Mitochondrial Replacement Techniques: Ethical, Social, and Policy Considerations

DETAILS

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Mitochondrial Replacement Techniques

ETHICAL, SOCIAL, AND POLICY CONSIDERATIONS

Committee on the Ethical and Social Policy Considerations of Novel Techniques for Prevention of Maternal Transmission of Mitochondrial DNA Diseases

Board on Health Sciences Policy

Institute of Medicine

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COMMITTEE ON THE ETHICAL AND SOCIAL POLICY CONSIDERATIONS OF NOVEL TECHNIQUES FOR PREVENTION OF MATERNAL TRANSMISSION OF MITOCHONDRIAL DNA DISEASES

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the report's conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by **Enriqueta C. Bond,** QE Philanthropic Advisors, and **Ellen Wright Clayton,** Vanderbilt University. They were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Preface

The proposed investigation and potential clinical use of mitochondrial replacement techniques (MRT) raises a novel collection of ethical, social, and policy issues. At the request of the U.S. Food and Drug Administration (FDA), the National Academies of Sciences, Engineering, and Medicine convened a committee with diverse interdisciplinary expertise and a range of backgrounds to examine and analyze these issues, make recommendations regarding whether and how to go forward with MRT, and elaborate principles for initial clinical investigations involving these novel techniques for avoiding some types of inherited mitochondrial DNA (mtDNA) diseases.

This report presents the consensus conclusions and recommendations of this diverse group of experts, each of whom brought her or his expertise and perspectives. As in the case of other ethics-related Academies studies, the subject did not lend itself to the typical approach of collection of data, but instead relied largely on conceptual considerations and analysis, as well as reference to existing practices and policies. The resulting recommendations reflect the committee's assessment of the ethical, social, and policy issues at the core of MRT and its articulation of the conditions and principles that should govern any clinical investigations of these techniques. The committee's deliberations were informed by information provided by FDA, input from a range of stakeholders, and presentations by invited experts at a public workshop. The recommendations that resulted from these deliberations are intended to be general enough to be applied as the science related to MRT evolves, but with enough specificity to address questions related to undertaking the first human investigations of these techniques.

This report would not have been possible without the dedicated, diligent, and skilled work of the Academies staff: Anne Claiborne, Rebecca English, Morgan Boname, and Michael Berrios. Erin Hammers Forstag also provided invaluable support to the committee. The committee gratefully acknowledges their truly tireless efforts. Lastly, thanks to my committee colleagues for their careful review of information; their thoughtful consideration of the many arguments and perspectives presented; and most of all their unflagging patience as we considered, and often reconsidered, our recommendations for addressing the ethical, social, and policy issues and challenges posed by human investigations of MRT. It was truly a privilege to work with them all.

Jeffrey P. Kahn, *Chair*
Committee on the Ethical and Social Policy Considerations of
Novel Techniques for Prevention of Maternal Transmission
of Mitochondrial DNA Diseases

Contents

Abstract

At the request of the U.S. Food and Drug Administration (FDA), the National Academies of Sciences, Engineering, and Medicine assembled an ad hoc committee tasked with developing a consensus report regarding ethical, social, and policy considerations related to mitochondrial replacement techniques (MRT), which entail modification of human oocytes and zygotes to prevent the transmission of mitochondrial DNA (mtDNA) diseases from mother to child. These diseases vary in presentation and severity, but lead to morbidity and in some cases premature death. MRT would be used to prevent the transmission of mtDNA diseases by creating an embryo with nuclear DNA (nDNA) from the intended mother and nonpathogenic mtDNA provided by a woman, using techniques that would modify either an oocyte (egg) or zygote (fertilized oocyte).

MRT, if effective, could satisfy the desire of women to have a genetically related child with a significantly reduced risk of passing on mtDNA disease. The techniques, however, have a unique combination of characteristics that raises a novel collection of ethical, social, and policy issues. These include that MRT would (1) create embryos that if transferred would result in offspring with genetic material from two women of different maternal lineage,[1] a novel intervention never approved by U.S. federal

[1] Every individual has genetic material from many individuals and ancestors. For instance, due to the matrilineal nature of the inheritance of mtDNA, each individual has genetic material from their mother, grandmother, great-grandmother, etc. Therefore, MRT is unique in that it would involve combining the genetic material of two women of different maternal lineage—nDNA from the intended mother who carries a pathogenic mtDNA mutation and mtDNA provided by a woman without pathogenic mutations in her mtDNA. In the instance

regulatory authorities[2]; (2) constitute modifications in the mitochondrial genome that could be heritable (i.e., could be passed down through future generations) if MRT were carried out to conceive female offspring, due to the matrilineal inheritance of mtDNA, and the effects of those modifications (whether beneficial or deleterious) could persist indefinitely; (3) entail genetic modification of which any resulting effects would not, at this time, be reversible[3]; and (4) constitute a genetic modification that would affect every cell type of the resulting individual, thus affecting the total organism rather than being confined to a specific organ system. In considering the ethical, social, and policy issues raised by this unique combination of characteristics, the committee examined (1) parental motivation to access MRT to produce genetically related children, taking into consideration the adequacy and availability of alternative approaches to creating families for women with a known risk of transmitting pathogenic mtDNA; (2) ethical, social, and policy concerns related to genetic modification of germ cells and the germline; (3) downstream social implications of MRT such as expanded clinical applications and potential enhancement; (4) implications of MRT for identity, kinship, and ancestry; and (5) the creation, manipulation, and possible destruction of human gametes and embryos that would be involved in MRT research or clinical application.

The committee identified significant and important distinctions between modification of mtDNA and nDNA that matter for an analysis of the ethical, social, and policy issues of genetic modification of germ cells and the germline. Among them, MRT is different from any technology that could be applied to the nuclear genome in that it would entail replacement of pathogenic mtDNA with unaffected mtDNA, as opposed to targeted genomic editing of either mtDNA or nDNA. Also, while mtDNA plays a central role in genetic ancestry, traits that are carried in nDNA are those that in the public understanding constitute the core of genetic relatedness in terms of physical and behavioral characteristics as well as most forms of disease. Moreover, while some forms of "energetic enhancement" (such as selecting for mtDNA to increase aerobic capacity) might hypothetically

where some level of mtDNA from the intended mother is carried over to the embryo created by MRT, this embryo would also contain mtDNA from two women of different maternal lineage.

