The impact of next-generation sequencing technology on preimplantation genetic diagnosis and screening

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Largely because of efforts required to complete the Human Genome Project, DNA sequencing has undergone a steady transformation with still-ongoing developments of high-throughput sequencing machines for which the cost per reaction is falling drastically. Similarly, the fast-changing landscape of reproductive technologies has been improved by genetic approaches. Preimplantation genetic diagnosis and screening were established more than two decades ago for selecting genetically normal embryos to avoid inherited diseases and to give the highest potential to achieve stable pregnancies. Most recent additions to the IVF practices (blastocyst/trophectoderm biopsy, embryo vitrification) and adoption of new genetics tools such as array comparative genome hybridization have allowed setting up more precise and efficient programs for clinical embryo diagnosis. Nevertheless, there is always room for improvements. Remarkably, a recent explosion in the release of advanced sequencing benchtop platforms, together with a certain maturity of bioinformatics tools, has set the target goal of sequencing individual cells for embryo diagnosis to be a realistically feasible scenario for the near future. Next-generation sequencing technology should provide the opportunity to simultaneously analyze single-gene disorders and perform an extensive comprehensive chromosome screening/diagnosis by concurrently sequencing, counting, and accurately assembling millions of DNA reads. (Fertil Steril® 2013;99:1054–61. ©2013 by American Society for Reproductive Medicine.)

Key Words: Next-generation sequencing (NGS), array comparative genome hybridization (CGH), embryo, PGD/PGS, trophectoderm biopsy, vitrification

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Recent advances in genetics and genomics have significantly affected biomedical sciences and how we face unsolved health problems (1). Improvements in our understanding for many common and rare conditions allow a better management of these disorders, thus offering patients and their families a better quality of life. Reproductive sciences, a fast developing field of medicine, is closely following all these changes in genomics to clinically integrate the most effective strategies for personalized fertility treatments. Thus assisted reproduction technology (ART) has incorporated genetic tools for genetic testing of preimplantation embryos, which was initially performed to diagnose patients who were known to carry high risk for monogenic disorders (2) or chromosomal structural abnormalities (3). It was then applied to treat fertility patients with increased risk for aneuploid embryos. Preimplantation genetic screening (PGS) was introduced into clinical practice for screening and discarding aneuploid embryos, thus improving the chance of healthy conceptions after infertility treatment with poor prognoses, such as advanced maternal age, repeated implantation failure, and recurrent miscarriage (4, 5). Request for PGS for chromosomal abnormalities is now a daily demand in IVF clinics. The initial preimplantation genetic diagnosis (PGD)/PGS standard technique for detecting chromosomes was fluorescence in situ hybridization (FISH), with the use of specific probes for the chromosomes most commonly involved in aneuploidy. The presence or absence of a normal pair of

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chromosomes could be identified, thereby selecting for transfer only chromosomally normal embryos. Choosing embryos with normal chromosomes should increase implantation rate and live-birth rate and reduce miscarriage rate. However, FISH-based PGS results were untenable by some reports (6–8).

Owing to these less favorable outcomes, a more comprehensive, genome-wide profiling technique was needed that did not require any prior information of the chromosomal aberration. This new technique, comparative genome hybridization (CGH), enables the assessment of all chromosomes by comparing test DNA with normal control DNA (9, 10). However, CGH had important limitations—time for diagnosis, human resources, less skilled IVF protocols for biopsy and freezing, and less robust protocols for whole-genome amplification (WGA)—which limited the adoption of CGH in the clinical practice of ART. Before long, array CGH (aCGH) was developed, an improved technology for the detection of chromosomal imbalances (11). Coupled with WGA, it enabled researchers to analyze the very limited amount of genetic material in a single cell, requiring less time than WGA followed by conventional CGH (12). In addition, some microarray platforms offer the advantage of testing embryos for combined chromosomal and single-gene disorder (SGD) diagnosis (13). This simultaneous approach is essential because even at a lower frequency than PGS, PGD for SGD is still an important indication in IVF clinics. According to the European Society for Human Reproduction and Embryology PGD consortium (14) SGD diagnosis represents ~17% of recorded PGD data. Furthermore, setting up new SGD-PGD protocols is a constant request, as demonstrated by a yearly increase in the number of reported diseases (15). Even with new methods such as WGA approaches, setting up new determinations requires a trained laboratory and dedicated time for assessing and validating each case. As an illustration, we began our department for SGD-PGD in 2002 and since then >100 different conditions have been analyzed (Supplemental Table 1; Supplemental Fig. 1; both available online at www.fertstert.org) with continual yearly additions. An enormous amount of work is necessary for almost every condition, because all SGDs require assessment and validation for every new test, i.e., detection of a mutation or mutations together with linked haplotype by polymorphic markers. Interestingly, comprehensive chromosome screening (CCS) by quantitative polymerase chain reaction (PCR) has demonstrated accurate aneuploidy diagnosis (16) and could also be used for a combined chromosomal and SGD screening. However, for conventional fluorescent PCR, a specific validation test should be performed for every new mendelian disease. Owing to these requirements, developing and validating a straightforward genomic approach capable of simultaneous analysis of all mentioned PGD/PGS indications is a necessity.

