Frequency of CCR5 Promoter Polymorphism Affecting the Rate of Progression to AIDS (59029-G/A) in Kuwaitis

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Key Words

Human immunodeficiency virus type I · CCR5 · 59029 G/A · Polymerase chain reaction · Genetic resistance · Allele frequency

Abstract

Objective: To estimate the frequencies of 59029-G and 59029-A alleles associated with slow and rapid progression to AIDS in Kuwaitis. **Methods:** The DNA was extracted from 109 blood samples of healthy unrelated Kuwaitis. The 59029-G/A polymorphism was detected by the polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) test. The amplification of a 268-bp fragment of the CCR5 gene encompassing the site of the polymorphism was followed by digestion with restriction endonuclease *Bsp* 1286I and sizing the DNA fragments by agarose gel electrophoresis. **Results:** Among 109 individuals genotyped, 10, 44 and 55 were 59029-A/A homozygous, 59029-G/G homozygous and 59029-G/A heterozygous, respectively. The frequency of the G allele was 0.66 (95% CI: 0.59–0.72) while the frequency of the A allele was 0.34 (95% CI: 0.28–0.41). **Conclusions:** The frequency of the AIDS-‘protective’ 59029-G allele in Kuwaitis is significantly higher than the frequency of the ‘rapid-progression’ 59029-A allele. Moreover, the frequency of the 59029-G allele in Kuwaitis is among the highest reported. However, the difference from the 59029-G allele frequencies of other ethnic groups in some cases is not statistically significant.

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Introduction

The rate of progression of human immunodeficiency virus (HIV) infection to AIDS varies among individuals. Most HIV-infected individuals develop AIDS within 10 years of infection, but approximately 5% of HIV-positive subjects remain clinically and immunologically stable for 10–15 years or apparently do not progress to AIDS at all. Such individuals are referred to as ‘slow progressors’ or ‘long-term non-progressors’. On the other hand, there is a group referred to as ‘exposed uninfected’ individuals – those who are repeatedly exposed to HIV but remain uninfected [1–3].

Factors accounting for long-term non-progression may include infection with an attenuated strain of HIV, genetic polymorphisms in the host and stronger than usual virus-specific immune responses [4]. There is strong evidence for such a role for host genetic factors. Most of the genetic polymorphisms conferring resistance against HIV infection and/or delayed progression to AIDS are located in the genes encoding some of the chemokine receptors and chemokines. The most important among them are CCR5 Δ 32 [5, 6], CCR5 59029 G/A [7], CCR2–64I [8, 9] and SDF1–3’A [10].

The CCR5 Δ 32 mutation is a 32-base-pair (bp) deletion in the open reading frame of the CCR5 gene. The CCR5 gene containing Δ 32 encodes the non-functional HIV-1 co-receptor [5]. As a result CCR5 Δ 32 homozygotes are completely protected against HIV-1 infection with M-tropic strains [11], while in HIV-1-positive CCR5

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$\Delta 32$ heterozygotes the progression to AIDS is delayed on average for 2 years [5, 6]. CCR5 $\Delta 32$ is quite common in Caucasians and found at lower frequencies in the Middle East, but it is very rare among native Africans, Amerindians and East Asians [5, 6, 11, 12]. The allele frequency in Caucasians is about 10%, and approximately 1% of this population is homozygous. In Kuwaitis the allele frequency of CCR5 $\Delta 32$ is approximately 1%, and about 2% of Kuwaitis are heterozygous for the mutation [13].

The CCR2-64I is a single nucleotide polymorphism leading to a conservative amino acid substitution – valine to isoleucine at position 64 in the first transmembrane domain of CCR2. This allele is associated with delayed progression to AIDS by 2–3 years in both heterozygous and homozygous subjects [8, 9, 14]. The CCR2-64I mechanism of protection is not known. It has been suggested that CCR2-64I may be linked to another polymorphism that affects CCR5 expression [8, 15]. The differential dimer interaction of CCR2-64I and wild-type CCR2 with CCR5 peptides inside T cells may also affect the availability of CCR5 for HIV [16]. The CCR2-64I allele is found in all racial groups in the USA with the following frequencies: Caucasians = 10%, Afro-Americans = 15%, Hispanics = 17%, Asians = 25% [8]. The allele frequency of CCR2-64I in Kuwaitis is 12% [17].

The SDF1-3'A polymorphism is G $\rightarrow$ A substitution at position 809 in the 3'-untranslated region of the gene encoding for the stromal derived factor 1 (SDF1), the ligand for chemokine receptor CXCR4 [10]. The latter is a co-receptor for T-tropic (X4-tropic) strains of HIV. The protective effect of the SDF1-3'A allele is recessive. Thus, it is observed only in SDF1-3'A homozygotes. The possible mechanism of action could involve increased SDF1 leading to blockade of CXCR4 [7, 10]. The SDF1-3'A allele is found in all racial groups in the USA with the following frequencies: Caucasians = 21%, Hispanics = 16%, Asians = 26%, Afro-Americans = 6% [10]. The allele frequency of SDF1-3'A in Kuwaitis is 26%, and approximately 6% of Kuwaitis are SDF1-3'A homozygous [17].

