Author’s response to reviews

Title: Expanded carrier screening and preimplantation genetic diagnosis in a couple who delivered a baby affected with congenital factor VII deficiency

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Author’s response to reviews:

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To the Editor of BMC Medical Genetics

Dear Dr. Pasini,

Thank you very much for your comments on our manuscript “Expanded carrier screening and preimplantation genetic diagnosis in a couple who delivered a baby affected with congenital factor VII deficiency” (MGTC-D-17-00016) and for allowing us to revise the manuscript. We also appreciate the insightful suggestions from the reviewers. We have responded to all the comments provided by the reviewers in a point-by-point manner.
The manuscript has been revised according to your and the reviewers’ suggestions (see the accompanying list of main changes and the copy of the original manuscript marked with changes).

We believe that these recommended modifications have improved our manuscript. We are grateful for your consideration of our manuscript, and we hope that it is now suitable for publication in BMC Medical Genetics.

All authors have reviewed the final revised version of the manuscript and approve it for publication. The authors have no conflicts of interest to declare.

Sincerely,

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Response to the comments from the reviewers

Reviewer 1

(1) In general, this seems like nice work with appropriate follow up (and a good result for the couple), but is not groundbreaking: the authors apply well-understood, off-the-shelf kits for WGA, mutation detection, STR locus sizing, and aneuploidy detection, and apply them to more than one condition at a time. My main concern with the paper is whether or not it contributes novelty to the literature.
Response: We appreciate this valuable advice. Indeed, the series of molecular genetic analyses performed is not novel. However, in this study, we successfully implemented PGD for congenital FVII deficiency and PGD after ECS to exclude CF for the first time. Additionally, our work provides a clear example of using ECS combined with PGD to avoid the delivery of offspring affected not only by identified monogenically inherited diseases but also by other potential monogenic pathologies and aneuploidy.

(2) PGD was clearly valuable in this case (carrier/compound het couple for F7 and carrier/carrier couple for CFTR). I would suggest that the conclusion or message of the paper be retargeted at this point: if PGD were not performed, we would expect a 3/8 chance of a fetus unaffected by either condition (50% chance unaffected for F7 times 75% chance unaffected by CF), so in this case the use of PGD significantly improved the reproductive outcome for the couple.

Response: Thank you for your suggestion. This statement has been added to the conclusion in the revised manuscript. “If PGD were not performed, we would expect a 3/8 chance that the foetus would be unaffected by either condition (50% chance of being unaffected for F7 multiplied by a 75% chance of being unaffected by CF); thus, the use of PGD significantly improved the reproductive outcome for the couple in this case.”

(3) However, in order for the study to actually provide generalizable information to the scientific literature beyond "we did this", at the very least more information needs to be provided about the methods. In particular, since the assay relies on PCR enrichment for a variety of loci, primer sequences used should be provided for each locus analyzed. Additionally, there are references to a variety of "standard" (L104) or "previous" (L85) methods, with very little description of methods in this paper. It would be valuable to include a brief summary of the analysis performed for aneuploidy analysis by PGM as well as the bioinformatics performed for ECS (in particular, filtering and QC criteria).

Response: We appreciate this important suggestion. The primer sequences used for mutation detection and linkage analysis are shown in Table 1 in the “Methods” in the revised manuscript.

Additionally, a brief summary of the ECS bioinformatics and PGM aneuploidy analyses has been provided in the “Methods” in the revised manuscript, as follows.
“Briefly, the Illumina analysis pipeline (CASAVA1.8) was employed for base calling. We then separated each barcoded dataset and removed the low-quality data using in-house scripts. The sequencing reads were subsequently aligned to the reference human genome (hg19) using the Burrows-Wheeler Aligner. Single-nucleotide variants and small insertions or deletions were identified using the Genome Analysis Toolkit (Broad Institute), while deletion or duplication of exons in genes was detected using an in-house pipeline. Variants were annotated with in-house scripts.”

“Briefly, the WGA products were purified with Agencourt® AMPure® XP beads (BeckMan), connected with an Ion Xpress™ Barcode (Life Tech), and subsequently sequenced on the Ion PGM™ platform (Life Tech), according to a standard protocol (https://ioncommunity.thermofisher.com/community/protocols-home). The raw sequencing data included approximately 0.5 M reads, which were mapped to the reference human genome (hg19) with a coverage rate of approximately 1%. Chromosomal copy number variation (CNV) analysis was performed for all samples using the Celloud cloud server (http://www.celloud.org/), offered by JBRH (Beijing, China). The applied analysis pipeline was similar to that in a previous study.”