Advances in DNA sequencing have made it increasingly practical to generate large amounts of sequence data cost-effectively with the use of high-throughput next-generation sequencing (NGS) technologies. The collection of technologies included in this new paradigm continue to evolve, and further improvements in technology robustness and workflow, as well as finding new ways to reduce costs, will favor the adoption of NGS into clinical diagnosis. Eventually the advantages of NGS will be brought to PGD/PGS patients. Herein we review how current changes in IVF practice for embryo assessment and biopsy, emerging and improvements in random whole genome amplification methods, as well as advances in sequencing technologies and occurrence of new platforms, open NGS to PGD/PGS programs.

IVF PRACTICES FAVORING THE IMPLEMENTATION OF NGS-BASED PGD/PGS

A key element for IVF success is to implement a good embryo assessment tool. Detailed embryo morphology examination, together with extended embryo culture and blastocyst transfer, was adopted by IVF clinics in an attempt to increase implantation and gestation rates, and it also facilitated the transition to single-embryo transfer (SET). The use of coculture media (17) or sequential embryo culture media (18) resulted in >50% blastocyst development with a simultaneous increase in implantation rates compared with those obtained with the transfer of cleavage-stage embryos. In addition, the possibility of selecting a normal embryo by culturing to blastocyst was suggested (19); however, further data indicated that this additional culturing does not select against chromosomally abnormal embryos at the preimplantation stage of embryo development (20) and that a high number of genetically abnormal embryos still exist (21).

In this situation, the challenge for the embryologist is trying to identify the most viable embryos to prioritize them for transfer, thus improving the clinical efficiency of SET. Further methods for selecting a normal blastocyst, including genetic techniques, were warranted. The rationale behind the use of PGD/PGS for this purpose is that only euploid embryos would be candidates for transfer, resulting in more live births. Additionally the number of miscarriages should be reduced by eliminating the transfer of chromosomally abnormal embryos. However, using cleavage-stage embryos, PGS by traditional chromosome screening methods mostly failed to show improvements in IVF outcome (6–8, 22).

Trophectoderm Biopsy

All along, cleavage-stage aspiration has been the predominant method of cell removal in the PGD field (14). However, the well documented chromosome mosaicism that exists in early human embryos (23) suggests that a biopsied cell may not be truly representative of the rest of the embryo. Mosaicism could complicate the accuracy of diagnosis, thus contributing to the failure of PGS to show a benefit. Another explanation for failed PGS could be the inconsistency of the technique used for diagnosis. Recently, a prospective, randomized, blinded, and paired comparison between single-nucleotide polymorphism (SNP) microarray-based 24-chromosome aneuploidy screening and FISH-based aneuploidy screening found a poor predictive value of cleavage-stage and FISH for aneuploidy determination in morphologically normal blastocysts (24). Taken together, this evidence makes it clear that FISH-based technology is mainly inadequate for the diagnosis of aneuploidy in early human embryos. To overcome the difficulties seen by

FISH-based PGS studies, and thanks to the advancements in IVF practice, a new biopsy approach—blastocyst biopsy—was clinically evaluated as a useful tool to obtain high embryo implantation rates (25).

By means of trophectoderm biopsy, more cells, and therefore more DNA, are available for analysis compared with cleavage-stage biopsy. This fact facilitates the implementation of techniques requiring a certain amount of DNA, thus helping to avoid pitfalls associated with a limiting technique and reducing the misdiagnosis rate. Still, recent investigations found a total of 17% of mosaic embryos (a mixture of diploid and aneuploid cell lines) at the blastocyst stage (26). The fate of these embryos is unclear. One should acknowledge that some diploid-aneuploid mosaic blastocysts may be viable and might be incorrectly discarded if aneuploid cells were analyzed. Moreover, because novel genetic diagnostic tools are based on expensive technology, the new time point of biopsy often reduces the number of samples analyzed, which results in a more time and cost-effective procedure.

