Indications and management of preimplantation genetic testing for monogenic conditions: a committee opinion

Practice Committee and Genetic Counseling Professional Group of the American Society for Reproductive Medicine, American Society for Reproductive Medicine, Washington, D.C.

This statement is offered to update and expand on the prior American Society for Reproductive Medicine preimplantation genetic testing (PGT) opinion, elucidate the current clinical and technical complexities specific to PGT for monogenic conditions, assist providers in supporting patient understanding of and access to this technology, and offer considerations for the development of future clinical and laboratory guidelines on PGT for monogenic conditions. (Fertil Steril® 2023;120:61-71. ©2023 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

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In 2008, the American Society for Reproductive Medicine Practice Committee published its first opinion on preimplantation genetic testing (PGT) with the stated purpose of "addressing issues relating to the safety, accuracy, and overall efficiency of preimplantation genetic [testing]" (1). Since this time, the landscape of this testing—now termed preimplantation genetic testing for monogenic conditions (PGT-M)—has changed in both complexity and frequency of use. Data from the European Society for Human Reproduction Preimplantation Genetic Diagnosis Consortium demonstrate a consistent increase in the number of in vitro fertilization (IVF)/PGT-M cycles performed each year (2), and similar trends are seen elsewhere (3). This dramatic growth can likely be attributed to a number of factors including the following: an increasing number of recognized IVF/PGT-M candidates because of the wider use of expanded carrier screening and genetic diagnostic testing in pediatrics, oncology, cardiology, and neurology; improved patient and provider awareness; higher IVF utilization; and broadening insurance coverage. Although the application of all types of PGT has increased simultaneously, PGT-M is by far the most complex form of PGT because of the need for patient-specific genetic counseling, variant (mutation) confirmation, case review, and laboratory test customization—all ideally occurring before initiation of the IVF cycle. Unique technical challenges can arise during test preparation and analysis, and the interpretation and management of PGT-M results can be complex. Furthermore, the current lack of PGT-M-specific guidelines in the United States has led to differences between clinical and laboratory practices (4).

Determining whether PGT-M will be efficacious for a given patient requires consideration of both the clinical indication and technical factors. With PGT-M now being technically feasible for most inherited Mendelian conditions of known genetic etiology, analytic limitations are perhaps less burdensome than the evolving complex clinical scenarios and patient requests. Of note, PGT-M is not regulated in the United States, unlike in other countries. Health care professionals generally apply clinical guidelines to determine when PGT-M should be offered.

Some physicians may feel obligated to recommend or require PGT-M for all cases in which a reproductive risk has been identified. Although providers should consider the possibility of PGT-M to mitigate reproductive risk in such cases, it should not be a requirement of fertility treatment and should always be presented as optional. Patients, too, may feel a sense of responsibility to pursue PGT-M, whether to stop the transmission of a condition to future generations or simply to ensure that their child is born free of the condition. However, patients may alternatively decline to pursue PGT-M for a variety of reasons. They may feel that a particular reproductive risk is tolerable or they may decide that the benefit of PGT-M is outweighed by financial or logistic challenges. A nondirective approach when offering PGT-M is crucial to support patient autonomy. Alternatives to PGT-M as a means of

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addressing reproductive genetic risk should be outlined for the patient, including prenatal and/or postnatal testing, gamete donation, embryo donation, and adoption.

INDICATIONS FOR PGT-M

The initial application of PGT-M was primarily to prevent the transmission of severe, untreatable, or life-threatening childhood-onset conditions. Today, however, the use of the technology extends to a much broader range of genetic conditions for which support of PGT-M is less clear or even controversial, including those with mild to moderate phenotypes and later age of onset and of much greater clinical variability and/or reduced penetrance (5). A survey of laboratory genetic counselors in 2021 found that all participants believed that PGT-M should be allowed for conditions of lower penetrance, citing patient autonomy as a primary consideration (4). At this time, no guidelines exist to offer direction to those involved in determining when to offer or decline to perform PGT-M, although some ethical arguments exist (6). As such, physicians, genetic counselors, and PGT laboratories may develop internal policies regarding PGT-M availability within their institution. If certain types of conditions or clinical scenarios are deemed not appropriate for PGT-M, the patient should be informed of the policy as early in the process as possible.

The following are various PGT-M indications stratified into 4 categories on the basis of age of onset, condition severity, penetrance, and the expected impact of PGT-M on overall risk reduction. Table 1 shows the examples of each category (7–9).

Traditional/Pediatric Indications

Most PGT-M cases are performed for childhood-onset, lethal and/or severe conditions that lack effective treatment. Most providers agree that PGT-M should be available for these conditions (10).

