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GENETICS

OneGene PGT: comprehensive preimplantation genetic testing method utilizing next-generation sequencing

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Abstract

Purpose Preimplantation genetic testing for monogenic disorders (PGT-M) allows early diagnosis in embryos conceived in vitro. PGT-M helps to prevent known genetic disorders in affected families and ensures that pathogenic variants in the male or female partner are not passed on to offspring. The trend in genetic testing of embryos is to provide a comprehensive platform that enables robust and reliable testing for the causal pathogenic variant(s), as well as chromosomal abnormalities that commonly occur in embryos. In this study, we describe PGT protocol that allows direct mutation testing, haplotyping, and aneuploidy screening.

Methods Described PGT protocol called OneGene PGT allows direct mutation testing, haplotyping, and aneuploidy screening using next-generation sequencing (NGS). Whole genome amplification product is combined with multiplex PCR used for SNP enrichment. Dedicated bioinformatic tool enables mapping, genotype calling, and haplotyping of informative SNP markers. A commercial software was used for aneuploidy calling.

Results OneGenePGT has been implemented for seven of the most common monogenic disorders, representing approximately 30% of all PGT-M indications at our IVF centre. The technique has been thoroughly validated, focusing on direct pathogenic variant testing, haplotype identification, and chromosome abnormality detection. Validation results show full concordance with Sanger sequencing and karyomapping, which were used as reference methods.

Conclusion OneGene PGT is a comprehensive, robust, and cost-effective method that can be established for any gene of interest. The technique is particularly suitable for common monogenic diseases, which can be performed based on a universal laboratory protocol without the need for set-up or pre-testing.

Keywords Preimplantation genetic testing · Next-generation sequencing · Monogenic disorders · Aneuploidy

Introduction

Preimplantation genetic testing for monogenic disorders (PGT-M) represents a very early form of prenatal genetic testing to exclude embryos that inherited pathogenic variant(s) linked to severe monogenic disorders. The key advantage of PGT-M is that women do not have to face the risk of pregnancy termination, since they have conceived from an unaffected embryo. PGT-M is performed in conjunction with in vitro fertilization, embryo culture, and subsequent biopsy of cellular material from the embryo [1]. Blastocyst biopsy is becoming more widely used in PGT-M practice [2], which also has a positive impact on diagnostic yield and accuracy by reducing amplification failure rate and allele drop-outs (ADO) as compared to blastomere biopsy [3].

Some centres for assisted reproduction perform polar body biopsy to avoid removing embryonic cells. This approach allows PGT-M in countries where regulations have prohibited embryo biopsy (e.g. Germany) and in some other countries where polar body biopsy is the only procedure permitted (e.g. Italy) [4, 5].

The methods used for PGT-M generally utilize analysis of short tandem repeat (STR) markers or genome-wide single-nucleotide polymorphisms (SNPs). STR markers are examined using fluorescently labelled primers that are generally co-amplified in a multiplex PCR fashion. Using

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DNA samples of the mother, father, and reference sample (usually an affected relative in the family), length polymorphisms of STRs can be used to establish linkage with normal and mutated alleles. This approach enables low-cost linkage analysis, which might be improved by direct pathogenic variant detection [6]. Genome-wide SNP technologies utilize SNP arrays such as karyomapping [7, 8] or NGS. Recently, several NGS-based platforms have emerged that enable combined PGT-A and PGT-M, e.g., OnePGT technique [9], GENType [10], or others [11, 12]. The advantage of genome-wide SNP technologies might be the simultaneous detection of chromosomal aneuploidies that are frequently observed in human embryos [13]. Remarkably, the aneuploidy frequency may exceed 50% in human IVF embryos derived from patients with advanced maternal age (≥ 38 years), recurrent pregnancy loss (≥ 2), or recurrent implantation failure (≥ 2 cycles) [14]. On the other hand, genome-wide SNP technologies are expensive platforms in general, since linkage analysis is performed genome-wide for all human genes, whereas PGT-M usually targets only one gene.

The goal of our work was to develop and clinically validate a robust yet cost-effective PGT-M approach that incorporates linkage analysis, direct mutation analysis, and aneuploidy detection in a single assay. Such an assay would enable the most informed decision on embryo transfer. For this task, we have introduced OneGene PGT technique. The method utilizes whole genome amplification (WGA) with a co-amplification of highly heterozygous SNPs within and around the gene of interest. Moreover, chromosome abnormalities are detected by CNV analysis using whole genome sequencing reads with the help of dedicated software.

