Check for updates

The first clinical validation of whole-genome screening on standard trophectoderm biopsies of preimplantation embryos

Yuntao Xia, Ph.D.,[a] Maria Katz, M.Sc.,[a] Dhruva Chandramohan, Ph.D.,[a] Elan Bechor, Ph.D.,[a] Benjamin Podgursky, M.Sc.,[a] Michael Hoxie, B.S.,[a] Qinnan Zhang, Ph.D.,[a] Willy Chertman, M.D.,[a] Jessica Kang, B.S.,[b] Edwina Blue, B.S.,[b] Justin Chen, B.S.,[b] Justin Schleede, Ph.D.,[a] Nathan R. Slotnick, M.D., Ph.D.,[a] Xiaoli Du, Ph.D.,[a] Robert Boostanfar, M.D.,[b] Eric Urcia, M.Sc.,[b] Barry Behr, Ph.D.,[c] Jacques Cohen, Ph.D.,[d] and Noor Siddiqui, M.Sc.[a]

[a] Laboratory Department, Orchid Health, Palo Alto, California; [b] HRC Fertility-Encino, Encino, California; [c] Department of Obstetrics and Gynecology - Reproductive Endocrinology and Infertility, Stanford University, Sunnyvale, California; and [d] A.R.T. Institute of Washington, Bethesda, Maryland

Objective: To validate the performance of our laboratory-developed whole-genome screening assay within clinical preimplantation genetic testing environments.
Design: Perform a laboratory-developed whole-genome assay on both cell lines and trophectoderm biopsies, subsequently employing the next-generation sequencing procedure to reach a sequencing depth of 30X. Adhere to the Genome Analysis Toolkit best practices for accuracy, sensitivity, specificity, and precision calculations by comparing samples with references. Our assay was then applied to cell lines and biopsies harboring known pathogenic variants, aiming to ascertain these changes solely from the next-generation sequencing data, independent of parental genome information.
Settings: Clinical laboratory.
Patients: Coriell cell lines and research embryos with known chromosomal or genetic variants. Research trophectoderm biopsies from a couple that are heterozygous carriers for distinct variants in the same autosomal recessive gene (*HOGA1*).
Intervention: Not applicable.
Main Outcome Measures: Accuracy, sensitivity, specificity, and precision were assessed by comparing the samples to their references. For samples with known variants, we calculated our sensitivity to detecting established variants. For the research embryos, noncarrier, carrier, and compound heterozygous states of inherited *HOGA1* variants were distinguished independently of parental samples.
Results: Amplification of DNA from cell lines and embryos yielded success rates exceeding 99.9% and 98.2%, respectively, although maintaining an accuracy of >99.9% for aneuploidy assessment. The accuracy (99.99%), specificity (99.99%), sensitivity (98.0%), and precision (98.1%) of amplified genome in the bottle (reference NA12878) and embryo biopsies were comparable to results on genomic DNA, including mitochondrial heteroplasmy. Using our assay, we achieved >99.99% sensitivity when examining samples with known chromosomal and genetic variants. This encompassed pathogenic *CFTR*, *BRCA1*, and other variants, along with uniparental isodisomies and microdeletions such as DiGeorge syndrome. Our research study identified noncarrier, carrier, and compound heterozygous states within trophectoderm biopsies while simultaneously screening for 1,300 other severe monogenic diseases.
Conclusion: To our knowledge, this is the first clinical validation of whole-genome embryo screening. In this study, we demonstrated high accuracy for aneuploidy calls (>99.9%) and genetic variants (99.99%), even in the absence of parental genomes. This assay demonstrates advancements in genomic screening and an extended scope for testing capabilities in the realm of preimplantation genetic testing. (Fertil Steril Rep® 2024;5:63–71. ©2024 by American Society for Reproductive Medicine.)
Key Words: Preimplantation genetic testing, whole-genome screening, in vitro fertilization, next-generation sequencing, clinical validation

Received August 25, 2023; revised January 4, 2024; accepted January 5, 2024.
Next-generation sequencing data will be made available on request.
Correspondence: Yuntao Xia, Ph.D., Laboratory Department, Orchid Health, 9 Laboratory Dr, Durham, North Carolina 27709 (E-mail: yuntao@orchidhealth.com).