Serious Adult-Onset Conditions

Over the last decade, roughly a quarter of PGT-M cycles in the United States were performed for an adult-onset condition (11, 12). The American Society for Reproductive Medicine has issued a statement generally supporting the use of the technology for such conditions "when the conditions are serious and when there are no known interventions...or the available interventions are either inadequately effective or significantly burdensome" (13).

Mild Conditions or Indications of Limited/Questionable Risk Reduction

Genetic variants may be identified in a patient or family for which the utility of PGT-M may be either limited or questionable. These include cases in which the risk of offspring is very low or not increased above that of the general population, conditions of very low penetrance or mild severity, and variants of uncertain significance (VUSs).

The term VUS is used when the genetic change in question lacks sufficient clinical evidence to categorize it as either pathogenic or benign, in terms of its impact on gene function and condition expression. Requests for PGT-M for a VUS are typically made when a VUS is suspected to be causative of a condition phenotype that has manifested in a patient or family. Patient counseling should emphasize that testing an embryo for a VUS can only identify the presence or absence of the variant. Therefore, if the condition in question has a different etiology, PGT-M would not reduce the risk of the condition. Notably, most VUSs are eventually reclassified as benign variants (14). For these reasons, PGT-M laboratories may have different policies and procedures regarding VUS handling, including specific consenting procedures (4, 15). Whether or not to offer PGT for a VUS may depend on a variety of factors including how the VUS was identified, supporting classification evidence, whether it tracks with the condition in the patient and family, associated recurrence risks, supporting clinical documentation, and the patient's risk tolerance.

Indications for Which PGT-M Is not Recommended

Preimplantation genetic testing for monogenic conditions may be requested by patients in situations where there is very little or no clinical utility. For example, carrier screening may identify a couple who carry variants in the same gene; however, the specific combination of variants is not causative of an adverse phenotype (e.g., pseudodeficiency variants). A couple in which only 1 partner carries an autosomal recessive condition typically has an extremely low risk of an affected child (typically $<1\%$); however, they may remain concerned about the residual reproductive risk that exists because of technical limitations and rare de novo mutations. In such cases, PGT-M can only be used to identify the presence or absence of the carrier parent's variant; it cannot confirm or deny the presence of a second variant. Therefore, such testing can only provide information about the carrier status of the embryo and cannot determine whether the embryo is affected. Although this information could theoretically be used to rank embryos and reduce relative reproductive risk, the absolute risk reduction would be minimal. Therefore, PGT-M is not recommended in these cases.

Additional indications include the following.

Human leukocyte antigen. PGT-M may be offered to patients who have a child with genetic immunodeficiency or hemoglobinopathy and are in need of a human leukocyte antigen (HLA)–compatible sibling to facilitate hematopoietic stem cell transplantation (16, 17). The goal of PGT-M in this case is to identify an embryo that is an HLA match for the affected (recipient) child. Generally, each embryo has a 1-in-4 (25%) chance to be a complete HLA match for a full sibling. However, HLA cases are often performed in conjunction with PGT-M for the condition with which the child is affected, often reducing the number of embryos available for transfer. Human leukocyte antigen matching for cases in which transplantation is not currently a recognized treatment (e.g., cerebral palsy) is more controversial.

TABLE 1

Suggested Categorization of PGT-M indications.

Category	Details	Condition examples
Childhood-onset, lethal or severe condition		Tay-Sachs disease (*HEXA*), sickle cell disease (*HBB*), spinal muscular atrophy (*SMN1*), classic cystic fibrosis (e.g., p.F508del in *CFTR*)
Serious adult- onset conditions		Hereditary breast and ovarian cancer syndrome (heterozygous *BRCA1* and *BRCA2*), Huntington disease (*HTT*), hereditary hypertrophic cardiomyopathy (multiple genes)
Mild conditions or indications of limited or questionable risk reduction	Low penetrance or susceptibility genes	Late-onset Alzheimer's disease susceptibility (*APOE*-e4), ankylosing spondylitis susceptibility (HLAB27), factor V Leiden or prothrombin thrombophilia (*F2* and *F5*)
	Mild genetic variants	5T allele or other modifying variants associated with cystic fibrosis related conditions (*CFTR*)
	Carrier status for autosomal recessive conditions with carrier manifestations	Carrier status for autosomal recessive Alport syndrome (*COL4A3* and *COL4A4*), Gaucher disease (*GBA*), ataxia-telangiectasia (*ATM*)
	Mild, common, and/or treatable conditions	Hereditary hemochromatosis (*HFE*)
	Variants appearing to be de novo in an affected child	The recurrence risk when both partners are negative is low (often approximately 1%) but not eliminated because of the possibility of gonadal mosaicism in a parent (7). Some conditions may have higher estimated gonadal mosaicism risks (8, 9)
	Variants of uncertain significance	
Indications for which PGT-M is not recommended	Autosomal recessive carrier status without manifestations of symptoms	Most autosomal recessive conditions
	Combination of variants not associated with disease	Risk of homozygosity for biotinidase deficiency variant D444H, nephrotic syndrome type 2 (*NPHS2*) variant R229Q, alpha-thalassemia (*HBA1/HBA2*) silent carrier, galactosemia (*GALT*) Duarte variant
		Common variants for methyltetrahydrofolate reductase deficiency (*MTHFR*)
	Pseudodeficiency alleles	Specific variants in *GAA*, *HEXA*, *GALT*
	Somatic-only variants	Variants identified in tumor testing that are not confirmed in the germline

