Hindawi
BioMed Research International
Volume 2023, Article ID 8823863, 8 pages
https://doi.org/10.1155/2023/8823863

Research Article

Prevalence of CCR5 Delta 32 Genetic Variant in the Turkmen Population of Golestan Province, Northeast of Iran

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Received 30 April 2023; Revised 23 May 2023; Accepted 14 June 2023; Published 21 June 2023

Academic Editor: Kazim Husain

The 32 bp deletion in the chemokine receptor (C-C motif) 5 gene (CCR5Δ32) is a natural loss of function polymorphism that prevents the protein from locating on the cell surface. This genetic variation acts as a double-edge sword in the pathogenesis/defense mechanism of different health conditions, such as viral infections, autoimmune diseases, and cancers. Here, we evaluated the prevalence of the CCR5Δ32 polymorphism in the Turkmen population of Golestan province, northeast of Iran. Blood samples were collected from 400 randomly selected Turkmen populations (199 women and 201 men), and genomic DNA was extracted. Characterization of CCR5Δ32 genotypes was performed by PCR using primers flanking the 32-nucleotide deletion in the *CCR5* gene. The amplified DNA fragments were visualized on 2% agarose gel electrophoresis with cybergreen staining under UV light. All individuals were of Turkmen ethnicity and lived in the Golestan province, northeast of Iran. The mean age of all participants was 35.46 years, with a 20-45 year range. All the studied subjects were healthy without any severe conditions such as autoimmune disease and viral infections. All individuals had no history of HIV infection. The PCR product visualization showed that all the samples are at the 330 bp size, which means the CCR5Δ32 allele was utterly absent from the study population. The presence of the CCR5Δ32 allele among Turkmens may be attributed to the admixture with European descent people. We conclude that the CCR5Δ32 polymorphism may be absent in the Iranian Turkmen population, and further studies with a large population are needed.

1. Introduction

The chemokine receptor (C-C motif) 5 (CCR5), also known as CD195, is a heptahelical surface protein belonging to the superfamily of GPCRs (G-protein coupled receptors), which cognate chemokine (C-C motif) ligands such as CCL5, CCL3, CCL4, CCL2, and CCL3L1 [1, 2]. CCR5 is expressed in nonhematopoietic peripheral tissues, the central nervous system (CNS), and a vast array of bone marrow-derived cells, including T lymphocytes, monocyte/macrophages, granulocytes, dendritic cells, and natural killer cells [3]. The receptor plays a vital role in the host defense mechanism and inflammation by recruiting immune cells via directing chemotaxis (cell migration) along the chemokine gradient [4, 5]. Moreover, the receptor acts as a learning, plasticity, and memory suppressor [6] and closes the temporal window for memory linking [7].

CCR5Δ32 (rs333) is a 32-base pair deletion in the coding region of the *CCR5* gene on the human chromosome 3, which results in a frameshift in the protein sequence leading to the expression of truncated CCR5 and aborting its localization on the cell surface [8, 9]. CCR5Δ32 heterozygous (WT/Δ32) individuals have shown a decreased expression of functional CCR5 on the cell surface compared to CCR5 wild-type cells. However, people with homozygous CCR5Δ32 (Δ32/Δ32) have no CCR5 on their plasmatic membrane [10–12]. The most abundant CCR5Δ32 allele frequency is observed in the Caucasian population (European descent) (~10%), while this allele is nearly absent in Africans, Native Americans, and Asians [13–15]. In the Iranian population,

the frequency of CCR5Δ32 ranges from 0.0033 to 1.6 due to the diversity of ethnicities in Iran [16, 17].

