Reproductive BioMedicine Online (2015) 31, 62–70

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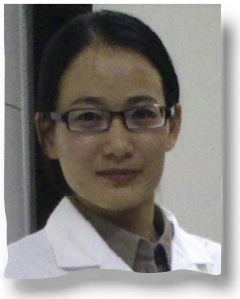
ARTICLE

The clinical utility of next-generation sequencing for identifying chromosome disease syndromes in human embryos

Junmei Fan [a,1], Li Wang [a,1], Hui Wang [a], Minyue Ma [a], Shufang Wang [b], Zhongyu Liu [a], Genming Xu [c], Jianguang Zhang [c], David S Cram [c,*], Yuanqing Yao [a,*]

[a] *Department of Obstetrics and Gynecology, Chinese PLA General Hospital, 28 Fuxin Road, Haidian District, Beijing 100853, China;* [b] *Department of Hematology, Chinese PLA General Hospital, 28 Fuxin Rd, Haidian District, Beijing 100853, China;* [c] *Berry Genomics, C., limited, Building 9, No 6. Jingshun East Street, Chaoyang District, Beijing 100015, China*

* Corresponding authors. *E-mail addresses:* david.cram@berrygenomics.com (DS Cram); yqyao_ghpla@163.com (Y Yao). [1] These authors contributed equally to this work.



Junmei Fan obtained her MSc in Obstetrics and Gynecology with a thesis on placental disease at Shanxi Medical University, China, in 2011. She now is a PhD student at the Department of Obstetrics and Gynecology, Chinese PLA General Hospital. Her clinical major is in reproductive medicine, with a research focus on next-generation sequencing technologies for pre-implantation genetic diagnosis.

Abstract Next-generation sequencing is emerging as a reliable and accurate technology for pre-implantation genetic diagnosis (PGD) of aneuploidies and translocations. The aim of this study was to extend the clinical utility of copy number variation sequencing (CNV-Seq) to the detection of small pathogenic copy number variations (CNVs) associated with chromosome disease syndromes. In preliminary validation studies, CNV-Seq was highly sensitive and specific for detecting small CNV in whole-genome amplification products from three replicates of one and five cell samples, with a resolution in the order of 1–2 Mb. Importantly, the chromosome positions of all CNV were correctly mapped with copy numbers similar to those measured in matching genomic DNA samples. In seven clinical PGD cycles where results were obtained for 34 of 35 blastocysts, CNV-Seq identified 18 blastocysts with aneuploidies, one with an aneuploidy and a 4.98 Mb 5q35.2-qter deletion associated with Sotos syndrome, one with a 6.66 Mb 7p22.1-pter deletion associated with 7p terminal deletion syndrome and 14 with no detectable abnormalities that were suitable for transfer. On the basis of these findings, CNV-Seq displays the hallmarks of a comprehensive PGD technology for detection of aneuploidies and CNVs that are known to affect the development and health of patient's embryos.

http://dx.doi.org/10.1016/j.rbmo.2015.03.010
1472-6483/

KEYWORDS: aneuploidy, chromosome disease, copy number variation (CNV), next generation sequencing (NGS), whole genome amplification (WGA)

Introduction

Aneuploidy, defined as a change in normal chromosome copy number, is the major genetic cause of IVF failure in infertile patients undergoing assisted reproductive treatment (Wilton, 2002). In patients with a poor prognosis for pregnancy such as couples in which the female partner is of advanced maternal age (>35 years) or has experienced multiple implantation failure, the frequency of aneuploid embryos in the cohort can sometimes exceed 50% (Harper et al., 2012; Wilton, 2002). Chromosome, chromatid non-disjunction during either meiosis I and II in gametes, during mitosis in cleavage divisions of the pre-implantation embryo, or both (Kuliev and Verlinsky, 2004; Kuliev et al., 2011; Nagaoka et al., 2012), is largely responsible for the formation of whole chromosome aneuploidies such as trisomies and monosomies. Segmental imbalances or partial aneuploidies can also arise in the early embryo by mechanisms such as breakage-fusion-bridge cycles (Voet et al., 2011). Most monosomies are embryonic lethal causing either growth arrest or implantation failure (Kuliev and Verlinsky, 2004; Wilton, 2002). On the other hand, most embryos with trisomies fail to implant (Kuliev and Verlinsky, 2004). In those aneuploid embryos that do implant, the resulting fetus can develop during the first trimester of pregnancy but usually succumbs by spontaneous abortion. Occasionally, some fetuses with Turner and Down's syndrome can reach full term (Hassold and Hunt, 2001) and are viable.

