European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 08, 2020

Comparative Analysis Of The Frequency Of Occurrence Of The Ccr5del32 Functional Allele

Kadirov Jonibek Fayzullaevich[1], Rizaev Jasur Alimdjanovic[2], Khudaykulova Gulnara Karimovna[3], Rakhmatullaeva Shakhnoza Bakhadyrovn[4], Muminova Makhbuba Teshaevna[5]

[1]*Tashkent State Dental Institute*
[2]*Samarkand State Medical Institute*
[3,4,5]*Tashkent Medical Academy*

Abstract: ***A promising area of modern medicine dealing with the problem of the human immunodeficiency virus (HIV) is the study of the role of chemokine receptors in the pathogenesis of HIV, such as CXCR-4 and CCR-5. A different role of the CCR5del32 polymorphism of the CCR5 gene in representatives of different ethnic groups has been established, which may cause different susceptibility to HIV.Purpose of the study is to carry out a comparative assessment of the frequency of occurrence of the functional allele "CCR5del32" of the CCR5 gene among different ethnic groups.To identify the delta32 deletion of the CCR5 gene, we used the RFLP approach of polymerase chain reaction followed by detection of the results by electrophoresis in 2% agarose gel.The obtained results of molecular genetic studies on the CCR5-Delta32 marker of the CCR5 gene are representative. The allelic variant "CCR5-Delta32" of the CCR5 gene within the studied populations is distributed unevenly and is most represented in the sample of Europeans. Comparative meta-analyzes show that the frequency of its occurrence among Europeans is significantly higher than in other populations of the world, including in comparison with our population.The relatively low concentration of the protective CCR5Δ32 allele in our population may lead to increased susceptibility to HIV-1 infection.***

Keywords: human immunodeficiency virus (HIV), chemokine receptors, CXCR-4, CCR-5, polymerase chain reaction, CCR5del32 allele, protective allele

1. INTRODUCTION

The Republic of Uzbekistan faced the problem of HIV infection somewhat later than other countries. The first case of HIV infection in the Republic of Uzbekistan was registered in 1987. Since 1999, there has been an increase in the registration of new cases of HIV infection in the Republic of Uzbekistan. As of 1.01.2015, the number of people living with HIV in the Republic of Uzbekistan was 30315 (in 2013-24121, in 2014-28250) [1]. According to the " Decree of the President of the Republic of Uzbekistan dated December 26, 2008 No. PP-1023 "on additional measures to improve the effectiveness of countering the spread of HIV infection in the Republic of Uzbekistan", the priority measures are " Study and implementation of safe methods for the prevention, diagnosis and treatment of HIV / AIDS»

Conducting these studies is impossible without a thorough study of the pathogenetic mechanisms of HIV infection and determining the role of various factors and links of immunity in the formation of this pathology. In this regard, a promising direction of modern HIV medicine is to study the role of chemokine receptors in the pathogenesis of HIV.

Chemokines and chemokine receptors play a key role in regulating the directed migration of white blood cells in blood and tissues, and are also involved in the pathogenesis of many diseases. The study of chemokine receptors in the context of the pathogenesis of HIV infection was started in the 80-90s of the last century, when a program was initiated to search for genes whose polymorphic variants could influence the process of HIV infection and the development of infection.

A breakthrough in understanding the pathogenesis of HIV is associated with the identification of chemokine receptors such as CXCR-4 and CCR-5.

Experiments with cell lines revealed a number of other chemokine receptors that are used by certain subspecies of the virus (CCR-3, CCR-2, CCR-8, CCR-9, STRL-33, Gpr 15, Gpr 1, APJ, Chem R 23 and CX 3 CR1). Despite this, CCR-5 and CXCR-4 are the main co-receptors for HIV in vivo. Natural ligands of these coreceptors can block the entry of a viral particle into the cell (MIP-Ia, MIP-Ib, RANTES binds to CCR-5, SDF-1 to CXCR-4, MCP-1 to CCR-2 via MCP-5, MCP-3, and MCP-4 to CCR-3). Chemokines using CCR-5 block the R5 subspecies of HIV-1, and those using CXCR-4-X4 block the HIV-1 subspecies.