It is well known that the CCR5Δ32 genetic variation could affect some human diseases. The human immunodeficiency virus 1 (HIV-1) needs the CD4 receptor and at least one coreceptor, usually CCR5, for entry or infectivity of cells [18]. As the CCR5Δ32 heterozygous genotype (WT/Δ32) promotes a decreased expression of functional CCR5 on the cell surface, this genotype has slight protection against HIV infection progression. On the other hand, since there is no CCR5 on the cell membrane of people with homozygous genotype, this polymorphism (Δ32/Δ32) shows strong protection against HIV-1 infection [19, 20]. CCR5Δ32 genetic variation also leads to a protective effect on *Streptococcus pneumoniae* [21], *Staphylococcus aureus* [22], Dengue virus [23], and a severe form of coronavirus disease 2019 (COVID-19) [24]. In contrast, the CCR5Δ32 polymorphism is associated with the disease severity of West Nile Virus (WNV) [25], influenza virus [26], tick-borne encephalitis (TBE) [27], and chronic hepatitis B virus (HBV) infection [28]. Additionally, CCR5Δ32 is related to impaired brain function, atherosclerosis development [29, 30], and many inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA) [31] and SLE [32]. Here, we aimed to evaluate the prevalence of CCR5Δ32 polymorphism in the Turkmen population of Golestan province, northeast of Iran. The population of Golestan province consists of different ethnic groups such as the Fars, Mazni, Azeri, Baloch, Sistani Persians, and Turkmen [33]. About 1.5 million Turkmen live in Iran, and this population mainly lives in the northeast of the country, which is located near the border of Iran-Turkmenistan. Turkmens value their traditions and cultural roots very highly, have preserved them in their families, and have their way of life and customs [34]. Moreover, studying populations using molecular techniques is very important and helpful for their characterization [35, 36]. Conservation of genetic diversity in population requires the proper performance of conservation superiorities and sustainable handling plans based on universal information on population structures, including genetic diversity resources among and between populations [37, 38]. Genetic diversity is essential for genetic improvement, preserving populations, evolution, and adapting to variable environmental situations [39, 40]. On the other hand, determining gene polymorphism is essential in populations [41, 42] to define genotypes and their associations with health and performance [43–45]. Hence, we aimed to evaluate the prevalence of CCR5Δ32 polymorphism in the Turkmen population of Golestan province, northeast of Iran.

2. Materials and Methods

This cross-sectional study was performed on 400 randomly selected Turkmen populations (199 women and 201 men) from different laboratories in Gonbad-e Kavus, Golestan, Iran. The mean age of all participants was 35.46 years, with a 20-45 year range. The sample size was calculated considering the frequency of this allele in northeastern Iran and the Turkmen population in Iran. The population living in Golestan province consists of many races and ethnicities, such as Fars, Turks, and Turkmen. Unlike the previous study, which was conducted in the same province on different ethnicities [46], the inclusion criteria of this study were the Turkmen ethnicity of the Turkmen parents and grandparents. All individuals were of the exact geographical origin with Turkmen ethnicity, and none were related. The geographical location of the Golestan province is shown in Figure 1. All subjects were informed of the purpose of the study, and informed consent was obtained from all participants. The current study was approved by the Science and Bioethics Committee of Golestan University of Medical Sciences (IR.GOUMS.REC.1400.332).

Blood samples were aseptically collected via venipuncture from each study participant into sterile vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. Following collection, the samples were immediately transferred to a refrigerated environment set to a temperature of 4°C until processed for DNA extraction. The genomic DNA was extracted following the manufacturers' instructions (Pioneer, Pishgam, Iran). Spectrophotometric analysis was performed (including optical density (OD) 260/OD 280 and OD 260/OD 230 measurements) to determine the concentration and quality of the extracted DNA (DeNovix Inc., USA). Characterization of CCR5Δ32 genotypes was performed by polymerase chain reaction (PCR) using forward primer 5′-TCCTGACAATCGATAGGTACCTGG CT-3′ and reverse primer 5′-GCCTCTTCTTCTCATTTCG ACACCGA-3′ flanking the 32-nucleotide deletion in the *CCR5* gene which was designed by Donyavi et al. [47]. The primers were used to amplify the 330 bp segment of the wild-type allele and the 298 bp segment of the mutant allele of CCR5. PCR amplification was carried out in a 20 μl reaction containing 1x Amplicon PCR Master Mix, 250 ng of extracted genomic DNA, and 0.5 μM of each primer. The thermal cycle profiles were as follows: initial denaturation for 6 minutes at 94°C and 35 cycles of 94°C for 35 seconds, 65°C for 40 seconds, and 72°C for 45 seconds, followed by a final extension at 72°C for 5 minutes. Finally, the DNA fragments were visualized on 2% agarose gel electrophoresis with cybergreen staining under UV light.

3. Results

A total of 400 people participated in this study, consisting of 201 men (50.2%) and 199 women (49.8%). The mean age of female and male subjects was 34.86 and 36.54 years, respectively. All the studied subjects were healthy, and based on the completed questionnaires, they had no severe conditions such as autoimmune disease and viral infections. All individuals had no history of HIV infection. The details of the demographic data are presented in Table 1.

