Author’s response to reviews

Title: Prevalence of CCR5delta32 in Northeastern Iran

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Author’s response to reviews:

Matteo Pasini, PhD.
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Thank you very much for considering our manuscript for publication in BMC Medical Genetics. We thank the reviewer for their careful reading of the manuscript and their constructive remarks. Based on your valuable advice and with respect to the reviewer’s constructive comments, we have revised our manuscript substantially. Additionally, we have taken the reviewer’s comments on board which have been cited point by point in this letter. There are some texts that have been removed/added to the manuscript which makes it more informative.

We hope this is accepted.

Yours sincerely

Zahra Meshkat, PhD

Reviewer reports:
Daniela Zanetti (Reviewer 1): Comments to the Authors

The authors present an interesting study about a 32-base pair deletion (Δ32) in the open reading frame (ORF) of C-C motif chemokine receptor 5 (CCR5). The CCR5Δ32/Δ32 seems to be
protective against the HIV infection, while the CCR5Δ32 heterozygote genotype (CCR5Wild/Δ32) considerably hinders the onset of AIDS but is not quite protected against it. The authors aim to assess the frequency of CCR5Δ32 in the healthy Iranian population.

Response: We thank the reviewer for this encouraging point.

The analysis is generally well-conceived and executed, and the paper is easy to follow. However, the study needs additional analysis and a deeper discussion in order to be suitable for publication.

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

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 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? Is the prevalence of HIV-1 in Iran higher than in Europe and in 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.

Response: Thank you for raising this important point. Population studies have been indicated that ethnicity and genetics are different in a different region of Iran. In our study, prevalence is the same as that of reported in East Asia, while is lower than that in the Europeans. It is indicated that by a systematic review Caucasians as compared to other populations are less susceptible to HIV infection due to the expression rates of CCR5Δ32 while the rest of the populations experience. Based on your advised comments, we design a table in articles.

2. The authors affirmed that the samples are not in HWE (P-value = 0.0199) without discussing the possible reason of this result. This point needs further explanations.

Response: We thank the reviewer for this suggestion. This bit has now been more explained and highlighted in the text.

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.

Response: Thank you for your comment. The new table is added to the text.

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?
Response: Thank you for your comment. We have reworded this part and unnecessary information have been removed.

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?
Response: Thank you for your comment. I agree with you. Based on your good points, it has now been removed.

Minor comments:

1. What does the term Caucasian mean in this context? Europeans? The reference 17 did not use the term Caucasian.
Response: Thank you for your comment. It has now been clarified.

2. Page 5
   discuss the origin of these genotypes
Response: Thank you for your constructive comment. It is revised in the text.

3. Page 6:
   In addition, the bioinformatics analysis indicated that mutated proteins lost three alpha helices, as the results of this changes degraded in the cells.
   Nevertheless, modeling indicated that the truncated protein also have the required domains for virus attachment and these domains did not show major conformational alterations with the wild type ones, so we can conclude that displaying the truncated protein on the cell surface may be a possible way of virus entry.
Response: Thank you for your comment. It is revised in the text.

4. Page 6 and 7:
   In addition, the bioinformatics…………. which may be considered as research area to the prevention of HIV infection
   Please, add some references for all this section.
Response: Thanks for pointing out this issue. The text is corrected.

5. Page 8:
   whereas some side effects such as drug-drug interactions, substantial toxicity, difficulties in adherence, and increased costs remain.
Response: Thank you for your comment. The text is corrected.

6. Page 8: an higher susceptibility
   should be taken to prevent
Moreover, these findings provide a vision (what does the term "vision" mean for the authors?) for scientists to define future research in the field of immunobiology of HIV-1 in Iranian population.
Response: Thank you for raising this important point. The text is corrected. It is now changed to “New understanding”.

7. The authors should revise the language of all the manuscript, especially the discussion.
Response: Thank you for your comment. They are revised in the text.

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.
Response: We thank the reviewer for this valuable suggestion. We have now added some more explanation to clarify this point.
The MASHAD study comprises a cohort of 9704 individuals aged 35-65 years using a stratified cluster random sampling design. 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.

Kyungtaek Park (Reviewer 2): Major

1. According to Shahbazi et al (Cell Mol Neurobiol, 2009), allele frequency of CCR5-delta32 in northeast Iran was estimated as 0.09. Moreover, Solloch et al (Hum. Immunol., 2017) estimated the frequency in Iran as 0.042. These are quite different from your result.
Response: Thank you for raising this important point.