[2] U.S. federal regulatory authorities have never approved a cell-based product that involves genetic material from two women of different maternal lineages, as would MRT. In the case of unapproved cytoplasm transfer in the late 1990s/early 2000s, FDA halted the application of these techniques and asserted the agency's jurisdiction in reviewing and approving any clinical applications of the techniques. To the committee's knowledge, there was no application to FDA to pursue cytoplasm transfer techniques, and therefore, MRT represents a unique opportunity for U.S. regulatory review.

[3] Only in highly hypothetical future technologies would genetic modifications introduced by MRT be reversible. The committee refers to the irreversibility of MRT in this report as it reflects the current state of science and the ethical analysis that accompanies MRT today.

be possible through MRT, they appear to be far fewer and more speculative relative to what might be possible in modifications of nDNA. In the committee's judgment, none of these distinctions are meant to imply that mtDNA is unimportant from the perspective of health, genetic relatedness, or potential energetic enhancement, but that its modification is meaningfully different from that of nDNA.

The committee concludes that the most germane ethical, social, and policy considerations associated with MRT could be avoided through limitations on the use of MRT or are blunted by meaningful differences between the heritable genetic modification of nDNA and that introduced by MRT. Therefore, the committee concludes that it is ethically permissible to conduct clinical investigations of MRT, subject to certain conditions and principles laid out in this report.

The committee's recommendations regarding potential clinical investigations and regulatory oversight of MRT include that minimizing the risk of harm to the child born as a result of MRT is the primary value to be considered in assessing the ethics of the balance of benefits and risks in clinical investigations of MRT.

Accordingly, the committee recommends that any initial MRT clinical investigations focus on minimizing the future child's exposure to risk while ascertaining the safety and efficacy of the techniques. The recommended restrictions and conditions for initial clinical investigations include

- limiting clinical investigations to women who are otherwise at risk of transmitting a serious mtDNA disease, where the mutation's pathogenicity is undisputed, and the clinical presentation of the disease is predicted to be severe, as characterized by early mortality or substantial impairment of basic function; and
- transferring only male embryos for gestation to avoid introducing heritable genetic modification during initial clinical investigations.

Following successful initial investigations of MRT in males, the committee recommends that FDA could consider extending MRT research to include the transfer of female embryos if clear evidence of safety and efficacy from male cohorts, using identical MRT procedures, were available, regardless of how long it took to collect this evidence; preclinical research in animals had shown evidence of intergenerational safety and efficacy; and FDA's decisions were consistent with the outcomes of public and scientific deliberations to establish a shared framework concerning the acceptability of and moral limits on heritable genetic modification.

For any initial and subsequent investigations of MRT, FDA, research institutions, investigators, and institutional review boards should also pay close attention to best practices for consent in research and special atten-

tion to communicating the novel aspects of MRT research to potential participants.

The committee recommends adherence to the following principles for oversight of MRT investigations and, if applicable, future clinical use:

- *Transparency*: Regulatory authorities should maximize timely public sharing of information concerning the MRT activities and decisions within their jurisdiction. FDA should encourage sponsors to commit to depositing of protocols and deidentified results in public databases.
- *Public engagement*: Regulatory authorities should incorporate ongoing exploration of the views of relevant stakeholders into the overall plan for review and possible marketing of MRT and should support opportunities for public meetings to gather these views.
- *Partnership*: FDA should collaborate with other regulatory authorities within and outside the United States to improve the quality of the data available for the assessment of benefits and risks.
- *Maximizing data quality*: FDA should require that sponsors have adequate resources, use appropriate designs, and plan studies that enable cross-referencing and pooling of data for assessments of benefits and risks.
- *Circumscribed use*: FDA should use the means at its disposal to limit the use of MRT to the indications, individuals, and settings for which it is approved, and should engage the public in a fresh ethical analysis of any decision to broaden the use of MRT.
- *Long-term follow-up*: FDA should require that sponsors design, fund, and commit to long-term monitoring of children born as a result of MRT, with a plan for periodic review of the long-term follow-up data.

Summary[1]

Mitochondrial replacement techniques (MRT) are designed to prevent the transmission of mitochondrial DNA (mtDNA) diseases from mother to child. These diseases vary in presentation and severity, but common symptoms include developmental delays, seizures, weakness and fatigue, muscle weakness, vision loss, and heart problems, leading to morbidity and in some cases premature death. The goal of MRT is to prevent the transmission of these serious diseases by creating an embryo with nuclear DNA (nDNA) from the intended mother and mtDNA from a woman with nonpathogenic mtDNA through modification of either an oocyte (egg) or zygote (fertilized oocyte). While MRT, if effective, could satisfy the desire of women seeking to have a genetically related child without the risk of passing on mtDNA disease, the techniques raise ethical, social, and policy issues.

The U.S. Food and Drug Administration (FDA) would regulate MRT under its authority to regulate "human cells or tissues that are intended for implantation . . . into a human" (21 CFR 1271). Under guidance issued by FDA, any clinical use of MRT would require an Investigational New Drug (IND) application. If approved, an IND allows clinical investigations in humans to begin. FDA's Cellular, Tissue and Gene Therapies Advisory Committee met in February 2014 to discuss MRT. At this time, the FDA committee received public comments that reflected concern about certain ethical, social, and policy issues surrounding MRT. Because these issues

[1] This summary does not include references. Citations for the discussion presented in the summary appear in the subsequent report chapters.

were beyond the purview of the FDA committee, FDA requested that the National Academies of Sciences, Engineering, and Medicine convene a consensus committee to consider the ethical, social, and policy issues raised and develop recommendations to inform the agency's consideration of MRT-related INDs.

This report presents the results of the committee's deliberations. In accordance with the statement of task provided by FDA, the committee addressed the foundational question of whether it is ethically permissible for clinical investigations of MRT to proceed.