Remarkably, although the results of single-cell aCGH (27, 28) and early single-cell NGS-based research (29) were encouraging, limited amounts of DNA may impede the establishment of accurate PGD programs based on single-cell sequencing. This was demonstrated by Navin et al. (29), who showed that the presence of sparse sequence coverage can translate into a nonviable clinical diagnosis scenario, giving a poor prognosis for clinical implementation of NGS-based PGD/PGS using a single-cell approach. Blastocyst biopsy should remove this limitation, because more cells can be analyzed at the same time, thus favoring a NGS-based embryo diagnosis.

Vitrification

One of the main limitations for PGD based on blastocyst biopsy is that it would likely require cryopreservation of the blastocyst to provide sufficient time for analysis. The recent introduction of vitrification as a new effective method to cryopreserve blastocysts (30) has become an important advancement in IVF laboratories. Vitrification is an ultrarapid cooling technique that is now widely used, replacing the conventional cryopreservation methods based on slow cooling.

Vitrification has high survival rates even after blastocyst biopsy (31, 32). This result was of great value for early PGD approaches based on trophectoderm biopsies, because it allowed an unlimited amount of time for the diagnosis. Moreover, the vitrified embryos can be transferred in a "nonstimulated" cycle where the endometrium can be optimal for implantation, resulting in a higher pregnancy rate (33–35).

But not all PGS approaches for comprehensive evaluation involve cryopreservation. Previously described CCS by quantitative PCR (16) offers the opportunity to analyze trophectoderm biopsies in time for the fresh transfer of a euploid blastocyst.

Althought NGS workflows may improve to eventually allow fresh embryo transfer after blastocyst biopsy, current analytic workflow (36) prevents having results in time for a viable clinical situation where biopsied blastocysts may be transferred without cryopreservation, which underscores the importance of vitrification.

WHOLE GENOME AMPLIFICATION

In addition to the above-described IVF practice, before clinical application of NGS-based PGD/PGS, an adaptation and validation of a WGA method to NGS-specific requirements will be crucial for obtaining a highly representative nonbiased WGA product. The ideal WGA approach should then give a high yield with consistent representation of the original template and complete coverage of the genome.

Different WGA methods have been described for single-cell analysis, but two main types are available: PCR-based ones and multiple displacement amplification (MDA)–based ones. Among the PCR-based methods, primer extension preamplification (PEP) and degenerate oligonucleotide–primed PCR (DOP-PCR) have been the most used. In reality, PEP was applied only in cases of PGD for monogenic disorders (37). The WGA methods applied to comprehensive screening in PGS have been DOP-PCR (38–41), but also MDA (42).

The first reports of WGA using DOP-PCR applied to PGS (both CGH and aCGH) showed 89%–93% of samples properly amplified with a reliable diagnosis (38–41). After that, other authors proposed MDA as a more reliable method for WGA with high yield and fidelity. MDA is an isothermal method that gives relatively unbiased amplification and amplification products higher in size than PCR-based methods (43). Currently, commercially available PCR-based WGA methods have improved the WGA step for PGD/PGS, achieving 97%–99% of amplification efficiency when applied to single blastomeres (28, 44).

This improvement in WGA techniques that results in almost 100% of properly amplified samples paves the way to the introduction of NGS in the preimplantation field, providing the opportunity to obtain more information that can be used for better embryo selection. This is because with the use of NGS we will be able to analyze DNA more extensively. However, before introducing NGS in preimplantation clinical use, more validation studies will be needed to determine if fold coverage is affected by allele dropout–related artifacts, which would affect NGS results.

NEXT-GENERATION SEQUENCING TECHNOLOGIES

In the early 1990s a new breakthrough entered the field of DNA sequencing, the development of capillary array electrophoresis (45). In 1998, improvements in automation and computing led to the commercialization of the first 96-capillary sequencers, then called high-throughput sequencing. The automated Sanger methodology is now referred to as a "first-generation technology."

Over the past decade new sequencing technologies have forced a redefinition of the "high-throughput" term. New technologies, collectively referred as NGS, outperform Sanger or dideoxy chain termination sequencing (46) in capacity in daily throughput, with a huge reduction in sequencing costs per megabase. The National Human Genome Research Institute has tracked the costs associated with DNA sequencing

(47). As seen in Figure 1, there is sudden and significant cost reduction beginning in January 2008, when sequencing centers moved from Sanger-based to NGS-based DNA sequencing technologies.