Shahbazi et al (Cell Mol Neurobiol, 2009), allele frequency of CCR5-delta32 is in not northeast Iran and it is in north of Iran with different ethnicity and population (Golestan province). Abdolmohammadi et al (APJCP, 2016), allele frequency of CCR5-delta32 in the same location (Golestan province) was estimated as 0.072. In this line, as we discussed in the discussion, the allele frequency in the north of Iran (Golestan) in higher than another place in Iran. Since Golestan Province is already located in the southeast of Caspian Sea (north of Iran), it is supposed to show a higher rate of this polymorphism but due to the presence of different ethnicities living in this region (like Turkmen), the mutant genotype (CCR5Δ32) is more prevalent (6). Historical data suggests that the more combination of Eastern population of Iran with the Mongol invaders and other Eastern nations could have diluted the CCR5Δ32 allele prevalence. The high prevalence of the CCR5Δ32 allele in the northern and the north-western population of Iran can be contributed to the age of the Vikings or can be due to the less combination with attackers whose allele prevalence was less than that population.
The different result may due to genetic diversity among Iranian population. As we discussed in the manuscript, Iran is an ethnically diverse country, consisting of different groups including Pars, Turk, Kurd, Arab, Turkmen, Baloch, and Lur (9). A long-standing belief of historians is that the most current Iranians are Aryan but during the history, they have been encountered with different foreigners e.g. Macedonians (334 to 331 BC), Arabs (7th century), Turks (10th century), and Mongols (13th to 15th centuries). Also, as a country located between Asia and Europe, Iran has played a key role in connecting various populations along the Silk Road (10). Investigation of genetic systems has been indicated a conclusive heterogeneity among these populations. Comparison of gene frequencies with the few available samples of Iranian populations demonstrated an intra-ethnic and extensive overall genetic diversity in the Iranian plateau. The genetic variation reflect the differences in the structure of these populations, the analysis of which is further attempted in the accompanying paper (9). Moreover, the complete mtDNA sequence analysis revealed an extremely high level of genetic diversity in the Iranian populations studied which is comparable to the other groups from the South Caucasus, Anatolia and Europe (11).

2. How to collect samples from the Mashhad cohort study? Please specify it in Method including whether 400 samples had cardiovascular disease history.
Response: Thank you for your constructive comment.
In the present study, the DNA samples (400 samples) of the Mashhad cohort study (number 85134 of Mashhad University of Medical Sciences; Khorasan) were randomly selected. Notably, the selected samples were collected from the HIV-negative humans from the North East of Iran (Khorasan, Iran). There were 27 clusters in this project, which 15 samples of each cluster were randomly selected. Altogether, it made 400 samples, which were almost age- and sex-matched that were included in this study.

Minor
1. The title would better be changed like "Prevalence of CCR5delta32 in Northeastern Iran" because there is no functional studies and no association studies between the allele and HIV-1 in Result.
Response: Thank you for your comment. We have reworded this part and unnecessary information have been removed.

2. Prevalence of CCR5delta32 is 0.01625 not 0.01625% as I saw. Please correct it.
Response: We thank the reviewer for this suggestion. It is revised in the text.

3. When I calculated p-value according to the information of Table 2, the value was 0.0199871. If I correct, you should change from p-value 0.0199 (main text) or 0.019 (Table 2) to 0.0200 or 0.020.
Response: Thank you for raising this important point. The ambiguous sentence has now been reworded.

4. How to calculate p-values of each genotype form in Table 2?
Response: Thanks for pointing out this issue. We used a basic statistical analysis for genetic case-control studies (12).
A p value less than 0.05 is considered as significant.

Normal and variant allele frequencies:

\[ p = \frac{[2 \times \text{Observed (Homozygote normal)} + \text{Observed (Heterozygote)}]}{2 \times N}, \]

\[ N = \text{Observed (Homozygote normal)} + \text{Observed (Heterozygote)} + \text{Observed (Homozygote variant)}; \quad \text{and } q = 1 - p. \]

Hardy-Weinberg expectation is calculated as:

\[ \text{Expected (Homozygote normal)} = p^2 \times N \]

\[ \text{Expected (Homozygote variant)} = q^2 \times N \]

\[ \text{Expected (Heterozygote)} = 2 \times p \times q \times N \]

Pearson's chi-square test state is obtained using:

\[ \chi^2 = \sum \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}} \]

5. In Figure 2, there is no result from CCR5-delta32 homozygotes. It is better to be included to compare with others.

Response: Thank you for your good suggestion. A new figure is added to the text.

Figure 2: The gel electrophoresis of PCR amplified DNA with CCR5\(\Delta32\) allele.

Lanes 1, and 2, wild type (CCR5\(\text{Wild/Wild}\)); lane 3: mutant type (CCR5 \(\Delta32/\Delta32\)); lane 4: heterozygous (CCR5 \(\text{Wild}/\Delta32\)); Ladder: 100 bp DNA size marker; N: negative control.

6. References seem to be omitted in the second paragraph from back in Discussion. Please check it.

Response: Thank you for your comment. It is revised in the text.

Best regards,

References