The committee concludes that the most germane ethical, social, and policy issues could be avoided through limitations on the use of MRT or are blunted by meaningful differences between the heritable genetic modification of nDNA and that introduced by MRT. **Therefore, the committee concludes that it is ethically permissible to conduct clinical investigations of MRT, subject to certain conditions and principles laid out in this report.**

BOX S-1
Statement of Task

An ad hoc committee of the Institute of Medicine will conduct a study to develop a report that will inform the U.S. Food and Drug Administration in consideration of review of applications in the area of genetic modification of eggs and zygotes for the prevention of mitochondrial disease specific to mitochondrial DNA. These include maternal spindle transfer, pronuclear transfer, and polar body transfer but could also encompass other technologies not currently proposed.

The development of novel techniques in this area raises complex ethical and social policy issues, including

- Whether manipulation of mitochondrial content should be considered germline modification (defined as *human inheritable genetic modification*) in the same way and with the same social and ethical implications as germline modification of nuclear DNA, or whether, from a social and ethical perspective, it should be viewed differently from germline modification of nuclear DNA.
- The implications of manipulating mitochondrial content both in children born to women as a result of participating in these studies and in descendants of any female children.
- Ethical issues in providing "consent" or "permission" to accept risks on behalf of a child who does not exist.
- Ethical and social issues that arise if a child is born with genetic material from three individuals.

STUDY CHARGE AND APPROACH

To address its charge (see Box S-1), the committee held both open and closed meeting sessions and a public workshop. The committee also solicited public comments to gather expert and public opinion on such issues as the ethics of heritable genetic modification (i.e., germline modification), patient perspectives, the role of religion, and how to conduct an ethically acceptable investigation of MRT.

The committee's analysis included discussion of whether the appropriate approach should be (1) to begin from a permissive perspective that would support going forward unless restrictions are justified, or (2) to begin from a restrictive or precautionary perspective that would support restrictions on going forward until risks have been sufficiently managed or controlled, or prohibit going forward at all based on fundamental ethical, social, and policy concerns. The committee used an approach that recognizes important aspects of liberal democratic theory, which acknowledges the acceptability of individual interests and desires and the autonomy of

Taking into consideration these ethical and social policy issues, the committee's report will address the conduct of clinical investigations of these novel techniques, including the foundational question of whether safeguards such as specific measures and public oversight could adequately address the social and ethical concerns, or whether those concerns preclude clinical investigations. In addition, the report will specifically examine:

- The circumstances under which clinical investigations of the technology for the prevention of mitochondrial disease might be conducted ethically, including implications for the concept of "informed consent" and other aspects of the enrollment and tracking of participants during and after the trial.
- Whether, and how, the existence of alternative approaches to prevent the transfer of mitochondrial disease from mother to child (e.g., adoption, egg donation, or preimplantation genetic diagnosis for mitochondrial mutations for which it would be informative) should factor into the assessment of allowing these trials to proceed.
- Whether it is advisable to establish controls that would distinguish between genetic modification to prevent transmission of mitochondrial disease (therapeutic/prevention purposes) and genetic modification to enhance desired traits (enhancement purposes). What controls could be effective at maintaining this distinction, particularly for first-in-human clinical investigations?

parental decision making in a society capable of deliberation, transparency, and the rule of law, along with an optimism about scientific knowledge. The committee applied this approach with a healthy skepticism as to whether foundational concerns about some of the ethical, social, and policy issues raised by MRT could be addressed at all.

ETHICAL, SOCIAL, AND POLICY CONCERNS SURROUNDING MRT

While recognizing that review of the safety and efficacy of MRT would ultimately be FDA's purview, the committee worked to learn about the latest science on mitochondrial genetics and MRT to inform its ethical analysis. The committee examined the scientific context in which MRT is proposed, and concluded that the field of mitochondrial genetics is characterized by complexities that make predicting the behavior of mtDNA difficult and uncertain. A thorough understanding of the state of the science related to the unknowns of mtDNA genetics and MRT is important for informing the benefit and risk assessment entailed in potential regulatory decisions regarding if, when, and how to proceed with MRT in first-in-human clinical investigations.

In addition to the developing scientific context underlying MRT, the techniques have a unique combination of characteristics that raises a novel collection of ethical, social, and policy issues. First, MRT would create embryos that if transferred would result in offspring with genetic material from two women of different maternal lineage,[2] a novel intervention never before approved by U.S. federal regulatory authorities.[3] Second, if MRT were carried out to conceive female offspring, the resulting mtDNA modifications would be heritable (i.e., could be passed down through generations) in female offspring due to the matrilineal nature of the inheritance

[2] Every individual has genetic material from many individuals and ancestors. For instance, due to the matrilineal nature of the inheritance of mtDNA, each individual has genetic material from their mother, grandmother, great-grandmother, etc. Therefore, MRT is unique in that it would involve combining the genetic material of two women of different maternal lineage—nDNA from the intended mother who carries a pathogenic mtDNA mutation and mtDNA provided by a woman without pathogenic mutations in her mtDNA. In the instance where some level of mtDNA from the intended mother is carried over to the embryo created by MRT, this embryo would also contain mtDNA from two women of different maternal lineage.

[3] U.S. federal regulatory authorities have never approved a cell-based product that involves genetic material from two women of different maternal lineage, as would MRT. In the case of unapproved cytoplasm transfer in the late 1990s/early 2000s, FDA halted the application of these techniques and asserted the agency's jurisdiction in reviewing and approving any clinical applications of the techniques. To the committee's knowledge, there was no application to FDA to pursue cytoplasm transfer techniques, and therefore, MRT represents a unique opportunity for U.S. regulatory review.

of mtDNA, and the effects of those modifications (whether beneficial or deleterious) could persist indefinitely. Third, the effects of the genetic modification performed on oocytes or zygotes, once carried out, would not, at this time, be reversible.[4] Fourth, the genetic modification would affect every cell type in the resulting individual, thus affecting the total organism rather than being confined to a specific organ system.