With the advent of NGS techniques emerge an opportunity to perform cost efficient genetic testing by sequencing in different clinical scenarios. In the PGD/PGS arena, it may contribute to definitive improvements in the genetic assessment of embryos before transfer to the uterus.

NGS Concepts

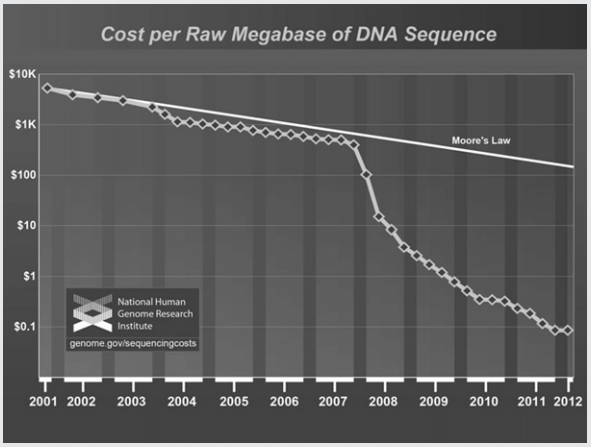
The core philosophy of massive parallel sequencing used in NGS is adapted from shotgun sequencing (48–50), developed to sequence longer sections of DNA. NGS technologies read the target DNA templates randomly. The target DNA or entire genome is broken into small pieces and then those DNA pieces are ligated to designated adapters for random reading during in-parallel DNA synthesis. The "read length" corresponds to the actual number of continuous sequenced bases. The read lengths are much shorter than with Sanger sequencing, which is why NGS results are called "short reads." Because most current NGS technologies produce short reads, coverage is an important factor. "Coverage of sequencing experiment" generally refers to the number of reads that overlap or cover the targeted genomic region (calculated as number of reads per read length, all divided by target size). For example, tenfold coverage (also reported as 10×) of a genome or a given genomic region means that, on average, each base of the genome or within the targeted region has been sequenced at least ten times. It may also be referred as "read depth" or "sequencing depth," meaning the number of reads that add information about a specific nucleotide. This depth varies quite a lot depending on the target DNA or genomic region. Consequently, an average sequencing depth of 10× leaves a lot of small portions of a target DNA unsequenced whereas other bases are represented by a lot more than ten sequences. Referring to the assembly, coverage means the percentage of the targeted sequence covered in the data itself.

Sufficient coverage is critical for accurate assembly of the genomic sequences, because short reads create many sequences that cannot be interpreted or "mapped" to any reference DNA. For example, some reads are too short and may match with many different regions of the target DNA or entire genome, so they cannot be accurately assembled. It is also worth noting that sequencing depth together with efficient algorithms for precise assembly of DNA sequences may be used to infer copy number, given a sufficient number of reads. For example, on average, a triploid region will get 1.5× more reads than a diploid region. This capability is key to designing NGS-based PGS approaches to use for early embryo aneuploidy screening.

Because of the massively parallel approach, each NGS run gives an exceedingly large number of sequence reads, and this capacity continues to increase at a rapid pace. Although this capacity is adequate or even necessary for sequencing complex genomes or large-scale applications, focusing on lower-complexity samples may result in sequence overreading with no increase in data quality. A solution to making NGS assays as efficient and affordable as possible is to analyze multiple samples in a single run. This is accomplished by using barcode analysis by sequencing ("Bar-seq").

Readers are referred to other sources for additional technical aspects related to NGS technologies such as barcoding, emulsion PCR, enrichment, assembly, etc. (51–54).

FIGURE 1



Yearly evolution of the cost of DNA sequencing (per megabase). The data from 2001 through October 2007 represent the costs of generating DNA sequence with the use of Sanger-based chemistries and capillary-based instruments ("first-generation" sequencing platforms). Beginning in January 2008, the data represent the costs of generating DNA sequence with the use of "second-generation" (or "next-generation") sequencing platforms. (www.genome.gov/sequencingcosts.)