OneGene PGT is a comprehensive, robust, and cost-effective technique that can be established and validated for any gene of interest. At our IVF centre, we use the technique for genes that are common indications for PGT-M (such as *CFTR*, *HBB*, *HTT*, *FMR1*, *BRCA1*, *BRCA2*, and *GJB2*).

Material and methods

Embryo biopsy and whole genome Amplification (WGA)

Laser-mediated trophectoderm biopsy was carried out on day 5 or 6 of embryo culture. On average, five to ten cells were biopsied and subsequently transferred to and stored in 1–2 µl of sterile 1 × PBS/ − 60–80 °C in 200 µl DNA- and RNA-free PCR tubes. Each sample was then amplified using multiple displacement amplification (MDA) according to the manufacturer's manual (REPLI-g Advanced DNA Single Cell Kit, Qiagen, Germany). WGA was performed in PCR box equipped with a set of dedicated pipettes and lab tools; the operator was wearing a lab coat and gloves to prevent cell or DNA contamination. The presence of a DNA smear typically greater than 10 kb, which is a sign of successful amplification, was checked by a 1% agarose/0.5 × TBE buffer gel electrophoresis. Amplified DNA was stored at − 20 °C for long periods or at 4 °C for short periods of time.

Multiplex PCR, direct pathogenic variant detection, library preparation, and next-generation sequencing

Multiplex PCR

For selected genes, highly heterozygous SNP markers were selected within and around the gene of interest (see Supplement A for a list of SNP markers and their population frequencies for each gene).

Primer pairs were designed for each SNP marker using the Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Each primer pair was checked for the production of a single specific amplicon by a 2% agarose/0.5 × TBE buffer gel electrophoresis. Validated primer pairs were combined into a single pool (stock concentration of a single forward/reverse primer was 1.85 µM in the pool). Subsequent Multiplex PCR was performed using the 2X Multiplex HotStart PCR Master Mix (Biotechrabbit, Germany). Final forward/reverse primer concentrations were 0.33 µM for each primer pair in a PCR reaction. Approximately, 50–80 ng of genomic DNA or WGA was used as a template.

Multiplex PCR was performed for the male partner, female partner, reference sample, and amplified embryos.

Direct pathogenic variant detection

A single forward and reverse primer is added to the multiplex PCR mix at a final concentration of 0.72 µM enabling amplification of the pathogenic variant. A technically challenging mutation tests such as triplet repeat expansion mutations for *FMR1* and *HTT* gene might be performed as an additional test on MDA product.

Library preparation

Library preparation was performed separately for the male partner, female partner, reference sample (usually an affected relative in the family, if available), and for individual embryos tested. For the male partner, female partner and reference sample, 50 ng of multiplex PCR product each and, for the individual embryos, 1 µg of WGA product enriched with 50 ng of multiplex PCR product was used as a template for library preparation. Library preparation for NGS was performed using the PG-Seq™ kit (PerkinElmer, USA) according to the manufacturer's protocol.

Next generation sequencing (NGS)

The barcoded libraries were denatured, neutralized, and diluted to a final concentration according to the MiSeq, NextSeq System Denature, and Dilute Libraries Guide (Illumina, USA — support.illumina.com). Final libraries were sequenced with single-end 1 × 150 bp reads using the MiSeq or NextSeq system (Illumina, USA). The minimum reads yield per sample is required to be 500 K, and the optimal read yield is recommended to be 1 M.

Data analysis

OneGene PGT tool

Demultiplexed fastq files were analysed using an in house developed genetic tool, which enables mapping and genotype calling of individual SNPs and targeted pathogenic variant. Sequencing reads were aligned to the human genome version 19 using BWA-MEM algorithm [15]. Variants used for genotyping were identified using the VarDict software [16]. Informative (heterozygous) SNPs present in the male/female partner were linked to the pathogenic variant using Mendelian rules of inheritance. As a rule, the informative SNP must be heterozygous in a male or female partner with the pathogenic variant. Complete Mendelian rules used for SNP informativity identification are described in [17, Table 1]. The final haplotype was visualized for each embryo in relation to the reference sample (Supplement C—OneGene PGT tool showing haplotypes for each individual embryo). For the subsequent analysis of chromosome abnormalities, the fastq files corresponding to demultiplexed individual embryos were converted to bam files and analysed using the Nexus Copy Number software (BioDiscovery) according to the manufacturer's protocol applying parameters: Significance Threshold ≥ 1.0E − 7; max contiguous probe spacing (Kbp) ≥ 50,000; min number of probes per segment ≥ 3; high gain ≥ 3.7 and big loss ≥ 0.3.