Fertil Steril Rep® Vol. 5, No. 1, March 2024 2666-3341
© 2024 The Author(s). Published by Elsevier Inc. on behalf of American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
https://doi.org/10.1016/j.xfre.2024.01.001

Preimplantation genetic testing (PGT) reduces the number of cycles needed to achieve a live birth (1–3). However, current methods can only assess ploidy status or screen for specific mendelian variants for which parents are known to be carriers, in which case DNA samples from both parents are required. However, in cases where donor eggs or donor sperm are used, it may be difficult to gain access to donor DNA to evaluate carrier status (4). In addition, small copy number variations (CNV) such as DiGeorge are not screened in regular PGT for aneuploidy (PGT-A) because the deletion size falls below the sensitivity threshold (approximately 2 Mb). Uniparental disomy (UPD) (such as Prader-Willi and Angelman) is also not regularly screened in PGT-A as low-pass next-generation sequencing (NGS) data cannot accurately capture β-allele frequency. To our knowledge, no other clinical whole-genome sequencing (WGS) is currently performed because of poor coverage and high allelic dropout rates (5). Here, we present a validation study on our latest whole-genome screening assay using 58 embryos, corresponding biopsies, and 41 cell lines. We sought to measure the performance of this new assay against the current clinical standard in ploidy analysis, variant detection using WGS, and screening for mitochondrial genetic disorders, microduplications, and microdeletions, as well as UPD from a single biopsy.

MATERIALS AND METHODS

Sample Collection

All cell lines used in this study were purchased from Coriell (Camden, NJ, USA). DNA was extracted from Coriell, not the Orchid laboratory. A critical cell line used in whole-genome sequencing for preimplantation genetic testing (PGT-WGS) validation is NA12878. It is from the International HapMap project, and Genome in a Bottle (GIAB) has currently characterized its whole-genome, enabling the translation of whole human genome sequencing to clinical practice and innovations in technologies. The cell line was made by transfecting Epstein-Barr virus (EBV) into B lymphocytes from donated blood, which is a common approach to immortalizing EBV-bearing lymphoblastoid cell lines. Other cell lines, such as NA17942, were made in a similar manner. A patient was diagnosed with DiGeorge syndrome at the age of 6 years. Then EBV was transfected into B lymphocytes from donated blood to establish the immortal cell line (NA17942). All cell line information can be accessed by searching cell line identifications on the Coriell website.

Embryo samples were all donated to research and provided by the fertility centers (wIRB number 20215134). All embryos were created using intracytoplasmic sperm injections. Embryos from days 5–7 were biopsied following standard standard operating procedure from clinics. No special SOP is needed. Embryologists at the in vitro fertilization laboratories prepared three samples per embryo. They took two biopsies from each embryo, and the third sample was the rest of the whole embryo. Each biopsy consisted of approximately five cells. We used the whole embryo as our reference and compared each of the biopsies to the corresponding embryo in the whole-genome variant analysis. A 200 μL polymerase chain reaction tube with 3 μL of cell buffer was used for each sample. A clinical case study was done under wIRB number 2022264.

Next-Generation Sequencing and Genomic Data Analyses

Samples were processed in the Orchid laboratory (certified: CAP No. 9234146 and CLIA No. 34D2260214). DNA or biopsies were amplified using a laboratory-developed protocol. DNA sizes after whole-genome amplification (WGA) were first confirmed by running 1%–2% Agarose E-Gel (Invitrogen, Waltham, MA, USA). After size determination, 250–500 ng of DNA was used for library preparation with the KAPA HyperPlus kit per manufacturer's instructions. Dual-index UMI adapters (Integrated DNA Technologies, San Diego, CA, USA) were used in the ligation. Library concentration was quantified using the Qubit 4 dsDNA HS. Library sizes were measured using the Agilent 4150 Tapestation Genomic ScreenTape assay (Agilent Technologies, Santa Clara, CA, USA). Sequencing runs were performed on a MiniSeq for low-pass aneuploidy screening and a NovaSeq6000 for 30X WGS per manufacturer's instructions. Biologic repeats instead of technical repeats were used in the ploidy analysis, because we took multiple samples from the same embryo and compared the ploidy results to previous clinical PGT-A results. In summary, 58 embryos and corresponding biopsies and 41 cell lines were tested following our whole-genome workflow, and 123 embryo samples were tested following our PGT-A workflow.

Next-generation sequencing data were processed using a combination of the Gencove Sentieon-based human genome pipeline and an in-house pipeline following Genome Analysis Toolkit (GATK) best practices (6). Genomic Variant Call Formats were generated for each sample and jointly called as part of a larger cohort. The Genome Analysis Toolkit VariantRecalibrator (VQSR) was run on the resulting callset. Mitochondrial DNA was analyzed using GATK without VQSR. Sensitivity, precision, and others were calculated using the Real Time Genomics packages. NxClinical was used for ploidy analysis. Samples with $<$30% of a chromosomal abnormality were reported as euploid, whereas samples with $\geq$80% of a chromosomal abnormality were reported as aneuploid. All data were aligned to GRCh37 with a bin size of 500 kbp. Embryos were excluded from the analysis on the basis of the following criteria: the whole embryo (source of truth) and biopsy were in agreement with each other but were all inconsistent with the previous clinical third-party PGT-A results, and no relevant mosaicism evidence can be found. Two embryos were thus excluded from statistical analysis accordingly, and details are shown in Supplemental Figure 1 (available online). We observed a loss of Chr5 in our samples, whereas the previous clinical PGT-A report showed a loss of Chr5 and also showed a gain of Chr18. To rule out mosaicism, we sequenced the whole embryo (the source of truth) and did not observe trisomy on Chr18 (Supplemental Fig. 1). The other case was previously

reported as a complex aneuploid (XY, +5, +13, +20), whereas we saw an euploid XY (Supplemental Fig. 1).