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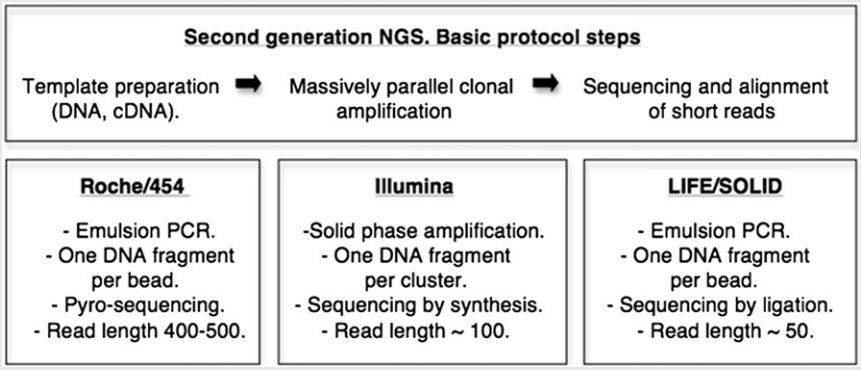
Basic Technical Aspects of NGS Platforms

Current NGS technologies are essentially grouped into second-generation (2G) and third-generation (3G) approaches. 2G platforms removed the in vivo bacterial cloning stage of the Sanger methodology and sped up sample preparation by with the use of either "emulsion PCR" (Roche-454; Life Technologies–Solid) or "bridge PCR" (Illumina) for clonal target amplification. All 2G approaches use a "cyclic array" process in which dense arrays of DNA features are derived by iterative cycles of enzymatic manipulation followed by image-based data collection (55). They also miniaturize the sequence derivation stage by with the use of strepavidin beads (Roche-454), flow cells (Illumina), or glass surfaces (Life Technologies–Solid). All 2G technologies are engineered to be massively parallel regarding operations and output (Fig. 2). Unlike the 2G technologies, which rely on PCR to grow clusters of a test DNA, attaching those clusters to a solid surface, and then cyclically being sequenced and imaged in a phased approach, 3G technologies interrogate single molecules of DNA without the need for synchronization, thereby overcoming issues related to the biases introduced by PCR amplification and dephasing.

FIGURE 2



Second-generation NGS technologies flow chart including basic methods for the leading commercial 2G NGS platforms.

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3G high-throughput NGS technologies are expected to offer advantages over 2G NGS, namely: 1) higher throughput; 2) longer read lengths; 3) higher accuracy; 4) less starting DNA; 5) faster turnaround time; and 6) lower cost. However, as found in 2G technologies, data analysis is still complex, owing to large data volume. In addition, 3G technologies need to solve signal-processing challenges.

Recently, sequencing platforms have been tailored toward low-scale applications, with footprints, workflows, reagent costs, and run times approaching the needs of small clinical laboratories. Three different benchtop high-throughput sequencing instruments are currently available that offer modest setup and running costs. 1) The 454 GS Junior (Roche) is based on emulsion PCR and pyrosequencing similarly to the biggest 454 GS FLX machine. 2) The Ion Torrent PGM (Life Technologies) also exploits emulsion PCR for clonal amplification. Remarkably, it is founded on semiconductor technology based on the well characterized biochemical process in which a nucleotide is incorporated into a strand of DNA by a polymerase, resulting in a release of hydrogen ion (H^+) as a byproduct. The PGM sequencing-by-synthesis approach relies on a modified silicon chip—a device that includes a high-density array of micromachined wells, an ion-sensitive layer beneath the wells, and beneath that a proprietary ion sensor—to detect pH changes induced by the H^+ release. Using an Ion sensor avoids the use of scanning, cameras, and light (Postlight sequencing technology; www.iontorrent.com). 3) The Illumina Miseq is based on existing Solexa sequencing-by-synthesis chemistry but with reduced run times compared with the biggest Hiseq 1000 and 2000 machines, made possible by a smaller flow cell, reduced imaging time, and faster microfluidics. Readers are encouraged to follow a recent comparison of performance for these benchtop instruments by Loman et al. (56).

This section was meant to provide a basic explanation of the technical aspects of the leading commercially available 2G platforms and corresponding benchtop instruments. Interested readers may follow a detailed review on this topic elsewhere (57). Nevertheless, one must be cautious with available information in the context that current 2G/3G NGS approaches are subject to constant ongoing refinements in hardware, software, and supporting methodologies.

Single-Cell Sequencing and Fundamentals of NGS-Based PGD/PGS

Applying NGS techniques to individual cells is in its early stages. The DNA work has mostly involved sequencing the relatively simple genomes of single-cell microbes. The much larger and more complex genomes of human cells have been a bigger challenge. Nevertheless, valuable data is obtained in pathologic scenarios where genetic heterogeneity is common. Navin et al. (29) used single nuclei and performed WGA with NGS and accurately quantified genomic copy number for individual tumor cells. Moreover, single-cell sequencing from individual sperm cells have uncovered recombination hot spots throughout the entire genome (58).