PGT-M = preimplantation genetic testing for monogenic conditions.

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Hemolytic disease of the newborn. Preimplantation genetic testing for monogenic conditions may be used to avoid maternal-fetal blood group incompatibility when the individual carrying the pregnancy has been previously sensitized; examples include RhD, Kell, and Anti-E sensitization. Selecting embryos with a compatible genotype reduces the risk of hemolytic disease of the fetus and newborn. Typically, the antigen genotyping results of the sperm and egg provider must be known to determine whether PGT-M is indicated and feasible.

Nondisclosure. Nondisclosure testing may be performed for patients who are known to be at risk of an adult-onset condition and want to reduce the reproductive risk without determining their own genetic status. This approach "recognizes the right of the parent not to know whether they are themselves affected while enabling them to have children not affected by the condition" (18). Conditions for which nondisclosure PGT-M is commonly performed include Huntington disease (19), spinocerebellar ataxias, and other monogenic neurodegenerative conditions.

Polygenic and multifactorial conditions. Historically, PGT for conditions of polygenic inheritance (caused by the interaction of multiple genetic changes) or multifactorial origin (caused by the interaction of multiple genetic and environmental factors) has not been widely available. Because there are insufficient data sets from which to derive the risks of polygenic disorders, current genetic testing for these conditions remains inaccurate and unreliable. However, the recent introduction of clinically available PGT for polygenic conditions invites an exploration of the analytic/clinical validity and clinical utility of this testing. This critical analysis is beyond the scope of this PGT-M document.

Regardless of the true reproductive risk and disease severity, patients who request PGT-M generally do so because they perceive that it will reduce risk and/or provide them with peace of mind. Patients' interest in PGT-M is likely influenced by the counseling they have received regarding their risks, their personal experience with the condition in question, the availability of possible treatments and interventions for the condition, and any pre-existing need to use IVF for

underlying fertility issues. Although some PGT-M laboratories decline cases with milder or lower risk indications, others may accept these cases with a requirement of documented genetic counseling, formal approval from the ordering provider, and/or additional consent forms. Clinical genetic counselors with expertise in ART as well as clinicians with expertise in the condition of concern can provide valuable guidance in reviewing the appropriateness of a PGT-M request.

CLINICAL CONSIDERATIONS

Given the growing number of PGT-M indications, IVF providers are increasingly encountering patients who may be identified as PGT-M candidates. Clinics should be aware of the various considerations involved in offering PGT-M, selecting a PGT-M laboratory, and pretest and posttest counseling, as well as the challenges that may be encountered.

Patients may present for IVF for the purpose of PGT-M on the basis of known genetic risk or may be identified as PGT-M candidates during the course of their fertility care. Any patient who expresses interest in PGT-M should be offered consultation with a genetic counselor. Both laboratory-based and clinic-based genetic counselors may facilitate this process; however, the differences in the scope of practice between clinic-based and laboratory-based genetic counselors should be recognized when determining appropriate patient management (20). Ideally, patient counseling should initially be provided by a clinic-based genetic counselor (i.e., a genetic counselor unaffiliated with a PGT laboratory, where conflicts of interest may be perceived) to discuss the natural history of the condition, any known genotype-phenotype correlations on the basis of the specific variant(s) detected, and the feasibility of PGT-M, as well as other reproductive options. Genetic counselors positioned within the fertility clinic are also able to provide process-specific information about PGT-M within the context of the clinic's own IVF protocols and can tailor recommendations to an individual's fertility history. Laboratory-based genetic counselors are best positioned to handle the technical aspects of the PGT-M process and provide tailored pretest and posttest counseling relevant to the laboratory's PGT technology. After thoroughly reviewing the case, a laboratory genetic counselor will confirm and explain the technical feasibility of PGT-M, required samples for test development, and estimated turnaround time of the process and serve as the patient's key contact during test development and after results reporting.