RESULTS

Validation on Aneuploidy Screening Using Cell Lines and Human Embryos

Ploidy analysis was initially validated by assessing the accuracy of aneuploidy screening. A total of 73 samples from eight commonly used Coriell cell lines were used first to validate our aneuploidy screening (Supplemental Fig. 2, available online). These cell lines covered common karyotypes seen in PGT-A, including euploid men and women and autosomal and sex chromosome aneuploidy. We diluted the DNA to approximately 20–50 pg, which is equivalent to the amount of DNA in embryo trophectoderm biopsies. Whole-genome amplification was then performed, followed by low-pass sequencing (approximately 0.01–0.05X) for aneuploidy evaluation with a bin size of 500 kbp on the genome in the analysis. Overall, we achieved >99.9% successful amplification and >99.9% accuracy (Supplemental Fig. 2B). Notably, we obtained >1,500 ng of DNA for all samples, which is higher and more robust than most WGA approaches.

Next, we extended our PGT-A validation to donated human embryos with a larger variety of karyotypes (Fig. 1; Supplemental Table 1, available online). We obtained 2–3 samples per embryo, on which we performed WGA and ploidy analysis. We first evaluated the consistency of these samples by comparing our CNV analysis to the previous clinical PGT-A reports. Among the samples, we observed a mosaic embryo with two samples being deletion of the short arm of chromosome 2 (-2p) and one sample being monosomy 2 (-2) (No. 55–57); and a mosaic embryo with one sample being mosaic trisomy 19 (+19) and one sample being trisomy 19 (+19) (No. 105–106). Considering the previous PGT-A results reported the same chromosome and the unknown accuracy of the original 2017 PGT-A testing technology, our PGT-A results are acceptably consistent. Two samples (marked indeterminate, Supplemental Table 1) have >8% mitochondrial reads in the NGS data, negatively impacting our CNV calling; however, because high levels of mitochondrial reads often suggest embryo viability issues, the clinical impact of processing samples contaminated by high levels of mitochondrial DNA may be limited (7). Viability is generally higher with fresh embryos compared with banked embryos because banked embryos suffer from multiple freeze-thaw cycles and additional rounds of biopsies. Regardless, we achieved >99.9% accuracy overall, with 1.6% indeterminate among 123 embryo samples.

Low-level mosaicism in embryos has been reported to be compatible with a successful pregnancy and live birth, especially when the aneuploidy mosaicism level is <40% (8). Therefore, our algorithm was designed to capture mosaicism >30%. We performed a dilution series from 50%–10% mosaicism, generated by mixing aneuploid (47, XY, +18) and euploid (46, XX) DNA. As seen in Supplemental Figure 3B-Batch 1 (available online), mosaic samples above 30% could be visualized and were captured successfully by the algorithm. To confirm the results, we performed another mosaicism dilution using a mixture of NA09288 (47, XY, +9) and euploid (46, XX) DNA and found the same result (Supplemental Fig. 3B-Batch 2). Additionally, 24 cell line mixtures with 30%–35% aneuploidy mosaicism were tested, and 100% of them were successfully marked during analysis (Supplemental Fig. 3B-Batch 3).

Validation of Whole-Genome Sequencing Using Cell Lines and Human Embryos

Although multiple clinical laboratories have demonstrated good performance in PGT-A (9), to our knowledge, none have attempted validation on PGT-WGS. DNA derived from WGA to date has poor quality and lowered sequence fidelity compared with genomic DNA and has not been used for embryo screening under clinical conditions (10). Our results using a laboratory-developed WGA protocol show that our assay achieves comparable sensitivity and specificity to genomic DNA.

