Frequencies of chromosome-specific mosaicisms in trophoectoderm biopsies detected by next-generation sequencing

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Objective: To examine the chromosome-specific frequencies of mosaicism detected by next-generation sequencing (NGS) compared with constitutional aneuploidy.
Design: Retrospective cross-sectional review of NGS results from trophectoderm biopsies analyzed by per-chromosome prevalence of mosaicism and constitutional aneuploidy.
Setting: Private fertility clinic.
Patient(s): A total of 378 patients who underwent preimplantation genetic screening by NGS for routine clinical indications from February 2016 to April 2017.
Intervention(s): None.
Main Outcome Measure(s): Aneuploidies and mosaicisms were tabulated per chromosome, and whole-chromosome and segmental mosaicisms were also analyzed.
Result(s): NGS results were analyzed from 1,547 blastocysts. Mosaicism was detected as the sole abnormality in 17.5% (n = 270) of samples but were also found in 196/634 aneuploid embryos, so the overall incidence of mosaicism per biopsy was 30.1%. Mosaicism did not statistically vary when stratified by maternal age. The mean rate of overall mosaicism per chromosome was 2.46%. When whole chromosome and segmental mosaicisms were compared, unequal frequencies were found in several chromosomes. Trisomy was more frequently detected as whole-chromosome mosaicism, although monosomy was more frequently seen in segmental mosaicism. Aneuploidy and mosaicism displayed different patterns of distribution in various chromosomes.
Conclusion(s): Mosaicism is unequally detected in various chromosomes and appears distinct from the distribution pattern of constitutional aneuploidy. Whole chromosome and segmental mosaicisms are also differentially detected. These results contribute to the study of mosaicism, illuminating a differential pattern of detection across the genome. (Fertil Steril® 2018;109:857–65. ©2018 by American Society for Reproductive Medicine.)
Key Words: Mosaicism, PGS, CCS, aneuploidy, NGS

Mosaicism is the coexistence of two or more cell lines with differing chromosomal complements. Caused by mitotic segregation errors during somatic division, it can be generalized or isolated to specific organ systems and associated with pathology, but it is also found in healthy individuals (1). Mosaicism was documented when G-banding was used to investigate karyotypes in 6–8-day-old blastocysts (2) and by fluorescence in-situ hybridization (FISH) at the cleavage stage (3). After the significant limitations of FISH for preimplantation genetic screening (PGS) were recognized, newer molecular cytogenetic techniques, such as single-nucleotide polymorphism (SNP) array, array comparative genome hybridization (aCGH), and quantitative polymerase chain reaction (PCR), were introduced for 24-chromosome copy number analysis. Most recently, next-generation sequencing (NGS) has been applied to PGS as a potentially more efficient and affordable technique (4). In contrast to the other molecular techniques, which are relatively less

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sensitive to mosaicism, NGS has been reported to detect mosaicism in ~30% of trophectoderm (TE) specimens (5). The detection limit for the proportion of aneuploid cells appears to be dependent on the NGS protocol and has been reported to be as low as 10%–16%, although lower detection limits are associated with greater diagnostic inaccuracy (6). According to the Preimplantation Genetic Diagnosis International Society (PGDIS), embryos with <20% aneuploid cells may be considered euploid and >80% aneuploid cells suggest constitutional aneuploidy; thus, embryos with aneuploid percentages of 20%–80% could be classified as mosaic (7). Consequently, NGS has catalyzed a renewed interest in the phenomenon of embryonic mosaicism, stirring controversy about the significance of these findings, especially regarding the viability of mosaic embryos and the possible manifestations of the aneuploid cell lines.

A few publications have demonstrated that a minority of mosaic blastocysts can result in live birth, albeit with lower implantation rates and higher spontaneous abortion (SAB) rates (6, 8, 9). However, given the relative novelty of NGS-detected mosaicism in PGS blastocysts, long-term follow-up has yet to be reported. Pathologic manifestations of mosaicisms are well documented, creating understandable concern regarding the outcomes of mosaic embryo transfers. On the other hand, there is no deterministic evidence to predict how embryonic mosaicism will manifest, and in fact, reasonable evidence to suggest that many embryonic mosaicisms may be clinically irrelevant owing to mechanisms such as self-correction, apoptosis, or preferential allocation (10). Furthermore, there is concern that the increased frequency of mosaicism may be an artifact of NGS technology and may lead to the discarding of potentially viable embryos (11).