QC metrics

An important addition to the OneGene PGT method is the QC metrics, which automatically calculates the successful call rates of SNPs, spread of heterozygous SNPs, ADO rates, and miscalls for each embryo. The QC metrics can identify embryos with poor sequencing data. It also confirms the inheritance of parental SNP alleles in embryos and verifies the DNA trio samples. The recommended parameters are as follows:

Call rate: More than 80% of SNPs should be successfully detected with at least 30-fold coverage.

Spread of heterozygous SNPs: Heterozygous SNPs should have ideally equal coverage for allele one and two. The high coverage spread at heterozygous loci refers to skewed WGA and more noisy data. Aneuploidy calling may be limit if a higher spread value is observed.

ADO rate: If three or more SNP loci show ADO (expected heterozygous but detected homozygous), haplotype calling should be performed with caution.

Miscalls: If more than one SNP shows miscall (presence of a SNP variant that is not expected), haplotype calling should be performed with caution and the miscalled SNPs should be queried.

Results

The OneGenePGT platform was implemented for the most common monogenic diseases for which a PGT-M was performed in our laboratory. The most frequent indications for

Table 1 Number of informative SNP markers and their distribution for individual genes analysed by the OneGene PGT platform

Gene	BRCA1	BRCA2	CFTR	FMR1	GJB2	HBB	HTT
PGT-M cycles performed	11	4	20	11	3	8	9
Number of informative SNPs (2 Mb left flanking)							
Total number of SNPs	43	28	38	28	43	39	46
Informative SNPs (average)	21.0	17.5	19.0	11.7	15.3	12.3	23.2
Min	6	15	14	8	13	3	12
Max	33	22	24	16	17	18	32
Number of informative SNPs (2 Mb right flanking)							
Total number of SNPs	33	39	35	26	49	28	48
Informative SNPs (average)	18.6	13.8	15.1	10.6	22	13.8	26.3
Min	8	11	8	4	20	6	14
Max	29	19	22	16	23	25	44

The table shows the total number of available SNP markers in the 2 Mb left and 2 Mb right flanking region of each gene. The average number of informative SNPs was calculated from the performed PGT-M cycles. The number of informative SNPs serves as the basis for a linkage analysis identifying haplotypes linked to a pathogenic or wild-type allele

PGT-M in our laboratory included cystic fibrosis (OMIM: #219,700; *CFTR* gene), sickle cell anaemia and beta thalassemia (OMIM: #603,903, #613,985; *HBB* gene), breast and ovarian cancers 1 and 2 (OMIM: #604,370, #612,555, *BRCA1*, *BRCA2* genes), Huntington's disease (OMIM: #143,100, *HTT* gene), fragile X syndrome (OMIM: #300,624, *FMR1* gene), and autosomal recessive deafness, 1A (OMIM: #220,290, *GJB2* gene). Schematic overview of the OneGene PGT technique is described in Fig. 1, and the total number of SNPs and the number of informative SNPs for each gene are shown in Table 1. The OneGene PGT platform underwent thorough validation which consisted of direct pathogenic variant testing, haplotype identification, and aneuploidy calling. The validation was performed on the basis of the re-analysis of the original DNA samples. Direct pathogenic variant testing of 83 DNA samples isolated from peripheral blood or trophectoderm samples showed 100% concordance between the OneGene PGT platform and Sanger sequencing (Supplement B). For linkage analysis, 51 embryos derived from specific PGT-M cases were re-analysed by OneGene PGT using DNA samples from the male partner, female partner, reference sample, and amplified embryos. We observed 100% correlation of linkage analysis between OneGene PGT and karyomapping (Supplement B). The final part of validation was the assessment of chromosomal abnormalities. In total, 39 MDA samples originally analysed by karyomapping were reanalysed using the OneGene PGT platform. We observed 100% concordance for full aneuploidy detection; however, some samples showed partial discordance caused mainly by mosaic findings (Supplement B). To further validate the sensitivity of our OneGene PGT platform for aneuploidy detection, a series of mixing experiments was performed (euploid and aneuploid samples) to determine the level of mosaicism that could be reliably detected (Fig. 2). Based on the results obtained from the mixing experiments, we can conclude that mosaicism could be reliably detected if it exceeds 50% in the trophectoderm sample.