Particular attention is paid to the chemokine receptor CCR5. It is known that it is involved in the pathogenesis of HIV infection. When injected into the human body, the human immunodeficiency virus must interact with cellular receptors on the surface of lymphocytes. The main one is CD4. the HIV Co-receptor is CCR5, which provides a more dense adhesion and the most effective penetration of the virus into the cell. A mutation in the CCR5 gene was found to reduce the risk of HIV infection by losing the functional activity of this protein. If infection does occur, the patients ' health remains satisfactory for a long time, the level of viral RNA is low, and the damage to the immune system is insignificant [3]. The most well-known polymorphism of the CCR5 gene, which is a deletion of 32 nucleotide pairs in the coding region and leads to the synthesis of a shortened and functionally inactive version of the receptor due to a shift in the reading frame. However, in heterozygotes, CCR5 expression is reduced, and in homozygotes, a functional block of this receptor is observed due to changes in its structure due to mutation.

A number of studies have established a different role of CCR5del32 polymorphism in representatives of different ethnic groups, which may cause different susceptibility to HIV. The mutant allele was detected with a frequency of 10-15% in European populations and in white Americans, while it was not detected in indigenous populations of Africa and Japan [9]. In the indigenous population of continental Asia, deletion was observed much less frequently than in Europe – its frequency in some populations did not exceed 5%, and in most populations it was absent at all [2].

It should be emphasized that in the Republic of Uzbekistan to date, no fundamental research has been conducted on the pathogenetic mechanisms of HIV infection, including the role of chemokine receptors, and this predetermined the purpose and objectives of this study.

2. PURPOSE OF RESEARCH

To conduct a comparative assessment of the frequency of occurrence of the CCR5del32 functional allele in different ethnic groups.

3. MATERIALS AND METHODS

The polymorphic variant of the CCR5-delta32 gene is a deletion of 32 nucleotide pairs (CCR5del32 mutation) at position 794-825 of the CCR5 gene (chemokine (C-C motif) receptor 5) (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996). In this work, we studied a total of 280 individuals, of which we formed and studied 2 study groups. The first group consisted of HIV-infected patients (General group, n=186) identified on the territory of the Republic, the second group consisted of conditionally healthy donors of Uzbek nationality (control group, n=94).

To identify the CCR5 delta32 deletion, the restriction fragment length polymorphism (RFLP) method was used – one of the variants of the polymerase chain reaction with subsequent detection of the results by electrophoresis in 2% agarose gel.

Synthesized systems of oligoprimes and reference DNA positive samples were kindly presented by the head of the laboratory of the MGC research Institute of AIG named After D. O. Ott (St. Petersburg), PhD Aseev M. V. the Design of special-purpose oligoprimes was carried out using the computer programs “Oligo " and "Primer v5. 0" and the bioinformatic database NCBI (http://www.ncbi.nlm.).

Structure used oligoprimers.:

CCR5-D32-F:5`CTTCATTACACCTGCAGTC3`,

CCR5-D32-R:5`TGAAGATAAGCCTCACAGCC3`.

To isolate genomic DNA, we used commercial qiaamp DNA Mini Kit (QIAGEN) and Ampliprime RIBOT-prep, in accordance with the attached protocols.

4. RESULTS AND DISCUSSION

Tables 1 and 2 present the results of calculations of the deviation of theoretical and empirical frequencies of the distribution of alleles and genotypes of the delta32 polymorphism of the CCR5 gene for RCV in the General group of HIV patients and population samples. As can be seen from the tables, the occurrence of the CCR5-delta32 mutation variant among both patients and conditionally healthy donors was low.

Table 1.