Single-cell MDA products (29, 43) cannot capture the entire genome; typically the coverage is between 40% and 90% (59). For some genomic applications it may be sufficient but it limits the application of MDA to a tiny group of cells. Current scenario in PGS/PGD may solve MDA limitations with trophectoderm biopsy.

Two recent works have evaluated NGS for preimplantation embryo assessment. Yin et al. (60) applied NGS to identify aneuploidies and unbalanced chromosomal rearrangements after blastocysts biopsy. Approximately 75% of the embryos were from couples with structural chromosomal abnormalities. For the analysis of the WGA products, they set low-coverage whole genome sequencing and used a purposely created algorithm. WGA products were detected with an average 0.07× sequencing depth and 5.5% coverage of the human genome. They compared NGS data with results obtained by SNP array. A total of 38 blastocysts were investigated. Sixty eight percent (26/38) of embryos were found to be euploid and 32% (12/38) to contain chromosomal abnormalities. Results were confirmed by the SNP array. For the embryos with unbalanced chromosomal rearrangements, NGS-based approach provided higher accuracy in some

regions. The whole procedure required up to 10 days, thus requiring cryopreservation.

A targeted NGS-based PGD of monogenic diseases (61) has also been investigated. The authors developed a semiconductor technology–based targeted NGS, including a barcoding protocol for the sequencing of the trophectoderm samples. NGS-based genotype predictions were compared with results from the same embryos with the use of two independent methods of PGD. Results were perfectly consistent with the two more conventional methodologies, with 100% reliability. Interestingly the procedure was completed in less than 24 hours. Nonetheless, to avoid cryopreservation in the majority of PGD cases, a much more rapid method may be necessary. Another possibility is to investigate the performance of this protocol with single cells. The sustained technical improvement for the benchtop sequencing platforms could generate modest sequencing data in just a few hours, eventually making fresh transfer possible in the near future. Another remarkable aspect of this study was the ability to evaluate several samples on the same sequencing device—Ion 316 chip—by the use of barcoding (51). This technical aspect provides an opportunity to model the possible NGS throughput capacity of a single instrument and chip. Using a higher-capacity sequencing chip—Ion 318—it is theoretically possible to evaluate genotyping information for >100 samples on a single device. A recent cost analysis (56) estimated that running a 318 chip would cost US$625. As many as 80 samples could be run in that chip, making the costs of NGS similar to current methodologies.

Further improvements and new developments in NGS technologies, currently using WGA products from trophectoderm biopsies, can produce extensive coverage representing accurately all significant genomic regions and thus limiting artifacts for analysis of genetic diseases, providing a realistic clinical PGS/PGD scenario. Further studies with large sample sizes are needed to outline potentials for routine clinical use of NGS-based preimplantation embryo assessment.

DISCUSSION

Intensive efforts are continuously applied in ART settings to improve live birth rates. Studies designed to elucidate the true value of the practical IVF methods have resulted in changes in embryo manipulation procedures (i.e., timing for embryo biopsy, embryo freezing methods) and embryo assessment techniques. This picture includes the use of improved PGS/PGD tools for characterizing the genome of viable embryos.

In parallel, the development of molecular methods to improve analysis of limited starting material (62) and advances in genomic tools—microarray and sequencing technologies—have made it increasingly practical to generate large amounts of genomic data cost-effectively. Furthermore, continued rapid changes in NGS technologies have been coupled with increasing expansion of applications for sequence data. To date, biologic applications for NGS have mostly focused on de novo sequencing, resequencing and metagenomics. Development of small affordable NGS instruments put these techniques closer to clinical settings that demand low-throughput applications.

It seems reasonable to acknowledge that the joint appearance of new methods for embryo manipulation and genomic DNA random amplification and the surge of new sequencing technologies and instruments might influence the adoption of new methods for genetic testing of embryo biopsy material. In this scenario, NGS-based PGD/PGS might be the decisive extensive comprehensive screening and diagnostic technology.

Along with the benefits offered by these technologies, there are a number of challenges, both technical and ethical (63), that must be addressed and solved before NGS technologies enter the clinical arena of embryo diagnosis. One limitation will be the interpretation of the massive sequence data generated by NGS technologies. Background polymorphisms must be distinguished from potentially disease-causing mutations and copy number variations. By selective recovery and subsequent sequencing of genomic loci of interest, generated data and efforts for analysis can be reduced significantly compared with a whole-genome sequencing approach. It is reasonable to expect that at their early approaches, NGS-based PGD/PGS should be focused on target genome resequencing to provide extensive CCS that includes single-gene mutation diagnosis but avoids the challenge to interpret and counsel patients on the large multitude of rare variants.