Our results indicate that the extracted DNA samples had high purity levels. Specifically, all samples yielded values above the acceptable threshold of 1.5 for the ratio absorbance of 280/260, indicating the absence of contaminants such as RNA or other organic substances. We examined the optical absorption ratio of 260/230 to evaluate protein

FIGURE 1: The geographical location of Golestan province, Iran. The majority of the Turkmen population of Iran lives in Golestan province, which are mainly scattered in the cities of Gonbad-e Kavus, Bandar-e Turkman, Gorgan, and Turkmen Sahara.

contamination, which also yielded favorable results. Specifically, all samples had values within the acceptable range of 2.0 to 2.2, indicating the absence of protein contaminants. These findings demonstrate the effectiveness of our DNA extraction method in isolating high-quality DNA samples for downstream applications.

The presence of CCR5 genetic variations was investigated in all samples using the PCR method via specific primers. The primers are designed to amplify the 330 bp segment of the wild-type allele and the 298 bp segment of the mutant allele of CCR5. The PCR product visualization on 2% agarose gel electrophoresis with cybergreen staining under UV light showed that all the samples are at the 330 bp size, which means the CCR5Δ32 allele was utterly absent from the study population. The PCR results of 17 samples with positive and negative controls are shown in Figure 2.

4. Discussion

The frequency of the CCR5Δ32 allele in the world is approximately equal to 3% [48]. This allele is mainly present in European countries (about 10%). The highest frequency of this allele has been reported in the surrounding area of the Baltic and White Sea and the central regions of Russia (>15%) [49]. On the other hand, this polymorphism is very rare in North Africa, the Middle East, and Central Asia. Also, CCR5Δ32 is absent in East and Southeast Asian populations, natives of America, Oceania, and sub-Saharan Africa [50]. In the Iranian people, the frequency of CCR5Δ32 is estimated at 1.6%, which varies in different ethnicities living in this country [16, 17]. By the end of 2021, 38.4 million HIV-infected individuals worldwide have been detected, with the highest frequency observed in South and West Africa (20.6 million infected) and the lowest in the Middle East and North Africa (18,000 infected) [51]. By the end of 2018, 38,996 people were diagnosed with HIV infection in Iran [52].

In the present study, the frequency of the CCR5Δ32 allele in healthy Turkmen people was not found. This is consistent with other studies from Iran [16, 17, 46, 53, 54] and other countries [55–62]. In contrast to our research, Trecarichi et al. have shown a significantly higher frequency of Δ32 in the healthy control group compared to HIV-positive people. Moreover, Philpott et al. reported a two-fold higher frequency of the Δ32 genotype in the healthy control compared with HIV-1-seropositive people. Many factors contribute to this diversity report, such as variations in ethnicity, which may play a significant role in the population. Multiple linear regression analyses by Thomas et al., adjusted for age and race, showed a significant negative association between HIV risk duration and CCR5 expression on monocytes [63]. Another study by Meditz et al. showed that CCR5 expression is reduced in the lymph nodes of HIV-1-infected women compared with men but does not mediate sex-based differences in viral loads [64].

The discussed origin of the CCR5Δ32 allele is still controversial. Beyond the proposed theories, Sabeti et al. indicated that this polymorphism became frequent in the European population due to neutral evolution. However, the authors have suggested the possible role of selective pressure in the historical period as an element in CCR5Δ32 frequency [65, 66]. The racial distribution of HIV risk raises the possibility that differences in the distribution of the CCR5Δ32 allele or other heritable host factors/mutations may influence the rate of transmission or the speed of the epidemic in different racial groups [67]. Genetically, Iranians are considered close to North Indians, Greeks, and specific European populations such as Italians, Germans, and British, which can be related to the following factors: (1) Indo-European same ancestor population (Aryans); (2) significant genetic admixture of Iranians with their neighbors in different eras; and (3) connecting the eastern and western Eurasian populations through the Silk Road network, which led to migrations and genetic admixture along this network [53]. Recent data on allele and haplotype frequencies of human leukocyte antigen (HLA) class II have confirmed the similarity in the genetic ancestry of Iranians, Greeks, and Italians [68, 69]. Historical evidence suggests differences between the population of Iran and Europeans of the same ancestry, known as Indo-Europeans. Around 2000 BC, an Indo-European tribe named Aryans invaded central Asia and occupied Iran, Iraq, the north of India, and Afghanistan. The significant difference between the western and eastern migrations of Indo-Europeans is that they mixed genetically with similar populations in the west. In contrast, in the east, they mixed with others. Genetically, together have produced a primarily mixed population, and the Arabs gradually diluted the primary Indo-Aryan traces over the centuries [69]. The rate of progression to AIDS varies among individuals infected with HIV-1, and it has been shown that CCR5Δ32 confers almost complete resistance to HIV-1 infection in homozygotes and partial protection against HIV disease progression in heterozygous adults.