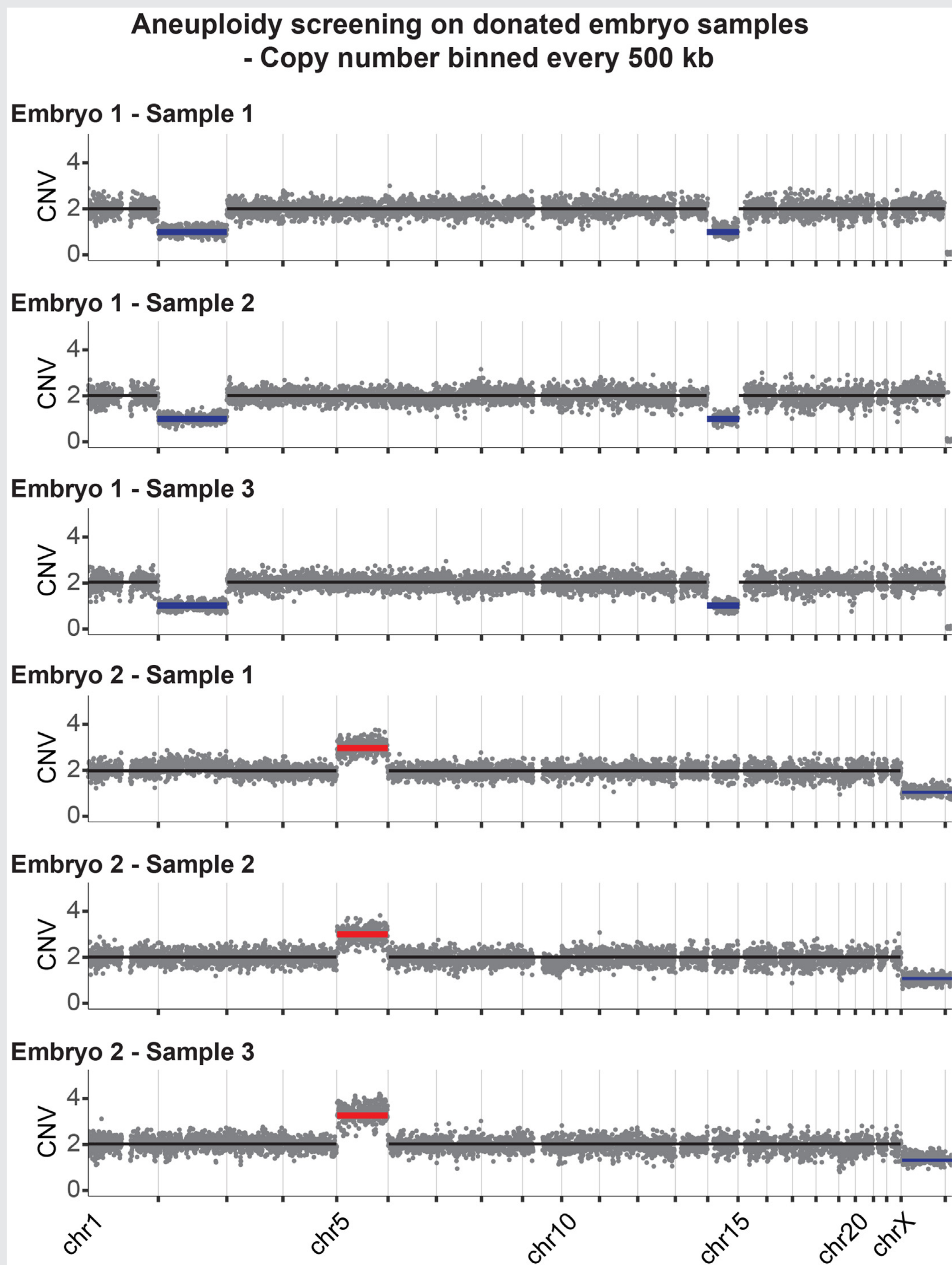
We used the National Institute of Standards and Technology (NIST) GIAB (NA12878) and its variants as the data analysis reference. Both genomic DNA and amplified DNA from NA12878 were analyzed and compared with benchmark calls from GIAB (NIST v4.2.1). As shown in Supplemental Tables 2 and 3 (available online), accuracy and specificity at the whole-genome level are 99.99% for both amplified DNA and genomic DNA. We observed an average of 99.8% genomic coverage in amplified DNA and the same in genomic DNA (gDNA) (Supplemental Tables 2 and 3). The precision of amplified DNA and gDNA are 98.1% and 97.8%, respectively, suggesting our amplified DNA does not generate more false positive calls than gDNA (Supplemental Table 2). Moreover, sensitivity reaches 98.0% in amplified DNA as compared with 98.9% in gDNA, indicating false negative calls in data are similar in magnitude as well (Supplemental Table 2). Therefore, our WGS on amplified DNA was validated against the variant list from NIST and its sequencing outcome is comparable with gDNA.

To validate the performance of biopsies of clinical embryos, we compared the results of the biopsies to those of their corresponding embryos. Using the whole embryo as the reference, we observed an average of 99.6% genomic coverage, 99.9% accuracy, 99.9% specificity, 98.0% precision, and 98.1% sensitivity (Table 1 & Table S4, available online). The demonstrated concordance allows us to treat the WGS of an embryo biopsy as an accurate reflection of the entire embryo's genome.

Validation on Mitochondrial DNA

A high proportion of pathogenic heteroplasmy has been correlated to mitochondrial diseases, including early-onset metabolic and degenerative diseases. Our validation of mitochondrial DNA focused on mitochondrial genome coverage, sensitivity, and precision by comparing biopsies to their embryos. Setting 15% as our reportable heteroplasmy threshold, we observed an average of 100% mitochondria DNA coverage, >99.9% accuracy, >99.9% specificity, 99.0%

FIGURE 1



Summary of preimplantation genetic testing for aneuploidy on embryo samples. Examples of copy number variation (CNV) results on embryo samples.

Xia. First clinical validation of PGT-WGS. Fertil Steril Rep 2024.