Although PGT-M is available for most monogenic conditions, providers should inform patients that there remain situations in which PGT-M may not be an option. Communicating limitations surrounding the feasibility of PGT-M is essential to manage patient expectations early in the IVF process and ensure that patients are aware of alternative reproductive options.

PGT-M Laboratory Selection

Given the many nuanced differences between PGT laboratories and the highly individualized nature of PGT-M, it can be challenging for IVF providers to determine the optimal laboratory for a specific case. Clinic-based genetic counselors may provide patient-centered recommendations for PGT-M laboratory selection. The factors to consider for PGT laboratory selection may include the following:

- Technical considerations and limitations depending on the genes and variants of interest and/or availability of genetic relatives to participate in PGT-M test development
- Turnaround time of PGT-M test development and whether the laboratory can accommodate IVF cycle start before its completion
- Laboratory requirements for molecular and/or clinical documentation of the diagnosis for the proband and family members
- Financial considerations such as insurance coverage, qualification for discounted or complimentary PGT-M, or cost-containment options such as "batching" (biopsies from multiple cycles to be tested simultaneously)

Depending on the reason cited, a PGT-M case that is not accepted by 1 laboratory could be referred to another laboratory for review. The test development of PGT-M is laboratory-specific. If the patient pursues PGT-M with a different laboratory, then test development must be repeated. Given the associated financial and timing implications, clinics are encouraged to work with the patient's original PGT-M laboratory.

Pretest Counseling

In vitro fertilization providers should ensure that their patients have a thorough understanding of the PGT-M indication and process and set expectations accordingly. The following aspects of PGT-M should be addressed with patients before beginning the process:

- The natural history of the condition and any known genotype/phenotype associations for the specific genetic variants of interest.
- The timeline for custom test development and need to postpone an IVF cycle until it has been completed or the risks of proceeding with an IVF cycle before completion.
- The potential need to involve relatives, to varying degrees, in the test development process and recognition that this may conflict with the patient or couple's preference regarding disclosure of their reproductive plans to relatives.
- The possibility of detecting an incidental finding during test development (which may or may not prevent successful PGT-M testing), such as a copy number variant (with potential clinical or reproductive significance), a finding discrepant with the result reported by an outside laboratory, or uncovering nonparentage, consanguinity or other misattributed biologic relationships.
- The unlikely but possible scenario that PGT-M test development cannot be completed despite initial acceptance of the case by the laboratory.
- The types of results that may be reported for their specific case and what types of information can and cannot be conveyed by a positive or negative result.
- The potential for inconclusive or reduced accuracy results.

- Expectations for the number and proportion of embryos available for potential transfer in a given cycle, on the basis of patient factors (maternal age, ovarian reserve, and availability of sperm), clinic factors (rates of fertilization, blastocyst utilization, and thaw survival), condition tested (mode of inheritance), and other tests being performed in conjunction (e.g., PGT for aneuploidy [PGT-A]).
- The expected accuracy of PGT-M results, including limitations inherent to PGT-M that may result in diagnostic error, and availability of prenatal diagnosis by chorionic villus sampling or amniocentesis to confirm the results of PGT-M with a higher accuracy during an established pregnancy (1, 2). Providers should recognize that decisions about prenatal testing can be nuanced and highly influenced by patient emotion and perception of risk. For patients using gestational carriers, the accuracy of PGT-M results and intended parents' desires for or against prenatal testing warrants additional counseling (21–23).
- Clinical policies on the management of positive PGT-M results, including which types of results would allow for embryos to be maintained in long-term cryostorage or transferred, with the recognition that some patients may prefer to use PGT-M results to rank or prioritize embryos for transfer rather than as a determination for which embryos to discard (24, 25).

Posttest (Results) Counseling

Because PGT-M can be performed for hundreds of different genes and thousands of different genetic variants, results are often highly nuanced given the differences in Mendelian inheritance patterns, pathogenicity, penetrance, or variable expression of particular variants. For example, the carriers of autosomal recessive or X-linked conditions may be asymptomatic or may be at risk of developing symptoms of the condition or have other health risks (referred to as "manifesting carriers").

In vitro fertilization providers should have a clear understanding of how the selected PGT-M laboratory reports test results. Preimplantation genetic testing laboratories may use different terminology to indicate positive or negative results, including "carrier," "affected," "unaffected," "wild type," "normal," "negative," "heterozygous," and "homozygous."

