Author’s response to reviews

Title: Preimplantation genetic testing for a family with Usher syndrome through targeted sequencing and haplotype analysis

Authors:

Haining Luo (luohn163@163.com)
Chao Chen (chenchao4@genomics.cn)
Yun Yang (yangyun@genomics.cn)
Yinfeng Zhang (tjzyf101545@163.com)
Yuan Yuan (yuanyuan@genomics.cn)
Wanyang Wang (wangwanyang@bgi.com)
Renhua Wu (wurh@genomics.cn)
Zhiyu Peng (pengzhiyu@genomics.cn)
Ying Han (mujiangjun@163.com)
Lu Jiang (jianglu@genomics.cn)
Ruqiang Yao (36401754@qq.com)
Xiaoying An (anxiaoying@genomics.cn)
Weiwei Zhang (475117757@qq.com)
Yanqun Le (leyanqun@bgi.com)
Jiale Xiang (xiangjiale@genomics.cn)
Na Yi (yina@bgi.com)
Hui Huang (huanghui@genomics.cn)
Wei Li (liwei10@bgi.com)
Yunshan Zhang (tjzys@hotmail.com)
Jun Sun (sunjun@genomics.cn)
Dear Editors and Reviewers:

We appreciate the detailed and valuable comments from you and reviewers. Those comments are all valuable and very helpful for revising and improving our paper. The manuscript has been carefully revised and the major revisions were indicated using track changes in revised manuscript. The point-by-point response to the comments and suggestions were listed as below. We sincerely hope this manuscript will be finally acceptable to be published on BMC Medical Genomics.

Responds to the reviewer’s comments:

Reviewer 1

Comment 1: The application of MPS in PGD is recently described by some other authors (Xiang 2015, Zhou 2018). Also other methods based on haplotyping of embryo’s are known and applied (Renwick 2010, Handyside 2010, 2015). In the introduction or discussion these papers have to be mentioned and also the differences, advantages and disadvantages of MPS based PGD compared to these methods.

Response: Thank you for your comments. As the reviewer’s good advice, we carefully read the recommended reference and added related literature in the reference section. We compared the difference between NGS based PGT and other technologies used to PGT such as FISH, aCGH and SNP array in the introduction. At the same time, compared with published NGS-based PGT, the advantages and disadvantages were also added in the introduction. (Line 56-86, Page 3).

Comment 2: In the introduction authors mention that PGD is possible by mutation analyses or linkage analysis. However, mutation analyses is usually combined with haplotyping to prevent misdiagnosis due to ADO.

Response: Thank you for your comments. Just like what the referee said, PGT-M is carried out by directly analyzing the mutation of interest combined with short tandem repeat (STR) linkage analysis to prevent misdiagnosis due to ADO. We corrected this point in revised manuscript and the detailed revision can be found in Line 59-61, Page 3.

Comment 3: Methods section is very long and contains a lot of technical details, authors can skip part of it, which will make the paper easier to read. More or less the same applies to the results section, that is many details and overlap with the methods section.
Response: Thank you for your comments. As the reviewer's good advice, the technical details in the method and results section were simplified and the overlap content was deleted. (Line 94-153, Page 4-6).

Comment 4: In discussion authors mention an increased risk on chromosomal anomalie after PGD, however this is not proven. Prenatal testing is merely ment for detection of a misdiagnosis after PGD as described earlier for review see Wilton et al).

Response: Thank you for your comments. We have made correction according to the reviewer’s comments. The content involving an increased risk on chromosomal anomalie after PGT was deleted in discussion. We have read the literature recommended by reviewers carefully. It is noteworthy that invasive prenatal diagnosis is warranted in all cases underwent PGT to avoid misdiagnosis. The previous studies have reported that allele dropout, contamination, mosaicism and inappropriate probes or primers were main causes of misdiagnosis via PGT. Chromosomal mosaicism affects up to 50% of early human embryos at the cleavage stage. (Wilton et al. 2009). Chromosomal mosaic rates have been estimated to be as high as approximately 20% at the blastocyst stage biopsy. (Dokras et al. 2018). Biopsy was performed using trophoblast cells rather than inner cell mass which was the true fetal sample. Accordingly, invasive prenatal testing for aneuploidy and chromosomal imbalanced arrangements was carried out to avoid the misdiagnosis due to the chromosomal abnormalities. Couples electing to have PGT-M do so generally to avoid the chance of having an affected pregnancy and undergo invasive prenatal diagnosis to confirm the accuracy of PGT procedure and chromosomal diseases after enough genetic counseling. (Line 256-269, Page 10-11).

Comment 5: Usher syndrome is autosomal recessive disorder. In this familie 3 generations are affected, which is eally exceptional. Do the authors have an explanation for this? Please mention in the discussion.

Response: Thank you for your comments. As reviewer suggested that the detail explanation was added in the discussion. (Line 221-238, Page 9-10).

Reviewer 2

Comment 1: The authors did not describe a biopsy procedure; this is all I see "...11 embryos were subsequently biopsied at the blastocyst stage". I think that more details will help.

Response: Thank you for your comments. As reviewer suggested that a biopsy procedure was added in the method section. (Line 119-127, Page 5). 21 mature oocytes underwent intracytoplasmic sperm injection (ICSI) and 11 embryos were obtained. Blastocysts biopsy was performed in embryos of grade 3 or higher according to the Gardner’s grading scale on day 6. Approximately five to ten trophectoderm cells were biopsied from each blastocyst. Embryos were vitrified immediately after blastocyst biopsy. Blastocysts biopsy cells samples underwent
multiple displacement amplification (MDA) using REPLI-g Midi kit (Qiagen) according to the instruction.

Comment 2: The clinical importance of this study is not clear. The c.10740+7G>A SNP in USH2A may or may not be pathogenic. However, the methodology looks promising thus the study looks attractive.

Response: Thank you for your comments. According to ACMG 2015 guideline, the c.10740+7G>A SNP in USH2A gene was classified to uncertain significance. Detailed evidence is as follows: PM2, this SNP at extremely low frequency which is below 0.5% in Exome Sequencing Project, 1000 Genomes and ExAC. PP4, patient’s phenotype and her family history are highly specific for Usher Syndrome with a single genetic etiology. Previous studies also supported the correlation between the USH2A gene and Usher Syndrome. In this case, affected patients were found in two generations. The clinical symptoms of patient’s father appeared at 30 years when she had been born. Her father was found to carry compound heterozygous mutations in the USH2A gene. Despite the low carrying rate of pathogenic mutation in normal population, the patient still inherited one pathogenic mutation from her mother who carried a heterozygous mutation in USH2A gene. Low-probability events increase pregnant women's determination to do PGT to avoid the Usher Syndrome in the third generation. In summary, although the clinical significance of c.10740+7G>A is not clear, this variant was predicted to affect the splicing process by MutationTaster, so the pathogenicity can’t be excluded. Since the family already suffers Usher Syndrome for two generations, the patient was unable to tolerate even a little risk for the third generation to avoid the Usher Syndrome. The young couple has a strong desire to receive PGT to prevent the embryo from inheriting c.10740+7G>A mutation from the husband after enough genetic counseling.

Comment 3: "...mutation taster..." is not good, the name of the program is "MutationTaster", the authors should add a reference.

Response: Thank you for your comments. We have revised and added a reference based on your advice (reference 18).

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked with revision mode. We appreciate for editors and reviewers’ warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Sincerely Yours.
Jun Sun