

May 14, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2906-review.doc).

Title: Fiber-FISH analyses as a diagnostic application for orientation of the microduplications

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**Name of Journal:** World Journal of Medical Genetics

**ESPS Manuscript NO:** 2906

The manuscript has been improved according to the suggestions of reviewers:

- 1 Format has been updated
- 2 Revision has been made according to the suggestions of the reviewer

[1] For Reviewer 02495239

1) I believe that fiber FISH is a very specialized method and generally not routinely available. This should be stated, or else refuted.

> Thank you very much for your comment. As you suggested, fiber-FISH would not be adaptable for routine examinations. Rather, it is a specialized method for research work. This has been added to the text as follows; “In terms of the clinical point of view, detection of microduplication orientation is not a suitable strategy for clinical analyses. Thus, fiber-FISH analysis is a specialized method for research work.” (pp6, L24-pp7, L2)

2) Please resolve these two diverse statements: - Text: mechanisms of microduplications, page 4: However, intra-chromosomal exchange only creates microdeletion and not microduplication. - Text: standard FISH, page 5: Compared to such intra-chromosomal duplications,...

> We appreciate your suggestion and have now revised them to, “intra-chromosomal duplications”, as mentioned in the section on Standard FISH was changed into “interstitial duplications” to prevent misunderstanding (pp5, L9).

3) For clarity, please use uniform descriptions throughout: - Text: mechanisms of microduplications, page 4: (1) inter-chromosomal, (2) inter-chromatid, and (3) intra-chromosomal - Figure legend 1: (1) inter-chromosomal exchange, (2) inter-chromatid exchange, and (3) intra-chromatid exchange. - Figure 1: (1) inter-chromosomal (2) intra-chromosomal (3) intra-chromatid

> Thank you very much for your comment. We are sorry to have confused you, and these were mistypes. Based on your suggestion, we have revised them (pp4, L17-22).

4) A graph could be considered to assist fig. (3), due to the weak resolution (especially 3B).

> Thank you very much for your recommendation. Accordingly, we have improved the resolution of Figure 3.

5) What is the importance (or possible consequences) of the orientation of microduplications for the authors? Since non-allelic homologous recombination is not assumed as the mechanism for inverted microduplications, what may be the cause of this finding? Does a diagnostic or clinical advantage arise with the knowledge of microduplication orientation? Are there other examples concerning the importance of microduplication orientation?

> As you have noted, detection of the orientations of microduplications does not confer

clinical advantages. However, no one can explain the mechanism of inverted microduplications presently. For a better understanding of the mechanism of chromosomal abnormalities, analysis of the orientations of microduplication will be important in the future. We hope that this makes it clearer.

[2] For Reviewer 00503405

The manuscript is original, well presented and of great technical / clinical importance. The only confusing thing for me is that the manuscript runs under the category of "Filed of Vision", where commentaries are required about articles published other, high-ranked scientific journals, but this manuscript is not a commentary, but a short report. The figures/tables are clear and help the understanding of the text. The references are relevant and up-to-date. I suggest to accept it for publication.

> Thank you very much for your encouragement. If "Field of Vision" is not suitable for publication of our manuscript, the category is open to be changed depending on the decision of the editor.

[3] For Reviewer 00069966

The manuscript "Fiber-FISH analysis as a diagnostic application for orientation of the microduplication" by Yamamoto T et al is acceptable for publication after carried out the completion of ref.no.6,12

> Thank you very much for your suggestion. We have revised the reference style accordingly.

[4] For Reviewer 00053419

1. The value of the method to help our understanding of the mechanisms underlying the chromosomal aberration is clear. However, Is the assessment of the orientation something that may benefit the management of patients?

> Thank you very much for your comment. As you suggested, detection of the orientations of microduplications is not a clinical strategy. For a better understanding of the mechanism of chromosomal abnormalities, analysis of the orientations of microduplication will be important. We have therefore added to the text the following explanation; “In terms of the clinical point of view, detection of microduplication orientation is not a suitable strategy for clinical analyses. Thus, fiber-FISH analysis is a specialized method for research work (pp6, L24-pp7, L2).”

2. The resolution of the figures should be improved, mainly for figure 3.

> Thank you very much for your suggestion. According to the suggestion, we have improved them.

3. Refs 6 and 12 should be completed.

> Thank you very much for your suggestion. We revised them accordingly.

[5] For Reviewer 00506358

1. The manuscript need re-organized in the way that follows common practice as Abstract, Introduction, Methods, results and discussion or combination of results and discussion.