The results involving variants associated with milder phenotypes, later onset, reduced penetrance, or uncertain significance may warrant additional counseling. It is essential that providers recognize these critical details so that patients can make truly informed decisions about the use or storage of their embryos. Although laboratory genetic counselors can provide guidance about results interpretation, patients may benefit from additional consultation with a genetic counselor or health care professional who has expertise applicable to the variant or condition of interest.

Clinical Challenges

Testing of patient relatives. The frequent necessity to involve patient relatives in the PGT-M process may pose logistic challenges for the IVF clinic. In some cases, the PGT laboratory may only need a deoxyribonucleic acid (DNA) sample (typically in the form of a buccal swab) from these relatives and can arrange for sample collection and shipping of the specimen directly with the patient. However, in other cases, relatives may require molecular testing through a separate diagnostic laboratory before the PGT laboratory can accept their DNA samples. Because PGT laboratories are unable to order outside clinical testing (20), it can be challenging to accomplish this task. The patient's IVF physician may elect to order the necessary testing for a patient's relatives; however, this may raise additional challenges associated with diagnostic laboratory selection, as well as counseling, consenting, and potential medical management for these relatives who are not themselves patients of the IVF clinic. Therefore, the patient's IVF physician may alternatively choose to refer patient relatives to a medical genetics provider.

Donor gamete recipients. Additional challenges may arise for patients using donor gametes because a DNA sample from the donor is always required by the PGT laboratory to perform PGT-M. The time point at which the donor's DNA sample is needed may be laboratory- or case-specific. In some cases, the donor's DNA may be needed before initiation or completion of test development, whereas in other cases, this DNA may only be needed to process the embryo biopsies and obtain final results. Preimplantation genetic testing laboratories may also have differing requirements for the type of DNA sample required from a donor (i.e., blood, buccal, or semen). Patients should be made aware of such considerations and their impact on test development or results timing, as well as the possibility that the necessary DNA samples may not always be available from all gamete donors because this may inform donor selection. Patients must additionally be made aware that if they select a new donor at any point, their PGT-M test will need to be updated to incorporate a DNA sample from the new donor.

Nondisclosure PGT-M. Although nondisclosure testing accounts for a small proportion of all PGT-M performed, it is exceptionally complex and sensitive (Table 2). Cases in which a direct nondisclosure approach is used (i.e., the PGT laboratory does not disclose whether embryos are excluded because of a positive PGT-M result vs. chromosomal aneuploidy) can be challenging for the IVF clinic. In these situations, patients may involuntarily predict their own genetic status on the basis of the proportion of embryos available for transfer, and whether these predictions are accurate, this opposes the purpose of nondisclosure testing and can cause additional anxiety. Therefore, it is traditionally recommended that patients pursuing direct nondisclosure testing do not learn any details of their IVF cycle outcome, including the number of oocytes retrieved, number of zygotes formed, or number of biopsied embryos, to avoid these potentially stressful statistical predictions. In vitro fertilization providers are encouraged to discuss the patient's preferences for cycle outcome disclosure before initiation of IVF, agree on which outcome measures will and will not be disclosed to the patient, and determine how the plan can be logistically executed within the clinic. In contrast, indirect (exclusion-based) testing does not require

TABLE 2

Nondisclosure preimplantation genetic testing for monogenic conditions methods

	Nondisclosure/direct	Exclusion-based/indirect
Advantages	• Embryos are only excluded from transfer if they are affected with the variant of interest and/or aneuploid.	• The disease status of the at-risk individual is not known to any party and, therefore, cannot be accidentally disclosed.
Disadvantages	• The disease status of the at-risk individual is determined by the laboratory and, therefore, may be accidentally disclosed. • Logistic complications involved with the nondisclosure of cycle outcome measures to avoid prediction of patient status.	• Embryos are excluded on the basis of a 50% risk of disease (using linkage analysis to determine the affected and unaffected haplotypes) rather than the presence of disease; therefore, embryos may be unnecessarily excluded if the patient did not inherit the variant from their affected parent.

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any additional confidentiality considerations between the IVF clinic, PGT laboratory, and patient because the patient's genetic status is not known by any of these parties. In such cases, embryos that inherit the at-risk allele (rather than clearly affected embryos) are identified, and patients should be informed of the option of continued cryopreservation of these embryos for possible future use, in the event that later predictive testing of the patient reveals negative results for the familial condition.