In current clinical practice, patients at high risk for producing aneuploid embryos can undertake pre-implantation genetic diagnosis (PGD) using either 24-chromosome array or real-time polymerase chain reaction (PCR) technologies (Handyside, 2013; Munne, 2012; Treff et al., 2012) to identify euploid embryos for transplantation to the uterus. More recently, next-generation sequencing (NGS) is emerging as a powerful technology to identify chromosomal abnormalities in oocytes (Hou et al., 2013) and embryos (Fiorentino et al., 2014a, 2014b; Wang et al., 2014a, 2014b, 2014c; Wells et al., 2014; Yin et al., 2013) at a much higher chromosomal resolution than arrays. Proof of concept NGS validation studies have now been conducted for a range of whole and partial aneuploidies (Fiorentino et al., 2014a; Wang et al., 2014b; Wells et al., 2014) and unbalanced Robertsonian and reciprocal translocations (Wang et al., 2014b; Yin et al., 2013). In a recent report (Wang et al., 2014c), we applied a NGS method called copy number variation sequencing (CNV-Seq) as the primary diagnostic method for a patient with repeated implantation failure, achieving an ongoing normal pregnancy that was confirmed by non-invasive prenatal diagnosis and has since resulted in a healthy live birth.

In addition to aneuploidies, copy number variations (CNVs) are common in the human and have an embryonic origin (Schaaf et al., 2011; Vanneste et al., 2012). Most (>99%) CNV is benign, with the remainder associated with clinically significant chromosome disease syndromes (Klopocki and Mundlos, 2011). Following successful diagnosis of chromosome disease syndromes in patients by CNV-Seq (Liang et al., 2014), we speculated that the same method, in conjunction with a robust whole-genome amplification (WGA) step, may have sufficient resolution for detection of CNV in low template DNA, such as the amount present in an embryo biopsy sample. In this study, we examined the feasibility of using CNV-Seq for identifying CNV in PGD embryos by first modelling an embryo biopsy sample using defined numbers of single cells with known CNVs.

Materials and methods

Study samples

The Ethics Committee of Chinese PLA General Hospital approved the clinical research study (S2013-092-02, 25 November, 2013), and patients provided written informed consent. For validation studies, three genomic DNA samples harbouring known chromosome disease CNVs (range of 6.52–93.02 Mb) identified by array comparative genomic hybridization were selected for the study. In addition, single lymphocytes were also sourced from five peripheral blood samples carrying small pathogenic CNVs (range 0.56–15.4 Mb) that were previously identified at the genomic DNA level by CNV-Seq (Liang et al., 2014). For clinical studies, CNV-Seq was used to analyse 34 embryos from seven patients undergoing PGD by blastocyst biopsy.

IVF procedures

For ovarian stimulation, GnRH-agonist long protocol was used. Triptorelin acetate (Ferring Pharmaceuticals, Kiel, Germany) was administered at a dose of 0.1 mg per day, starting from the mid-luteal phase (progesterone levels >30 nmol/l), to day 2 of the next menstrual cycle (serum oestradiol levels <50 pg/ml) and when follicular structures were less than 1 cm in diameter. A dose of 150–300 IU of recombinent FSH (Merck Serono Darmstadt, Germany) was then administered, based on women's weight, age, antral follicle counts and serum FSH levels. When at least three follicles had a mean diameter of 18 mm, 10,000 IU of HCG (Livzon, Zhuhai, Guangdong, China) was injected, and 34–36 h later, oocytes were retrieved by vaginal guided-ultrasound. Oocytes were harvested in G-GAMETE media (Vitrolife AB, Goteborg, Sweden) supplemented with 20% human serum albumin (Vitrolife AB, Goteborg, Sweden). After removal of granulosa cells, all mature oocytes were subjected to intracytoplasmic sperm injection. One-cell embryos were cultured to the blastocyst stage, biopsied and cryopreserved as previously described (Wang et al., 2014b).

Micromanipulation of single cells

Single lymphocytes were isolated from frozen peripheral blood samples. After thawing, 10 μl of blood was diluted 1:10 with 90 μl of phosphate buffered saline (PBS) and 100 μl erythrocyte lysing solution (Probe, Beijing, China) added,