TABLE 1

Validation results of the whole genome screening. Comparison of biopsies and their embryos in terms of genomic coverage, total SNV called, accuracy, specificity, precision, and sensitivity. The whole embryos were used as references.

Sample	Genomic Coverage	Total SNV	Accuracy	Specificity	Precision	Sensitivity
1	99.7%	3343804	99.995%	99.997%	97.8%	98.4%
2	99.6%	3327631	99.995%	99.997%	98.0%	98.1%
3	99.6%	3312139	99.995%	99.997%	97.9%	98.1%
4	99.4%	3279468	99.993%	99.997%	97.8%	97.1%
5	99.6%	3404155	99.995%	99.998%	98.4%	98.1%
6	99.7%	3414277	99.996%	99.998%	98.4%	98.4%
7	99.6%	3360682	99.995%	99.998%	98.4%	98.1%
8	99.6%	3163591	99.995%	99.998%	98.2%	98.2%
9	99.6%	3160113	99.995%	99.998%	98.3%	98.2%
10	99.6%	3304310	99.994%	99.996%	97.2%	98.0%
11	99.7%	3301716	99.994%	99.997%	97.5%	98.2%
12	99.7%	3368834	99.996%	99.998%	98.6%	98.2%
13	99.7%	3340483	99.995%	99.998%	98.8%	97.5%
14	99.6%	3270893	99.995%	99.997%	97.5%	98.4%
15	99.5%	3262566	99.995%	99.997%	97.6%	98.3%
16	99.6%	3274334	99.995%	99.997%	97.8%	98.2%
17	99.6%	3275227	99.995%	99.997%	97.7%	98.2%
18	99.6%	3254447	99.995%	99.998%	98.1%	98.4%
19	99.4%	3234717	99.995%	99.998%	98.2%	97.9%
20	99.7%	3274002	99.995%	99.997%	97.9%	98.5%
21	99.5%	3257361	99.995%	99.998%	98.1%	98.2%
22	99.6%	3270572	99.995%	99.998%	98.2%	97.9%
23	99.7%	3284833	99.995%	99.998%	98.2%	98.3%
24	99.6%	3237713	99.993%	99.997%	97.9%	97.0%
25	99.7%	3382545	99.996%	99.998%	98.3%	98.6%
Average	**99.6%**	**3294417**	**99.995%**	**99.997%**	**98.0%**	**98.1%**

Xia. First clinical validation of PGT-WGS. Fertil Steril Rep 2024.

sensitivity, and >99.9% precision (Supplemental Table 5, available online). Because mitochondrial DNA is known to be exclusively inherited from the mother, our assay has the potential to be used to perform maternity confirmation.

Detection of Specific Variants Using WGS without Parental Samples

We performed WGS on biopsies from embryos that previously underwent PGT-M, as well as on Coriell cell lines that carry specific pathogenic variants. Following our protocol with 30X sequencing, all variants detected by previous PGT-M assays were detected successfully in our WGS results without the use of parental genomes (Table 2). To further confirm our ability to capture pathogenic variants in WGS, Coriell cell lines were selected to represent common genetic disorders. Amplified DNA from picogram-level of Coriell cell line followed by 30X sequencing further confirmed our PGT-WGS assay has ability to detect all reported pathogenic variants regardless of the number of variants in one sample (Table 3) (11).

We demonstrate the value of this screening via a clinical research case (Supplemental Table 6, available online). Here, both parents carry different *HOGA1* variants as determined using carrier screening. Our embryo screening identified *HOGA1* status from WGS data directly, without the use of parental genomes or a custom probe design. In addition, the screening included an analysis of chromosomal abnormalities and pathogenic variants in approximately 1,300 genes related to monogenic neurodevelopmental disorders, birth defects, and hereditary cancers. Supplemental Table 6 includes a summary of these results. A deidentified sample report is included in Supplemental Figure 4 (available online). Please note that for our general screening, we only report affected embryos, meaning we do not report carrier status unless it was specifically requested on the test requisition form.

Common Microduplications and Deletions, Triploid, as well as Uniparental Disomy Screening

Besides variants, we also validated the screening for microduplications, microdeletions, and uniparental disomy, among others, that conventional PGT-A is unable to detect. Amplified DNA from picogram-level Coriell cell lines was used as a substitute because the availability of research embryos with the above genotypes is extremely rare. We covered a variety of CNV-related diseases, including but not limited to Cri-Du-Chat, Williams-Beuren, DiGeorge and Prader-Willi syndrome (Supplemental Table 7, available online). On the basis of limit of detection study, we were able to reproducibly detect CNV >400 Kb and UPD >20 Mb at a sequence depth of 30X WGS. Examples are shown in Supplemental Figure 5 and Supplemental Table 7 (available online).

TABLE 2

Variants in embryo samples that underwent PGT-M previously. PGT-WGS captured all reported variants in positive samples and was clean on negative samples.

