Author's response to reviews

Title: Global Population-specific Variation in miRNA associated with Cancer Risk and Clinical Biomarkers

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Author's response to reviews: see over
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Prof Xianquan Zhan
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Dear Professor Xianquan,

We are pleased to submit our revision of manuscript 2076023095128535 entitled "Global Population-specific Variation in miRNA associated with Cancer Risk and Clinical Biomarkers", by Rawlings-Goss et al. We very much appreciate the extremely thoughtful reviewer comments, which have helped to improve our manuscript. All major changes to the main text are highlighted in red in the revised manuscript. In the following, please find our point-by-point responses:

Reviewer’s Comment #1: Figure 1a and Figure 6a should not be included in the manuscript.

Authors’ Response #1: Both figures have been removed and renumbered

Reviewer’s Comment #2: In Figure 1b, are there any differences if the allele number of Pre-microRNA hairpins or Stem-Loop or Mature seed regions is divided by the number of their bases? Report as allele frequency

Authors’ Response #2: Yes, the paragraph below was added to the results:

“The number of alleles per base was calculated separately for each region of the miRNA (stem-loop, mature, and seed). In our dataset, allele frequencies were slightly higher in the stem-loop region compared to the mature miRNA and seed region (0.013, 0.011 and 0.011, respectively).”

Reviewer’s Comment #3: Figure 1c, Population doesn't mean Global group

Authors’ Response #3: Markers were added to the figure to indicate specific population locations.

a. Figure 1c is now Figure 1a
b. Figure 1 legend has been amended as follows:
c. “Variants per miRNA region. (A) The different colored circles on the world map indicate the geographic location of the 14 sampled populations. Populations from Africa are shown in red (Pygmy, Hadza, Sandawe, Yoruba, Luhya, and Maasai),
Asian populations in green (Gujarati Indians, Japanese, and Han Chinese), European populations in blue (Toscani Italians, Utah residents with Northern European ancestry), and recently admixed populations in stripes (African-Americans, Mexican-Americans and Puerto Ricans) (See Methods). Representative regions are enclosed by the thick black lines.”

**Reviewer’s Comment #4:** The explanation of Figure 5D is missing in Results.

**Authors’ Response #4:** We have now added the following to the Results section—Disease association analysis reveals links to multiple cancers (page 6):

“The hsa-mir-196a-2 T-allele at SNP rs11614913 has been significantly associated with increased risk for oesophageal cancer in non-smoking European males [57] but decreased risk for breast, lung and gastric cancers in Chinese populations [57]. In the present study, we observed a significantly lower frequency of the hsa-mir-196a-2 T-allele at SNP rs11614913 in Africans compared to non-African populations (FST = 0.41; p < 0.001) (Figure 5D).”

**Reviewer’s Comment #5:** Figure 5D, the font of y-axis should be adjusted to that of x-axis

**Authors’ Response #5:** The y-axis font has been adjusted to match the x-axis

**Reviewers’ Comment #6:** Use the plural form of miRNA as “miRNAs” throughout the paper and replace “microRNA” with the abbreviation “miRNA” consistently

**Authors’ response #6:** We addressed both reviewers’ comments to use the plural form of miRNA as “miRNAs” and to replace “microRNA” with the abbreviation “miRNA” consistently. Specifically, we replaced:

- Abstract, under subheading Results, line 6- “identify 7 PD-miRNA” with “identified 7 PD-miRNAs”.
- Abstract, under subheading Conclusion, line 3- “these miRNA” with “these miRNAs”.
- Background, line 5- “MiRNA function” with “MiRNAs function”
- Background, line 9- “miRNA” with “miRNAs”.
- Background, line 15- “miRNA” with “miRNAs”.
- Background, line 43- “1524 miRNA” with “1524 miRNAs”.
- Results, paragraph 3 (Population differentiation~), lines 15, 16 “PD-miRNA” with “PD-miRNA alleles” and line 18 with “PD-miRNAs.
- Results, paragraph 3 (Population differentiation~), line 23- “8 highly population-differentiated miRNA (HPD-miRNA)” with “8 highly population-differentiated miRNAs (HPD-miRNAs)”.