Expected and observed frequencies of distribution of CCR5-Delta32 polymorphic marker genotypes in the group of patients with HIV infection by RCV

Alleles	**The frequency of alleles**				
wt	0.99				
Δ32	0.01				
Genotypes	**Frequency of genotypes**		χ^2	**P**	**df**
	Observed	***Expected***			
wt/wt	0.98	0.98	0.000	0.9	1

Δ32/wt	0.016	0.016	0.000		
Δ32/Δ32	0.00	0.01	0.012		
in total	1.0	1.0	0.012		

In both groups, the empirical-actual distribution of the CCR5-delta32 mutation genotypes corresponded to the theoretical-expected distribution under the hardy-Weinberg equilibrium (PX, p=0.9, according to the exact Fischer test). The frequency of wild wt and mutational Δ32 alleles, respectively, was: 0.99/0.01 - in the group of patients with HIV and 0.98/0.02-in the control group.

In the General group of HIV patients, the empirical and theoretical frequencies of wt /wt, Δ32/wt, and Δ32/Δ32 genotypes were 0.98/0.98, 0.016/0.016, and 0.0/0.001, respectively, and the difference did not significantly differ from the hardy-Weinberg equilibrium at the 5% significance level. In the population sample, the empirical and theoretical frequency of genotypes was almost identical: 0.97/0.97, 0.032/0.031, and 0.0/0.0003 (P>0.05).

Table 2

Expected and observed frequencies of distribution of genotypes of the CCR5-Delta32 polymorphic marker in KG by RHV

Alleles	**The frequency of alleles**				
Wt	0.98				
Δ32	0.02				
Genotypes	**Frequency of genotypes**		χ^2	**P**	**df**
	Observed	***Expected***			
wt/wt	0.97	0.97	0.000	0.9	1
Δ32/wt	0.032	0.031	0.001		
Δ32/Δ32	0.00	0.0003	0.024		
Total	1.00	1.00	0.025		

In both groups, the empirical frequency of the homozygous variant Δ32/Δ32 of the CCR5 gene, which is highly resistant to HIV-1 infections, was Ho=0. It should be emphasized that among HIV-infected patients, homozygous Δ32/Δ32 variants of the CCR5 gene are very rare.

The results of the population study showed that the empirical distribution of genotypic variants of CCR5-delta32 corresponded to the theoretically expected one, i.e., in this case, both groups performed RCV. Both samples were characterized by low frequencies of the unfavorable allele Δ32 and the heterozygous genotype Δ32/wt, therefore, a low level of genetic variability of this mutation in our population. This low frequency of the ccr5delta32 allele makes the level of protection of our population from HIV infection low, even in groups with a high frequency of CCR5de132 (Δ32=0.02). It should be emphasized that in addition to the CCR5del32 gene, there are other genes (modifiers) that can affect the body's resistance to

HIV and these genes can also contribute to inter-population differences. However, the frequency of genotypic variants and the possible contribution of these genes to the development of HIV infection requires further research.

We conducted a comparative analysis of our results with the data of the world population [10].

The number of donor samples analyzed ranged from n =60 (DR Congo) to n=892652 (Germany). The frequency of deletion allelic and genotypic variants of CCR5de132 varied between these countries. As can be seen from the table, the frequencies of the Δ32 allele of the CCR5 gene varied from 0.164 (16.4%) in the Norwegian sample to 0.0 in donors from Ethiopia. The highest frequency of the homozygous CCR5-Δ32/Δ32 genotype with high resistance to HIV-1 infection was observed in European countries, such as the Faroe Islands - 0.023 (2.3%), Belarus - 0.0219 (2.19%) and Finland - 0.02 (2.0%), while in 28 populations (mainly conditionally healthy donors from Africa, Asia and South America), including our sample, none of the donors had this genotype.

Based on meta-analysis data and data on the frequency of the CCR5del32 allele in modern and historical populations in Europe and Asia, it was hypothesized that the natural selection factor for the CCR5-Δ32 allele variant seems to have been active for several thousand years.

