Author’s response to reviews

Title: Chromosome (re)positioning in spermatozoa of fathers and sons - carriers of reciprocal chromosome translocation (RCT)

Authors:

Marta Olszewska (marta.olszewska@igcz.poznan.pl)
Ewa Wiland (ewa.wiland@igcz.poznan.pl)
Nataliya Huleyuk (huleyuk@yahoo.com)
Monika Fraczek (monika.fraczek@igcz.poznan.pl)
Alina Midro (midro@umb.edu.pl)
Danuta Zastavna (zastavna.d@ihp.lviv.ua)
Maciej Kurpisz (kurpimac@man.poznan.pl)

Version: 1 Date: 09 Nov 2018

Author’s response to reviews:

Editorial Office
BMC Medical Genomics

Sir,

Thank you for an opportunity of revision of our manuscript coded as MGNM-D-18-00185 by Olszewska et al.. We have put every attention to amend it accordingly to thorough remarks of the reviewers. Please, note here below the detailed rebuttal to each comment disregarding (as recommended by Editor) Reviewer No 1 considering his/her remarks as tangential to the subject of study.
According to Referee No 2.

Vladimir Trifonov (Reviewer 2): The article by Olszewska et al "Chromosome (re)positioning in spermatozoa of fathers and sons - carriers of reciprocal chromosome translocation (RCT)" provides interesting data on the effect of chromosomal translocations on chromosome location changes in sperm nuclei. It is especially interesting that the authors use familial cases to demonstrate these effects. I found the article very interesting, but the text needs some improvement. The manuscript would benefit from extensive editing for language and style. I would also reduce the discussion section. Please find below my comments.

P1 "Sperm chromosome topology in family" Familial cases?

It is right to use “Familial cases” according to our ‘native’ Editor, however, due to lack of the obligation of the Journal for running title we decided to remove it from the title page.

P2L7 "Nuclear order of chromosomes" Nuclear arrangement of chromosomes?

It is now corrected and amended on P2L4.

P2L56 «chromosomal factors (karyotype, hyperhaploidy)» Do you define karyotype as one of chromosome factors? Do you mean type of rearrangement?

Amended on P2L25. In this paper we argue that karyotype is one of the chromosome factors. In case of the other meaning of expression ‘chromosome factors’, as structural, numerical, or more detailed description as: RCTs or Robs or inv, etc., more studies will be required for documentation.. At this moment there is no data concerning topology screening in human sperm cells of other types than RCTs or aneuploidies.

P3L22 "revealed prominent progress" re-phrase

P3L10: it has been re-phrased accordingly

P3L22 "closely determined" precisely determined? “Well- defined”

P3L10: “Well defined” expression has been inserted

P7L19 "Topology of the chromosomes 4, 7, 8, 9, 10, 11, 18, X and Y was performed" Topology was estimated?

P7L9: it has been amended accordingly

P15L27 "in nuclear ordering of chromosomes the critical role may play mechanisms physically governing genomic organization" What do you understand under genomic organization? Doesn't it include nuclear chromosome ordering?
In our view, genomic organization includes also nuclear chromosome order. We just wanted to put the light on the pure physical/mechanistic side of the elements included within the nuclear space.

"chromosome X" the term "X-chromosome" is more widely used

it has been amended upon recommendation of the Referee

"Specific apical positioning was also documented" Apical positioning of what?

it has been clarified in the appropriate sentence

What is "proper RCT"? Are there any improper RCTs?

it has been corrected and clarified as recommended

"in case of rearrangements with the same karyotype" please re-phrase! Do you mean chromosome rearrangements?

a sentence has been re-phrased accordingly

"presence of additional chromosome may be more important than a karyotype." please re-phrase!

this part of the text has been re-phrased as recommended

"chromosomes posses firm locations in sperm nucleus during the meiotic stages of spermatogenesis" please re-phrase, sperm cell is the final product of meiosis

also this line has been re-phrased accordingly

"The role of chromatin integrity components in sperm nuclear order should not be neutral, but most probably can be considered as secondary (playing a role in directioning of repositions) when looking into cases with the same karyotype (RCT)" How can this explain the existence of Robertsonian translocation polymorphisms in many mammalian species?

The existence of Robertsonian translocation polymorphisms has not been verified in the context of chromosomes’ topology, yet. Some papers concerning chromosomes’ topology in sperm cells of other species are available, however, no one has discussed yet the problem of the existence of polymorphisms. Previous reports were focused clearly onto chromosomes’ localization. One example is a paper published by Acloque H. et al. ‘Sperm Nuclear Architecture Is Locally Modified in Presence of a Robertsonian Translocation t(13;17)’; PLoS One, 2013: 8: e78005, where the authors revealed that in boar spermatozoa with Rob(13;17), the centromere from SSC13 and SSC17 fusion should behave like a SSC17 centromere and so the resulting fused chromosome would adopt a SSC17-like position. And that may be probably due to the differences between alpha-satellite sequences.
In our opinion, the existence of Rob polymorphism is a separate problem, not linked to the topology. It is rather some evolutionary issue. So, may be polymorphism in the meaning of the length of chromosome fragments may have some influence on topology (not checked, so far), but the issue of the existence per se – that should be another problem.

P22L7, L22 "All men were notified" … Patients and healthy volunteers were informed?

P22L3 and 9: All participants ‘patients and healthy control donors’ have been notified about the purpose of the study

To the Referee No 3.

Rui-zhi Liu (Reviewer 3): Based on the previous research, the authors of this manuscript further explored the positioning of nine chromosomes' centromeres was (re)arranged in their spermatozoa in fathers and sons - carriers of the same RCT. The authors showed that chromosomal factors have been superior ones influencing strongly chromosomal topology, when confronted with the sperm chromatin integrity components.

This is a very interesting research paper. But, there are two problems as follows:

1. In abstract, the authors described 13 carriers of 11 RCTs, including two familial RCT cases: \( t(4;5) \) and \( t(7;10) \), followed by the analysis of the eight control individuals. In fact, there are nine individuals. Please check.

The numbers analyzed were double- checked, and in the whole study were included 8 control individuals but 9 sets of analyzed chromosomal complements. Therefore number nine appeared in the paper.

2. Please check the probes used in experiments, 4-locus D3Z1(4P11-Q11) in slide preparation of FISH in page 6.

We accordingly corrected the probes used for D4Z1 (4p11.1-q11.1)

Overall, we are truly grateful for an opportunity of the manuscript correction. A paper has been additionally checked by the “native speaker” editing process. We sincerely hope that we have
been able to upgrade the quality of the manuscript as this should be suitable for publication in
your prestigious Journal.

Yours faithfully

Maciej Kurpisz, MD, PhD
Professor in medicine