Transfer of embryos with positive PGT-M results. Although most patients who pursue PGT-M do so with the intention of only transferring variant-negative embryos, some may not have success in obtaining such embryos and may decide to transfer those with positive results. This decision is more common when PGT-M is performed for conditions of later onset or variable disease presentation (25). Clinicians may elect to perform or decline transfer of variant-positive embryos (6, 24). For cases in which such transfers are permitted by the clinic, additional patient counseling about the implications of this decision is strongly encouraged.

TECHNICAL AND LABORATORY CONSIDERATIONS

Although PGT-M historically involved single cell analysis of polar bodies or blastomeres, it is now most commonly performed on trophectoderm biopsy samples. Intracytoplasmic sperm injection is typically recommended to minimize contamination from cumulus cells and extraneous sperm to reduce the chance of misdiagnosis. Blastocyst cryopreservation after biopsy is often required to accommodate the time needed to process the test results. Preimplantation genetic testing for aneuploidy is often able to be performed in conjunction with PGT-M on a single biopsy sample. Laboratories may vary in their approach as to whether both tests are performed simultaneously or sequentially, which may have differing financial implications. Genetic counseling is important for patients to understand the risks and benefits of adding PGT-A. Patients should understand that adding PGT-A may limit the number of embryos available for transfer and, in young patients, there is no proven benefit to PGT-A for improving live birth rates (ref: randomized controlled trial data).

Once the laboratory receives the biopsied samples, the first step is extraction of DNA followed by DNA amplification. Laboratories may differ in the type of amplification method used (targeted, multiplex, or whole genome).

Preimplantation genetic testing for monogenic conditions is generally performed by linkage analysis with or without direct variant analysis:

- **Linkage analysis** involves the examination of 1 or more unique DNA markers located adjacent to the variant of interest, which are inherited alongside the affected and unaffected alleles. To overcome the risks associated with allele dropout (ADO), which is preferential or failed amplification of 1 allele, linkage analysis has traditionally been considered the gold standard for PGT-M (26). Linkage analysis involves the analysis of DNA samples from patients and their biologic relatives to identify polymorphic markers, typically either short-tandem repeats or single nucleotide polymorphisms, to distinguish between the mutant and wild-type haplotypes linked to the disease variant. The linked markers must be informative, meaning that the egg source and sperm source must have sufficiently unique markers to enable tracking of the haplotypes in embryo samples. The number of informative linked markers needed to meet accuracy standards will vary depending on the laboratory.
- **Direct variant analysis** directly identifies the known genetic variant and may be performed through a variety of assays. Because of the small amount of genetic material available from an embryo biopsy, direct analysis alone carries a risk of ADO. The chance of ADO may vary depending on the type of biopsy (analysis of trophectoderm biopsy is associated with lower ADO rates than that of blastomere or polar body biopsy) and the type of DNA amplification performed (the ADO rates are higher after whole genome amplification than those after targeted amplification) (27). Because ADO can result in embryo misdiagnosis, linkage analysis is standardly performed simultaneously to reduce this risk.

To determine the best testing approach, the PGT laboratory reviews the following:

- Genetic testing laboratory report documenting the particular gene(s) and variant(s) of interest.

- Patient's personal and family history of the genetic condition(s) of interest.
- Information regarding the availability of relatives (typically parents, children, and/or prior pregnancies) to provide DNA samples for test development and documented genetic status of these relatives if available. If the genetic status of necessary relatives is unknown, genetic testing of these relatives may be required before case review can be completed. For deceased relatives or previous pregnancies, banked DNA samples (including those obtained from prenatal testing) may be useful. If all ideal samples are not available, the laboratory will determine whether test development can be performed using only those samples that are available.

Regardless of the test method, PGT-M always requires custom test development, optimally in advance of IVF initiation. Test development can take anywhere from a few weeks to a few months, with the specific timeline being case- and laboratory-dependent. In rare cases, test development may be unsuccessful because there may be challenges to amplifying the genetic variant or obtaining sufficient informative linked markers; in these cases, PGT-M cannot be performed.

After successful test development, obtaining results on embryo biopsy samples is highly likely but not guaranteed. A result may be uninformative or have reduced accuracy because of several possible factors, including ADO, poor DNA quality, contamination in the biopsy sample, or recombination. In these cases, rebiopsy of the embryo often yields a result. However, if the uninformative result was because of recombination alone, a new biopsy is unlikely to clarify the result.

Technical Challenges

Although PGT-M is available for most variants, there remain some cases for which PGT-M is not technically feasible. Generally, if linkage can be established during test development, it is frequently possible to successfully perform PGT-M. However, for cases in which linkage cannot be established before testing, PGT-M can be challenging and may ultimately not be performed successfully.