Discussion

PGT-M represents a key technique for prevention of transmitting severe genetic disorders to the offspring and thus reducing the total number of affected children born with single gene disorders. From the ESHRE data, there is a gradual increase in IVF cycles with indications for PGT-M [2, 18]. This trend will most likely continue as expanded carrier screening programmes and exome analysis are on the rise in genetic testing practice [19, 20]. The valued benefit of PGT-M is that the genetic testing is performed prior to pregnancy. With this approach, there is no risk of termination of pregnancy as only unaffected embryos are considered for transfer to the uterus. PGT-M is a technically demanding approach, because only a single cell or a few cells collected from the embryo are genetically tested. Since the DNA template in the biopsied embryonic sample is limited, WGA is commonly used to amplify the minute DNA template. The well-established WGA technologies are, for example, PicoPLEX, MDA, and MALBAC [21, 22]. From the quantitative comparison studies, no single method performed best across all measured criteria and the choice of the WGA method depends strongly on the intended use and particular application [23]. In PGT-M applications, a high degree of accuracy is required despite the limited amount of DNA available from embryo biopsy. Allele drop-out (ADO)

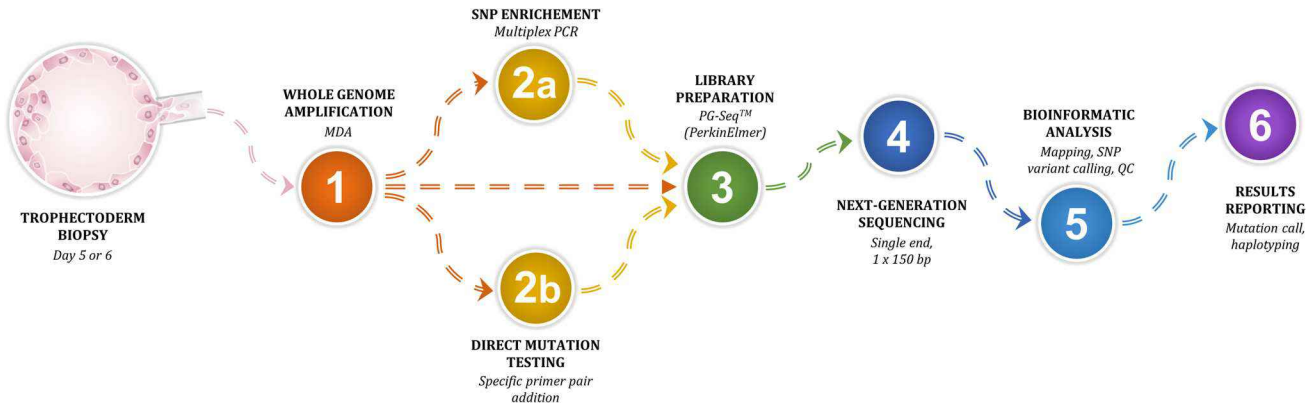

Fig. 1 Schematic overview of the OneGene PGT workflow. The embryo is subjected to a trophectoderm biopsy on day 5 or 6. In a further step, a trophectoderm sample is amplified by MDA. Multiplex PCR is used to further amplify SNP markers and a pathogenic mutation. The mixed MDA sample with enriched SNP markers from multiplex PCR is used as a template for library preparation with PG-SeqTM (step 3). The prepared library is sequenced. Finally, the NGS data are analysed using standard bioinformatics tools and informative SNP variants are linked with detected pathogenic variant (or wild-type allele). Using dedicated software (Nexus Copy Number software), a CNV is performed to detect chromosomal abnormalities

