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The frequency of CCR5 promoter polymorphisms and CCR5 Δ 32 mutation in Iranian populations

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ABSTRACT

Evidence showed that chemokines serve as pro-migratory factors for immune cells. CCL3, CCL4 and CCL5, as the main CC chemokines subfamily members, activate immune cells through binding to CC chemokine receptor 5 or CCR5. Macrophages, NK cells and T lymphocytes express CCR5 and thus, affected CCR5 expression or functions could be associated with altered immune responses. Deletion of 32 base pairs (Δ 32) in the exon 1 of the CCR5 gene, which is known as CCR5 Δ 32 mutation causes down regulation and malfunction of the molecule. Furthermore, it has been evidenced that three polymorphisms in the promoter region of CCR5 modulate its expression. Altered CCR5 expression in microbial infection and immune related diseases have been reported by several researchers but the role of CCR5 promoter polymorphisms and CCR5 Δ 32 mutation in Iranian patients suffering from these diseases are controversial. Due to the fact that Iranian people have different genetic backgrounds compared to other ethnics, hence, CCR5 promoter polymorphisms and CCR5 Δ 32 mutation association with the diseases may be different in Iranian patients. Therefore, this review addresses the most recent information regarding the prevalence as well as association of the mutation and polymorphisms in Iranian patients with microbial infection and immune related diseases as along with normal population.

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Introduction

Cytotoxic T lymphocytes, NK cells, DCs and macrophages play important roles in immune responses against foreign antigens, PAMPs and DAMPs (1). Previous investigations evidenced that chemokines serve as recruiters of the above-mentioned parts of immune system, through interaction with their receptors (2). Furthermore, CCR5 is a specific receptor for MIP-1α/CCL3, MIP-1β/CCL4 and RANTES/CCL5. It has also been reported that MCP-3/CCL7 bind CCR5 without producing any signal (3). Based on the potential intracellular signaling of CCR5/ligands axis which leads to activation of several transcription factors (see the next section), it seems that CCR5/ligands axis plays important roles in immune cell activation and migration response to microbes, microbial particles and self-antigens (Figure 1) (4). It has been documented that deletion of 32 nucleotides from exon 1 of the CCR5 gene (known as the Δ 32 mutation) causes a frame shift mutation at position 185 and in turn leads to dysfunction or declined expression of CCR5 receptor (4-8). The CCR5 Δ 32 mutation is localized on the second extracellular loop of the receptor, which is shown in Figure 1. Previous studies demonstrated that this mutation is prevalent and polymorphic in various ethnic populations (9, 10). Therefore, evaluation of CCR5 Δ 32 mutation could lead us to a better understanding of the main mechanisms responsible for progression of chronic microbial infections and also immune related diseases including autoimmune diseases. Further-more, previous investigations revealed that the functional polymorphisms within promoter region of CCR5 gene result in alteration of CCR5 expression (11). Moreover, in comparison with other countries, Iran is a vast country with multiple ethnic populations with different genetic backgrounds, hence, CCR5 promoter polymorphisms and especially CCR5 Δ 32 mutation associations with the chronic microbial infections and immune related diseases may vary in Iranian patients. On the other

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side, several investigations identified that CCR5 serves as a co-receptor for HIV; hence, the genetic factors including CCR5 promoter polymorphisms and CCR5 Δ 32 mutations that affect CCR5 expression can be significantly associated with HIV restriction. Therefore, Iranian researchers have evaluated genetic variations in general population and specific patients. Therefore, the main aim of this review was to collect the available information on the status of CCR5 Δ 32 mutation and the polymorphisms within promoter region of CCR5 gene in Iranian general population and in patients suffering from some chronic microbial infections and immune related diseases.

Evidence acquisition

A literature search in various international and Iranian scientific databases namely Medline, PubMed, Web of Science, Web of Knowledge, Scopus, ProQuest, Google Scholar, Iranian Scientific Information Database (www.sid.ir) and Iranian Magazines Database (www.magiran.com) was conducted using appropriate combinations of medical terms for CCR5, mutation, polymorphisms and Iran, within the maximal date range until 2013. To retrieve more relevant and updated results, some advanced search measures such as Controlled Vocabulary (MeSH), Boolean operators, Truncations, limits and field searching were used.

Introduction to CCR5

CCR5 is defined as a seven transmembrane-spanning α-helix architecture protein belonging to GPCRs family and its gene is located on the short arm of chromosome 3 (3p21.31) (12). Evidence demonstrated that several pro-inflammatory cytokines (13, 14) upregulate CCR5 expression in the immune cells via action of NF-κB transcription factor (15) and CREB pathways (16). CCR5 as a member of the heterotrimeric GPCRs family increases cytoplasmic Ca^{2+}, in one side while, inhibits cAMP production in the other side and thus results in activation of crucial enzymes, including PI3-kinase, MAP kinases and other tyrosine kinase cascades such as FAK and Pyk2 (17). These molecules play important roles in immune cell migration and activation (17). Additionally, it has been suggested that CCR5/ligands axis is crucial for immune cells proliferation and expression of inflammatory cytokines through activating PKB, MAPK family, Rho GTPase and JAK/STAT (ERK1/2, p38, and SAPK/JNK) pathways (Figure 1) (18, 19).