(4) Finally, there are a number of typographical and grammatical errors in the paper that should be corrected before final acceptance/publication. A short list of examples (certainly incomplete): "challanging" L48; "who are" at L51 should be "are"; "both were carrier of another gene" L60 should be "both were carriers of pathogenic variants in another gene"; "is a carrier" L73 vs "harbors a heterozygous mutation" L74; "previos" L85; "form" L117; "crypt recessive" L158; "necesscity" L171;

Response: We apologize for the errors in the English language of the manuscript. We have made the following changes in the typographical and grammatical errors in our revised manuscript.

"challanging" (L48), "who are" (L51), "both were carrier of another gene" (L60), "previos"(L85), "form"(L117), "crypt recessive" (L158) and "necessity" (L171) have been changed to "challenging", "are", "both were carriers of pathogenic variants in another gene", "previous", "from", "cryptic recessive" and "necessity", respectively, in the revised manuscript.

We have also replaced "is a carrier" with "harbours a heterozygous mutation" (L74).
Reviewer: 2

(1) As a general comment, the authors would benefit considerably by utilizing a professional language editing service. In its current form, the manuscript contains a large number of stylistic and grammatical errors, which often negatively affects the clarity of the manuscript.

Response: We apologize for the errors in the English language of the manuscript. To improve the readability of our manuscript, we have used a reputable English language editing service, American Journal Experts (http://bit.ly/AJE_BS). The editing certificate has been uploaded in the online Submission system, and the certificate verification key number is 1EE9-F185-631E-7DFD-197P.

Detailed comments:

(1) Line 43: One of the references is too old, dating to 1985, when many of the currently known recessive disorders were not yet characterized. This source should not be relied upon when assessing the overall burden of recessive diseases.

Response: Thank you for your suggestion. The indicated reference has been replaced with a recent citation (Verma IC et al. Semin Foetal Neonatal Med. 2015; 20: 354-363), and corresponding modifications regarding the effect of recessive disease have been made in the “Background” section of the revised manuscript.

(2) Lines 50-53: Given the context, the authors use a very conservative estimate (1-2%) for the proportion of at-risk couples in the general population. The CGT Igenomix ECS test, which was utilized in this study, identifies 5% of all couples as carriers (Martin et al. 2015; Website of Igenomix).

Response: Thank you for your suggestion. Indeed, Gabriel A et al. reported that a total of 23,453 individuals, not 23,453/2 couples, were referred for routine recessive disease carrier screening and that the members of 127 “carrier couples” were both heterozygous carriers for the same condition, accounting for 1.09% of half of all individuals [2]. However, Martin et al. reported that 7 in 138 couples, or approximately 5%, were at high risk because the partners shared recessive mutations in the same gene [3]. Thus, the estimate of “approximately 5 in 100 infertile couples” rather than “1-2 in 100 couples” is referenced in the “Background” section of the revised manuscript (Lines 50-53).
In general, we suggest that the authors use 'both members of the couple' or 'both reproductive parents' throughout the paper, as opposed to 'two couples', or 'both couples'. This would avoid linguistic confusion over the number of couple(s) tested.

Response: We appreciate this important suggestion. We have replaced “two couples” and “both couples” with “both members of the couple” in the revised manuscript anywhere.

While the authors discuss a 'customized panel', based on the number of genes and disorders they provide, it appears that the panel they used was virtually identical to the standard CGT Igenomix panel. Therefore, we recommend that they mention how customization was performed.

Response: We apologize for this error. A misunderstanding resulted from our unclear description. The panel used in our study was actually the standard CGT Igenomix panel, which was designed by BGI-Tianjin and is currently a commercial panel.

“The panel was designed to screen 547 genes associated with 623 monogenic disorders and includes recessive and X-linked diseases with severe and highly penetrant phenotypes as well as high-prevalence monogenic diseases with moderate phenotypes.” To provide a clear description of the panel, we have made some modifications in the revised manuscript (Line 81).

A verb is missing at the end of the sentence

Response: We apologize for making this error in English language. We have replaced “research” with “study” in the revised manuscript (Line 85).

For the reader unfamiliar with Ion PGM platform, could the authors briefly mention which chromosomal aneuploidies are tested?