In considering the ethical, social, and policy issues raised by the unique combination of characteristics of MRT, the committee examined parental motivation to access MRT to produce genetically related children, taking into consideration the adequacy and availability of alternative approaches to creating families for women with a known risk of transmitting pathogenic mtDNA. The committee also considered ethical, social, and policy concerns related to genetic modification of germ cells and the germline; unintended downstream social implications of MRT; the implications of MRT for identity, kinship, and ancestry; and the creation, manipulation, and possible destruction of human gametes and embryos that would be involved in MRT research or clinical application. The committee addressed as well the key differences between nDNA and mtDNA as they relate to the foundational question of whether it is ethically permissible for clinical investigations of MRT to proceed.

Availability of Alternatives

At present, prospective mothers[5] at risk for transmitting mtDNA disease to their offspring must choose among reproductive options that allow for varying degrees of nuclear genetic connection between the child and the prospective parents with variable risk of transmitting mtDNA disease. These options include unassisted sexual reproduction, preimplantation genetic diagnosis (PGD), oocyte or embryo donation, adoption, or childlessness. Unassisted sexual reproduction and PGD both would preserve the genetic relationship to prospective parents but are not viable options for reliably preventing transmission of mtDNA disease. In the case of oocyte

[4] Only in highly hypothetical future technologies would genetic modifications introduced by MRT be reversible. The committee refers to the irreversibility of MRT in this report as it reflects the current state of science and the ethical analysis that accompanies MRT today.

[5] This report uses the term "prospective parents" (including prospective mother or father) to mean those people who are interested in accessing MRT and would be clinically suited for MRT to prevent transmission of mtDNA disease. The report uses the term "intended parents" (including intended mother and father) to refer to the people who have entered the process of undergoing MRT, should clinical investigations be approved. The intended mother is the contributor of nDNA to the MRT oocyte or zygote and is the intended social mother; the intended father, if applicable, may or may not contribute sperm for the MRT oocyte or zygote, but is the intended social father.

donation, children have no genetic relationship to the prospective mother; in the case of embryo donation or adoption, children have no genetic relationship with either of the prospective parents.

Parental Desire to Pursue MRT

Although prospective offspring born as a result of MRT would lack an mtDNA connection with prospective mothers, MRT could satisfy a deeply held desire on the part of these mothers to have a child who bears an nDNA connection to them. MRT would not treat an existing person for a disease, illness, or condition, so its pursuit does not address a medical need per se. While pursuit of reproductive goals and desires deserves to be respected within the bounds of options made available through research and clinical settings, the responsibilities of professionals and the oversight process necessarily also include the protection of the health and well-being of a child created through use of these techniques. In the committee's judgment, the desire of prospective parents to have children who are at significantly reduced risk of manifesting serious mtDNA disease and with whom they have an nDNA connection is justifiable, and clinical research on the use of MRT could be permitted within limits. These limits would be focused on protecting the health and well-being of the children who would be born as a result of MRT.

Genetic Modification of Germ Cells and the Germline

For the purposes of this report, "genetic modification" means changes to the genetic material within a cell. In the case of MRT, the genetic modification is the combination of mtDNA from one woman with the nDNA of another woman of different maternal lineage within an oocyte or zygote. While there is no direct modification or editing of the mtDNA sequence itself,[6] this novel combination would not occur in unassisted sexual reproduction or in other assisted reproductive technologies. "Germline modification" is defined by the statement of task provided by FDA to this committee as "human inheritable genetic modification."[7] Using these definitions, the committee finds that MRT results in the genetic modification of germ cells, but that it constitutes heritable genetic modification (germline modification) only if used to produce female offspring because mtDNA is solely mater-

[6] While there is not direct gene editing of the nucleotide sequence of mtDNA through MRT, the overall frequencies of mtDNA alleles within the population are altered.

[7] The committee has adopted the shorter synonym "heritable" (instead of "inheritable") in this report.

nally inherited, and therefore MRT to produce male offspring would not constitute heritable genetic modification (germline modification).

As a form of genetic modification of germ cells, MRT raises concerns about interference with nature, "playing God," eugenics, and the potential impact on persons with disabilities. Some contend that international treaties or country-specific laws against germline modification would be violated by MRT. In the committee's judgment, although a number of ethical, social, and policy concerns have been raised about human genetic modification, whether heritable or not, through the use of MRT, these concerns warrant significant caution and the imposition of restrictions rather than a blanket prohibition on the use of MRT to prevent transmission of serious mtDNA disease.

Unintended Downstream Social Implications of MRT

As a result of the regulatory context and the social and market forces that drive the uptake of innovative reproductive technologies in the United States, concerns exist about the expanded use of MRT should it be approved by FDA, including its use for scientifically unproven or potential enhancement purposes. For instance, female idiopathic or age-related infertility is a likely candidate for expanded use of MRT, one that would significantly enlarge the pool of possible patients. The committee does not suggest an absolute limit on any eventual applicability of MRT to other conditions or diseases, but rather believes FDA and relevant professional societies need to take a cautious approach, with deliberate attention to ethical, social, and policy issues, in considering any uses of MRT beyond the primary indication of preventing transmission of serious mtDNA disease. To this end, the committee concluded that federal regulations would be needed and principled professional society guidelines interpreting the regulations would be helpful to limit the use of MRT to the prevention of transmission of serious, life-threatening mtDNA diseases and to prevent slippage into applications that raise other serious and unresolved ethical issues.

Identity, Ancestry, and Kinship

MRT would result in a novel combination of, and interaction between, mtDNA and nDNA different from that which would otherwise be the case, with potential implications for identity, kinship, and ancestry. In the committee's view, an mtDNA provider's genetic contribution would connect her to the resulting child through the sharing of an aspect of their lineage or ancestry. The novel combination of mtDNA and nDNA that would result from MRT blurs traditional notions of relatedness in ways that may undermine intergenerational connections and lineage as measured by mtDNA. In

the committee's view, however, the contribution of genetic material from two women of different maternal lineage does not form a basis for prohibiting initial investigation of MRT; rather, it is a matter for reflection by families considering undertaking MRT and for societal discussions related to conceptions of identity, kinship, and ancestry.

