P-12 Successful PGD for Trinucleotide Repeat Diseases of Huntington's Disease, Spinocerebellar Ataxia 3 and Fragile X Syndrome

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Objectives: Many neurological diseases are known to be caused by expansion of trinucleotide repeats, such as Huntington's disease (HD), Myotonic dystrophy (DM), spinocerebellar ataxia 3 (SCA3) and fragile X syndrome (FXS) have been applied suitable for preimplantation genetic diagnosis (PGD).

Methods: We performed the pre-clinical test of single cell PCR-PGD protocols with lymphocytes and the clinical PGD applications for biopsied blastomeres to detection of the respective polymorphic trinucleotide repeats in cases of HD, SCA 3 and FXS. Fluorescent nested PCR (fnPCR) method was used for HD and SCA 3 cases, and combining fnPCR and multiple displacement amplification (MDA) method for the FXS case, respectively. The MDA products were used to amplify the number of CGG repeats in addition to amelogenin for gender selection and two semi-informative linked markers in the PGD for FXS case.

Results: As a result of the pre-clinical test, amplification rates were 94.9% and 94.7% and allele dropout (ADO) rates were 17.1% and 5.6% in cases of HD and SCA3, respectively. In case of FXS using MDA method, amplification rate of CGG repeats was 84.2% and ADO rate was 31.3%. In the clinical PGD cycles, embryo biopsy was performed on 33 embryos. Successful diagnosis rate was 93.4% (31/33) and 10 embryos (32.3%) were diagnosed as unaffected. Among them, one embryo was transferred for HD, and three embryos for SCA 3 and FXS case, respectively. One clinical pregnancies of SCA 3 and one chemical pregnancy of FXS were achieved, and the result of PGD in SCA 3 case was confirmed by amniocentesis.

Conclusion: Our data presented mutiplex fluorescent PCR and MDA method successfully applied to PGD for trinucleotide repeat diseases. Various advanced methods for amplification of minuscule DNA sample could improve the sensitivity and reliability of PGD for complicated single gene disorders.