Family	Sample	Previous PGT-M Reports	Gene	Disease	Detected in Orchid WGS?
Family 1	Sample 1	c.2279G>A, Carrier	TGM1	Lamellar Ichthyosis	Yes, Carrier
	Sample 2	c.2279G>A, Carrier	TGM1	Lamellar Ichthyosis	Yes, Carrier
	Sample 3	c.2279G>A, Carrier	TGM1	Lamellar Ichthyosis	Yes, Carrier
	Sample 4	c.2279G>A, Carrier	TGM1	Lamellar Ichthyosis	Yes, Carrier
	Sample 5	c.2279G>A, Carrier	TGM1	Lamellar Ichthyosis	Yes, Carrier
	Sample 6	c.2279G>A, Carrier	TGM1	Lamellar Ichthyosis	Yes, Carrier
	Sample 7	Negative			No
	Sample 8	Negative			No
	Sample 9	Negative			No
Family 2	Sample 10	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 11	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 12	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 13	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 14	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 15	Negative			No
	Sample 16	Negative			No
	Sample 17	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 18	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 19	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 20	c.901-1G>A, Affected Male	ABCD1	X-linked Adrenoleukodystrophy	Yes, Affected Male
	Sample 21	c.901-1G>A, Carrier Female	ABCD1	X-linked Adrenoleukodystrophy	Yes, Carrier Female

Note: PGT = preimplantation genetic testing; WGS = whole-genome sequencing.

Xia. First clinical validation of PGT-WGS. Fertil Steril Rep 2024.

DISCUSSION

We validated the performance of our PGT-WGS in this study. The screen included PGT-A, uniparental isodisomy, pathogenic microduplication and microdeletions, gene panels, and mitochondrial heteroplasmy. Remarkably, the DNA genomic coverage, sensitivity, and specificity from amplified material were broadly equivalent to assays performed on the current clinical gold standard, unamplified genomic DNA. On the basis of equivalent coverage, sensitivity, and specificity, this technique should make it possible to screen embryos before implantation for chromosomal and genetic disorders. Potential benefits of the new technique may include increasing the likelihood of a successful pregnancy (on the basis of the fraction of mitochondrial reads) and reducing the likelihood of birth defects or pediatric or adult developmental disorders.

To ensure accurate and relevant identification of genetic causes of severe, highly penetrant monogenic diseases, we scored 1,300 genes associated with well-studied conditions (12). These gene selections are based on American College of Medical Genetics and Genomics panels for applicable embryo screening and are grounded in extensive research spanning decades, including familial analysis and cohort studies (13). For many of these genes, functional investigations have established strong links between them and specific disorders. Considering that humans possess over 20,000 genes, our approach takes a conservative stance by concentrating on the 1,300 genes that have undergone a comprehensive review and have been unequivocally linked to severe disease. This targeted gene selection ensures that we prioritize clinically meaningful implications for patients when making decisions about embryo transfer. The same concept applies to microduplications and microdeletions. Instead of a general screening, we have selected approximately 50 regions on the basis of the literature where there are known clinically relevant microdeletion and microduplication syndromes. For example, DiGeorge syndrome occurs at 22q11.2, and Cri-Du-Chat occurs at 5p-. Traditional carrier screening, for example, requires genes to have a confirmed relationship between the detected mutations and a well-defined disorder to provide valuable information to patients and allow reproductive decision-making. It is crucial to strike a balance between the amount of data provided and the complexity it presents, as an excess of information can pose challenges when patients are asked to make reproductive decisions. We similarly prioritize diagnostic precision, giving high accuracy in identifying the genetic causes of severe monogenic diseases. Although noninvasive PGT-A is becoming possible (14), initial attempts on noninvasive PGT-WGS showed severe allele dropout, making it not suitable for clinical use currently.

In addition, polygenic risk scores are also easily computable with our method because of the high genomic coverage and accuracy of variants. Some polygenic risk scores use 6–7 million single nucleotide polymorphisms in total (15), which is difficult to achieve on single nucleotide polymorphism array chips without relying heavily on statistical imputation. The theoretical limits of genome imputation as applied to rare variant detection are a topic of open research (16).

Whole-genome sequencing (WGS) is a powerful molecular technique that involves decoding >99% of the DNA sequence of an individual, providing a comprehensive

TABLE 3

Variants in Coriell cell lines that possess known variants. PGT-WGS captured all reported variants in positive samples.

