SUMMARY

- We report findings from a study assessing the performance and reproducibility of a new high-resolution method for fragile X preimplantation genetic diagnosis (PGD).
- The study utilized a set of well-characterized lymphoblastoid fragile X cell lines and novel methods that were tested by 2 different laboratories.
- A combination of whole genome amplification (WGA) and the AmplideX® PCR/CE FMR1 Kit (Asuragen) can detect repeat expansions from 1-5 fragile X cells.

INTRODUCTION

PGD methods are increasingly used to detect chromosomal abnormalities and genetic disorders relevant to in vitro fertilization (IVF). One such disorder is fragile X syndrome (FXS), the most common form of inherited intellectual disability. An estimated 1.5 million women in the US are fragile X carriers yet most are unaware of their carrier status. Currently, the identification of FXS by PGD is mainly limited to low-cell-count biopsies used in PGD. The study utilizes a set of well-characterized lymphoblastoid fragile X cell lines as a model for the low cell count biopsies used in PGD.

METHODS

Lymphoblastoid cells from 5 fragile X cell lines (one normal (NOR), two premutations (PM), and two full mutations (FM)) were tested by 2 different laboratories. Samples were also assessed using direct cell inputs into FMR1 PCR compared to matched cell equivalents of purified gDNA. Using an optimized WGA-based protocol, normal and expanded genotypes were detected from 1-5 cells in less than 48 hr on 3 different days.

RESULTS

- Across 2 laboratory sites, 92% of full-mutation cell lines with 5-cell inputs were accurately detected. Results were more consistent when intact cells rather than purified gDNA were used as the input into WGA.
- A combination of whole genome amplification (WGA) and the AmplideX® PCR/CE FMR1 Kit can genotype fragile X expansions from a single cell without WGA.

CONCLUSIONS

- A combination of WGA and the AmplideX PCR/CE FMR1 Kit can genotype fragile X expansions from 1-5 fragile X cells.
- Across 2 laboratory sites, 92% of full-mutation cell lines with 5-cell inputs were accurately detected. This result augurs PGD applications for FXS using OS/6 trophectoderm clinical biopsies.
- A further benefit of the approach is that it generates microgram quantities of WGA DNA amenable for other IVF-related genetic tests.
- In preliminary studies, a modified AmplideX PCR technology from a single cell without WGA showed a repeat-primed profile extending to >55 CGGs for expanded samples with clear distinction from normal sample or reaction without template (NTC) and a turnaround time <6 hours. In total, 5 cell lines were tested and all expanded alleles were detected with no dropouts.

REFERENCES

1. Chen L, Hadd A, Sah S, Filipovic-Sadic S, Krosting J, Sekinger E, Pan R, Hagerman PJ, Stenzel TT, Tassone F. An information-rich CGG template (NTC) and a turnaround time <6 hours. In total, 5 cell lines were tested and all expanded alleles were detected with no dropouts.

Figure 1. Overview of study design across 2 sites.

Figure 2. Comparison of intact cell inputs and cell-equivalent inputs of purified gDNA. WGA of intact cells demonstrated superior sensitivity, repeatability, and reproducibility after FMR1 PCR compared to matched cell equivalents of purified gDNA.

Figure 3. Summary of detection rates. FM alleles were accurately detected in 92% of replicate runs across the two sites when 5 cells were input into WGA. PCR and 3-cell inputs into WGA. FM alleles were detected at least 100 replicates, and in 5 cells at least 10 replicates were detected.

Figure 4. Example of sporadic random allele dropouts in WGA from intact cells. In a two-step amplification process, random allele dropouts occur during the WGA step as confirmed by controls used in the PCR step and consistent with previous publications. In addition, genotyping of a normal gDNA aliquot replicated random dropout in PCR.

Figure 5. Feasibility of direct PCR without WGA to detect expanded FMR1 alleles from single cells. Modified AmplideX PCR technology shows a repeat-primed profile reaching 105 CGGs for expanded samples with clear distinction from normal sample on reaction without template (NTC) and a turnaround time <6 hours. In total, 5 cell lines were tested and all expanded alleles were detected with no dropouts.