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ARTICLE

Validation of next-generation sequencing for comprehensive chromosome screening of embryos

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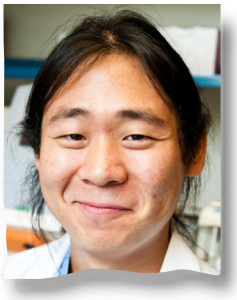

Allen Kung obtained his BSc degree in Biology at Pennsylvania State University. In 2011 he started clinical work in the field of preimplantation genetic diagnosis at Reprogenetics under the supervision of Santiago Munné. He has now moved onto becoming a clinical research associate for the company, being involved in many research projects as well as randomized clinical trials. His newest specialty is working on next-generation sequencing technology.

Abstract Massively parallel genome sequencing, also known as next-generation sequencing (NGS), is the latest approach for preimplantation genetic diagnosis. The purpose of this study was to determine whether NGS can accurately detect aneuploidy in human embryos. Low coverage genome sequencing was applied to trophectoderm biopsies of embryos at the blastocyst stage of development. Sensitivity and specificity of NGS was determined by comparison of results with a previously validated platform, array-comparative genomic hybridization (aCGH). In total, 156 samples (116 were blindly assessed) were tested: 40 samples were re-biopsies of blastocysts where the original biopsy specimen was previously tested for aCGH; four samples were re-biopsies of single blastomeres from embryos previously biopsied at the cleavage stage and tested using aCGH; 18 samples were single cells derived from well-characterized cell lines; 94 samples were whole-genome amplification products from embryo biopsies taken from previous preimplantation genetic screening cycles analysed using aCGH. Per embryo, NGS sensitivity was 100% (no false negatives), and 100% specificity (no false positives). Per chromosome, NGS concordance was 99.20%. With more improvement, NGS will allow the simultaneous diagnosis of single gene disorders and aneuploidy, and may have the potential to provide more detailed insight into other aspects of embryo viability.

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Introduction

Chromosomal abnormalities are a major contributor to human reproductive failure. Embryonic aneuploidy is associated with spontaneous abortion, with up to 70% of first-trimester miscarriages being aneuploid (Carp et al., 2001; Daniely et al., 1998; Garrisi et al., 2009; Hassold et al., 1980; Hodes-Wertz et al., 2012; Menasha et al., 2005; Munne et al., 2005; Qumsiyeh et al., 2000). In addition, embryonic aneuploidy is also associated with implantation failure (Gianaroli et al., 1999; Munne et al., 1993, 1999, 2004; Scott et al., 2013a). Furthermore, there is a significant increase in embryonic aneuploidy with advanced maternal age. A 40% aneuploidy rate has been reported in blastocysts from egg donors, but this rises to 85% in blastocysts from women 43 and older (Ata et al., 2012). Therefore embryonic aneuploidy is likely to be the main factor responsible for the concomitant decrease in implantation rates with advancing maternal age.

Preimplantation genetic screening (PGS) was proposed as a method to improve assisted reproductive technology (ART) outcomes by distinguishing chromosomally normal embryos from those with potentially lethal forms of aneuploidy (Munne et al., 1993). The first generation of PGS strategies involved the use of fluorescent in-situ hybridization (FISH) to screen for chromosome abnormalities in polar bodies and cleavage stage biopsies (Colls et al., 2007; Gianaroli et al., 1999; Magli et al., 2001; Munne et al., 1993, 1998, 1999; Rubio et al., 2013a; Verlinsky et al., 2005). However, FISH is limited to screening 3 to 12 chromosomes in each embryo biopsy specimen (Colls et al., 2009; Munne et al., 2010b). A 12-probe assay is only capable of identifying ~90% of the chromosomally abnormal embryos detectable by comprehensive chromosome screening (CCS) technologies (24-chromosome aneuploidy testing technologies) (Munne et al., 2010a). In addition, FISH accuracy is affected by variability in cell fixation skills (Velilla et al., 2002). More importantly, recent studies have shown that the implantation potential of cleavage-stage embryos can be hampered by the biopsy procedure, especially if performed under suboptimal conditions or by insufficiently skilled personnel (Mastenbroek et al., 2007; Munne et al., 2010b; Scott et al., 2013b). A meta-analysis of clinical trials using FISH to screen cleavage stage biopsied embryos showed no benefit (Mastenbroek et al., 2011), although such meta-analysis did not take into consideration biopsy conditions.