It should be emphasized that our data on the frequency of Δ32 allele (1.6%) is close to the India - 0.021 (2.1%), China 0.0047 (0.47%), Bangladesh - 0.015 (1.5%), Cameroon - 0.007 (0.7%), Somalia-0.021 (2.1%), Lebanon - 0.021 (2.1%), Jordan - 0.027 (2.7%), Ghana - 0.028 (2.8%), Egypt - 0.029 (2.9%), the democratic Republic (DR) Congo 0.025 (2.5%) and close with data from donors from Turkey - 0.034 (3.4%), Thailand-0.032 (3.2%), Morocco-0.033 (3.3%), South Korea - 0.032 (3.2%), Kenya-0.03 (3.0%), Indonesia - 0.035 (3.5%), Armenia 0.033 (3.3%), etc. A comparative analysis of the frequencies of the CCR5del32 functional allele revealed no statistically significant differences between the population of Uzbekistan and data from China, Bangladesh, Thailand, Indonesia, Korea, etc. (tables 3-11).

Comparable results were obtained by comparing the distribution of alleles in ethnically close Turkic-speaking groups (Uzbekistan, Turkey, and Azerbaijan). In our population, the frequency of the Δ32 allele was lower than in representatives from Turkey (0.016 vs. 0.034, with $\chi 2=1.9$; p=0.2) and Azerbaijan (0.016 vs. 0.039, with $\chi 2=1.8$; p=0.2). However, these differences were statistically insignificant (tables 3 and 4).

Table 3.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-Turkey)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		**Turkey**			
	Abs	%	Abs	%		
-	185	98.404	69624	96.6	1.9	0.2
+	3	1.59	2448	3.4		

It is interesting to note that in the Turkish population, a rare homozygous genotype CCR5-Δ32/Δ32 (80/36036; 0.22%), which is characteristic of the European population, was found to be highly resistant to HIV infection. On the contrary, significant inter-population differences were noted when comparing our data with those of the European population.

Table4.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-Azerbaijan)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		**Azerbaijan**			
	Abs	%	Abs	%		
-	185	98.404	146	96.05	1.8	0.2
+	3	1.59	6	3.95		

Comparative analysis of allele frequencies of the CCR5-Delta32 gene between the population sample of Uzbekistan and Italy (1.6% vs. 6.3%, respectively; χ2=6.9; p=0.008; OR=4.1; 95%CI1. 315-12. 93) and Czech populations (1.59% vs. 10.7%, respectively χ2=15.8; p<0.05; OR=7.4; 95%CI2. 335-23. 27) also revealed a significant 4.1 to 7.4 fold decrease in the protective allele Δ32 in our population compared to the corresponding populations (table 5).

Table5.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-Italy)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		Italy			
	Abs	%	abs	%		
-	185	98.4	11842	93.7	6.9	0.008
+	3	1.6	792	6.3		

Table6.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-Czech Republic)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		Czech			
	Abs	%	Abs	%		
-	185	98.404	1740	89.3	15.8	<0.05
+	3	1.6	208	10.7		

Table7.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-Nigeria)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		*Nigeria*			
	Abs	%	Abs	%		
-	185	98.404	306	95.63	2.8	0.09
+	3	1.6	14	4.37		

Table8.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-Iran)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		*Iran*			
	Abs	%	Abs	%		
-	185	98.404	3720	95.97	2.8	0.09
+	3	1.59	156	4.02		

Table9.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker in (Uzbekistan-Belarus)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		*Belarus*			
	Abs	%	Abs	%		
-	185	98.404	246	89.78	13.2	<0.05
+	3	1.59	28	10.22		

Table10.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker in (Uzbekistan-Yu.Korea)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		Korea			
	Abs	%	Abs	%		
-	185	98.404	172	95.56	2.6	0.1
+	3	1.59	8	4.44		

Table11.
Differences in the frequency of allelic and genotypic variants of the CCR5-Delta32 polymorphic marker (Uzbekistan-China)

Presence of the Δ32 allele	Number of alleles				χ^2	P
	Uzbekistan		*China*			
	Abs	%	Abs	%		
-	185	98.404	848	99.764	2.9	0.09
+	3	1.59	2	0.235		

Thus, the results of molecular genetic studies on the CCR5-Delta32 marker are representative. The allelic variant "CCR5delta32" of the CCR5 gene within the studied populations is distributed unevenly and is most represented in the sample of Europeans. Comparative meta-analyses show that the frequency of its occurrence among Europeans is significantly higher than in other populations of the world, including in comparison with our population [3,6,7,8,10,11]

5. CONCLUSION.

The comparatively low concentration of the protective CCR5Δ32 allele in our population may lead to increased susceptibility to HIV-1 infection.