Important Distinctions Between Modification of mtDNA and nDNA

In light of the relative and important, albeit different, scientific, medical, and social contributions of mtDNA and nDNA to health, well-being, and conceptions of identity, as well as the unique combination of characteristics of MRT as an approach, a central question for the committee was whether the sort of heritable genetic change resulting from MRT raises ethical, social, and policy issues comparable to those raised by heritable modification of the nuclear genome. In the committee's judgment, there are significant and important distinctions between modification of mtDNA and nDNA that matter for an analysis of the ethical, social, and policy issues of genetic modification of germ cells and the germline:

- MRT is different from any technology that could be applied to the nuclear genome in that it would entail replacement of pathogenic mtDNA with unaffected mtDNA, as opposed to targeted genomic editing of either mtDNA or nDNA. The replacement of whole, intact, and naturally occurring mitochondrial genomes represents a qualitatively different form of heritable genetic change from that resulting from any approach for modifying nDNA, which would likely involve editing rather than en bloc replacement of chromosomes—the closest parallel to MRT.
- While mtDNA plays a central role in genetic ancestry, traits that are carried in nDNA are those that in the public understanding constitute the core of genetic relatedness, in terms of physical and behavioral characteristics as well as most forms of disease.
- While some forms of energetic "enhancement" (such as selecting for mtDNA to increase aerobic capacity) might hypothetically be possible through MRT, they appear to be far fewer and more speculative relative to what might be possible in modifications of nDNA.

None of these distinctions are meant to imply that mtDNA is unimportant from the perspective of health, genetic relatedness, or potential energetic enhancement, but that its modification is meaningfully different from that of nDNA. In the committee's judgment, the significant and important distinctions between modification of mtDNA to prevent transmission of

mtDNA disease through MRT and modification of nDNA (1) have implications for the ethical, social, and policy issues associated with MRT and (2) could allow justification of MRT independent of decisions about heritable genetic modification of nDNA.

While significant ethical, social, and policy considerations are associated with MRT, the most germane of these issues can be avoided through limitations on the use of MRT or are blunted by meaningful differences between the heritable genetic modification introduced by MRT and heritable genetic modification of nDNA. Therefore, the committee concludes that it is ethically permissible to conduct clinical investigations of MRT. To ensure that clinical investigations of MRT were performed ethically, however, certain conditions and principles would need to govern the conduct of clinical investigations and potential future implementation of MRT.

INITIAL INVESTIGATIONS AND GOVERNANCE OF MRT RESEARCH IN HUMANS

Having addressed the foundational question of whether it is ethically permissible for clinical investigations of MRT to proceed, the committee considered the conditions and principles necessary to guide clinical investigations and oversight of MRT.

Centrality of Minimizing Risk to the Future Child

In discussing the benefits and risks of MRT, a weighing and balancing that would ultimately be the responsibility of FDA, the committee observed that proponents of MRT sometimes describe use of the techniques as either a preventive measure or a therapy for children with mtDNA disease. Because in vitro fertilization (IVF) techniques are required as part of the MRT process to create an embryo, MRT would not treat a preexisting person or prevent a likely medical condition in an already existing individual. Drawing on this observation, the committee finds that MRT has at least two potential benefits: (1) the subjective benefit accruing to prospective parents of having a child related to the prospective mother by nDNA (but not mtDNA), and thereby at reduced risk of manifesting mtDNA disease; and (2) the population level benefit in the reduction in the number of children who would be born with serious mtDNA disease as a result of access to this reproductive technology. The committee notes that neither of these potential benefits would accrue to the children who would be born as a result of MRT, heightening the need for and importance of an emphasis on minimizing risk to future children because risk would accrue to the child, while benefit would accrue to others. In contrast with the typical case in biomedical research where some individuals are asked

to consent to bear risks voluntarily to enable potential benefits that would be enjoyed largely or exclusively by others, in the case of MRT consent cannot play a role in ensuring understanding of and agreement with these conditions because the child is brought into existence as a result of the research in question. Therefore, the committee concludes that minimizing the risk of harm to the child born as a result of MRT is the primary value to be considered in assessing the ethics of the balance of benefits and risks in MRT clinical investigations.

Conditions for Clinical Investigations

The committee's recommendations for conditions for potential initial investigations of MRT focus on taking a cautious approach. Among the most potentially contentious of these conditions is that initial investigations be limited to transferring male embryos for gestation, a condition based on the need to take a cautious approach to any pursuit of MRT. Because of the scientific uncertainties associated with these novel techniques and because MRT in female embryos would result in heritable genetic modification, the committee believes that a cautious approach to MRT in the U.S. research context is required, including a restriction to male embryos in initial clinical investigations. Sex selection for medical reasons is generally accepted; for instance, PGD was initially introduced to enable selection of female embryos to avoid X-linked disorders. While there is ethical debate about the acceptability of sex selection, the restriction recommended by the committee is predicated not on selection of one sex over another, but on the need to proceed slowly and to prevent potential adverse and uncertain consequences of MRT from being passed on to future generations. In addition, preclinical research to study intergenerational effects of MRT could continue while at the same time allowing families to use MRT to have male children with a significantly reduced risk of mtDNA disease. Other conditions noted in Recommendation 1 below also address risk minimization and the safety of MRT in the setting of a potential initial clinical investigation.

