Author’s response to reviews

Title: Prevalence of CCR5delta32 in Northeastern Iran

Authors:

Amir Tajbakhsh (Tajbakhsha921@mums.ac.ir)
Mostafa Fazeli (FazeliM921@mums.ac.ir)
Mehdi Rezaee (RezaeiM911@mums.ac.ir)
Faeezeh Ghasemi (ghasemifaezeh2015@gmail.com)
Mastoureh Momen Heravi (MomenHM1@mums.ac.ir)
Aida Gholoobi (GholoobiA1@mums.ac.ir)
Zahra Meshkat (meshkatz@mums.ac.ir)

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Author’s response to reviews:

Matteo Pasini, PhD.
Editor-in-Chief

Valerio Napolioni, Ph.D.
Associate Editor
BMC Medical Genetics

Thank you very much for considering our manuscript for publication in BMC Medical Genetics. We would like to thank the referees and editors for evaluating our manuscript. Thank you very much for your valuable comments and considerate suggestions for our manuscript. These comments are helpful for improving our manuscript. We have tried to address all the reviewers’ concerns in a proper way and believe that our paper has improved considerably. We have marked green the relevant corrections in the current version of the paper. We would be happy to make further corrections if necessary and look forward to hearing from you soon.

We hope this is accepted.

Yours sincerely
Zahra Meshkat, PhD
Reviewer reports:

Daniela Zanetti (Reviewer 1): The authors did not address the majority of my previous comments, specifically the followings:
Reply: We would like to apologize for the misunderstanding and not making it clear.

Major comments:

1. The authors assessed that the frequency of CCR5Δ32 allele in the general population of North East of Iran has not been investigated, and that the low prevalence of CCR5Δ32 CCR5Δ32 allele in the Iranian population may result in the increased susceptibility to HIV-1.
Has the prevalence of HIV-1 in Iran been investigated before?
Reply: Thank you for your comment. Yes, it is. It is done throughout the text. We have added a new paragraph in this field.

Is the prevalence of HIV-1 in Iran higher than in Europe and in East Asia?
Reply: Thank you for your comment. No. it is not. It is done throughout the text. Based on the WHO, the prevalence of HIV-1 in Iran is lower than in Europe and East Asia.

The authors should analyze the allele frequency of the CCR5 locus in relation to the HIV-1 prevalence in Iran and in other countries.
Reply: Thank you for your comment. We try to do.

The authors added a table in the new version of the manuscript of CCR5Δ32 allele distribution in Iran, but my question was focused on the link between HIV-1 and CCR5Δ32 frequency.

Reply: We thank the reviewer for this valuable suggestion and agree that it would be interesting to carry out this study. However, in this case, it is outside the scope of the paper because we wanted to evaluate the prevalence of CCR5Δ32 in general population by the technique of stratified cluster random sampling. As there is no report in this region, this result will help to the global prevalence of this mutation. Moreover, I totally agree with your suggestions. As the prevalence of HIV-1 is very low, but HTLV1 is high, it will need to evaluate the association of CCR5Δ32 and HTLV1 or co-infection of HIV/HTLV1 in Khorasan, Iran. The result of our study will help to effective plane for future research in this field. The related data will be measured in the next step.

3. The Δ32 mutation at the CCR5 locus is a well-studied example of natural selection acting in humans. It would be interesting for the manuscript to perform some type of selection analyses (spatial ancestry analysis or maybe a geographical distribution of the minor allele frequency of the CCR5 locus) using the Iranian population together with the 1000 Genome Populations, if no other populations are available.
In addition to this, it would be interesting to compare the geographical allele frequency distribution of the CCR5 locus with the prevalence of HIV-1 in different countries. The two maps (allele frequency of CCR5 and prevalence of HIV-1) will be useful to discuss about the origin of CCR5 in Iran compared to other countries.
The authors added a table in the new version of the manuscript of CCR5Δ32 allele distribution in Iran, but my question was about natural selection and/or adding a plot comparing the geographical allele frequency distribution of the CCR5 locus with the prevalence of HIV-1 in different countries.

The points 1 and 3 would be helpful to support their conclusion about the low prevalence of CCR5Δ32 allele in the Iranian population and the increased susceptibility to HIV-1. Since the authors did not perform any functional study or observational association between the CCR5Δ32 allele and HIV-1, their conclusions are not supported by their data right now.

Reply: Thank you for your constructive comment. We have now updated our search and added recent publications in this field to the manuscript (highlighted).