There are other key drawbacks that need to be addressed before NGS is suitable for clinical services. The accuracy, for both base calling and alignment, needs further evaluation and validation studies. In addition, the present NGS protocol, even for targeted sequencing, is expensive and would not be easily applied to clinical PGS services. To overcome this, the use of "Bar-seq" will be necessary to process multiple samples in parallel. With the use of barcoding, DNA sequences of embryos from different patients will be analyzed at the same time, even for different sequencing data analysis requirements. Fortunately, the cost of multiplex barcode sequencing continues to decline as the number of reads per experiment increases.

Finally, with improvements in protocols still occurring and with the arrival of improved 3G NGS technologies (64) requiring a smaller amount of input DNA, longer sequence reads with greater accuracy, and faster turnaround time with less cost per analysis, a realistic scenario may be discovered where most clinical IVF settings could have access to NGS-based PGD/PGS.

CONCLUSION

Clearly, during the past decade we have seen intensive efforts to enter into a personalized reproductive medicine scenario by significantly improving and validating ART methods, i.e., use of optimized culture media, definition of best possible time point for biopsy, optimization of embryo assessment—by morphology and genetic status—and preservation. Moreover, controversial data have forced us to understand the advantages and limitations of the standard methods used for embryo genetic evaluation. In parallel, the explosive development of genomics technologies, including microarrays and advances in DNA sequencing, resulting in the advent of NGS provided the opportunity to implement comprehensive chromosome screening approaches.

Application of state-of-the-art IVF techniques coupled with genome embryo assessment by deep comprehensive diagnosis/screening methods using NGS technologies should result in high implantation and live birth rates. It seems to be the perfect momentum to give a definitive force to the worldwide practical application of SET.

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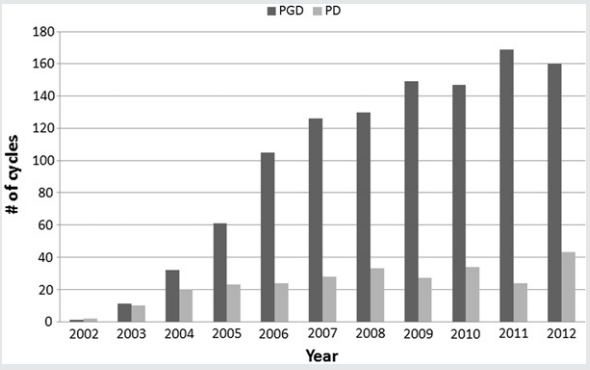
SUPPLEMENTAL FIGURE 1



Yearly development of new preimplantation genetic diagnosis (PGD) tests for single-gene disorders.

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SUPPLEMENTAL TABLE 1

List of genetic conditions analyzed in the preimplantation genetic diagnosis program for single-gene disorders.

Disease name	Gene ID
Autosomal recessive inheritance	
Spinal muscular atrophy	SMN1
Cystic fibrosis	CFTR
Beta thalassemia	HBB
Sickle cell anemia	HBB
Autosomal recessive polycystic kidney disease	PKDH1
Congenital disorder of glycosylation type 1A	PMM2
Congenital disorder of glycosylation type 1E	DPM1
Nonsyndromic hearing loss and deafness	GJB2
	GJB6
Metachromatic leukodystrophy	ARSA
Congenital adrenal hyperplasia	CYP21A2
Gaucher disease	GBA
Tyrosinemia type I	FAH
Familial hemophagocytic lymphohistiocytosis	PRF1
PCCA-related propionic acidemia	PCCA
PCCB-related propionic acidemia	PCCB
Mucopolysaccharidosis type I	IDUA
Mucopolysaccharidosis type IIIA	SGSH
L-CHAD deficiency	HADHA
Autosomal recessive osteopetrosis	TCIRG1
Severe combined immune deficiency	RAG2
Fanconi anemia, complementation group A	FANCA
Fanconi anemia, complementation group E	FANCE
Dystrophic epidermolysis bullosa	COL7A1
Junctional epidermolysis bullosa	LAMB3
	ITGB4
	LAMC2
Juvenile hyaline fibromatosis	CMG2
Spinal muscular atrophy with respiratory distress 1	IGHMBP2
Niemann-Pick disease type C	NPC1
Hypomagnesemia, renal, with ocular Involvement	CLDN19
Pompe disease	GAA
Zellweger syndrome	DBP
Phenilketonuria	PAH
Ataxia with central nervous system hypomyelination/vanishing white matter	EIF2B3
Mevalonicaciduria	MVK
Pyruvate kinase deficiency	PKLR
Lipoprotein lipase deficiency	LPL
Bardet-Biedl syndrome	BBS1
Autosomal dominant inheritance	
Myotonic dystrophy type 1	DMPK
Huntington disease	HD
Autosomal dominant polycystic kidney disease	PKD1
	PKD2
Neurofibromatosis 1	NF1
Lynch syndrome	MLH1
	MSH2
Spinocerebellar ataxia	SCA1
	SCA2
	SCA3
	SCA6
Multiple endocrine neoplasia type 2A	RET
Charcot-Marie-Tooth type 1A hereditary neuropathy with liability to pressure palsies	PMP22
Charcot-Marie-Tooth type 2A	MFN2
Charcot-Marie-Tooth type J/1B	MPZ
Charcot-Marie-Tooth type 2K	GDAP1
Familial adenomatous polyposis	APC
Hereditary multiple osteochondromatosis, type I	EXT1
Facioscapulohumeral dystrophy	FSHD
Tuberous sclerosis 1	TSC1
Tuberous sclerosis 2	TSC2
von Hippel–Lindau disease	VHL
Marfan syndrome	FBN1
Hereditary hemorrhagic telangiectasia	ENG
Hyperkalemic periodic paralysis	SCN4A
RhD isoimmunization	RhD