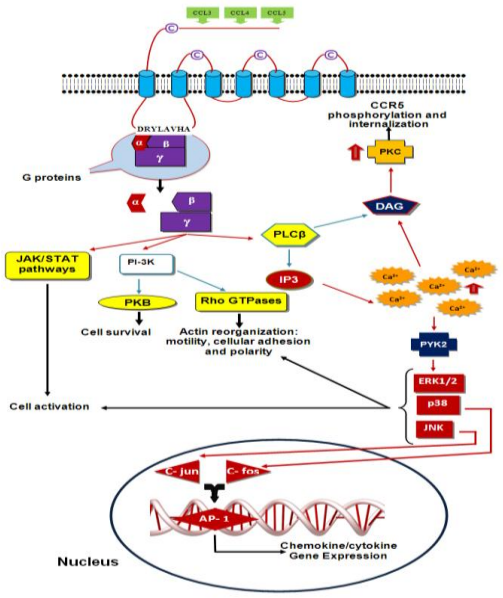

Figure 1. The structure of CCR5 molecule and its signaling pathways are shown. CCR5, as 7 transmembrane domains receptor, interaction with CCL3, 4 and 5 via the extracellular domain of CCR5 results in activation of intracellular pathways. The conserved amino acids sequences within the first intracellular loop (DRYLAVHA) play important roles in activation of G proteins (α, β and γ). The CCR5/ligand interaction results in G protein dissociation and starts intracellular signaling including JAK/STATs, phosphoinositide 3-kinase (PI-3K), proline rich tyrosine kinase 2 (PYK2), phospholipase Cβ (PLCβ), p38 and c-Jun N-terminal kinase (JNK), triphosphoinositol (IP3), diacylglycerol (DAG), protein kinase C (PKC), elevation of intracellular calcium ions (Ca^{2+}), extracellular signal-regulated kinase (ERK1/2), protein kinase B (PKB) and Rho GTPase. Adapted from Sorce *et al* (18)

The prevalence of CCR5 Δ 32 mutation among Iranian populations

Since CCR5 plays important roles in the induction of appropriate immune responses as well as HIV infection, several investigators have evaluated CCR5 Δ 32 mutation in Iranian populations (Table 1).

Table 1. The prevalence of CC5 delta32 mutation in Iranian populations

Condition	CCR5 prevalence (%)		Regions	Ref
	Patients	Controls		
Head and neck cancer	2.2	3.1	Shiraz	22
Alzheimer	4.5	5	Tehran	23
Alzheimer	12.5	8.6	Eastern Azerbaijan	25
Occult HBV	0	3	Rafsanjan	26
Chronic HBV	0	1	Rafsanjan	27
Asthma	0	1.5	Rafsanjan	10
Type 2 diabetes	0	1	Rafsanjan	28
Multiple sclerosis	0	1	Rafsanjan	29
Multiple sclerosis			Tehran	30
Homozygotes	15	2		
Heterozygotes	6	13		
Behcet			Shiraz	24
Female	9	4.7		
Male	2.91	4.3		