It may not be possible to establish linkage if relatives of known genetic status are unavailable to provide DNA samples or if samples from necessary relatives are uninformative for any of the following reasons:

- Variant has occurred de novo in an affected patient.
- The affected patient is mosaic or chimeric for the variant or has a history of bone marrow transplant.
- The patient's parents both carry the same genetic variant as the patient.
- The couple or their parents are consanguineous.
- There are misattributed biologic relationships (e.g., nonparentage).

If linkage cannot be established with available family members, PGT-M relies on the ability to directly detect the genetic variant. In some cases, direct detection of genetic variants may be particularly challenging or not feasible (Table 3) (28–34). If the particular variant is conducive to direct detection, then embryo biopsy samples may be used to establish linkage during the testing phase; however, the ability to successfully make a diagnosis may be reliant on a minimum number of embryo biopsies showing the mutant haplotype. Several PGT laboratories may accept samples from immature or unfertilized oocytes (in the case of a maternal genetic variant) or arrested embryos to increase the chance of successfully establishing linkage. If linkage cannot be established because of a small sample number, then results may be reported as inconclusive. In these cases, it is recommended to undergo an IVF cycle to create additional embryos, and the previous embryos may be retroactively diagnosed once linkage is established. Some laboratories may alternatively attempt to establish linkage for paternal variants during test development by detecting the genetic variant directly in single (haploid) sperm.

If a couple is requesting PGT-M for a likely de novo variant identified in a previous child or pregnancy, linkage cannot be established. Some laboratories may attempt direct variant analysis if DNA from the affected offspring is available as a positive control for test validation. However, the PGT-M results must be interpreted with caution because a negative result may be caused by ADO rather than the true absence of the variant. For this reason, the results in these cases are often reported with reduced accuracy. An alternative strategy may be to identify which embryo haplotypes match those of the affected child, with the intention of transferring embryos with both opposite haplotypes. Given that the risk of an affected embryo in these cases is often very low (often $<1\%$), this strategy may unnecessarily eliminate a large proportion of unaffected embryos. It is, therefore, essential to recognize the limited utility of PGT-M in these situations.

Ideally, intent to undergo PGT-M is known before embryo biopsy. However, some patients may not be identified as PGT-M candidates until after embryo biopsies have been processed for PGT-A. In these cases, the PGT laboratory may have remaining amplified DNA from the original biopsies, and depending on the method of amplification initially used, it may be possible to attempt PGT-M without necessitating a rebiopsy of the embryos. However, in some cases, rebiopsy may be required. It should be noted that the remaining DNA from embryo biopsies is typically unable to be transferred between laboratories given interlaboratory differences in buffer solutions and amplification strategies.

SUMMARY

- Preimplantation genetic testing for monogenic conditions is an assisted reproductive technology available for most Mendelian conditions to optionally reduce the risk of a genetic condition in offspring.
- Preimplantation genetic testing for monogenic conditions can be performed for a wide range of indications, some of which are controversial.
- Although PGT-M can be performed for most monogenic variants, there remain some cases for which PGT-M is not technically feasible.
- The highly complex and individualized nature of PGT-M necessitates case review by a PGT laboratory followed by

TABLE 3

Types of genes and variants that may pose technical challenges (28–34).