The data obtained by us will also complement the international database (Allele Frequency Database)on the frequency of the CCR5del32 protective allele for various populations of the world. Knowledge of the regularities of the genetic profile of the CCR5del32 chemokine gene in ethnic Uzbeks can be used in the future to conduct scientific research on the relationship of this gene with various infectious diseases, and will also be useful for specialists in the field of population genetics to study the ethnogenesis of peoples or ethnic groups.

6. REFERENCES:

[1] Saidaliev S.S., Atabekov N.S. On the measures taken to counter the spread of HIV infection in the Republic of Uzbekistan. Infection, immunity and pharmacology, 2015, No. 6, pp. 6-10.

[2] Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, Donfield S, Vlahov D, Kaslow R, Saah A, Rinaldo C, Detels R, O'Brien SJ. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science. 1996 Sep 27;273(5283):1856-62. Erratumin: Science 1996 Nov 15;274(5290):1069

[3] Faure E, Royer-Carenzi M. Is the European spatial distribution of the HIV-1-resistant CCR5-Delta32 allele formed by a breakdown of the pathocenosis due to the historical

Roman expansion? Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases. 2008 Dec;8(6):864-874. DOI: 10.1016/j.meegid.2008.08.007.

[4] Faure E, Royer-Carenzi M. Is the European spatial distribution of the HIV-1-resistant CCR5-Delta32 allele formed by a breakdown of the pathocenosis due to the historical Roman expansion? Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases. 2008 Dec;8(6):864-874. DOI: 10.1016/j.meegid.2008.08.007.

[5] *Hum Immunol*. 2017; 78 (11-12): 710-717. doi:10.1016/j.humimm.2017.10.001**;** Ni J, Wang D, Wang S. The CCR5-Delta32 Genetic Polymorphism and HIV-1 Infection Susceptibility: a Meta-analysis. *Open Med (Wars)*. 2018; 13:467-474. Published 2018. doi:10.1515/med-2018-0062

[6] Hütter G, Blüthgen C, Elvers-Hornung S, Klüter H, Bugert P. Distribution of the CCR5-delta32 deletion in Southwest Germany. *Anthropol Anz*. 2015;72(3):303-309. doi:10.1127/anthranz/ 2015/0479).

[7] Ni J, Wang D, Wang S. The CCR5-Delta32 Genetic Polymorphism and HIV-1 Infection Susceptibility: a Meta-analysis. *Open Med (Wars)*. 2018;13:467-474. Published 2018. doi:10.1515/med-2018-0062.

[8] Ongadi B, Obiero G, Lihana R, Kiiru J. Distribution of genetic polymorphism in the CCR5 among Caucasians, Asians and Africans: a systematic review and meta-analysis. Open J Genet. 2018;08:54–66. DOI: 10.4236 / ojgen.2018.83006.

[9] Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, Muyldermans G, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi Y, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature. 1996 Aug 22;382(6593):722-5.

[10] Solloch UV, Lang K, Lange V, Böhme I, Schmidt AH, Sauter J. Frequencies of gene variant CCR5-Δ32 in 87 countries based on next-generation sequencing of 1.3 million individuals sampled from 3 national DKMS donor centers. *Hum Immunol*. 2017;78(11-12):710-717. doi:10.1016/j.humimm.2017.10.001.

[11] Tajbakhsh, A., Fazeli, M., Rezaee, M. *et al.* Prevalence of *CCR5delta32* in Northeastern Iran. // *BMC Med Genet***20,** 184 (2019). https://doi.org/10.1186/s12881-019-0913-9;