Contributing to the controversy is the fact that there are several presentations of mosaicisms in TE cells, including whole-chromosome, segmental (or partial), and complex. It has been proposed that some mosaic embryos may be more suitable to transfer than others, as a function of the specific chromosomes that are affected and the degree of mosaicism defined by the percentage of aneuploid cells (7, 12).

Although the general prevalence of mosaicism in TE has been reported, there is a paucity of published data regarding how mosaicism affects specific chromosomes, as has been reported with constitutional aneuploidy (13–15). Also, the frequency of whole-chromosome, segmental, and complex mosaicisms as detected by NGS is not well documented. Therefore, we reviewed the NGS results of our PGS cohort to examine the patterns and prevalence of chromosome-specific mosaicisms in TE samples. In addition, because aneuploidy and mosaicism are attributed to different mechanisms, we compared the chromosome-specific aneuploidies rates with those of mosaicisms to determine if distinct distributions are exhibited. We hypothesized that documenting the chromosome-specific frequencies of mosaicism may elucidate the clinical significance of mosaicism and provide insight to the biologic and technical factors that lead to its detection.

METHODS

We performed a retrospective cross-sectional review of all NGS results in patients presenting for PGS from February 2016 to March 2017. Ovarian stimulation was performed with a combination of highly purified urinary gonadotropin (Menopur; Ferring) and recombinant FSH (Puregon; Merck) in an antagonist protocol (Orgalutran; Merck). Final maturation was triggered with 5–10 IU hCG or GnRH agonist (Decapeptyl; Ipsen) according to physician discretion. Transvaginal oocyte retrieval was performed 35 hours after trigger. Oocytes were denuded and intracytoplasmic sperm injection was performed 4 hours after retrieval and cultured to day 3 in Global-Plus media (Life Global). Assisted hatching of viable cleavage stage embryos was performed on day 3 with the Zilos-tk Zona Infrared Laser Optical System (Hamilton-Thorne), and culture was continued in individual droplets until day 5 or 6, at which time biopsy was performed on blastocysts with a viable grade, generally considered to be 3CC or greater according to Gardner scoring (16). In HEPES supplemented with 20% serum, micromanipulation and biopsy were performed with the use of laser pulses to release five to ten TE cells for analysis, which were transferred into microcentrifuge tubes and shipped to Reprogenetics, Los Angeles.

According to Reprogenetics protocol, PGS was performed as follows. Whole-gene DNA amplification (WGA) from the TE cells was performed with the Sureplex Amplification System (Illumina). WGA was followed by tagmentation of DNA and subsequent PCR reaction of adapter sequences for amplification of insert DNA to add index sequences. Dual-indexed sequencing of pooled libraries was performed on Illumina MiSeq with the use of the Veriseq PGS system (Illumina). Copy number analysis was made with the use of Bluefuse Multi software (Bluefuse/Illumina). Mosaic calls were made when 20%–80% of the cells were aneuploid. Validation of mosaicism detection has been previously documented with a series of mixing experiments using known proportions of euploid and aneuploid cell lines, ranging from 0 to 100% (6).

Statistical analysis was performed with the use of R v. 3.3.2. Comparisons of equal frequency were performed with the use of chi-square goodness-of-fit test. When stratified by age group, chi-square test of independence was performed to determine whether age was related to other factors. One-way analysis of variance was performed when comparing mean error rate of different chromosome structures. In all cases, statistical significance was considered to be at $P<.05$. The study was approved by the University of British Columbia Institutional Review Board.

RESULTS

A total of 1,582 blastocysts were biopsied from 378 patients who underwent 448 egg retrievals. The average age of the patient was 36.54 (range 28–47). Thirteen TE specimens could not be processed owing to degraded DNA and 22 were reported as "no diagnosis," resulting in a 97.8% diagnosis rate (n = 1,547).

Of the embryos, 643 (41.6%) were classified as euploid, 634 (40.9%) aneuploid, and 270 (17.5%) mosaic. Furthermore, of the 634 aneuploid specimens, 196 were also affected by