CORRESPONDENCE

Detection of Structural Rearrangements in Embryos

TO THE EDITOR: Carriers of balanced chromosomal rearrangements (BCRs) are at risk for infertility, recurrent miscarriages, and abnormal offspring because of unbalanced rearrangements. A small subgroup of carriers is at risk for neurodevelopmental or neuropsychiatric conditions.[1] Preimplantation genetic testing for structural rearrangements (PGT-SR) allows for the preselection of embryos without chromosomal gains or losses.[2] However, next-generation, low-pass, whole-genome sequencing — the current standard for preimplantation aneuploidy (PGT-A) screening of cells from an embryo biopsy — cannot distinguish between an embryo that carries a BCR and one that does not and thus does not provide information to the prospective parents that would enable the prevention of vertical transmission of the BCR to future generations and the detection of cryptic potential disease-causing imbalances, complex rearrangements, or gene disruptions at breakpoint sites. Indirect approaches to genetic-linkage analysis used in PGT-SR, such as single-nucleotide polymorphism (SNP) array or sequencing-based haplotyping, are labor intensive, costly, and limited to familial cases of BCRs because DNA from family members who are known BCR carriers or from embryos with chromosomal unbalances resulting from BCR is required to enable haplotype phasing.[3] In addition, such approaches cannot detect potential disease-causing cryptic microdeletions or microduplications.[3] Another technique, mate-pair next-generation sequencing — also labor intensive — can delineate chromosomal structural rearrangements with abnormal phenotypes but may not perform well when the breakpoint is in a highly repetitive genomic region.[4]

We conducted a study to show that we can accurately discriminate embryos that carry a BCR from those that do not, as well as detect the presence of cryptic imbalances and complex rearrangements, using PGT-SR with the MinION long-read nanopore sequencer (Oxford Nanopore Technologies) (Fig. 1). This method involves the direct sequencing of long and ultra-long DNA strands (between tens and hundreds of thousands of kilobases) in real time, with minimal capital cost and user time, on a small hand-held platform.[5] Using this technology, we clinically validated a karyotype-guided PGT-SR protocol by means of low-coverage, long-read sequencing to identify breakpoints, which we manually reviewed and then validated by means of polymerase-chain-reaction and Sanger sequencing.

Eleven couples, with one partner who was a BCR carrier, underwent in vitro fertilization at the CReATe Fertility Centre in Toronto between 2016 and 2018. Of the 11 couples, 9 underwent in vitro fertilization with the use of PGT-SR be-

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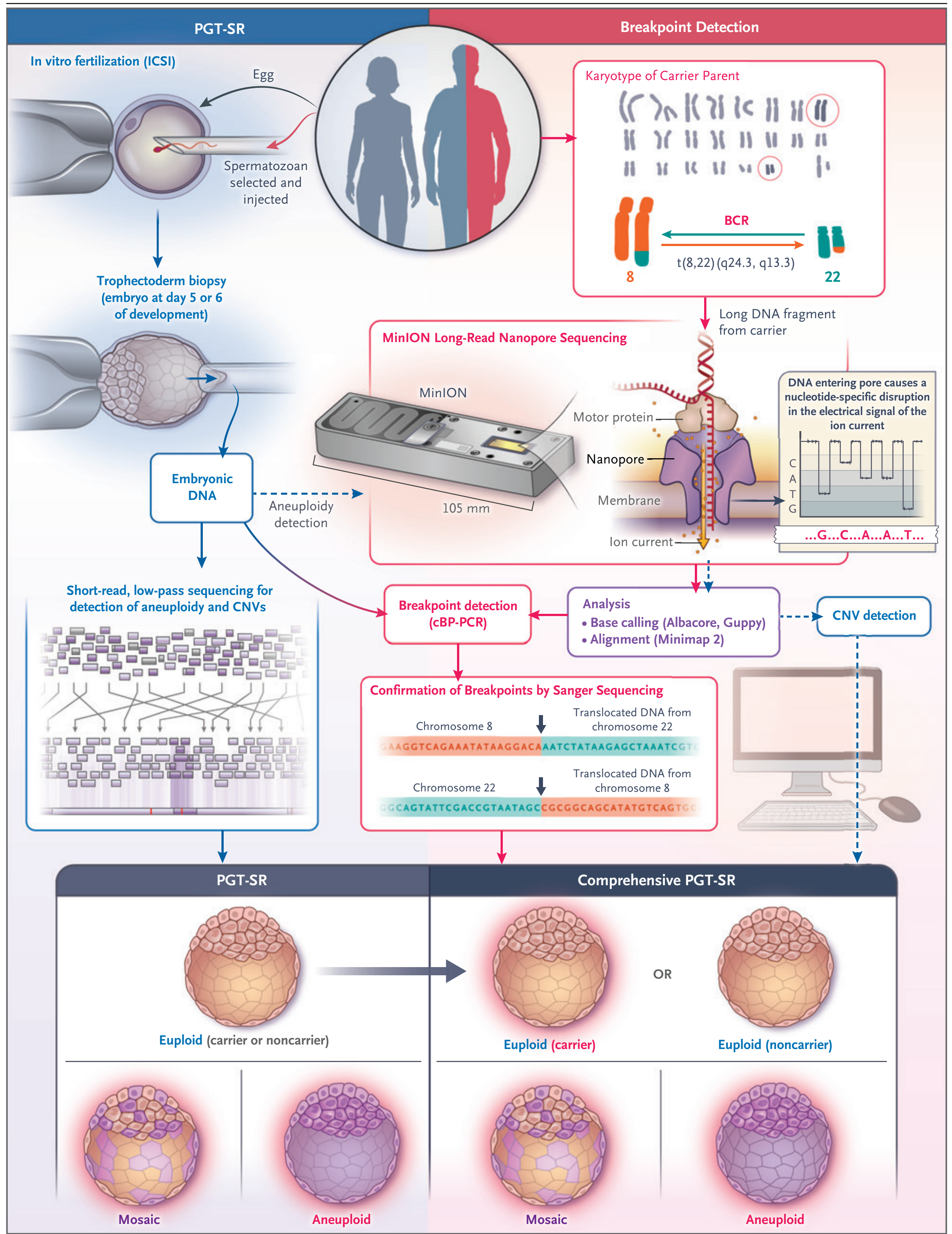