Recommendation 1: Initial clinical investigations of mitochondrial replacement techniques (MRT) should be considered by the U.S. Food and Drug Administration (FDA) only if and when the following conditions can be met:

- **Initial safety is established, and risks to all parties directly involved in the proposed clinical investigations are minimized. Because attempts to minimize risk and burden for one of the parties could interact with risk for another, minimizing risk to future children should be of highest priority.**

- **Likelihood of efficacy is established by preclinical research using in vitro modeling, animal testing, and testing on human embryos as necessary.**
- **Clinical investigations are limited to women who otherwise are at risk of transmitting a serious mitochondrial DNA (mtDNA) disease, where the mutation's pathogenicity is undisputed and the clinical presentation of the disease is predicted to be severe, as characterized by early mortality or substantial impairment of basic function.**
- **If the intended mother at risk of transmitting mtDNA disease is also the woman who will carry the pregnancy, professional opinion informed by the available evidence determines that she would be able to complete a pregnancy without significant risk of serious adverse consequences to her health or the health of the fetus.**
- **Intrauterine transfer for gestation is limited to male embryos.**
- **Clinical investigations are limited to investigators and centers with demonstrated expertise in and skill with relevant techniques.**
- **FDA has reviewed mtDNA haplogroup matching and if compelling, considered it as a means of mitigating the possible risk of mtDNA-nuclear DNA (nDNA) incompatibilities.**

Manipulation of Embryos

MRT would involve the creation, manipulation, and possible destruction of embryos not only in the preclinical research phase but also during clinical investigations and perhaps in clinical use. The creation, manipulation, and destruction of embryos for research purposes has long been controversial in the United States. While the creation of human embryos for research is not prohibited under federal law in the United States (although some states are more restrictive), neither FDA nor any other agency of the U.S. Department of Health and Human Services can financially support such research where embryos are destroyed, discarded, or subjected to risks with no prospect of medical benefit for the embryo. And even an agency request that data from such research be submitted in support of an IND to start first-in-human clinical investigations could well be controversial.

Recommendation 2: Ethical standards for the use of human embryos in research have been developed by the U.S. National Academies of Sciences, Engineering, and Medicine (the Academies), the U.S. National Institutes of Health (NIH), and the International Society for Stem Cell Research (ISSCR). These standards include the expectation of prospective independent review of research proposals. In light of concerns about the oocyte procurement and embryo manipulations necessary for

mitochondrial replacement techniques (MRT) preclinical and clinical research, regulatory authorities should ensure the ethical provenance of preclinical or clinical data submitted to the U.S. Food and Drug Administration (FDA) in support of an Investigational New Drug (IND) application. To the extent possible, regulatory authorities should ensure that sponsors adhere to ethical standards comparable to those developed by the Academies, NIH, and ISSCR. In preclinical research, nonviable human embryos should be used when possible. When use of nonviable human embryos is not possible, viable human embryos should be used only when required in the interest of developing the science necessary to minimize risks to children born as a result of MRT, and even then only in the smallest numbers and at the earliest stages of development consistent with scientific criteria for validity.

As data accrued from all sources on the benefits and risks of MRT, these data would need to inform the assessment of benefits and risks for potentially less beneficial or riskier investigations. A cautious, staged approach would need to be taken in the design of both initial and subsequent investigations, for example, in determining eligibility for prospective mothers, numbers of participants, and pacing of investigations.

Recommendation 3: If the conditions of Recommendation 1 are met, the U.S. Food and Drug Administration (FDA) should ensure that the design and conduct of initial and subsequent clinical investigations of mitochondrial replacement techniques (MRT) adhere to the following principles and practices:

- **The health and well-being of any future children born as a result of clinical investigation protocols of MRT should have priority in the balancing of benefits and risks with respect to the design of investigations, eligibility of prospective mothers, numbers of participants, and pacing of investigations.**
- **Study designs of clinical investigation protocols of MRT should be standardized to the extent possible so as to minimize the number of variables and enable valid comparisons and pooling of outcomes across groups.**
- **Data from research or clinical practices outside FDA jurisdiction should be incorporated into FDA's analysis to enhance the quality of the assessment of benefits and risks.**
- **Clinical investigations should collect long-term information regarding psychological and social effects on children born as a result of MRT, including their perceptions about their identity, ancestry, and kinship.**

The question of whether and when to expand MRT research to include transfer of female embryos for gestation is complex and depends on factors that are presently uncertain and unknowable. In addition to sharing characteristics of MRT with male embryos, MRT involving female embryos would introduce intergenerational effects, whether they be positive or negative. The committee's view is that sufficiently robust evidence of the safety and efficacy of MRT in males would be necessary before introducing the additional risks associated with the potential intergenerational effects that would accompany transferring female MRT embryos, regardless of how long it took to collect this evidence. Sufficiently compelling evidence that would reach the level of confidence envisioned by the committee would include information from experience with numerous male children followed at least during their early childhood years, as well as evidence from animal models that showed no adverse intergenerational effects when MRT was used to produce female offspring.

If and when sufficiently compelling evidence of safety and efficacy from experience with male MRT offspring and preclinical data on intergenerational effects were obtained, moving to transferring female embryos would remain a controversial step in that it would entail heritable genetic modification. A productive public discussion and process has been initiated to establish a shared framework with respect to whether heritable genetic modification is acceptable and if so, under what circumstances and for what purposes. The committee believes that its analysis can aid this ongoing discussion and that any decision about moving forward with MRT with female embryos should be informed by this discussion, and should be consistent with the established shared framework in effect at that time concerning the acceptability of techniques that result in heritable genetic modification of human embryos.

Recommendation 4: Following successful initial investigations of mitochondrial replacement techniques (MRT) in males, the U.S. Food and Drug Administration (FDA) could consider extending research of MRT to include the transfer of female embryos if

- **clear evidence of safety and efficacy from male cohorts, using identical MRT procedures, were available, regardless of how long it took to collect this evidence;**
- **preclinical research in animals had shown evidence of intergenerational safety and efficacy; and**
- **FDA's decisions were consistent with the outcomes of public and scientific deliberations to establish a shared framework concerning the acceptability of and moral limits on heritable genetic modification.**

Informed and voluntary consent of those deemed research participants in MRT clinical investigations would be required pursuant to federal guidelines and applicable state laws and institutional practices. In addition, it would be important for MRT researchers and institutions, in consultation with local review committees or a central institutional review board, to consider current guidance and emerging best practices in determining appropriate compensation for gamete providers, taking into account the demands placed on a gamete provider by an MRT research protocol.

Recommendation 5: In addition to attention to best practices for consent in research, the U.S. Food and Drug Administration (FDA), research institutions, investigators, and institutional review boards should pay special attention to communicating the novel aspects of mitochondrial replacement techniques (MRT) research to potential research participants.

