Δ	1	CAGGAATCAT	CTTTACCAGA	TCTCAAAAAG	AAGGTCTTCA	TTACACCTGC
		60	70	80	90	100
CCR5-X91492	51	AGCTCTCATT	TTCCATACAG	TCAGTATCAA	TTCTGGAAGA	ATTTCAGAC
CCR-5-W/W	51	AGCTCTCATT	TTCCATACAG	TCAGTATCAA	TTCTGGAAGA	ATTTCAGAC
CCR-5-Δ/Δ	51	AGCTCTCATT	TTCCATACA-	-----	-----	-----
		110	120	130	140	150
CCR5-X91492	101	ATTAAAGATA	GTCATCTTGG	GGCTGGTCCT	GCCGCTGCTT	GTCATGGTCA
CCR-5-W/W	101	ATTAAAGATA	GTCATCTTGG	GGCTGGTCCT	GCCGCTGCTT	GTCATGGTCA
CCR-5-Δ/Δ	101	-TTAAAGATA	GTCATCTTGG	GGCTGGTCCT	GCCGCTGCTT	GTCATGGTCA

Fig. 2. DNA sequence comparison of the wild-type and Δ*accr-5* alleles of the human CCR-5 gene. CCR5-X91492 = Prototype sequence of the human CCR-5 gene (GenBank Accession Number X91492); CCR-5-W/W = partial sequence of the wild-type allele of the human CCR-5 gene; CCR-5-Δ/Δ = partial sequence of the Δ*accr-5* allele of the human CCR-5 gene.

The detection of the amplimers was carried out by minigel electrophoresis in 1.7% agarose (NA, Pharmacia, Uppsala, Sweden) and staining with ethidium bromide.

DNA Sequencing and Analysis
 PCR-amplified fragments of the CCR-5 gene were sequenced directly using the AV-66/AV-67 primers according to the manufacturer's (Applied Biosystems/Perkin Elmer, Foster City, Mich., USA) protocols using a Dye Terminator Cycle Sequencing Kit with AmpliTaq DNA Polymerase FS and the Applied Biosystems Model 373 automatic DNA sequencer. To minimize artifacts due to *Taq* polymerase mistakes, samples were prepared for sequencing as a pool of 10 separate PCR amplifications with the same target DNA and primers. The DNA sequences were aligned and compared with the help of the DNASIS program (Hitachi).

Results

Three possible outcomes were expected for the PCR amplification of the region of the human CCR-5 gene defined by selected primers: (1) a single 230-bp fragment in wild-type homozygotes (genotype w/w); (2) a single 198-bp fragment in Δ*accr-5* homozygotes (genotype

Δ*accr-5*/Δ*accr-5*), and (3) both the 230-bp and 198-bp fragments in a Δ*accr-5*/wild-type heterozygotes (Δ*accr-5*/w genotype).

In our set of samples, two of these patterns were observed: the pattern corresponding to w/w and that corresponding to the Δ*accr-5*/w genotypes (fig. 1). The vast majority of the samples tested in this study (345) revealed the 230-bp band only (w/w genotype). In 8 cases, the pattern consistent with genotype Δ*accr-5*/w was observed. To confirm the specificity of the PCR conclusively, we sequenced part of the 230-bp fragment containing the region of the Δ*accr-5* deletion. The DNA sequence generated was identical to the prototype sequence of the corresponding fragment of the CCR-5 gene (fig. 2) [10, 16]. Thus, the amplification of the single 230-bp band by our PCR indeed is diagnostic for the w/w genotype. To prove conclusively that the 198-bp band in the heterozygous samples represents the Δ*accr-5* allele, we sequenced the part of the 198-bp fragment framing the deletion. This was achieved by reamplification of the fragment (recovered from the agarose after electrophoretic separation by pricking with a syringe needle) fol-

Table 1. Frequency of the Δ ccr-5 deletion in the Kuwaitis and the Bedouins residing in Kuwait (data from this study) and some other ethnic groups (reported by others)

Group	Sample size	Δ ccr-5/w, n	w/w, n	Δ ccr-5/w allele frequency
Kuwait				
Kuwaitis	270	4	266	0.007
Bedouins	83	4	79	0.024
Total	353	8	345	0.011
Saudi Arabia [18]	241	10	231	0.020
Yemen [18]	34	0	34	0
W. Europe [10]	704	114	582	0.092 ¹
USA, white [17]	143	23	120	0.080
USA, black [17]	620	21	599	0.017
Africa [10]	124	0	124	0
Japan [10]	248	0	248	0
Venezuela [10]	46	0	46	0

¹ Eight Δ ccr-5/ Δ ccr-5 homozygotes in this group.

lowed by direct sequencing of the reamplified product. As expected, the sequence generated contained a 32-nucleotide deletion as compared with the sequence of the wild-type allele of the CCR-5 gene (fig. 2).

