Abstract: VFS spring meeting, Brussels, Belgium (May 2012)

Reliability of using pre-implantation genetic diagnosis in a heteroplasmic mitochondrial mouse model

J. Neupane[1], M. Vandewoestyne[2], B. Heindryckx[1], S. Ghimire[1], Y. Lu[1], C. Qian[1], D. Deforce[2], P. De Sutter[1]

[1]Department for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

[2]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium

Introduction

Pathogenic mitochondrial DNA (mtDNA) mutations are usually present in a heteroplasmic form and transmission of such disorders is exclusively through the maternal line. Hence, it is important to evaluate whether pre-implantation genetic diagnosis (PGD) is accurate and reliable in distinguishing healthy oocytes and embryos from affected ones by measuring heteroplasmic load in polar bodies (PB) and blastomeres. In this study, heteroplasmic mice, carrying genotypes of NZB and BALB mtDNA, were used as a model to investigate the reliability of PGD.

Materials and methods

From 5 mice containing heteroplasmic mtDNA, 92 samples were analysed. First PBs were biopsied from metaphase II (MII) oocytes, which were fertilized by intra-cytoplasmic sperm injection (ICSI) to analyse second PB and zygotes. Parts of the fertilized oocytes were further cultured to harvest blastomeres from 2-cell, 4-cell and 8-cell embryos. The heteroplasmic load was measured by restriction fragment length polymorphism (RFLP) method. Correlation was verified between the heteroplasmic load of first PB and ooplasm of MII oocytes; between first PB, second PB and zygotes; and between first PB, second PB and individual blastomeres of cleavage stage embryos. Inter-blastomere variation within an embryo was also analysed at different embryonic stages. Data were analysed using Wilcoxon's Signed Rank test to check the significant difference. $p<0.05$ was considered significant. Coefficient of correlation (r) was calculated by Pearson's correlation test.

Results

Our results showed that there was no significant difference in levels of heteroplasmy between ooplasm of a mature oocyte (n=10) and the first PB (n=10) ($r=0.92$); between first PB (n=10), second PB (n=10) and zygotes (n=10) (r=0.907 and 0.922 respectively); between first and second PBs (r=0.88); and between first PB (n=7) and blastomeres (n=28) (r=0.968). However, there was significant difference in heteroplasmic level between second PB (n=7) and their corresponding blastomeres (r=0.70). The difference in heteroplasmic level ranged from 0.09 to 15 % between MII oocytes and first PBs; 0.34 to 12.48 % between first and second PBs; 0.16 to 8.81 % between zygotes and first PBs; 0.29 to 8.82 % between zygotes and second PBs, respectively. The inter-blastomere variation ranged from 0.57 to 7.99 %.

Conclusion

These data show that first PBs are more reliable than second PBs in diagnosing heteroplasmy level in oocytes and embryos in our heteroplasmic mouse model. Moreover, compared to first PBs, blastomeres give better predictive values of heteroplasmy level in embryos. Currently,

there is still controversy about the reliability of using PGD for human mitochondrial disorders. Thus, it will be important to confirm our mouse results in human patients.