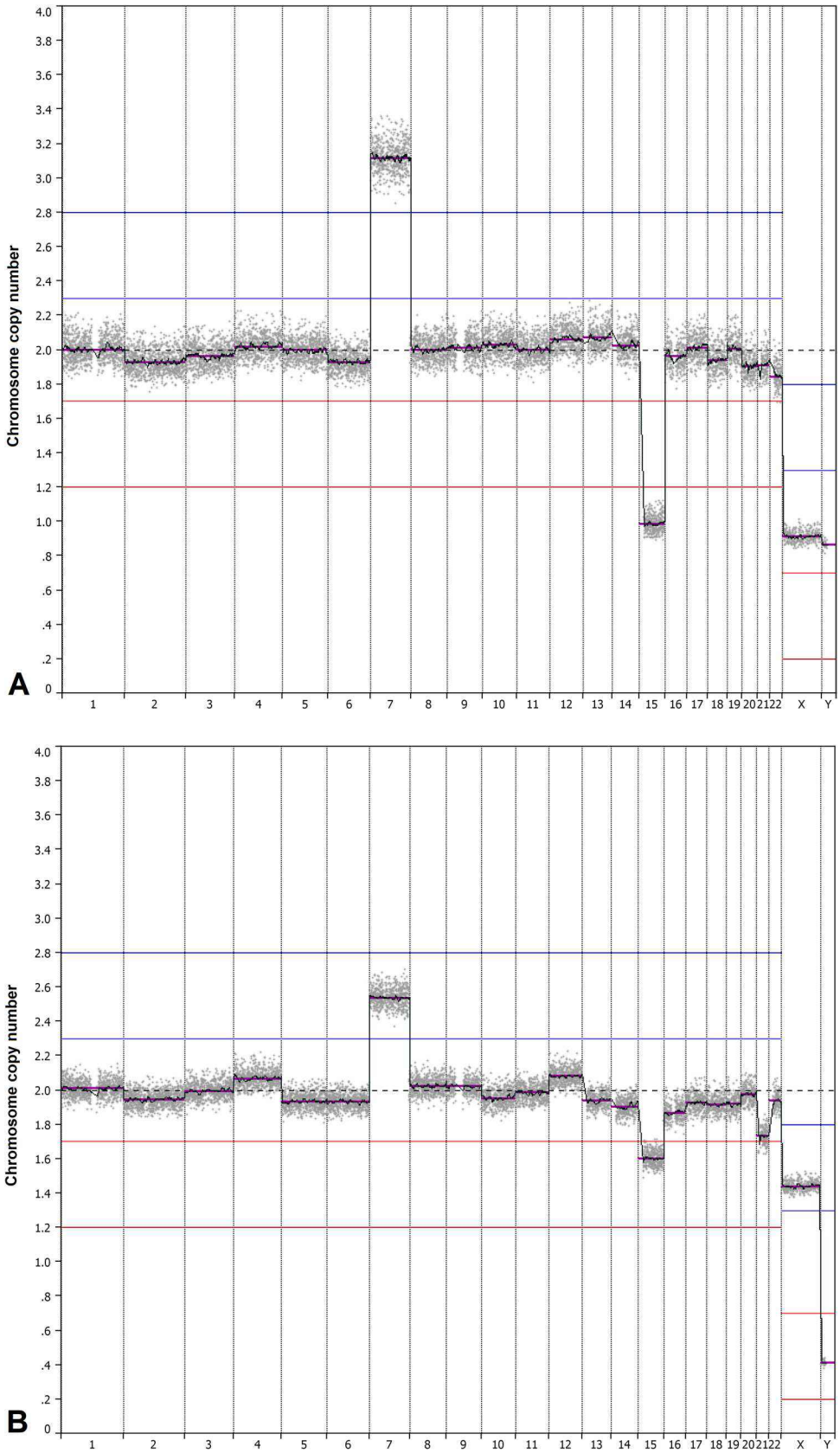

Fig. 2 Mixing experiments of euploid and aneuploid trophectoderm samples. Example of a mixing experiment to mimic a mosaic trophectoderm sample. Mixed sample was analysed using the OneGene PGT technique. **A** NGS chart shows the aneuploid trophectoderm DNA sample 46, XY + 7, − 15. **B** NGS chart showing analysis of aneuploid 46, XY + 7, − 15 and euploid 46, and XX trophectoderm DNA sample mixture in a ratio 1:1. Red lines indicate 1.7 and 1.2 copy number levels; blue lines indicate 2.3 and 2.8 copy number levels

and amplification failure are common drawbacks of WGA performed from a limited number of DNA samples as a source. ADO can be defined as amplification error affecting only one of the parental alleles in the tested sample. [24, 25] have described that ADO can occur in 5–50% of cases and is generally higher when blastomeres are tested, but decreases when trophectoderm samples are used for testing [26]. To achieve high diagnostic efficiency, it is recommended to exclude morphologically very poor embryos from the PGT-M procedure [27].

Here, we describe a new PGT-M platform — OneGene PGT. The technique incorporates direct mutation testing (with the exception of Huntington's disease), linkage analysis, and key quality control (QC) data to provide accurate and reliable results. Direct mutation testing is performed for the known disease-causing pathogenic variants in the family by adding a specific primer pair to the multiplex PCR reaction. To avoid misdiagnosis caused by ADO, OneGene PGT enables linkage analysis using 60–100 highly heterozygous SNP markers present within and around the gene of interest. Using the parental and family reference DNA samples, the OneGene PGT method automatically identifies haplotypes associated with the pathogenic variant or normal allele. An important addition to the OneGene PGT tool is the QC metrics, which automatically calculates SNP markers, successful call rates, and the spread of heterozygous SNP reads, ADO rates, and miscall rates for each embryo. The QC metrics can identify embryos with poor sequencing data. It also confirms the inheritance of parental SNP alleles in embryos. OneGene PGT uses SNP analysis/haplotyping only in the region of the affected gene and not genome-wide. This approach significantly reduces sequencing costs compared to the recently published NGS-based comprehensive PGT-A/PGT-M strategies [9–12, 28]. The limitation of OneGene PGT is that the platform has only been validated for several genes (*CFTR, FMR1, BRCA1, BRCA2*, *HTT, HBB, GJB2*); however, these genes account for 30% of all PGT-M indications in our IVF centre.