PGT-SR
Breakpoint Detection
In vitro fertilization (ICSI)
Egg
Spermatozoan selected and injected
Trophectoderm biopsy (embryo at day 5 or 6 of development)
Embryonic DNA
Aneuploidy detection
Short-read, low-pass sequencing for detection of aneuploidy and CNVs
Karyotype of Carrier Parent
BCR
t(8,22) (q24.3, q13.3)
8
22
Long DNA fragment from carrier
MinION Long-Read Nanopore Sequencing
MinION
105 mm
Motor protein
Nanopore
Membrane
Ion current
DNA entering pore causes a nucleotide-specific disruption in the electrical signal of the ion current
C
A
T
G
...G...C...A...A...T...
Breakpoint detection (cBP-PCR)
Analysis
• Base calling (Albacore, Guppy)
• Alignment (Minimap 2)
CNV detection
Confirmation of Breakpoints by Sanger Sequencing
Chromosome 8
Translocated DNA from chromosome 22
GAAGGTCAGAAATATAAGGACAAATCTATAAGAGCTAAATCGT
Chromosome 22
Translocated DNA from chromosome 8
GGCAGTATTCGACCGTAATAGCCGCGGCAGCATATGTCAGTG
PGT-SR
Comprehensive PGT-SR
Euploid (carrier or noncarrier)
OR
Euploid (carrier)
Euploid (noncarrier)
Mosaic
Aneuploid
Mosaic
Aneuploid

Figure 1 (previous page). Preimplantation Genetic Testing for Structural Rearrangements with the Use of Long-Read Nanopore Sequencing. The red arrows indicate the approach used for the delineation of balanced translocations and inversions after long-read DNA sequencing is performed with the MinION nanopore sequencer (Oxford Nanopore Technologies); such delineation allows for breakpoint confirmation by means of a polymerase-chain-reaction (PCR) assay customized to the breakpoint (cBP-PCR), together with single-base resolution of the breakpoint by means of Sanger sequencing. High-molecular-weight DNA extracted from balanced chromosomal rearrangement (BCR) carriers was fragmented, and libraries were prepared for long-read sequencing with the use of the SQK-LSK108 ligation kit (Oxford Nanopore Technologies). Long-read sequencing was performed on the MinION sequencer over 48 hours. The average length of the sequenced fragments was approximately 8 to 10 kb, with the longest reads reaching over 100 kb. Karyotype-guided computational analysis identified derivative reads and mapped breakpoints. The use of custom-designed PCR primers and Sanger sequencing yielded confirmation of breakpoints within 7 days after the sample was submitted. The blue arrows indicate the approach used for preimplantation testing for structural rearrangements (PGT-SR) with short-read sequencing, which can detect only chromosomal gains and losses (and not BCRs) in embryos. Cells from in vitro fertilized embryos of couples undergoing PGT-SR were biopsied on day 5 or 6 of development (before vitrification) to obtain DNA for aneuploidy (chromosomal copy-number variation [CNV]) detection and for the discrimination of embryos that do not carry a BCR from those that do with the use of the custom breakpoint PCR designed for the BCR-carrier parent. The blue dashed arrows indicate an approach in which embryonic DNA obtained from the same trophectoderm biopsy sample can also be used for aneuploidy detection by means of MinION sequencing and an algorithm for CNV analysis. ICSI denotes intracytoplasmic sperm injection.