Unfortunately about selection analysis, we don’t access to someone expert in PLINK and SPA tools, so we begin to learn these tools for rendering suitable models for allele distribution in Iran or even Middle East maybe in next article, we attempt to learn these tools and software and gathering data from other parts of Iran and other countries to establish a new paper about this title, we would appreciate to having your guidance in this way.

However, approximately 0.8% of adults worldwide are living with HIV based on the latest data from the WHO (1). In regions and countries, the burden of the epidemic is different (1). the prevalence of adults living with HIV is 7.0%, 1.5%, 0.2%, 0.2%, 0.4%, 0.9%, 1.2% and <0.1% in Eastern and Southern Africa, Western and Central Africa, Asia and the Pacific, Western and Central Europe and North America, Latin America, Eastern Europe and Central Asia, The Caribbean, and Middle East and North Africa (1). Moreover, the prevalence of HIV among the general population in Iran remains low (1). In Iran, the main populations at risk of HIV infection are people who inject drugs, prisoners and sex workers (2). The general population category consisted mainly of research on blood donors in Iran (3). In Bagheri's systematic review, the prevalence of HIV in the general population was 0.00% (3). Importantly, in a study by Haghdoot et al. is indicated that a change in the prevalence of HIV infection from people who inject drugs to the general population. This shift may due to the enhancing rate of premarital and also extramarital sexual contact, particularly with female sex workers in Iran (4). It also demonstrated that the burden of HIV/AIDS was not distributed equally among different Iranian provinces, and in some provinces such as Kermanshah, Hormozgan, Lorestan, and Tehran it was more concentrated (5). Remarkably, no case with HIV infection was detected in the general population of Mashhad (6). Likewise, the prevalence of infection with HIV in the Iranian population of thalassemia and hemophilia and blood donors was low (7).

Furthermore, HIV/AIDS in Iran still is a taboo. Thence government tries to avoid publishing accurate data of HIV/AIDS prevalence. In this condition, researchers don’t able to perform an accurate comparison between HIV/AIDS prevalence and CCR5Δ32 allele frequencies in Iran. Beside genetic modifications, other critical immune factors that may prevent HIV-1 infection are certain chemokines and also their receptors. In this case, the CCR5 binding chemokines include CCL3, CCL4, and also CCL5 have a function as the main natural factors that act as a suppressor of HIV-1 (8). CCL3L1 up-regulation results in the down-regulation of CCR5 and following the internalization of receptor (9). The trans-activating function of Tax protein 2 is attributed to an increased secretion of CCL3L1 (8). During human T-cell lymphotrophic virus type 1 (HTLV)-1 and HTLV-2 infections with CCLs and CCRs, Tax1 and Tax2 may increase innate immunity in the extracellular environment, which may play a major role in regulating innate immunity during
co-infection with HIV/HTLV and inhibiting CCR5/HIV-1 (10). The CCL3L1 down-regulates CCR5 for the entry of HIV-1, resulting in a long-term non-development status in co-infected patients with the high infection of HTLV-1 and 2 (11). The most affected HTLV-1 cell is CD4+ T cell (12). HTLV-1 and -2 are main co-pathogens among HIV-infected patients (13). In this line, HTLV-2 and HTLV-1 infections can trigger the participation of innate HIV-1 immunity by modifying CCR5/HIV-1 binding and HIV-1 development in patients with co-infection (13). In this regard, CCR5 down-regulation was reported for lymphocytes from HIV-1/HTLV-2 co-infected individuals (13). High levels of co-infection with HIV-1/HTLV appear in HTLV-1-endemic regions, where HTLV-2 is transmitted by sharing the needle. In U.S. and European studies, individuals with HIV-1 and HTLV-2 co-infections were found to result in altered clinical outcomes, and also delayed development of AIDS (14, 15). In contrast, there are several reports were indicated that co-infection with HTLV-1/HIV-1 is associated with faster AIDS clinical progression and shorter survival time and also have more risk to progress myelopathies as well as neurological disease (13, 14, 16, 17). HTLV-1 is widespread in a variety of geographic regions, including Japan, the Caribbean, South America, Africa and Northeastern Iran (18-20). HTLV-1 is endemic in five Iranian provinces such as Khorasan Razavi (Mashhad), Northern Khorasan, Alborz, Eastern Azarbeyjan, and Golestan (20-22). However, there is no report of co-infection HTLV-1 and HTLV-2 infection with HIV in Mashhad in the general population (6, 23). Rahimi et al. indicated that HTLV-1/HIV co-infection may stimulate HIV replication and also could decrease the HTLV-I viral load, in infected cells in non intravenous drug users in Mashhad (21).