Several competing platforms have been described in the literature. OnePGT (Agilent Technologies, Inc.) uses genome-wide next-generation sequencing (NGS), which allows most genetic conditions to be traced within families and embryos. The advantage of this test is its universality, as genome-wide SNP markers are used to detect each individual genetic disorder, but at a higher cost comparing to our platform [9]. Another approach utilizes optimized reduced representation sequencing (RRS) by GENType, along with a novel analysis platform (Hopla), which enables cheap, accurate, and comprehensive PGT of blastocysts, even without the inclusion of additional family members; however, the technology requires prior diagnosis of at least one reference embryo by an independent technology [10]. SNP arrays coupled with siCHILD/haplarithmisis can be used for genotyping of human cell line and PGT-M samples. In brief, siCHILD relies on pedigree-based haplotyping analysis of genotypes from parents and phasing reference(s) to separate the two haplotypes of the parent(s) [11]. A novel technique for comprehensive PGT method called HaploPGT, combines reduced representation genome sequencing, read-count analysis, B allele frequency, and haplotyping analysis, to simultaneously detect different genetic disorders in one single test. However, the technique requires 80 million reads (80 M) of the sample genomic, which can only be achieved by using high-capacity sequencers [12]. PGD-SEQ™ (ThermoFisher Scientific) represents comparable technique to OneGene PGT. The advantage of the OneGene PGT is a direct detection of pathogenic variants by simple addition of a specific primer pair to the multiplex PCR. The only exceptions are technically challenging mutation tests such as triplet repeat expansion mutations for *FMR1* and *HTT*; however, both might be performed by an additional test on MDA product. Another advantage of OneGene PGT is the lower cost per sample compared to PGD-SEQ™.

It has been widely recognized that one of the greatest contributors to infertility is advanced maternal age (AMA), often defined as higher than 35 years [29]. AMA is linked with higher aneuploidy frequency in oocytes [30], which results in decreased implantation rates during IVF procedures [31] and a higher risk of miscarriage [32]. For that reason, preimplantation genetic testing for aneuploidy (PGT-A) has been adopted as a routine genetic testing in embryos. Indeed, PGT-A has been shown to significantly increase the chance for implantation, reduce miscarriage rate in AMA patients [33, 34], and diminish the advanced maternal age effect on pregnancy rates when euploid embryos are transferred [35]. Several research groups studied aneuploidy rates in blastocysts derived from egg donors and found that 39.1–53.2% of them are aneuploid [36, 37]. They further stated that 88.1% of aneuploidies in embryos derived from egg donors are of maternal origin [37]. From these data, we suggest that detection of chromosome aneuploidy might also be beneficial for couples undergoing PGT-M, with the aim to eliminate aneuploid embryos from the transfer. To assess aneuploidy detection performance of our newly introduced OneGene PGT platform, we performed a thorough validation based on the re-analysis of amplified samples. For these samples, the aneuploidy status was known since the samples had been previously analyzed by karyomapping. As a result of reanalysis, the OneGene PGT platform showed a high level of agreement with karyomapping, showing 100% concordance in detecting whole chromosome aneuploidies. Only a few samples exhibited discordant findings, and all discordant findings were related to the mosaicism issue and different resolution of the platform. Thus, we can conclude that the OneGene PGT platform reliably detects

whole chromosome trisomies or monosomies. Some NGS platforms are sensitive enough to reveal a mosaic composition of biopsied trophectoderm sample [38]. However, the detection of the mosaicism was not intended to validate using OneGene PGT. Only a single mixing experiment was performed to show that the technique is capable of detection of mosaic trophectoderm samples, but thorough validation is needed. From a clinical perspective, the main goal of OneGene PGT is to identify non-mosaic aneuploid embryos, as the transfer of mosaic embryos can still lead to successful implantation and birth of a healthy child in a significant number of cases [39]. Our previous work has shown that PG-Seq™ technology with the Nexus Copy Number software provides a practical resolution of 5–10 Mb (megabases) when 500 K sequencing reads were used for CNV analysis. Therefore, we suggest that OneGene PGT is capable of detecting segmental (subchromosomal) aneuploidies with similar resolution; however, the practical resolution has not been internally validated.

