Author’s response to reviews

Title: The Establishment and Application of Preimplantation Genetic Haplotyping in Embryo Diagnosis for Reciprocal and Robertsonian translocation carriers

Authors:

Shuo Zhang (chnszhang@163.com)
Caixia Lei (474981375@qq.com)
Junping Wu (wujunping-88@163.com)
Jing Zhou (noya0303@163.com)
Haiyan Sun (bettershy@sina.com)
Jing Fu (fujing_givf@163.com)
Yijuan Sun (yijuansss@163.com)
Xiaoxi Sun (steven3019@hotmail.com)
Daru Lu (drlu@fudan.edu.cn)
Yueping Zhang (jiaitg@163.com)

Version: 1 Date: 07 Aug 2017

Author’s response to reviews:

Responses to Reviewers’ Comments

Yuanjie Su (Reviewer 1):

Comments:

Title: "Establishment" or "Development" instead of Establishing
Response: Thanks for the valuable suggestion. As suggested, "Establishment" might be more suitable.

Page 5 line 48: remove "this corresponding"
Page 6 line 60: "member" instead of "number"
Response: Thank the reviewer very much for carefully reading our manuscript. We are very sorry for our carelessness. We have corrected these errors as suggested. Thanks again!

Page 9 line 5: Not clear about the sixth point, similar with Fifth point?
Response: Thanks for the valuable comment. We agree with the reviewer’s comment that the Sixth point is somewhat similar with Fifth point, then we decide to delete this sentence under careful consideration.

Table 3: Font need to be standardized
Response: Thank the reviewer very much for carefully reading our manuscript. We are very sorry for our carelessness. As suggested, we have standardized the Font.

Figure 1: It is better to color the Karyotype near the bottom to make it consistent; highlight informative SNP is also another detail to address.
Response: Thanks for the reviewer’s suggestion.
Actually, the “Karyotype near the bottom” means the “Haplotype” which is used to predict for the status of embryos’ chromosomes shown in the bottom line of Figure 1, and all the Haplotypes presented in Figure 1 are not colored. Therefore we think it may be not necessary to color the Karyotype.

General comments: This paper showed a successful POC of PGH to select structurally normal embryos for IVF. It can be very useful for the general public.
Response: Thank the reviewer very much for carefully reading our manuscript and the positive comments. PGH is an efficient method to distinguish between balanced and structurally normal chromosome embryos from reciprocal and Robertsonian translocation carriers. Our study has great clinical signification for these patients. More balanced translocation families would benefit from stopping the passing on of the translocation to their next generation.
However, there is a lack of details in the methods section to show the whole process. Figure 1 is not very helpful to piece the information together.

Response: Thanks for the reviewer’s suggestion.

In fact, as we described in “Method” section Page 5 line 27-61 and Page 6 line 1-13, these sentences have shown the whole process to distinguish balanced and structurally normal embryos including the selection criteria for informative SNPs (Page 5 line 35-48) and predictive criterion and process (Page 5 line 54-61 and Page 6 line 1-13). Figure 1 also shows the process of establishing haplotypes and distinguishing between balanced and structurally normal chromosome embryos through PGH analysis. Maybe our paragraph division caused confusion, now we have adjusted the paragraph division which could show the process more obviously. Please refer to Page 5 line 27.

In paper, author claimed 2-day is enough to get all the results. Does that include the collection of carrier, parent, family info also? Will the related cost and time make this system not as appealing as author described here? At the same time, it is not very clear why the Illumina platform is chosen and what are the other options out there for comparison, which might be more cost effective if the sole purpose is to differentiate balanced vs structural normal embryos.

Response: Thanks for the reviewer’s comment.

As we described, the total process could be finished within two days including the experiment and data analysis (Page 8 line 58-60 and Page 9 line 1). The collection of carrier, parent and family info should be collected before the IVF treatment cycle in our center and therefore the time of two days doesn’t include this part.

And, the key reason to choose the Illumina platform is that the BlueFuse®-Multi software (Illumina, Inc. San Diego, USA) could help do linkage analysis with the Genome-wide SNP genotype data very conveniently, while no the other platforms could meet this requirement at present. Also, the cost of Illumina platform is not very expensive and acceptable for most patients. As the rapid development of the SNP-array and NGS, we think more cost effective and convenient platforms will emerge to fulfil the requirement.

The two major references on PGH is 6-10 years old, should reference some newer updates in this field.

Response: Thanks for the reviewer’s valuable suggestion.
We agree with the reviewer that the two major references on PGH are not recent, while we search the literature again and no some newer publications are found. The progressing in this field is slow.

Also, to include more details about how to call informative SNP and how these SNPs can be used for other purposes can be meaningful for other researchers.

Response: Thanks for the reviewer’s suggestion.

The informative SNP should fulfil the selection criteria (Page 5 line 35-48) in all the genotyped SNPs, and these SNPs could be used to establish the haplotypes of every chromosome among the whole genome.

In most researches, STR markers are used for linkage analysis. While two limitations are obvious: one is that STR markers are less (about 10000) in genome and another is that it’s hard to get the genome wide STR markers information. While, SNPs markers are enough in genome and could be used to establish the haplotypes of any region among the whole genome. As we described in Page 10 line 25-27, “In each Mb distance, 6.6±1.4 SNPs could be used to establish haplotypes, the recombination less than 1 Mb also could be identified in the method”. We think these results would be meaningful for other researchers.

Naureen Aslam (Reviewer 2):

Comments:

1. It would be good if you clearly mentioned the difference between balanced and structurally normal chromosomes with example?

Response: Thank the reviewer very much for carefully reading our manuscript and the positive comment.

As we described in “Background” section Page 2 line 52-56, the difference between balanced and structurally normal chromosomes is that balanced translocation is a common structural chromosome rearrangement that occurs when an exchange of terminal segments happens between different normal chromosomes.

2. In the result section, 42 unbalanced or aneuploidy translocations were identified, whereas 26 were balanced or normal chromosomes. Is there any common biological pattern of these 42
unbalanced or aneuploidy translocations with respect to their parents genomes involved in this study?

Response: Thanks for the valuable suggestion.

As we described in “Result” section Page 6 line 46-50, of the 68 diagnosed blastocysts, 26 were balanced or normal, 25 blastocysts had translocation related abnormalities and 17 blastocysts showed de novo abnormalities unrelated to the translocation.

Among the 25 blastocysts with translocation related abnormalities, 11 are adjacent-1 segregation pattern, 3 are adjacent-2 segregation pattern, 6 are 3:0 segregation pattern and 5 are other irregular segregation pattern (the results of molecular karyotypes are listed in the Supplemental table S1). As we described in “Background” section Page 3 line 5-17, “A quadrivalent structure is formed at meiosis I through pairing of translocated chromosomes and the two corresponding normal chromosomes. This structure commonly undergoes one of the three modes of segregation: 2:2, 3:1 or 4:0”.

In addition, among the 17 blastocysts with abnormalities unrelated to the translocation, there is no regularly biological pattern.

3. Is there any hotspot chromosome in eleven families which are more prone to chromosome breakage or translocation?

Response: Thanks for the insightful suggestion.

Until now, no relative researches have been reported. Maybe this is a very good idea for our future research.