Sample	Variants Reported in Coriell	Gene	Disease	Detected in Orchid WGS?
NA09301	NM_003640.5:c.2204+6T>C, Het.	ELP1	Dysautonomia	Yes, Het.
NA19977	NM_022455.5:c.6450dup(p.Lys2151GlnfsTer15), Het.	NSD1	Sotos Syndrome	Yes, Het.
NA07500	NM_000038.6:c.4612_4613del(p.Glu1538IlefsTer5), Het.	APC	Familial Adenomatous Polyposis	Yes, Het.
NA14090	NM_007300.4:c.68_69del(p.Glu23ValfsTer17), Het.	BRCA1	Hereditary Breast and Ovarian Cancer	Yes, Het.
NA14170	NM_000059.4:c.5946del(p.Ser1982ArgfsTer22), Het.	BRCA2	Hereditary Breast and Ovarian Cancer	Yes. Het.
NA13715	NM_007300.4:c.5329dup(p.Gln1777ProfsTer74), Het.	BRCA1	Hereditary Breast and Ovarian Cancer	Yes, Het.
NA00998	NM_000492.4:c.1521_1523del(p.Phe508del), Hom.	CFTR	Cystic Fibrosis	Yes, Hom.
NA13591	NM_000492.4:c.1521_1523del(p.Phe508del), Het.	CFTR	Cystic Fibrosis	Yes, Het.
	NM_000492.4:c.350G>A(p.Arg117His), Het.	CFTR	Cystic Fibrosis	Yes, Het.
	NM_000410.4:c.187C>G(p.His63Asp), Hom.	HFE	Hereditary Hemochromotosis	Yes, Hom.
	NM_005957.5:c.665C>T(p.Ala222Val), Het.	MTHFR	Homocystinuria (MTHFR related)	Yes, Het.
NA10080	NM_000314.8:c.781C>T(p.Gln261Ter), Het.	PTEN	PTEN hamartoma tumor syndrome	Yes, Het.
NA13326	NM_000051.4:c.1179_1180del(p.Trp393Ter), Het.	ATM	Ataxia Telangiectasia	Yes, Het.
	NM_000051.4:c.4396C>T(p.Arg1466Ter), Het.	ATM	Ataxia Telangiectasia	Yes, Het.
	NM_024675.4:c.3508C>T(p.His1170Tyr), Het.	PALB2		Yes, Het.
	NM_000535.7:c.88C>A(p.Gln30Lys), Het.	PMS2	Lynch Syndrome	Yes, Het.
NA12932	NM_001079804.3:c.1441T>C(p.Trp481Arg), Het.	GAA	Glycogen storage disease Type 2	Yes, Het.
	NM_001079804.3:c.1326+1G>A, Het.	GAA		Yes, Het.
NA14646	NM_000410.4:c.845G>A(p.Cys282Tyr), Hom.	HFE	Hereditary Hemochromotosis	Yes, Hom.
NA22752	NM_000090.4:c.636+5G>A, Het.	COL3A1	Ehlers-Danlos syndrome	Yes, Het.
NA00325	NM_000402.4:c.653C>T(p.Ser218Phe), Het.	G6PD	G6PD Deficiency	Yes, Het.
NA12878	NM_000769.4:c.681G>A(p.Pro227=), Het.	CYP2C19	Clopidogrel resistance	Yes, Het.
NA11319	NM_001127328.2:c.178G>C(p.Ala60Pro), Hom.	ACADM	Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)	Yes, Hom.
NA07859	NM_000492.4:c.3302T>A(p.Met1101Lys), Hom.	CFTR	Cystic Fibrosis	Yes, Hom.
NA07415	NM_000368.5:c.993_994insA(p.Ser332IlefsTer9), Het.	TSC1	Tuberous Sclerosis Complex	Yes, Het.
NA21976	NM_000138.5:c.3444dup(p.Asn1149GlnfsTer10), Het.	FBN1	Marfan Syndrome	Yes, Het.
NA11906	MT:8344 A⇒G, Heteroplasmic, 39% from literature		MT-TK (myoclonic epilepsy with ragged-red fibers)	Yes, 45%
NA04368	ENST00000361381.2:c.1019G>A(p.Arg340His), Homoplasmic	MT-ND4	Leber Hereditary Optic Neuropathy	Yes, 100%
NA13741	ENST00000361899.2:c.467T>G(p.Leu156Arg), Homoplasmic	MT-ATP6	Mitochdonrial Complex V Deficiency	Yes, 97%
NA10742	ENST00000361381.2:c.1019G>A(p.Arg340His), Homoplasmic	MT-ND4	Leber Hereditary Optic Neuropathy	Yes, 100%

Note: PGT = preimplantation genetic testing; WGS = whole-genome sequencing.

Xia. First clinical validation of PGT-WGS. Fertil Steril Rep 2024.

understanding of their genetic makeup. In 2009, the first announcement of WGS was made. Clinical WGS has been available since 2014 and has been employed primarily to identify a genetic diagnosis for diseases in unhealthy individuals. Historically, WGS is ordered when a patient presents with unexplained or complex medical conditions that conventional tests have failed to diagnose accurately. Because the cost of testing has dropped and general population interest has spiked, more whole-genome data are available from healthy individuals. Supported by NIH funding, Genomes2-People is an example of a research team managing a number of NIH studies involving genetic testing in healthy populations, including BabySeq. Many companies have started switching from whole exome sequencing to WGS this year after Illumina launched NovaSeq X Plus sequencer, which brings the reagent cost of WGS from $1,000 per sample to $200, making PGT-WGS more attractive.