The trend in PGT is to offer a comprehensive platform enabling reliable testing of single-gene disorders in families combined with aneuploidy testing. The OneGene PGT platform meets these requirements and might be used as a comprehensive PGT-M method in the centres for assisted reproduction.

Abbreviations *ADO*: Allele drop-out; *CNV*: Copy number analysis; *MDA*: Multiple displacement amplification; *PGT-M*: Preimplanation genetic testing for monogenic diseases; *STR*: Short tandem repeat; *SNP*: Single nucleotide polymorphism; *WGA*: Whole genome amplification; *MPS*: Massively parallel sequencing; *NGS*: Next-generation sequencing; *IVF*: *in vitro* Fertilisation

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Author contribution Manuscript drafting: MH; manuscript editing: DK, KB, RN, VH; developing OneGene PGT method: MH, KB; participating in experiments, sample preparation and data collection: MH, KB, DK, RN, VH, MBa, MBo, KW; final approval of the manuscript: KV. All authors have critically reviewed the manuscript and have approved the final version submitted for publication.

Declarations

Ethics approval The study was approved by the Reproductive Medicine Ethics Committee of the Repromeda IVF centre. All DNA samples used for the method validation were collected with informed consent, allowing the laboratory to use them anonymously for research purposes.

Conflict of interest The authors declare no competing interests.

References

1. Lee I, Alur-Gupta S, Gallop R, Dokras A. Utilization of preimplantation genetic testing for monogenic disorders. Fertil Steril. 2020;114(4):854–60.
2. van Montfoort A, Carvalho F, Coonen E, Kokkali G, Moutou C, et al. ESHRE PGT Consortium data collection XIX–XX: PGT analyses from 2016 to 2017†. Hum Reprod Open. 2021;3:hoab024.
3. Forman EJ, Ferry KM, Gueye N-A, Smith RD, Stevens J, Scott RT. Trophectoderm biopsy for single-gene disorder preimplantation genetic diagnosis (PGD) is significantly more reliable than day 3 blastomere biopsy. Fertil Steril. 2011;96(3):S222.
4. Fiorentino F, Biricik A, Nuccitelli A, De Palma R, Kahraman S, et al. Rapid protocol for pre-conception genetic diagnosis of single gene mutations by first polar body analysis: a possible solution for the Italian patients. Prenat Diagn. 2008;28(1):62–4.
5. Tuffs A. Germany allows restricted access to preimplantation genetic testing. BMJ. 2011;343:d4425.
6. Gutiérrez-Mateo C, Sánchez-García JF, Fischer J, Tormasi S, Cohen J, et al. Preimplantation genetic diagnosis of single-gene disorders: experience with more than 200 cycles conducted by a reference laboratory in the United States. Fertil Steril. 2009;92(5):1544–56.
7. Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara N, et al. Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet. 2010;47(10):651–8.
8. Natesan SA, Bladon AJ, Coskun S, Qubbaj W, Prates R, et al. Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. Genet Med. 2014;16(11):838–45.
9. Renwick P, Gonzalez J, Hewitson H. Evaluation and implementation of onepgt for monogenic conditions. Reprod Biomed Online. 2019;39:e26-27.
10. De Witte L, Raman L, Baetens M, De Koker A, Callewaert N, et al. GENType: all-in-one preimplantation genetic testing by pedigree haplotyping and copy number profiling suitable for third-party reproduction. Hum Reprod. 2022;37(7):1678–91.
11. Masset H, Ding J, Dimitriadou E, Ardeshirdavani A, Debrock S, et al. Single-cell genome-wide concurrent haplotyping and copy-number profiling through genotyping-by-sequencing. Nucleic Acids Res. 2022;50(11):e63.
12. Xie P, Hu X, Kong L, Mao Y, Cheng D, et al. A novel multifunctional haplotyping-based preimplantation genetic testing for different genetic conditions. Hum Reprod. 2022;37(11):2546–59.
13. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet. 2001;2(4):280–91.
14. Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray–based comprehensive chromosome screening in infertile patients. Fertil Steril. 2011;96(3):638–40.
15. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv, 2013; https://doi.org/10.48550/arXiv.1303.3997.
16. Lai Z, Markovets A, Ahdesmaki M, Chapman B, Hofmann O, et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. Nucleic Acids Res. 2016;44(11):e108.
17. ESHRE PGT-M Working Group, Carvalho F, Moutou C, Dimitriadou E, Dreesen J, et al. ESHRE PGT Consortium good practice recommendations for the detection of monogenic disorders†. Hum Reprod Open. 2020;2020(3):hoaa018.
18. Coonen E, van Montfoort A, Carvalho F, Kokkali G, Moutou C, et al. ESHRE PGT Consortium data collection XVI–XVIII: cycles from 2013 to 2015†. Hum Reprod Open. 2020;2020(4):hoaa043.
19. Lazarin GA, Haque IS. Expanded carrier screening: a review of early implementation and literature. Semin Perinatol. 2016;40(1):29–34.