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SUPPLEMENTAL TABLE 1

Continued.

Disease name	Gene ID
Duffy isoimmunization	Duffy
Kell isoimmunization	Kell
HPA Isoimmunization	HPA5
Spastic paraplegia 3A	SPG3A
Aniridia	PAX6
Blepharophimosis, ptosis, and epicanthus inversus	FOXL2
Treacher-Collins	TCOF1
Ehlers-Danlos syndrome type I	COL5A1
Optic atrophy type 1	OPA1
Osteogenesis imperfecta	COL1A1
Familial amyloid polyneuropathy type I	TTR
Holt-Oram syndrome	TBX5
Blackfan-Diamond anemia	RSP19
Central core disease	RYR1
Myotonia congenita, autosomal dominant (Thomsen)	CLCN1
Retinitis pigmentosa	RHO
Hidrotic ectodermal dysplasia 2	GJB6
Emery-Dreifuss muscular dystrophy	LMNA
Early-onset primary dystonia	TOR1A
Cadasil	NOTCH3
Supravalvular aortic stenosis	ELN
Papillary renal carcinoma	MET
Hereditary hearing loss and deafness	MIR96
Saethre-Chotzen syndrome	TWIST
Epidermolysis bullosa simplex	KRT5
Pseudoachondroplasia	COMP
Familial cerebral cavernous malformation	KRIT1
Craneofacial dysostosis	FGFR2
Hereditary paraganglioma-pheochromocytoma syndrome	SDHD
Blau syndrome	CARD15
Best vitelliform macular dystrophy	VMD2
Familial hypertrophic cardiomyopathy	MYBPC3
Familial hypertrophic cardiomyopathy	TNNT2
Limb-girdle muscular dystrophy	LGMD1B
Parkinson disease	SNCA
Breast-ovarian cancer, familial	BRCA2
Li-Fraumeni syndrome	TP53
Adult syndrome	P63
Caveolinopathy	CAV3
X-linked inheritance	
Fragile X syndrome	FMR1
Hemophilia A	FVIII
Hemophilia B	FIX
Duchenne-Becker muscular dystrophy	DMD
Alport syndrome	COL4A5
Adrenoleukodystrophy	ABCD1
Incontinentia pigmenti	IKBKG
Hypohidrotic ectodermal dysplasia	EDA
Ornithine transcarbamylase deficiency	OTC
Mucopolysaccharidosis type II	IDS
Agammaglobulinemia X-linked	BTK
X-linked mental retardation associated with fragile site FRAXE	FRAXE
Norrie disease	NDP
Hyper IgM syndrome X-linked	CD40L
Juvenile retinoschisis X-linked	XLRS
Spinal and bulbar muscular atrophy	AR
Myotubular myopathy X-linked	MTM1
Charcot-Marie-Tooth X-linked	GJB1
Chronic granulomatous disease X-linked	CYBB
Choroideremia	CHM
Hypophosphatemia X-linked	PHEX
Lesch-Nyhan syndrome	HPRT
MECP2 duplication syndrome	MECP2
Retinitis pigmentosa	RPGR
Leri-Weill dyschondrosteosis	SHOX
Lowe syndrome	OCRL

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