- **For individuals who provide gametes, consent processes should reflect**
  - **the range of MRT procedures contemplated for preclinical and/or clinical investigations and the general ethical, social, and policy considerations surrounding MRT;**
  - **the management of incidental findings, should they arise;**
  - **appropriate compensation, with sensitivity to socioeconomic status;**
  - **the prospect of future contact between individuals who provided their gametes and children born as a result of MRT; and**
  - **the management of residual eggs and embryos.**
- **For intended parents, consent processes should reflect**
  - **information on the MRT research protocol, with focus on the implications for the health and well-being of resulting children;**
  - **alternative ways of becoming parents that can avoid maternal transmission of mitochondrial DNA (mtDNA) disease;**
  - **the management of and potential restrictions on access to embryos created through MRT (e.g., if initial investigations are limited to male embryos);**
  - **preimplantation and prenatal genetic diagnostic tests that would be incorporated into clinical investigation protocols;**
  - **the importance of long-term follow-up and how it would be part of the experience of any child born as a result of MRT; and**
  - **the challenges of maintaining patient privacy given intense media interest in MRT.**

- **For children born as a result of MRT, consent processes should reflect assent (and eventual consent) for monitoring and research procedures to be performed after birth, up to and including seeking informed consent from the children upon their reaching the legal age of consent.**

MRT would require special considerations across the trajectory of regulation and oversight—from preclinical studies to authorization of an IND, potential approval for clinical use, and postmarketing surveillance. These considerations could be addressed through the following guiding principles for oversight.

Recommendation 6: The U.S. Food and Drug Administration's (FDA's) overall plan for review and possible approval and subsequent marketing of mitochondrial replacement techniques (MRT) should incorporate the following elements:

- ***Transparency*: Regulatory authorities should maximize timely public sharing of information concerning the MRT activities and decisions within their jurisdiction. FDA should encourage sponsors to commit to depositing protocols and deidentified results in public databases.**
- ***Public engagement*: Regulatory authorities should incorporate ongoing exploration of the views of relevant stakeholders into the overall plan for review and possible marketing of MRT and should support opportunities for public meetings to gather these views.**
- ***Partnership*: FDA should collaborate with other regulatory authorities within and outside the United States to improve the quality of the data available for the assessment of benefits and risks.**
- ***Maximizing data quality*: FDA should require that sponsors have adequate resources, use appropriate designs, and plan studies that enable cross-referencing and pooling of data for assessments of benefits and risks.**
- ***Circumscribed use*: FDA should use the means at its disposal to limit the use of MRT to the indications, individuals, and settings for which it is approved, and should engage the public in a fresh ethical analysis of any decision to broaden the use of MRT.**
- ***Long-term follow-up*: FDA should require that sponsors design, fund, and commit to long-term monitoring of children born as a result of MRT, with a plan for periodic review of the long-term follow-up data.**

1

Introduction

Mitochondrial replacement techniques (MRT) are novel procedures designed to prevent the maternal transmission of mitochondrial DNA (mtDNA) diseases. Such diseases are rare, yet can be severely debilitating, progressive, and often fatal in infancy or childhood. While MRT could provide a reproductive option for women at risk of passing on mtDNA disease to their children, it raises a series of complex ethical and social questions that have implications for public policy.

STUDY BACKGROUND AND CONTEXT

Origin of the Study

On February 25-26, 2014, the U.S. Food and Drug Administration (FDA) convened a meeting of its Cellular, Tissue and Gene Therapies (CTGT) Advisory Committee to discuss "oocyte modification in assisted reproduction for the prevention of transmission of mitochondrial disease or treatment of infertility" (FDA Cellular Tissue and Gene Therapies Advisory Committee, 2014). The oral comments received by FDA from members of the public at this meeting revealed substantial concern among certain commenters about the perceived ethical, social, and policy implications of the proposed techniques, which entailed issues outside the scope of the advisory committee's discussion. In response, FDA requested that the Institute of Medicine produce a consensus report addressing these issues and how they might influence the conduct of clinical investigations for MRT; the charge to the committee is presented in Box 1-1. FDA indicated that

BOX 1-1
Charge to the Committee

Ad hoc committee of the Institute of Medicine will conduct a study to develop a report that will inform the U.S. Food and Drug Administration in consideration of review of applications in the area of genetic modification of eggs and zygotes for the prevention of mitochondrial disease specific to mitochondrial DNA. These include maternal spindle transfer, pronuclear transfer, and polar body transfer but could also encompass other technologies not currently proposed.

The development of novel techniques in this area raises complex ethical and social policy issues, including

- Whether manipulation of mitochondrial content should be considered germline modification (defined as *human inheritable genetic modification*) in the same way and with the same social and ethical implications as germline modification of nuclear DNA, or whether, from a social and ethical perspective, it should be viewed differently from germline modification of nuclear DNA.
- The implications of manipulating mitochondrial content both in children born to women as a result of participating in these studies and in descendants of any female children.
- Ethical issues in providing "consent" or "permission" to accept risks on behalf of a child who does not exist.
- Ethical and social issues that arise if a child is born with genetic material from three individuals.

it will take the recommendations of this report into consideration in its review of future Investigational New Drug (IND) applications for clinical investigations of MRT.

mtDNA Disease

Mitochondria are microscopic organelles present in almost every cell type of the human body. Although they are now recognized as having myriad functions, their main role is the production of cellular energy through a process termed oxidative phosphorylation. The majority of a cell's genes and DNA are housed in its nucleus; the mitochondria contain only 37 genes, all of which encode for molecules essential to oxidative phosphorylation.