20. Capalbo A, Valero RA, Jimenez-Almazan J, Pardo PM, Fabiani M, et al. Optimizing clinical exome design and parallel gene-testing for recessive genetic conditions in preconception carrier screening: translational research genomic data from 14,125 exomes. PLoS Genet. 2019;15(10):e1008409.
21. Biezuner T, Raz O, Amir S, Milo L, Adar R, et al. Comparison of seven single cell whole genome amplification commercial kits using targeted sequencing. Sci Rep. 2021;11(1):17171.
22. 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. 2016;16(1):79–102.
23. de Bourcy CFA, De Vlaminck I, Kanbar JN, Wang J, Gawad C, Quake SR. A quantitative comparison of single-cell whole genome amplification methods. PLoS ONE. 2014;9(8):e105585.
24. Chen CK, Yu HT, Soong YK, Lee CL. New perspectives on preimplantation genetic diagnosis and preimplantation genetic screening. Taiwan J Obstet Gynecol. 2014;53(2):146–50.
25. Volozonoka L, Perminov D, Korņejeva L, Alkšere B, Novikova N, et al. Performance comparison of two whole genome amplification techniques in frame of multifactor preimplantation genetic testing. J Assist Reprod Genet. 2018;35(8):1457–72.
26. Unsal E, Aktuna S, Aydin M, Ozer L, Baltacı V. Improved IVF success of combined PGT-M and PGT-A applications. Reprod Biomed Online. 2019;39:e69-70.
27. Magli MC, Gianaroli L, Ferraretti AP, Lappi M, Ruberti A, Farfalli V. Embryo morphology and development are dependent on the chromosomal complement. Fertil Steril. 2007;87(3):534–41.
28. Chen S, Yin X, Zhang S, Xia J, Liu P, et al. Comprehensive preimplantation genetic testing by massively parallel sequencing. Hum Reprod. 2021;36(1):236–47.
29. Kemper JM, Vollenhoven BJ, Talmor AJ. Preimplantation genetic testing for aneuploidy: a review. Obstet Gynecol Surv. 2019;74(12):727–37.
30. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, et al. Aneuploidy across individual chromosomes at the embryonic level in trophectoderm biopsies: changes with patient age and chromosome structure. J Assist Reprod Genet. 2014;31(11):1501–9.
31. Schaeffer E, Porchia L, López-Luna A, Hernández-Melchor D, López-Bayghen E. Aneuploidy rates inversely correlate with implantation during in vitro fertilization procedures: In Favor of PGT. Mod Med Genet Genomics. 2018; IntechOpen: London, UK, 2018; pp. 1–19
32. Jia CW, Wang L, Lan YL, Song R, Zhou LY, et al. Aneuploidy in early miscarriage and its related factors. Chin Med J (Engl). 2015;128(20):2772–6.
33. Yang Z, Liu J, Collins GS, Salem SA, Liu X, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012;5(1):24.
34. Scott RT, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril. 2012;97(4):870–5.
35. Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril. 2013;100(6):1695–703.
36. Haddad G, Deng M, Wang CT, Witz C, Williams D, et al. Assessment of aneuploidy formation in human blastocysts resulting from donated eggs and the necessity of the embryos for aneuploidy screening. J Assist Reprod Genet. 2015;32(6):999–1006.
37. Sills ES, Li X, Frederick JL, Khoury CD, Potter DA. Determining parental origin of embryo aneuploidy: analysis of genetic error observed in 305 embryos derived from anonymous donor oocyte IVF cycles. Mol Cytogenet. 2014;7:68.
38. Popovic M, Dhaenens L, Boel A, Menten B, Heindryckx B. Chromosomal mosaicism in human blastocysts: the ultimate diagnostic dilemma. Hum Reprod Update. 2020;26(3):313–34.
39. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril. 2021;115(5):1212–24.