CONCLUSION

To our knowledge, this is the first clinical validation of whole-genome embryo screening. In this study, we demonstrated high accuracy for aneuploidy calls (>99.9%) and genetic variants (99.99%), even in the absence of parental genomes. This assay demonstrates advancements in genomic screening and an extended scope for testing capabilities in the realm of PGT.

Acknowledgments

The authors acknowledge HRC Fertility for their assistance in the study, especially Bar Sverdlov, Nadia Deratani, and Christopher Valdez. The authors thank Alex Lagunov and Jason E. Swain, Ph.D., from CCRM for sample collection. The authors thank Michael Feinman, M.D., and Jerry Launchbury, Ph.D., for insightful discussion and feedback. The authors also thank Jonathan Kort, M.D., from RMA for patient recruitment and discussion.

CRediT Authorship Contribution Statement

Yuntao Xia: Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Katz:** Writing – review & editing, Writing – original draft, Conceptualization. **Dhruva Chandramohan:** Formal analysis, Data curation. **Elan Bechor:** Methodology, Formal analysis, Data curation. **Benjamin Podgursky:** Supervision, Software, Formal analysis, Data curation, Conceptualization. **Michael Hoxie:** Methodology. **Qinnan Zhang:** Writing – review & editing, Visualization, Methodology. **Willy Chertman:** Writing – original draft. **Jessica Kang:** Methodology. **Edwina Blue:** Methodology. **Justin Chen:** Methodology. **Justin Schleede:** Methodology, Conceptualization. **Nathan R. Slotnick:** Supervision, Conceptualization. **Xiaoli Du:** Supervision, Conceptualization. **Robert Boostanfar:** Supervision, Resources, Methodology. **Eric Urcia:** Supervision, Resources, Conceptualization. **Barry Behr:** Supervision, Resources, Conceptualization. **Jacques Cohen:** Writing – review & editing, Writing – original draft, Conceptualization. **Noor Siddiqui:** Supervision, Resources, Conceptualization.

Declaration of Interests

Y.X., M.K., D.C., E.Bechor., B.P., M.H., Q.Z., W.C., J.S., N.R.S., X.D., N.S. are employees of Orchid Health, a clinical preimplantation genetic testing laboratory. J.K., E. Blue, J. Chen, R.B., E.U., J. Cohen have nothing to disclose. B.B. is a scientific advisor to Orchid.

REFERENCES

1. Keltz MD, Vega M, Sirota I, Lederman M, Moshier EL, Gonzales E, et al. Preimplantation Genetic Screening (PGS) with Comparative Genomic Hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcomes while lowering multiple pregnancies and miscarriages. J Assist Reprod Genet 2013;30:1333–9.
2. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril 2017;107:1122–9.
3. Forman EJ, Tao X, Ferry KM, Taylor D, Treff NR, Scott RT Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod 2012;27:1217–22.
4. Sacchi L, Albani E, Cesana A, Smeraldi A, Parini V, Fabiani M, et al. Preimplantation genetic testing for aneuploidy improves clinical, gestational, and neonatal outcomes in advanced maternal age patients without compromising cumulative live-birth rate. J Assist Reprod Genet 2019;36:2493–504.
5. Zheng YM, Wang N, Li L, Jin F. Whole genome amplification in preimplantation genetic diagnosis. J Zhejiang Univ Sci B 2011;12:1–11.
6. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. Curr Protoc Bioinformatics 2013;43:11:10.1–11.10.33.
7. Dickson D, Tao T, Qin W. Exploring mitochondrial DNA content as a novel biomarker to improve embryo implantation potential: a review. Biomed Technol 2024;5:82–6.
8. Abhari S, Kawwass JF. Pregnancy and neonatal outcomes after transfer of mosaic embryos: a review. J Clin Med 2021;10:1369.
9. Treff NR, Zimmerman R, Bechor E, Hsu J, Rana B, Jensen J, et al. Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform. Eur J Med Genet 2019;62:103647.
10. Huang L, Ma F, Chapman A, Lu S, Xie XS. Single-cell whole-genome amplification and sequencing: methodology and applications. Annu Rev Genomics Hum Genet 2015;16:79–102.
11. Kaplun L, Krautz-Peterson G, Neerman N, Stanley C, Hussey S, Folwick M, et al. ONT long-read WGS for variant discovery and orthogonal confirmation of short read WGS derived genetic variants in clinical genetic testing. Front Genet 2023;14:1145285.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.

13. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2021;23:1381–90.
14. Navarro-Sánchez L, García-Pascual C, Rubio C, Simón C. Non-invasive preimplantation genetic testing for aneuploidies: an update. Reprod Biomed Online 2022;44:817–28.
15. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet 2018;50:1219–24.
16. Si Y, Vanderwerff B, Zöllner S. Why are rare variants hard to impute? Coalescent models reveal theoretical limits in existing algorithms. Genetics 2021;217:iyab011.