Based on the controversially results in studies, more investigation is needed to evaluate HTLV prevalence, especially HTLV-1 and its influence on the viral load of HIV as well as AIDS development in co-infected patients in endemic area such as Khorasan, Iran. Even though there are no findings of the prevalence co-infection with HTLV/HIV in Iran, which can due to low prevalence of HIV in this area, patients need screening for potential clinical manifestations, particularly neurological diseases. To our knowledge and based on the results of previous studies, we could not find any association between prevalence of HIV and HTLV-1 infection and CCR5Δ32 in Iran. The complete and accurate information according to the prevalence of HIV can help health authorities to design more successful plans in the general population (6). Since the prevalence of HIV in Mashhad remains low, the implementation of health policies, public awareness, free HIV counseling and testing services appear to have led to this low prevalence (6).

4. Page 7: Some studies have shown the relation between CCR5Δ32 allele and MS disease. Can the authors discuss the possible links between CCR5Δ32, MS and HIV-1?

Reply: Thank you for your comment. Few studies have focused on genetic susceptibility of Iranian general population to HIV-1, while other Iranian studies have focused on other diseases such as Multiple Sclerosis (MS) (24, 25). MS was used because it has been studied in some parts of Iran in connection with CCR5Δ32, in which there are no reports for CCR5Δ32 in general populations. There has also been a relationship between MS and the frequency of this mutation in populations. Some studies have shown the relation between CCR5Δ32 allele and MS. It is probable that this mutated allele is involved in MS prevalence, which it has been proposed that there was an association between the MS and the Vikings movements (26, 27). The Vikings
probably were not the origin of this allele, but acted as a reservoir and spread it for a period of four hundred years of domination over the waterways and Seven Seas.

5. Can the authors explain this sentence?
An application of this research can be identified the most appropriate individuals to work with HIV-1 in the laboratories, in which we could enroll personnel having this mutation to reduce the risk of HIV-1 infection in the laboratories.
What does it mean "to work with HIV-1 in the laboratories"? Is there perhaps an high risk of HIV-1 infection in the laboratories? Could the authors contextualize this affirmation?

Rely: No, there is an high risk of HIV-1 in reference laboratories, which are related to main key population, but not in general laboratories. There is a low risk of HIV-1 infection for HIV-1 laboratory and affiliated workers as we all health care workers that are prolonged laboratory exposure to concentrated virus and also exposure to experiencing needle stick injuries (28, 29). It is suggested that strict biosafety level 3 containment as well as practices is needed for work with HIV-1, particularly concentrated HIV-1 (28). Although the frequency of HIV in general population is low, it is higher in the high-risky populations such as persons who inject drugs, prisoners, and sex-workers. In this regard, the laboratories dealing with the latter group of populations are exposed to danger. Moreover, the lack of biosafety level 3 containment is another risk factor. Thus, the laboratories and the staffs doing research on HIV are at risk, and most of the staffs are not interested to work in such a risky environment due to unwanted incidents. So, it is reasonable to employ the staffs, carrying the mutation (CCR5Δ32), for working in such risky environments and blood samples.

The authors did not explain their previous affirmations, but they simply removed their previous sentences indicating a very poor effort in increasing the value of the manuscript.
Reply: We thank the Referee for having raised this issue. Unfortunately, this point was not clear in our original manuscript. We would like to apologize for the misunderstanding and not making it clear. The paper has now revised to explain this better. We also have reformulated our conclusions and tried to better convey our thoughts and priorities concerning future work in this field.

Minor comments:

1. What does the term Caucasian mean in this context? Europeans? The reference 17 did not use the term Caucasian.
The authors did not explain the reason why they are using the term Caucasian. The term European would be more appropriate.

Reply: Thank you for your comments and suggestion that allowed us to improve the quality of the manuscript. We have changed those parts in the text.
In other parts, in this paper Caucasians have been used as maternal population for Indo-Europeans population including European and Indo-Persian population, there are some evidence for relationship confirmation between these populations such as ancient DNA analysis and languages, based on the Indo-European steppe hypothesis, some population in north
Caucasus including Yamnaya pastoralist cultures originates Indo-European population which that spread along Europe and some part of Iran plateau and India (30-33), therefore in this paper we used Caucasians term to point to both population such as European and Indo-Persian which that phylogenetically related populations.

8. Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.
What about the 400 samples genotyped? I think that the authors generated and analyzed new data in the current study.
The authors added a description of their data for the Reviewers, without changing any section in the manuscript and without giving any reference in the Availability of data and materials section.

Reply: We thank the reviewer for this suggestion. This bit has now been more explained and highlighted in the text. We have added the reference of MASHAD study in the availability of data and materials section. All samples and data were collected from MASHAD study. As it is mentioned in this manuscript, the MASHAD study started in 2010 and will continue until 2020. The total population in the city of Mashhad was estimated using the national Iranian census in 2006. Participants were drawn from three regions in Mashhad, located in the north-eastern Iran, using a stratified cluster random sampling technique. Each region was divided into nine sites centered upon Mashhad Healthcare Center divisions. Households with individuals of eligible age between 35 and 65 years were identified and the local population authorities provided families with an information brochure of the study (34). It is worth mention that we selected healthy individuals without HIV infection or cardiovascular events. Thus, cardiovascular events are not a limitation of our study. For the purpose of this study, the following key data were also extracted from Mashhad cohort study (34).

Kyungtaek Park (Reviewer 2): 1. [Related to responses of major comments] As you said, the difference of CCR5-delta32 frequency between this study and others might be due to diverse ethnicity in Iran population. However, as geographical distance between Golestan and Mashhad province is close, it is not easy to believe genetic distance between the two province is not close. It is much better to present supporting data that the two province has systematic genetic difference.

Reply: Thanks for pointing out this issue. The ethnic composition of Khorasan and Golestan provinces is quite different. In Golestan province, Turkmen ethnicity resides while in Khorasan the dominant ethnic group is Persian. Many ethnic groups settled in north and northeast of Iran including Turkmens in Golestan province, Turks in Northern Khorasan and Persian in Razavi Khorasan. Turkmens have ethinical, religious and cultural differences with other ethnic groups that cause minimizing of genetic mixture with them, in addition to this, Turks and Turkmens are different phylogenetic origins with Persian neighbors (35). So these reasons maybe cause different in genetic characteristics and allele frequency between these populations who are neighbor geographically.

Mehrjoo et al. by a genome-wide association study indicated that there is a distinct genetic variation and also heterogeneity of the Iranian population comprising 1021 unrelated individuals
from 11 major Iranian ethnic groups living in Iran such as Turkmen and Persian (36). They demonstrated that “Iranians, present distinct genetic variation consistent with long-standing genetic continuity, harbor high heterogeneity and different levels of consanguinity, fall apart into a cluster of similar groups and several admixed ones and have experienced numerous language adoption events in the past. Iran is an important source for human genetic variation in Western and Central Asia” (36).

Figure: Ancient DNA samples from 45,000 (Upper Palaeolithic)–3350 BCE in the context of extant Iranian ethnic groups.

Moreover, Rodriguez-Rodriguez et al (Arthritis Res Ther, 2011) showed that patients with cardiovascular events had lower frequency of CCR5-delta32. Mashhad cohort study aimed to investigate cardiovascular events (Ghayour-Mobarhan et al, International Journal of Public Health, 2015) and samples of your study were from this cohort study. So, relative low frequency of CCR5-delta32 of your study might be due to samples with cardiovascular events. Please specify whether 400 samples had cardiovascular event history.

Reply: Thank you for your comment. It has now been clarified.

As it is mentioned in the manuscript, we collected healthy individuals. At first, Mashhad study was designed to investigate 10000 healthy individuals. After 7 years, these people followed up for incidence of cardiovascular disease. Just for record, only 235 individuals had an incidence of cardiovascular disease (not involved in our study).

We selected healthy individuals without HIV infection or cardiovascular events. Thus, cardiovascular events are not a limitation of our study. For the purpose of this study, the following key data were also extracted from Mashhad cohort study (34).

2. [Related to Table 2]
Please correct typos in Table 2 such as 7/573 and 0.019 and match significant figures.
Reply: We apologise for this typographic error. The table is now amended.

Also, it seems that p-values of each genotype form were calculated with one degree of freedom, which is wrong. It is better to remove p-values of each genotype form.
Reply: Thank you for raising this important point. We do hope that with changes in the table this is clarified.

Best regards,

On behalf of all the authors
References

1. UNAIDS. 2019 Global AIDS Update: Communities at the Centre; July 2019. UNAIDS. AIDSinfo website; accessed July 2019 aahauoUC.