l. Results, paragraph 4 (Messenger RNA target~), lines 1, 2, 4, 7, 15, 16 and 36 “HPD-miRNA” with “HPD-miRNAs”.

m. Results, paragraph 4 (Messenger RNA target~), line 27- “8 miRNA” with “8 miRNAs”.

n. Results, paragraph 5 (Disease association~), lines 2, 3, 8, 9 and 14- “PD-miRNA” or “miRNA” with “PD-miRNAs” or “miRNAs”, respectively.

o. Discussion, paragraph 1 (Population Differentiation~), lines 12 and 16 - “HPD-miRNA” with “HPD-miRNAs”.

p. Discussion, paragraph 2 (The role of miRNA~), lines 3 and 9- “miRNA” with “miRNAs”.

q. Discussion, paragraph 3 (Conclusions and Future Directions), line 3- “HPD-miRNA” with “HPD-miRNAs”.

r. Discussion, paragraph 3 (Conclusions and Future Directions), lines 8 and 9- “miRNA” and “PD-miRNA” with “miRNAs” and “PD-miRNAs”, respectively.

s. Discussion, paragraph 3 (Conclusions and Future Directions), lines 17 and 18- “microRNA” with “miRNA”.

t. Materials and Methods, paragraph 2 (Nucleotide Diversity~), line 2- “Pre-microRNA” with “Pre-miRNA”.

u. Materials and Methods, paragraph 2 (Nucleotide Diversity~), lines 3, 5 and 9- “miRNA” with “miRNAs”.

v. Materials and Methods, paragraph 5 (Disease Association), line 5- “31 miRNA” with “31 miRNAs”.

w. Figure Legends, Figure 3, line 9- “Population differentiated miRNA (PD-miRNA)” with “Population differentiated miRNAs (PD-miRNAs)”.

x. Figure Legends, Figure 3, line 11- “Highly population differentiated miRNA (HPD-miRNA)” with “Highly population differentiated miRNAs (HPD-miRNAs)”.

y. Figure Legends, Figure 4, lines 4 and 6- “Highly population differentiated miRNA (HPD-miRNA)” and “Seven PD-miRNA” with “Highly population differentiated miRNAs (HPD-miRNAs)” and “Seven PD-miRNAs”, respectively.

z. Figure Legends, Figure 4, line 10- I would recommend that “HPD-miRNA” with “HPD-miRNAs”.

**Reviewers’ Comments #7: Additional language corrections**

**Authors’ Response #7:** All additional language corrections were made as recommended by the reviewers. Specifically:
aa. Results, paragraph 2 (MicroRNA conservation~), line 2- “levels nucleotide diversity” was replaced with “levels of nucleotide diversity”.

bb. Results, paragraph 5 (Disease association~), line 16- “Figure 6” was deleted

c. Discussion, paragraph 1 (Population Differentiation~), line 21- “often results” was replaced with “often result”.

dd. Discussion, paragraph 1 (Population Differentiation~), line 28- “miRNA” and “observe” was replaced with “miRNAs” and “observed”, respectively.

e. Discussion, paragraph 2 (The role of miRNA~), line 52- “vary” was replaced with “varies”.

ff. Figure Legends, Figure 3, line 7- “in B” was replaced with “in A”.

gg. Figure Legends, Figure 4, line 9- “were” was replaced with “was”.

hh. Figure Legends, Figure 6, line 2- “affect” was replaced with “effect”.

ii. P5 line 9: “More than half” was changed to “About two-thirds”.

jj. P12 “Author Contributions Abbreviations” was replaced with “Author Contributions” because abbreviations are listed in a separate paragraph below

kk. Figure 2A- “HZ” was replaced with “Hz”.

ll. Figure 3C, the y-axis label- “Africa and NonAfrica” was replaced with “African and Non-African”.

mm. Figure 4B, Target Gene Enrichment- “The list of HPD-miRNA gene targets are” was replaced with “The list of HPD-miRNA gene targets is”.

Reviewers’ Comment #8: Figure 2 a very interesting analysis results, the nucleotide at miRNA variants just above its at Exon, like report inconsistent with others

Authors’ Response #8: This comment appears to be a statement only.