Challenging case	Description	Example
Triplet repeat conditions	Often only feasible by linkage analysis, requiring involvement of family members of known genetic status (28). Direct assessment of the specific repeat number is not available at all laboratories.	• Fragile X syndrome (FMR1) • Huntington disease (HTT) • Myotonic dystrophy types I and II (DMPK and CNBP) • Spinocerebellar ataxia (multiple genes)
Large deletions	Often only feasible by linkage analysis, requiring involvement of family members of known genetic status. Some laboratories may alternatively perform direct detection using informative SNPs within the deleted region. For large deletions (typically minimum of >3 to 5 Mb), a PGT-A/PGT-SR approach may enable direct detection.	• 22q11.1 deletion (DiGeorge) syndrome • Multiexon deletions, commonly found in certain genes (e.g., DMD) • Contiguous gene deletions
Intragenic inversions	Only feasible by linkage analysis, requiring involvement of family members of known genetic status (29).	• Hemophilia A (F8) common inversion
Fusion genes	Only feasible by linkage analysis, requiring involvement of family members of known genetic status.	• Glucocorticoid-remediable aldosteronism (*CYP11B1/CYP11B2*)
Telomeric genes	Only feasible by linkage analysis, requiring involvement of family members of known genetic status. Informative markers are typically only available on 1 side of the gene; therefore, undetected recombination events may yield an incorrect diagnosis (30).	• Facioscapulohumeral dystrophy (*D4Z4*)
Duplications of uncertain phase	Only feasible by linkage analysis, requiring involvement of family members of known genetic status. Duplications detected by microarray are frequently in tandem (side by side); however, less commonly, the duplicated copy may be located elsewhere in the genome. In the latter scenario, PGT-M via linkage may result in misdiagnosis given that the region targeted is based on the assumed tandem location of the duplication. In some cases, FISH testing of the proband may confirm if the duplication is in tandem; however, FISH testing is not widely available and may be further limited by the chromosomal region of interest.	• *MECP2* duplication syndrome • 16p11.2 duplication
mtDNA variants	PGT-M is not widely available for mtDNA variants. In cases where PGT- M may be offered by estimating the mutant load in an embryo biopsy, it is unknown whether this mutant load is representative of the remainder of the embryo or of the future child because mitochondrial populations can shift during fetal development (31).	• Leber hereditary optic neuropathy • Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)
Recombination	If a relative being used to establish linkage is recombinant within the region of interest, linkage may be established incorrectly. Depending on the availability of other relatives' DNA samples during test development, this situation may not be detected until embryos are tested.	• HLA matching; if the child being matched is recombinant within the HLA region, finding an HLA- matched embryo is virtually impossible because recombination would need to occur in the exact same location
Pseudogenes or pseudogenic regions	Gene-homologous genomic regions may interfere with detection of variants in the gene of interest (32–34). In most cases, PGT-M is only feasible by linkage upfront, requiring involvement of family members of known genetic status.	• Gaucher disease (*GBA*) • Congenital adrenal hyperplasia (*CYP21A2*) • Autosomal dominant polycystic kidney disease (*PKD1*) • Spinal muscular atrophy (*SMN1*) • Lynch syndrome (*MSH1* and *PMS2*) • Cowden syndrome (*PTEN*)

DNA = deoxyribonucleic acid; HLA = human leukocyte antigen; FISH = fluorescence in situ hybridization; mtDNA = mitochondrial DNA; PGT-A = preimplantation genetic testing for aneuploidy; PGT-M = preimplantation genetic testing for monogenic conditions; PGT-SR = preimplantation genetic testing for structural chromosomal rearrangements; SNP = single nucleotide polymorphism.

Practice Committee and Genetic Counseling Professional Group of the American Society for Reproductive Medicine, American Society for Reproductive Medicine, Washington, DC. Fertil Steril 2023.

customized test development, which should be completed before an IVF cycle is started.

- Clinic-based and laboratory-based genetic counselors have different scopes of practice, and collaboration yields the most effective support for both the patient and IVF clinic.

CONCLUSIONS

- Preimplantation genetic testing for monogenic conditions should be offered if a significant reproductive risk is identified. Acceptance of PGT-M by patients should be optional.

- Preimplantation genetic testing should not be offered for autosomal recessive carrier status without manifestations of symptoms, combination of variants not associated with disease, pseudodeficiency alleles, or somatic-only variants.
- Patients should have genetic counseling about the condition and all reproductive options before PGT-M is performed.
- Patients may also benefit from genetic counseling about PGT-M results, particularly when making embryo transfer decisions.
- Given technical limitations that may result in embryo misdiagnosis, prenatal testing should be offered for pregnancies conceived using PGT-M to confirm the embryo testing results and screen for other fetal anomalies unrelated to the indication for PGT-M.
- Although PGT laboratory genetic counselors support providers and patients in the PGT-M process, IVF clinics should consider employing genetic counselors to result in smoother case management, more efficient workflows, and improved patient experiences.

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Indicaciones y manejo de los estudios genéticos preimplantatorios para enfermedades monogénicas: una opinión del Comité

Comité de Práctica y Grupo Profesional de Asesoramiento Genético de la Sociedad Americana de Medicina Reproductiva, Sociedad Americana de Medicina Reproductiva, Washington, DC.

Esta declaración se ofrece para actualizar y ampliar la opinión anterior de la Sociedad Americana de Medicina Reproductiva sobre los estudios genéticos preimplantatorios (PGT), dilucidar las complejidades clínicas y técnicas actuales específicas del PGT para enfermedades monogénicas, ayudar a los proveedores a apoyar el entendimiento de los pacientes y el acceso a esta tecnología y ofrecer consideraciones para el desarrollo de futuras pautas clínicas y de laboratorio sobre el PGT para enfermedades monogénicas.