For instance, Gharagozloo *et al* have evaluated the frequencies of CCR5 Δ 32 mutation in normal population of southern Iran and reported a 0.0146 frequency for CCR5 Δ 32 mutation alleles among the population (20). To the best of our knowledge, the study by Gharagozloo and colleagues is the unique investigation assessing CCR5 Δ 32 mutation in Iranian general population, but several researchers have evaluated this mutation among Iranian individuals with specific diseases or conditions. Azmandian *et al* have evaluated the role of CCR5 Δ 32 mutation in both acute (AR) and DGF kidney transplant rejection in 100 donor/recipient pairs. Their results showed that CCR5 Δ 32 mutation was neither associated with AR nor DGF in Iranian donor and recipient kidney transplantation (21). In another study, Khademi and colleagues identified the frequency of CCR5 Δ 32 mutation in 156, 125 and 31 Iranian patients with malignant head and neck cancer, squamous cell carcinoma and salivary gland tumors, respectively, in comparison to 262 healthy controls (22). Interestingly, their results also revealed that CCR5 Δ 32 mutation was not prevalent in Iranian patients with malignant head and neck cancer, squamous cell carcinoma and salivary gland tumors as well as healthy controls (22). Following evaluation of 156 patients with late-onset Alzheimer's disease (AD) and 161 control subjects, Khoram *et al* reported that CCR5 Δ 32 mutation was uncommon in both AD patients and healthy controls (23). Another study on the Persian race (Shiraz, Iran) showed that CCR5 Δ 32 mutation was not associated with Behcet's disease (BD) compared to a large healthy control population (24). This study reported that CCR5 Δ 32 (380 cases) mutant allele rate was significantly higher in female patients than female control individuals (24); hence, the authors suggested that CCR5 Δ 32 mutation may be a potential risk factor for BD in Iranian women. Ardabili *et al* have assessed the mutation in 160 patients with AD and 163 healthy controls from north-west of Iran (province of Eastern Azerbaijan) and reported that CCR5 Δ 32 mutation was not associated with the disease in the Iranian population of the region (25). Our research team has also done several studies on Iranian patients. Accordingly, our previous studies on the 57 Iranian patients with occult HBV infection (OBI) revealed that none of patients carried the mutation, while 3% of healthy controls had heterozygotic form of CCR5 Δ 32 mutation (26). Our results from the Iranian patients with chronic HBV infection (CHI) have also demonstrated that neither CHI patients nor healthy controls had homozigotic form of CCR5 Δ 32 mutation (27). Interestingly, the study revealed that homozigotic form of CCR5 Δ 32 mutation was observed in 3 control subjects out of 300 (1%) Iranian healthy controls (27). Our study in the Iranian asthmatic patients also showed that CCR5 Δ 32 mutation was not prevalent in patients suffering from this disease (10). The results were also repeatedly reported when the mutation was assessed in the Iranian patients with type-2 diabetes with and without nephropathy (28) and multiple sclerosis (29). Interestingly, our results revealed that the mutation was rarely prevalent in Iranian healthy controls e.g. general population (10, 28, 29). In contrast to our and other studies, Shahbazi and colleagues reported that 37 out of 254 (15%) patients with multiple sclerosis (MS) and 8 out of 380 (2%) healthy controls were homozygous for the CCR5 Δ 32 mutation (30). Their results also demonstrated that the prevalence of heterozygotic forms of CCR5 Δ 32 mutation in MS patients and healthy controls were 6% and 13%, respectively (30). To explain this discrepancy between our result and that reported by Shahbazi *et al*, several background reasons could be proposed as follow: Iran is a vast country with different ethnic groups and the patients included in Shahbazi *et al* study were genetically different, since most of them were Turkmen people from northern Iran.

Based on the above studies done by Iranian researchers, except for Shahbazi *et al* (30), it appears that the rate of CCR5 Δ 32 mutation is low and irrelevant to infectious and immune-related diseases in Iranian populations.

Status of CCR5 polymorphisms in Iranians

Previous studies confirmed that variations in promoter region (-59029, -59353 and -2459) of CCR5 gene could influence the expression of this chemokine receptor. Therefore, probably a significant association between this polymorphism and immune-related and infectious diseases could be speculated. In contrast to CCR5 Δ 32 mutations; there is not enough information on the other polymorphisms in Iranian populations. To the best of our knowledge, there are only two published articles regarding the polymorphisms in Iranian populations.

First, Abdi *et al* evaluated 163 renal transplant recipients regarding outcome of renal transplantation and the polymorphism within -59029 region (31). Their results demonstrated that polymorphism was not associated with the outcome of the transplantation (31). Second, Omrani *et al* conversely reported that the polymorphic region of -59029 within CCR5 was significantly associated with the survival rate of renal allografts (32).

According to the results of the most recent reports which are considered here, it seems that the potential effects of polymorphisms within promoter region of CCR5 on the outcome of immune-related conditions in Iranians is controversial, because the data of the two studies was inconsistent and their sample sizes were too low to give a definite conclusion. Additionally,

according to the facts that polymorphisms within promoter region of CCR5 may be associated with its expression and that the ethnicity is an important factor, which alters the effects of genetic variations, it seems that the effects of genetic variations on the expression of CCR5 in Iranian populations need further evaluation. Moreover, due to the fact that CCR5 plays important roles in migration and activation of immune cells in the fight against microbes, it may be hypothesized that the polymorphisms within CCR5 gene may be associated with several immune- related conditions in Iranian patients. More investigations would improve our knowledge in this field.

Conclusion

Based on the present data it is likely that CCR5 Δ 32 mutations is not prevalent in Iranian normal population, and accordingly, studies were unable to find out its relationship with infectious and immune-related diseases the population. Therefore, it is reasonable to conclude that impaired immune responses during chronic infectious diseases in Iranian patients are not related to CCR5 Δ 32 mutation. It could also be concluded that uncontrolled immune responses during immune-related diseases including autoimmune responses and inflammation during diseases (e.g. graft rejection and asthma) are not related to CCR5 Δ 32 mutation in Iranians. Additionally, because CCR5 is a HIV co-receptor, high prevalence of CCR5 Δ 32 mutations in the populations can lead to decreased HIV infections. Therefore, according to the reviewed studies, it may be concluded that Iranian populations are at a high risk for HIV infection and susceptible to be affected by a serious course of the disease. Due to the paucity of studies on the polymorphisms of promoter region of CCR5 within Iranian populations, it seems to be impossible to reach a clear conclusion concerning the relationship between polymorphisms and infectious or immune related diseases in Iranian populations and more studies are required.

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