The most recently identified polymorphism in the chemokine receptor genes affecting the rate of progression to AIDS is referred to as CCR5 59029-G/A. It is a single nucleotide polymorphism, G versus A at position 59029 in the CCR5 promoter region [7]. Both 59029-G and 59029-A alleles are quite common in all racial groups tested (in the USA). The allele frequencies of 59029-A are Caucasians = 57%, Afro-Americans = 43%, Asians = 47% and Hispanics = 68% [7]. The frequencies of 59029-G and 59029-A alleles in Arabs were not known.

Table 1. The size of the bands (bp) characteristic of different genotypes

Mutation	Genotype		
	A/A	A/G	G/G
59029-G/A	268	268, 130	130

The 59029-G allele is associated with slower progression to AIDS – about 4 years delay in 59029-G homozygotes as compared with 59029-A homozygotes. In heterozygotes (59029-G/A genotype) the rate of progression to AIDS appears to be intermediate [7]. The mechanism of protection may be related to reduction in CCR5 mRNA production in the presence of the 59029-G allele [7]. It is of importance that the protective effect of 59029-G in homozygotes is twice as strong as those of the CCR5 $\Delta 32$ or CCR2-64I alleles and is independent of protection conferred by other variants [7].

This study was aimed at an estimation of the frequencies of 59029-G and 59029-A alleles in Kuwaitis.

Materials and Methods

DNA Samples

Blood specimens were collected from 109 healthy unrelated Kuwaitis. The DNA was extracted from leucocytes using the 'salting out' method [18].

Genotyping for 59029-G/A Polymorphism

The polymorphisms were determined according to McDermott et al. [7] using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). The primers 59029-S (5'-CCCGTGCCCATAGTTAAACTC-3') and 59029-AS (5'-TCA-CAGGGCTTTTCAACAGTAAGG-3') were used for amplification of the 268-bp fragment of the CCR5 gene encompassing the site of the 59029-G/A polymorphism. The reaction mixture included standard PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂), 50 μ M of each of deoxynucleoside triphosphates, 10 pmol of each primer, 0.75 unit of AmpliTaq Gold DNA polymerase and approximately 500 ng of genomic DNA in a total volume of 25 μ L. Thermocycling (10 min at 94°C, then 30 cycles at 94°C, 55°C and 72°C, 30 s at each temperature) was performed in a Perkin-Elmer System 9600. After amplification by PCR the product was digested by the restriction endonuclease *Bsp*1286I [recognition/cutting sites: 5'-G(G/A/T)GC(C/A/T) $\downarrow$ C-3'//3'C $\downarrow$ (C/T/A)CG(G/T/A)G-5']. The fragments obtained after digestion were sized by agarose gel (1.4%) electrophoresis. The sizes of the bands characteristic of different genotypes are presented in table 1.

Fig. 1. Typical result of PCR-RFLP genotyping. Lane 1: fragment size markers (50, 100, 200, 300, 400, 500, 700 and 1,000 bp); lane 2: 59029 G/A genotype (130-bp and 268-bp bands); lane 3: 59029 A/A genotype (268-bp band); lane 4: 59029 G/G genotype (130-bp band).

Computation of Confidence Intervals for Allele Frequencies

Computation of 95% confidence intervals for allele frequencies was done using the option 'confidence interval for proportions' of the program StatMate Version 1.0 (GraphPad Software Inc., San Diego, Calif., USA).

Results and Discussion

Among the 109 individuals genotyped for the 59029-G/A polymorphism, 10, 44 and 55 were identified as 59029-A/A homozygous, 59029-G/G homozygous and 59029-G/A heterozygous, respectively. The frequency of the G allele was 0.66 (95% CI: 0.59–0.72) while the frequency of the A allele was 0.34 (95% CI: 0.28–0.41). The typical result of PCR-RFLP genotyping is shown in figure 1.

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The frequency of the 59029-G allele in Kuwaitis is significantly higher than the frequency of the 59029-A allele. Moreover, the frequency of the protective allele (59029-G) in Kuwaitis is among the highest reported. At the same time, the difference from the 59029-G allele frequencies in other ethnic groups in some cases is not statistically significant. The nature of selective factors contributing to such an extensive accumulation of the 59029-G allele in Arabs, represented by Kuwaitis, remains unknown. The most plausible possibility is selective advantage provided by the 59029-G allele with respect to resistance to infectious diseases that might have had a great impact on the survival of ancient Middle Eastern populations. In this framework, a potential link between CCR5 59029-G/A genotypes and susceptibility/resistance to such diseases as plague and smallpox has to be explored.

Conclusion

This study provided the first data on the frequency of 59029-G/A polymorphisms in Arabs. Genotyping HIV-positive Kuwaitis for these polymorphisms as well as a prospective study of their impact on the pace of progression to AIDS in Arabs is currently in progress. The preliminary results of this study show that the frequencies of 59029-G and 59029-A alleles in HIV-positive Kuwaitis are not significantly different from those in healthy Kuwaitis.

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