> Thank you very much for your comment. This manuscript is not an original paper but a review. We have structured the manuscript according to the recommended style. We appreciate your understanding.

2. Abstract should very precisely summarize the result and conclusion. For example: “fiber-FISH analysis has the potential to reveal them” could be better if changed to “Our data indicated that fiber-FISH analysis has the potential to reveal the orientation of duplicated and triplicated segments of chromosomes”.

> Thank you very much for your comment. According to the suggestion, we revised it (pp2, L5-6).

3. It is difficult to separate which segment of the manuscript belongs to introduction (Background). Are these two segments “MECHANISM OF MICRODUPLICATIONS” and “STANDARD FISH” included as parts of Background?

> Thank you very much for your comment. This manuscript is not an original paper but a review. We constructed this manuscript according to journal style. We appreciate your understanding.

4. METHODS OF FIBER-FISH: “DNA fiber specimens can be prepared after separating chromatin structures by surfactants. To perform fiber-FISH analysis, traditional Carnoy fixation can be used.” What the statements “can be” mean? Did authors use these procedures? If DNA fiber was prepared this way, it should be stated as so “DNA fiber specimens were prepared after separating chromatin structures by surfactants. To perform fiber-FISH analysis, traditional Carnoy fixation was used. There are several time “can be” were used in the manuscript and should be revised.

> Thank you very much for your comment. As we mentioned earlier, this paper is a review article. Thus, this is not in the style of method description as generally seen in original papers. This style, “can be”, is according to the text books or the manuals provided by companies, as you can see in such materials. We appreciate your understanding.

[6] For Reviewer 00505755

<General comments>

(1) The research describes about microduplications and genomic copy number variations detected with methods such as fiber-FISH analysis.

> Thank you very much for your understanding.

(2) The research is considered as novel and innovative since tandem duplication is detectable with fiber-FISH analysis.

> Thank you very much for your assessment.

(3) Presentation of the manuscript is acceptable because the schematic representation of the mechanism of non-allelic homologous recombination helps the readers' comprehension, however, figure legends do not describe the figure content well. Table1 should also be revised as well, for instance copy number variation analysis using microarray may be added. Methods may be described more in detail by adding another section.

> Thank you very much for your suggestions. We have revised the figures legends and table 1, according to the suggestion.

(4) There is no remarkable concern in the ethics of the research.

> Thank you very much for your assessment.

<Specific comments>

(1) In page 3, line 8, abbreviation such as MIM may be explained somewhere in the article.

> Thank you very much for your suggestions. According to the suggestion, we have provided full spelling (pp3, L9).

(2) In page 4, line 15, "(2) inter-chromatid and (3) intra-chromosomal" are described as "(2) intra-chromosomal and (3) intra-chromatid" in figure 1. The terms should be unified in either way and please clarify the mechanism of intra-chromosomal exchange.

> Thank you very much for your suggestions. We are sorry to have confused you. There were many misspelling. According to the suggestion, we have revised them (pp4, L17-22).

(3) In page 5, line 7, the term “intra-chromosomal duplications” does not seem to be consistent with the explanation “intra-chromosomal exchange only creates microdeletion and not microduplication” in page 4, line 17.

> Thank you very much for your suggestions. We are sorry to have confused you. The term “interstitial duplication” has been used as a replacement (pp5, L9).

(4) In page 5, line 13, check whether “to be determine” should be “to determine” or not.

> Thank you very much for your suggestions. According to the suggestion, we have revised it (pp5, L15).

(5) In page 6, line 21, the paragraph starting with the sentence “If signals...” should have additional sentences. Otherwise, consider to merge the sentence in the different paragraph.

> Thank you very much for your suggestions. According to the suggestion, we have merged the paragraphs (pp6, L19).

(6) In figure 3 in page 13, the fluorescence in the results of fiber-FISH is not demonstrated well, so the resolution of the pictures may be increased.

> Thank you very much for your suggestions. According to the suggestion, we have improved the resolution of the pictures.

<Others>

- (1) Regarding the title of this manuscript, it is impossible not to use the abbreviation of FISH. As the word number for the title is restricted, the full spelling of FISH (fluorescent in-situ hybridization) cannot be used.
- (2) Regarding the reference list, we provided PubMed citation numbers and DOI.
- (3) Regarding the tables, we provided the Excel version.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Medical Genetics*.

Sincerely yours,

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