cause of a known BCR (seven reciprocal translocations, one pericentric inversion, and one paracentric inversion), and the other 2 couples involved patients in whom a balanced translocation was incidentally discovered after aberrations indicative of a BCR were detected on routine PGT-A analysis. MinION sequencing identified the precise breakpoints in all of the 11 BCR carriers (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Biopsy of the trophectoderm (four to six cells) was performed on 82 blastocysts from these 11 couples, followed by whole-genome amplification with the use of the SurePlex DNA Amplification System (Illumina) or the REPLI-g kit (Qiagen) on the trophectoderm DNA. Standard PGT-SR was performed on all the cell samples from the embryos by means of short-read next-generation sequencing–based testing with the VeriSeq PGS (preimplantation genetic screening) kit and the Karyomapping SNP array (Illumina) (Fig. S3). Of the 82 blastocysts, 27 (33%) were euploid and the rest 55 (67%) were aneuploid or unbalanced (Table S1).

Using a comprehensive approach of PGT-SR with MinION sequencing, we found that 11 of the 27 euploid embryos (41%) carried a BCR; the remainder (16 [59%]) tested negative. We were thus able to distinguish BCR carriers from noncarriers and map the breakpoints with single-base resolution in the embryos that were carriers. These results were confirmed through haplotype phasing and with the genotypes of the six children born to date from this cohort. Concurrent aneuploidy detection is also possible with the use of the MinION device, and the results from eight representative cases are presented in Figure S2 and Table S3. The results of this study show that a clinical application of long-read nanopore sequencing for preimplantation testing can distinguish embryos that carry a BCR from those that do not and thus can serve in the prevention of vertical transmission of BCRs to the offspring of BCR carriers.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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DOI: 10.1056/NEJMc1913370

Covid-19 and Kidney Transplantation

TO THE EDITOR: Kidney-transplant recipients appear to be at particularly high risk for critical Covid-19 illness due to chronic immunosuppression and coexisting conditions.[1] At Montefiore Medical Center, we identified 36 consecutive adult kidney-transplant recipients who tested positive for Covid-19 between March 16 and April 1, 2020. A total of 26 recipients (72%) were male, and the median age was 60 years (range, 32 to 77). Fourteen recipients (39%) were black, and 15 recipients (42%) were Hispanic. Twenty-seven recipients (75%) had received a deceased-donor kidney; 34 recipients (94%) had hypertension, 25 (69%) had diabetes mellitus, 13 (36%) had a history of smoking tobacco or were current smokers, and 6 (17%) had heart disease. Thirty-five of the patients (97%) were receiving tacrolimus, 34 (94%) were receiving prednisone, and 31 (86%) were receiving mycophenolate mofetil or mycophenolic acid.

The most common initial symptom was fever (in 21 patients [58%]), and diarrhea was observed in 8 patients (22%). Eight patients who were in stable condition without major respiratory symptoms (22%) were monitored at home, and 28 patients (78%) were admitted to the hospital. Twenty-seven of the hospitalized patients (96%) had radiographic findings that were consistent with viral pneumonia, and 11 (39%) received mechanical ventilation. Six patients (21%) received renal replacement therapy. At a median follow-up of 21 days (range, 14 to 28), 10 of the 36 kidney-transplant recipients (28%) and 7 of the 11 patients who were intubated (64%) had died. Two of the 8 patients who were monitored as outpatients died at home; both were recent kidney-transplant recipients who had received antithymocyte globulin within the previous 5 weeks (see the Supplementary Appendix, available with the full text of this article at NEJM.org).

Table 1 summarizes the initial laboratory results in the 28 hospitalized patients. Twenty-two (79%) were lymphopenic, 12 (43%) had thrombocytopenia, 19 (68%) had low CD3 cell counts, 20 (71%) had low CD4 cell counts, and 8 (29%) had low CD8 cell counts. Inflammatory markers were measured, and 10 patients (36%) had ferritin levels higher than 900 ng per milliliter, 13 (46%) had C-reactive protein levels higher than 5 mg per deciliter, 12 (43%) had procalcitonin levels higher than 0.2 ng per milliliter, and 16 (57%) had D-dimer levels higher than 0.5 μg per milliliter.

Although effective treatment of Covid-19 is currently unknown,[2] immunosuppressive management included withdrawal of an antimetabolite in 24 of 28 patients (86%). In addition, tacrolimus was withheld in 6 of the 28 severely ill patients (21%). Hydroxychloroquine was administered to 24 of these 28 patients (86%). Apixaban was administered to patients with D-dimer levels higher than 3.0 μg per milliliter. Six severely ill patients received the CCR5 inhibitor leronlimab (PRO 140, CytoDyn) on a compassionate-use basis, and 2 received the interleukin-6 receptor antagonist tocilizumab. Interleukin-6 levels were very elevated (range, 83 to 8175 pg per milliliter) when leronlimab was initiated (on day 0) in the 5 patients with elevated interleukin-6 levels; these levels decreased markedly 3 days later (range, 37 to 2022 pg per milliliter) (see Table S2 in the Supplementary Appendix). However, only the 1 patient who