Comparative genome hybridization (CGH) was the first technology to allow PGS of all 24 types of chromosome (Voullaire et al., 2000; Wells and Delhanty, 2000; Wells et al., 1999, 2002; Wilton et al., 2001) and the first to show improved ART outcome (Schoolcraft et al., 2010). Second-generation PGS is the combination of improvements in genetic testing and advances in embryological practice. These include extended embryo culture (Gardner and Lane, 2003; Gardner and Schoolcraft, 1999; Gardner et al., 1998), blastocyst biopsy following breach of the zona pelucida (McArthur et al., 2005) and, in some cases, vitrification of embryos, with transfer to the uterus taking place in a subsequent cycle (Cobo et al., 2012; Kuwayama, 2007; Kuwayama et al., 2005; Stehlik et al., 2005).

Currently, second-generation PGS can involve chromosomal testing using any of several alternative techniques. These PGS methods, including microarray comparative genomic hybridization (aCGH), single nucleotide polymorphism microarrays (SNP arrays), and quantitative polymerase chain reaction (qPCR) have been validated and applied clinically (Colls et al., 2012; Gutierrez-Mateo et al., 2011), (Schoolcraft et al., 2011), (Treff et al., 2012). Multiple randomized clinical trials (Forman et al., 2013; Rubio et al., 2013a, 2013b; Scott et al., 2013a; Yang et al., 2012) have shown a significant increase in implantation and ongoing pregnancy rates when second-generation PGS was used. Furthermore, the usual decline in the likelihood of embryo implantation with advancing female age is not observed when PGS is used and transfer is limited to euploid blastocysts. This confirms aneuploidy to be the principal cause of the well-documented age-related decline in embryo competence (Harton et al., 2013).

Next-generation sequencing (NGS) is the newest technique to be incorporated into second-generation PGS. NGS is fundamentally built around the ability to massively parallel sequence small DNA fragments, until the required depth of coverage (number of sequence reads covering a genome) is achieved. For CCS, only a low amount of coverage is needed to accurately assess aneuploidy (Wells et al., 2014).

Compared with the other PGS methods, NGS has the advantage that it could potentially test for both aneuploidy and monogenic diseases simultaneously. In contrast, the other PGS techniques only detect chromosome abnormalities. Even a very recent technique, karyomapping (Natesan et al. 2014), an SNP array-based technology, which can test for single gene defects and detects most aneuploidies of meiotic origin, fails to detect some mitotic chromosome abnormalities (Konstantinidis et al., 2014; Prates et al., 2014). Microarray CGH and SNP arrays are compatible with the processing of high sample volumes, but are constrained in terms of the number of samples that can be tested using an individual array slide, and each additional slide increases the cost of the test proportionally. While qPCR is the fastest technique, it is not ideally suited for high-throughput applications and is hampered by an extremely low resolution. For example, while aCGH can detect most imbalances caused by reciprocal translocations without any change of protocol (Alfarawati et al., 2012; Colls et al., 2012), application of qPCR for this purpose usually requires addition of extra sets of PCR primers, followed by costly re-validation of the test.

aCGH and SNP-arrays rely on whole-genome amplification (WGA) to generate sufficient DNA from biopsied cells for subsequent analysis, whereas qPCR utilizes a sequence-specific targeted approach. Any DNA amplification method applied to single cells risks the introduction of allele drop out (ADO: failure to successfully amplify both alleles of a locus). ADO is particularly problematic for preimplantation genetic diagnosis (PGD) of monogenic diseases, where failure to detect each copy of a mutant gene can potentially result in a misdiagnosis. Both WGA and targeted PCR approaches can be used to amplify sufficient DNA from the sample for subsequent PGD or PGS using NGS (Chen et al., 2014a, 2014b; Fiorentino et al., 2014a; Treff et al., 2013; Wang et al., 2014b; Yin et al., 2013).

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