Evaluation of Comprehensive Chromosome Screening Platforms for the Detection of Mosaic Segmental Aneuploidy

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ABSTRACT

Advancements in the efficacy of comprehensive chromosomal screening (CCS), namely, the ability to detect aneuploidy in all 24 human chromosomes, have established it as a cornerstone of infertility care. Recently, the development of high-throughput, massively parallel sequencing for use with CCS has led to highly accurate, simultaneous screening of multiple samples. Such advances have led to the ability to diagnose segmental aneuploidies when screening embryos for subchromosomal imbalances. Recent findings have shown that segmental aneuploidy may be commonly found in a mosaic state. Segmental aneuploidy within a mosaic embryo may be detectable using contemporary CCS on a single trophectoderm biopsy containing multiple cell lines from the blastocyst.

This study aimed to compare the specificity and sensitivity of 3 commercially available CCS platforms for detection of segmental aneuploidy observed in a mosaic sample. Two cell lines (a female line containing a 16.2-Mb deletion on chromosome 5 and a male cell line containing a 25.5-Mb deletion on chromosome 4) were used. These lines were mixed together at predetermined ratios of 6 total cells (0:6, 1:5, 2:4, 3:3, 4:2, 5:1, and 6:0). The 3 CCS methods examined were VeriSeq PGS, NexCCS, and SNP-array. Blinded computational segmental aneuploidy predictions were then made to evaluate the sensitivity and specificity of each platform, which were compared with expected results based on known karyotyping of the cell lines.

This study found the ability to predict an accurate abnormality was 17% with custom VeriSeq, 50% with custom NexCCS, and 50% with SNP-array. All 3 platforms had increased detection of segmental errors as the percentage of aneuploid cells increased in the mixture. Comparison of NexCCS and SNP found no significant differences at 17%, 33%, 83%, and 100% mosaicism; however, at 50% (P = 0.0093) and 66% (P = 0.0284), statistically significant differences were found. Comparison of VeriSeq and NexCCS found no significant differences at greater than 50%; however, at 17% (P = 0.119), 33% (P = 0.0119), and 50% (P = 0.0119), statistically significant differences were found. Comparison between SNP and VeriSeq found no statistically significant differences were found. Specificity was found to be 93% for SNP-array (P = 0.0126), and 100% for NexCCS and VeriSeq. When previously published custom VeriSeq analysis was applied, there was significantly improved sensitivity at detecting aneuploidy between 17% and 66% (P < 0.05), but an increase in the false-positive rate from 7% using SNP-array and 0% using NexCCS and VeriSeq to 67%.

This study indicates potential for NGS-based detection methods to diagnose segmental mosaicism within a single biopsy. The results also show that before implementation in a clinical setting, further research is needed to carefully evaluate custom analysis criteria, specifically to balance specificity and sensitivity, when considering detection criteria of segmental aneuploidy in a mosaic sample.

EDITORIAL COMMENT

(Widespread application to IVF of comprehensive chromosomal screening (CCS), that is, detection of aneuploidy in all 24 human chromosomes, has increased transfer pregnancy and decreased miscarriage rates. However, these new methods increasingly identify mosaicism in preimplantation embryo biopsies. Segmental aneuploidy, a condition in which part of a chromosome is abnormal, may be viable in some cases. Apparently, normal offspring have resulted from transfer of embryos with segmental mosaicism, albeit at reduced rates and with poorly understood risk of fetal or newborn anomalies. A number of platforms to conduct CCS on a single trophectoderm
biopsy are available, including VeriSeq PGS, NexCCS, and SNP-array. These methods may differ in their ability to accurately detect mosaicism. Zimmerman et al compared the specificity and sensitivity of 3 CCS platforms to detect segmental aneuploidy in cultured cells containing various mixtures of euploid and aneuploid cells. Aneuploid and euploid cell lines were mixed at different ratios, 0:6, 1:5, 2:4, 3:3, 4:2, 5:1, and 6:0, then analyzed by the 3 methods. Increasing the proportion of aneuploid cells increased sensitivity of all 3 assays. NexCCS and SNP were similar at 17%, 33%, 83%, and 100% mosaicism, but different at 50% and 66% mosaicism. VeriSeq and NexCCS were similar at greater than 50% mosaicism, but differed at 17%, 33%, and 50% mosaicism. SNP and VeriSeq did not differ at 83% or 100%, but did differ at 33%, 50%, and 66% mosaicism. Specificity was 93% for SNP-array, and 100% for NexCCS and VeriSeq. Thus, methods of analyzing segmental mosaicism differ in their sensitivity and specificity, thus explaining disparate results from different platforms.—DK

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**Risks of Ovarian, Breast, and Corpus Uteri Cancer in Women Treated With Assisted Reproductive Technology in Great Britain, 1991–2010: Data Linkage Study Including 2.2 Million Person Years of Observation**

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**ABSTRACT**

Assisted reproductive therapy such as exposure of women to supraphysiological levels of estradiol, exogenous gonadotropins, and multiple ovarian punctures are suspected to incur an increased risk of cancer. Studies investigating the carcinogenic effects of these exposures are largely inconsistent. Specific concern surrounds the potentially increased risks of breast, endometrial, and ovarian cancers.

This large population-based cohort study aimed to provide risk estimates for ovarian, breast, and corpus uteri cancer in women undergoing assisted reproduction. Data were obtained on all women undergoing assisted reproduction between 1991 and 2010 in Great Britain through the Human Fertilisation and Embryology Authority (HFEA). UK law mandates reporting of all assisted reproduction cycles to the HFEA, making this a very robust representation of the target population. Data on cancer prevalence in this population were obtained from the National Health Service Central Registers of England, Wales, and Scotland, and these were linked to HFEA records through a one-off linkage. The HFEA database was also used to obtain data on potential confounding variables including demographics, infertility diagnosis, parity, and treatment details. Follow-up was calculated from the date of first treatment to the date of any cancer diagnosis, death, emigration, or end of study. National incidence rates and person years of expected risk were used to calculate expected cancer rates. Standardized incidence ratios (SIRs) were calculated by comparison of observed outcomes versus expected outcomes.

A total of 255,786 women contributed 2,257,789 person years of follow-up for analysis. Median follow-up duration was 8.8 years, and median age at first treatment was 34.5 years. Subjects had a median of 1.8 stimulated cycles with 50,485 (20%) having more than 2 stimulated cycles. No increase in risk of breast cancer overall (SIR, 0.98; 95% confidence interval