The data on the frequency of the Δ ccr-5 mutation in Kuwaiti citizens and Bedouins are presented in table 1. No subject having the Δ ccr-5/ Δ ccr-5 genotype was found among the individual samples investigated in this study. The frequency of Δ ccr-5 in the Bedouins was more than 3 times higher than among the Kuwaiti citizens. The data for other ethnic groups reported in the literature are presented in table 1 for comparison.

Discussion

The Δ ccr-5 deletion was reported for the first time in August 1996 [9]. Within a few months after this discovery, it was established that homozygous carriers of the mutation were absent among HIV-infected individuals [10, 17, 19]. Thus, it was concluded that

homozygosity for Δ ccr-5 confers resistance to HIV-1 infection. The resistance is profound, though not absolute and 1 case of HIV-1 infection in a Δ ccr-5 homozygote has recently been reported in Australia [11]. The data on the protective significance of heterozygosity for Δ ccr-5 are controversial. On the one hand, it appears to postpone the progression of asymptomatic HIV infection to AIDS [10, 17, 20, 21]. On the other hand, at least in one group of AIDS patients (Danish cohort of HIV-1-positive male homosexuals), the survival of the Δ ccr-5 heterozygotes was significantly shortened [22].

One of the interesting facts reported in the first publication on Δ ccr-5 was the absence of carriers of the mutation outside Europe and North America. About several hundred non-Europeans, none of them Arabs, were tested before our study, and no single individual positive for Δ ccr-5, even a heterozygote, was found [9, 10, 17].

One of the explanations for that was the relatively small number of individuals of each ethnic group tested and the nonrepresentation

of many large ethnic groups in the first studies. To close one such gap in the knowledge of ethnic distribution of Δ ccr-5 deletion, we undertook a survey of Δ ccr-5 in Arabs residing in Kuwait. We have shown here that the Δ ccr-5 deletion is not confined to populations of European descent. It is present in indigenous Arab populations of the Middle East, though its allelic frequency in Arabs is significantly lower than in Europeans.

There are two possible explanations for the origin of Δ ccr-5 in the Gulf Arab populations. First, the Δ ccr-5 deletion in Europeans and Arabs arose independently. This seems to be highly unlikely because there are no features in the genomic sequence of the wild-type CCR-5 gene suggestive of an increased probability of a specific deletion in the region of the gene where Δ ccr-5 is actually located. The alternative and more plausible explanation implies a relatively recent gene flow from Caucasians into indigenous Gulf Arab populations. Importantly, this hypothesis is testable by the identification and comparison of haplotypes (defined by polymorphisms in the genomic regions flanking the CCR-5 gene) associated with the Δ ccr-5-positive chromosomes in Europeans and Arabs.

Another interesting question related to the origin of the Δ ccr-5 is its timing. The mutation is clearly much more common in Europeans [9, 10], including East Europeans [23], which suggests that the mutation originates in ancient populations of Europe.

The much higher frequency of Δ ccr-5 in the Bedouins residing in Kuwait than in the Kuwaiti nationals deserves attention. The Bedouins belong to different tribes which are highly consanguineous groups, and it is possible that the frequency of Δ ccr-5 in some of the tribes can be quite high due to a 'founder' effect. Unfortunately, the information on the tribal origin of the Δ ccr-5-positive Bedouins identified in our study was not available. De-

spite the difficulties in collecting samples and suitable information, an attempt to identify a hypothetical ' Δ ccr-5 positive' among the Bedouin tribes seems worth pursuing.

After we had found Δ ccr-5 in the Arabs and some other non-Europeans, and reported these findings [12, 13], similar observations were reported by Martinson et al. [18]. These authors tested Arabs (241 Saudis and 34 Yemenis) among other ethnic groups. The frequency of Δ ccr-5 in the Saudis was similar to what we had found for our Bedouin sample. None of the Yemenis was positive for Δ ccr-5, though the size of the sample was too small to draw any conclusion. It has to be mentioned that our latest data show that Δ ccr-5 is present in Arabs from the Eastern Mediterranean (Egyptians, Syrians) and in Iranians. Moreover, the frequency of the mutations in Iranians seems to be higher than that in Arabs [Voevodin et al., unpubl. data].

In conclusion, the Δ ccr-5 mutation is definitely present in Arabs, but it is too rare to significantly influence the pace of HIV-1 spreading in the Arab world. At the same time, it is possible that the frequency of the Δ ccr-5 mutation is much higher in some Arabic tribes. In a broader context, knowledge of the distribution of Δ ccr-5 mutation allows better global and regional modeling of the HIV-1 epidemic. The CCR-5 molecule is also a very promising target for new anti-HIV/AIDS therapeutic and preventive interventions which, in some cases, have to be tailored according to the Δ ccr-5 status of the individuals.

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