Table 1: The demographic and clinical characteristics of participants.

	Male	Female	Total
Individuals	201	199	400
Mean of age (years)	36.54	34.86	35.46
Median of age (years)	38	35	37
Ethnicity	Turkmen	Turkmen	Turkmen
HIV/AIDS	Negative	Negative	Negative
Other viral infections*	Negative	Negative	Negative
Autoimmune disease	No background	No background	No background
History of HIV infection	No background	No background	No background
CCR5 genotype	WT-WT (100%)	WT-WT (100%)	WT-WT (100%)

*HBV, HCV, SARS-CoV-2, influenza A, and B.

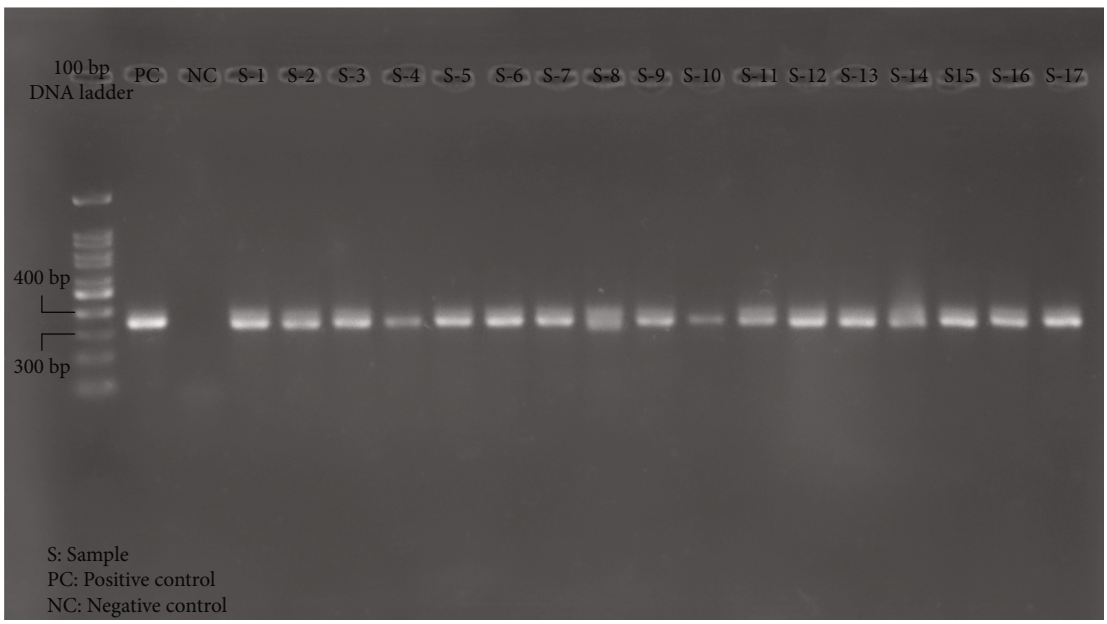

Figure 2: CCR5 genotype determination by agarose gel electrophoresis. Lane 1: 100 bp DNA ladder. Lane 2: positive control (PC). Lane 3: negative control (NC). Other lanes: wild-type genotype (CCR5/CCR5).

CCR5 (formerly CKR5) plays a crucial role in the chemotaxis of immune cells to inflammation sites and in mediating inflammatory responses. Beyond cell migration, CCR5 is involved in the surveillance of the immune cells, inflammation, and the pathogenesis of inflammatory diseases and cancers [3]. CCR5Δ32 is a 32 bp deletion in the *CCR5* gene on the second extracellular loop encoding region. This variation results in a frameshift in the CCR5 protein sequences, leading to a prematurely truncated protein lacking three transmembrane domains. CCR5Δ32 mutant protein has no function and does not localize on the cell surface [13, 70]. The CCR5Δ32 has received much attention due to its protective role against HIV infection and disease progression [20]. Beyond HIV infection, data has shown that this polymorphism is correlated to other conditions such as autoimmune diseases and pathogenic infections [3, 71].