Response: We appreciate this significant advice. A brief summary of the PGM aneuploidy analysis has been provided in the “Methods” section of the revised manuscript, as follows (Line103):
“Briefly, the WGA products were purified with Agencourt® AMPure® XP beads (BeckMan), connected with an Ion Xpress™ Barcode (Life Tech), and subsequently sequenced on the Ion PGM™ platform (Life Tech), according to a standard protocol (https://ioncommunity.thermofisher.com/community/protocols-home). The raw sequencing data included approximately 0.5 M reads, which were mapped to the reference human genome (hg19) with a coverage rate of approximately 1%. Chromosomal copy number variation (CNV) analysis was performed for all samples using the Celloud cloud server (http://www.celloud.org/), offered by JBRH (Beijing, China). The applied analysis pipeline was similar to that in a previous study.”

(7) Line 129: Do the authors mean that embryo number 3 was not a carrier of congenital FVII deficiency or CF, rather than not affected?

Response: We appreciate this important suggestion. Based on the PGD results, this sentence (line 129) was modified as follows in the revised manuscript: “embryo 3 was a carrier of congenital FVII deficiency but did not harbour either of the CFTR gene mutations”.

(8) Lines 139-143: The principal added value of this manuscript is a detailed description of the diagnostic trajectory undertaken by a high-risk couple. To form a more comprehensive opinion about the merits and disadvantages of the entire pathway, many readers would probably find it helpful if the authors also briefly discussed issues such as: genetic counseling, psychological challenges (if experienced by the couple) and costs. Alternatively, these issues can be reflected upon in the Discussion.

Response: Thanks for your suggestion. We have discussed genetic counselling and cost in the third and fourth paragraphs of the “Discussion” section in the revised manuscript, as follows:

“The ECS results and reproductive outcome may contribute to genetic counselling and fertility guidance for the other family members of the couple.”

“This strategy, of ECS followed by PGD and PGS, was employed in the couple evaluated in this study, resulting in a favourable reproductive outcome at a comparatively low cost. Most importantly, the two monogenic diseases were not transmitted to the offspring”.

(9) Line 152: This should state "caused by mutations in the F7 gene".

Response: Thank you for your suggestion. We have added “mutations in the” before “F7 gene” in line 167 in the clean version of our revised manuscript.

(10) Lines 178-180: The statement that the proposed protocol can decrease workload and costs cannot be directly inferred from the preceding discussion. We therefore invite the authors to further elaborate on how they arrived at this conclusion.

Response: Thank you for your suggestion. Under the double-factor PGD strategy (PGD for monogenic diseases and PGS for chromosome abnormalities) applied in our study, PGD for two monogenic diseases was performed first, and PGS was subsequently conducted for chromosomal CNVs. It has been reported that the aneuploidy embryo rate is 2% to 6% in women aged 26 to 37 (the age of the woman in our study was 34) [4]. The rate of receiving a foetus unaffected by either monogenic disease was 37.5% for the couple in our study (50% chance of being unaffected by F7 multiplied by a 75% chance of being unaffected by CF).

In addition, the cost of PGS and PGD for one embryo is 1500 RMB and 600 RMB, respectively, at our centre. If PGS were to be performed for all embryos, the cost would be 16,500 RMB (11 times 1500). However, the cost of receiving an embryo for transplantation is 11,100 RMB (600 multiplied by 11 plus 1500 multiplied by 3) in our study. Thus, we suggested that performing PGS for unaffected embryos after monogenic disorder detection under the double-factor PGD protocol could decrease workload and cost.

Main changes made to the manuscript

1. We have used a reputable English language editing service, American Journal Experts (http://bit.ly/AJE_BS). The editing certificate has been uploaded to the online Submission system, and the certificate verification key number is 1EE9-F185-631E-7DFD-197P.

2. The title “Expanded carrier screening and preimplantation genetic diagnosis for a couple who delivered a baby affected with congenital factor VII deficiency” has been changed to
“Expanded carrier screening and preimplantation genetic diagnosis in a couple who delivered a baby affected with congenital factor VII deficiency”.

3. The primer sequences used for the mutational detection and linkage analysis are shown in Table 1 in the “Methods” section in the revised manuscript.

4. Brief summaries of the ECS bioinformatics analysis and PGM aneuploidy analysis have been added to the “Methods” section in the revised manuscript.

References


