Author’s response to reviews

Title: BasePhasing: a highly efficient approach for preimplantation genetic haplotyping in clinical application of balanced translocation carriers

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Author’s response to reviews:

Re: Manuscript ID: MGNM-D-18-00246R1

Manuscript Title: Basephasing: a high performance cost effective method for preimplantation genetic testing of balanced translocations in clinical application

Dear editor,

Thank you for giving us the opportunity to resubmit our revised manuscript. We thank you and the reviewers for their interest in our manuscript, and for all of their helpful comments. We have addressed issues concerning our manuscript, and have revised the manuscript accordingly.

In the following few pages, please find our response to the reviewers’ concerns and critiques, point by point, in the order listed.

We hope that, with the following clarification and corrections, this revised manuscript could be acceptable for publication. We look forward to hearing from you.
With best regards,

Yours sincerely,

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Responses to Reviewer’s/Editor’s Comments

Yanwen Xu (Reviewer 1):

1. Line 29-33. Please state clearly that "The 5.3Mbp deletion was detected positively" in DNA products of what kind of cells.

Response: Thanks for the reviewer’s valuable suggestion. We have added the detail of cell line, please refer to “Abstract section, line 4, page 3”. Thanks again.

2. In discussion, authors stated that "8/9 of the gametes would be abnormal in couples with a reciprocal translocation, and 2/3 with a Robertsonian translocation", which is not accurate, for the proportions of abnormal gametes is not in accordance with theoretical segregation modes. Some segregation modes could not produce viable gametes.

Response: Thanks for the reviewer’s valuable comment. We agree with the reviewer that some segregation modes could not produce viable gametes. Here, we only consider the theoretical situation for discussion. Considering possible confusion, we have made the following adjustments to this description:

“Theoretically, only two kinds of gametes from alternate segregation, one with a normal karyotype and another with a balanced karyotype, could produce a viable conceptus in balanced translocation carriers, the other unbalanced gametes from other segregation patterns may lead to repeated miscarriage, infertility or newborns with congenital malformations”. Please refer to “Discussion section, line 18-22, page 10”. Thanks again.
3. In discussion, authors stated that "Second, precise translocation breakpoint location and personalized design was unnecessary, our method was universal for any kind of translocation." It should be more cautious for precise translocation breakpoint location will increase the accuracy of PGT. Meanwhile, authors stated that "Therefore, one limitation of our research was that the method didn't apply to these patients both with de novo translocation and without an unbalanced embryo. To our knowledge, no current methods could overcome this difficulty." But, at least MicroSeq-PGD could apply to the patient without a reference. And I was wondering whether recombination may bring any risk of misdiagnosis, since the nature of this method was indirect testing.

Response: Thank the reviewer very much for carefully reading our manuscript and the valuable suggestion.

We agree with the reviewer that precise translocation breakpoint location may help increase the accuracy of PGT. But the identification of breakpoint location, based on finding precise breakpoints, will fail in the highly repetitive and variable regions. What’s more, in fact, precise breakpoint position is not necessary for linkage analysis.

As reported, MicroSeq-PGD method applied the chromosome microdissection technique and NGS to identify precise translocation breakpoints for reference, then to distinguish between balanced and structurally normal embryos by combining junction-spanning PCR sequencing analysis in the breakpoints and linkage analysis. As we know, linkage analysis commonly required an unbalanced embryo as a reference for phasing haplotype. Considering more appropriate expression, we have made the following adjustment: “To our knowledge, no current methods could effectively overcome this difficulty.” Please refer to “Discussion section, line 6-7, page 12”.

In addition, as we described, the genome-wide haplotype could be established successfully with our method (Abstract section, line 4-5, page 3). Therefore, besides the haplotypes of the breakpoint regions, the haplotypes of the two whole chromosomes involved in the translocation and the two corresponding normal homologous chromosomes could be established simultaneously, which could show the condition of homologous recombination in the breakpoint regions. Thus, the prediction of PGH can avoid the risk of misdiagnosis from recombination.

Editor Comments:

1. Please change the Article Type to a Technical Advance, which better suits your manuscript.

Response: Thanks for the insightful suggestion.

We will change the “Article Type” when submitting the revised manuscript. Thanks again.

2. Please change the Materials and Methods heading to Methods.
Response: Thanks for the reviewer’s valuable comment.

We have already changed the “Materials and Methods” heading to “Methods”. Please refer to “Methods section, line 9, page 5”. Thanks again.

3. Please remove the attached Authorship Change form, it is no longer needed at this stage.

Response: Thanks for the insightful suggestion.

We will remove the attached “Authorship Change form” when submitting the revised manuscript. Thanks again.

4. Please upload the supplementary tables as Supplementary Material files when submitting your revision.

Response: Thanks for the reviewer’s valuable comment.

We will upload the supplementary tables as Supplementary Material files when submitting the revised manuscript. Thanks again.

5. Please include the ethics approval reference number in the Ethics approval and consent to participate section, if applicable.

Response: Thanks for the insightful suggestion.

We have already added the “ethics approval reference number”. Please refer to the “Ethics approval and consent to participate section, line 6, page 13”. Thanks again.

6. Please reorder the Declarations so that they come in the following order:

Abbreviations

Ethics approval and consent to participate

Consent for publication

Availability of data and material

Competing interests

Funding
Authors' contributions

Acknowledgements

Response: Thanks for the valuable comment.

We have already reordered the “Declarations” as required. Please refer to page 13-14. Thanks again.

7. Please note, the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared in the Funding section.

Response: Thanks for the insightful suggestion.

We have already added the role of the funding. Please refer to the “Funding section, line 16-21, page 13”. Thanks again.

8. At this stage, please upload your manuscript as a single, final, clean version that does not contain any tracked changes, comments, highlights, strikethroughs or text in different colours. All relevant tables/figures/additional files should also be clean versions. Figures (and additional files) should remain uploaded as separate files.

Response: Thanks for the insightful suggestion.

We will upload our manuscript and all relevant tables/figures/additional files as required. Thanks again.

Furthermore, we change the original title of the manuscript to “BasePhasing: a highly efficient approach for preimplantation genetic haplotyping in clinical application of balanced translocation carriers”, which seems more accurate in grammar. Thanks very much again!

Again, we express our sincere thanks to the reviewers and editors!