A protective effect of CCR5Δ32 allele variation on some conditions such as childhood asthma [72], type 2 diabetes (noninsulin-dependent diabetes mellitus) [73], hepatitis B virus (HBV) [74], liver inflammation in HCV infection [75, 76], osteomyelitis of *Staphylococcus aureus* infection [77], and toxoplasmosis [78, 79] has been reported. On the other hand, this polymorphism has been considered a risk factor for multiple sclerosis (MS) [80], symptomatic West Nile virus (WNV) infection [81], the severity of influenza virus [26], severe form of coronavirus disease-2019 (COVID-19) [24], *Streptococcus pneumoniae* infection [21], and tuberculosis [82].

The gradient distribution of the CCR5Δ32 polymorphism from north to south with the highest frequency in the Nordic population suggests a Scandinavian origin of this polymorphism. Subsequently, this variant spread to the European population through raids of the Vikings in the 8th-10th centuries [66, 83]. The CCR5Δ32 allele frequency around the world is estimated at approximately 3% based on the information obtained from various studies on more

Table 2: CCR5Δ32 allele frequency (%) distribution in the Iranian population living in different geographical locations.

Population	Sample size	Genotypes (%)			Δ32 allele frequency	Reference
		WT/WT	WT/Δ32	Δ32/Δ32		
Turkmen (north of Iran)	400	400 (100)	0 (0)	0 (0)	0%	Current study
Fars province (south of Iran)	395	384 (97.2)	11 (2.8)	0 (0)	1.4%	[53]
Urmia city (northwest of Iran)	200	186 (97.89)	4 (2.105)	0 (0)	1.05%	[54]
Mashhad city (northeast of Iran)	400	388 (97)	11 (2.75)	1 (0.25)	1.6%	[16]
Golestan province (southeast of the Caspian Sea)	300	291 (97)	9 (3)	0 (0)	1.5%	[46]
Tehran province (capital of Iran)	371	357 (96.2)	14 (3.8)	0 (0)	3.8%	[47]
Iran	530	523 (98.5)	6 (1.1)	1 (0.19)	1.1%	[17]

than 5000 samples [84, 85]. This genetic variation is primarily observed in Caucasian populations (of European ethnicity), where the average mutant allele frequency is about 10% of this population [13]. In contrast to the European population, CCR5Δ32 polymorphism is virtually absent in Asians, Sub-Saharan Africans, and Native American ethnicities [15]. The CCR5Δ32 allele frequency in Asian countries and Iran is about 2.06% of the population [17]. Table 2 displays the outcomes of prior investigations conducted on various populations and ethnicities residing in Iran, as well as the findings from our current study. This study is aimed at determining the distribution of the CCR5Δ32 polymorphism in the Turkmen population in the north of Iran. The CCR5Δ32 allele was utterly absent from our study population. In this study, we had a limitation on sample size and no access to HIV-infected patients in the Turkmen population. Also, due to the absence of mutant alleles (homozygous or heterozygous) among this study's samples, it was impossible to calculate the Hardy-Weinberg equilibrium. Further studies with large sample size are needed to investigate the frequency of CCR5 mutations and its association with diseases.

5. Conclusion

In conclusion, the recent study confirms that the CCR5Δ32 polymorphism is absent in the Turkmen population residing in northern Iran. The absence of this polymorphism is expected to persist in this community due to their cultural practices, regardless of whether its evolution was driven by environmental pressure or neutral evolution across various populations over decades.

Abbreviations

GPCR: G-protein coupled receptors
CCR5: The chemokine receptor (C–C motif) 5
RANTES: Regulated on activation, normal T cell expressed and secreted
MIP-1α/β: Macrophage inflammatory protein-1 alpha/beta
MCP-1: Monocyte chemoattractant protein-1
CCL3L1: Chemokine (C-C motif) ligand 3-like 1
DCs: Dendritic cells
NK cells: Natural killer cells
HIV-1: Human immunodeficiency virus 1
WNV: West Nile virus
HBV: Hepatitis B virus
RA: Rheumatoid arthritis
SLE: Systemic lupus erythematosus
NIDDM: Noninsulin-dependent diabetes mellitus.

Data Availability

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure

A preprint has previously been published [26].

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

A.T conceptualized and designed the study. E.N, S.D.H, and M.R collected samples and did the experiment. A.T and M.R analyzed the collected data. E.N, and MR drafted the manuscript. A.T, B.A, M.R, and E.N evaluated and edited the manuscript. All authors have read and approved the final manuscript. Elmira Norasi and Mostafa Rastegar are the first author.

Acknowledgments

The authors appreciate the financial support of the Research Deputy at Golestan University of Medical Sciences (112296).

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