Diseases that affect the mitochondria can be caused in three ways: an individual inherits a pathogenic mtDNA mutation from its mother; an in-

Taking into consideration these ethical and social policy issues, the committee's report will address the conduct of clinical investigations of these novel techniques, including the foundational question of whether safeguards such as specific measures and public oversight could adequately address the social and ethical concerns, or whether those concerns preclude clinical investigations. In addition, the report will specifically examine:

- The circumstances under which clinical investigations of the technology for the prevention of mitochondrial disease might be conducted ethically, including implications for the concept of "informed consent" and other aspects of the enrollment and tracking of participants during and after the trial.
- Whether, and how, the existence of alternative approaches to prevent the transfer of mitochondrial disease from mother to child (e.g., adoption, egg donation, or preimplantation genetic diagnosis for mitochondrial mutations for which it would be informative) should factor into the assessment of allowing these trials to proceed.
- Whether it is advisable to establish controls that would distinguish between genetic modification to prevent transmission of mitochondrial disease (therapeutic/prevention purposes) and genetic modification to enhance desired traits (enhancement purposes). What controls could be effective at maintaining this distinction, particularly for first-in-human clinical investigations?

dividual inherits a pathogenic nuclear DNA (nDNA) mutation from one or both parents that affects mitochondrial function; or an individual develops a de novo pathogenic mutation in either mtDNA or nDNA that affects mitochondrial function. MRT focuses only on the first type of causation—maternal transmission of pathogenic mtDNA mutations. During the process of sexual reproduction, the father's mtDNA is destroyed; only the mother's mtDNA is passed on to the child.[1] If a mother is homoplasmic for a pathogenic mutation (all of her mtDNA has the same mutation), all of her offspring will have the mutation. If a mother is heteroplasmic (some mtDNA is normal and some is mutated), her offspring will have varying levels of mutated, pathogenic mtDNA. The proportion of mutated, pathogenic

[1] There has been one reported case of paternal transmission of mtDNA in humans, but the vast majority of evidence points to sole maternal transmission. See, e.g., Filosto et al. (2003); Schwartz and Vissing (2002); and Taylor et al. (2003).

mtDNA may have clinical significance, with more severe disease generally being associated with a higher percentage of mutated, pathogenic mtDNA.

Given the complexity of mitochondrial biology, mtDNA diseases can vary markedly from patient to patient; however, they are often debilitating, progressive, and fatal at a young age. Common symptoms of mtDNA disease include muscle weakness, extreme fatigue, seizures, developmental delays, heart problems, and gastrointestinal disorders. Diagnosis can be complicated, as these diseases often share symptoms with other disorders, and testing requires an integrated approach that could include metabolic, muscle, and genetic tests. The prevalence of disease-causing mtDNA mutations is difficult to estimate, but an epidemiological survey in the North East of England suggests a minimum point prevalence of 1 in 5,000 (Gorman et al., 2015).

Mitochondrial Replacement Techniques

MRT is an in vitro fertilization (IVF) technique that involves removing an intended mother's nDNA from her oocyte or zygote, which contains mutated mtDNA, and transferring it into a female provider's oocyte or zygote, which contains nonpathogenic mtDNA and from which the nDNA has been removed.[2] The woman providing oocytes would have no personal or family history or genetic evidence of having mutated, pathogenic mtDNA. In this report, the term "MRT" encompasses both the transfer of the nuclear genetic material and the accompanying fertilization procedure that is necessary to produce a human embryo. These techniques could allow intended mothers to produce a child that would share their nDNA without passing on their pathogenic mtDNA. Three such techniques are most advanced in development: maternal spindle transfer (MST); pronuclear transfer (PNT); and, most recent, polar body transfer (PBT). (See Chapter 2 for more detailed description of these techniques.)

Maternal Spindle Transfer (MST)

In this technique, the nuclear chromosomes, which are grouped in a spindle formation, would be removed from both an oocyte provided by a woman with nonpathogenic mtDNA and the intended mother's oocyte. The intended mother's oocyte, containing mutated mtDNA, would be discarded. The intended mother's nuclear chromosomes would be inserted

[2] This report adopts the framing convention that the intended mother's pathogenic mtDNA is replaced with nonpathogenic mtDNA from an individual who provides an oocyte and thus constitutes "mitochondrial replacement." A proposed alternative framing is that the technique is a form of "nuclear transfer." This report does not contest that, procedurally, nuclear genetic material is moved; rather, the framing adopted emphasizes that mtDNA, rather than nDNA, is being weighted and selected for.

into the provided oocyte, which would contain nonpathogenic mtDNA. The oocyte would then be fertilized with the intended father's or another man's sperm. Following fertilization, the embryo would be grown in culture and subjected to diagnostic testing to ensure its quality and viability; the testing would include preimplantation genetic diagnosis (PGD) to confirm that the embryo had acceptably low or undetectable levels of the pathogenic mtDNA molecules. The resulting embryo(s) would be frozen until test results confirmed suitability for transfer and then transferred into the uterus of the intended mother (or gestational carrier, if needed).

Pronuclear Transfer (PNT)

In this technique, both an oocyte provided by a woman with nonpathogenic mtDNA and the intended mother's oocyte would be fertilized with sperm in vitro, creating two zygotes. The maternal and paternal pronuclei, which contained the nDNA, would be removed from both zygotes. The intended mother's enucleated zygote, containing pathogenic mtDNA, would be discarded. The pronuclei from the intended mother's zygote would be inserted into the enucleated zygote created with the provided oocyte and the intended father's (or another man's) sperm, which would contain nonpathogenic mtDNA. The resulting embryo(s) would then be grown, tested, and transferred as detailed above for MST.

Polar Body Transfer (PBT)

There are two versions of PBT. In polar body 1 transfer (PB1T), the intended mother's first polar body, which is a by-product of oogenesis, containing her nDNA and very little mtDNA, would be transferred to an oocyte provided by a woman with nonpathogenic mtDNA from which the nDNA had been removed. The reconstructed oocyte would then be fertilized, grown, tested, and transferred as detailed above for MST. In polar body 2 transfer (PB2T), both the intended mother's oocyte and an oocyte provided by a woman with nonpathogenic mtDNA would be fertilized. The intended mother's second polar body, containing nDNA and very little mtDNA, would be transferred to the zygote of the woman who provided the oocyte, from which the pronuclei had been removed. The resulting embryo(s) would then be grown, tested, and transferred as detailed above for MST.

Other Techniques and Developments

In addition to MST, PNT, and PBT, there are other current and potential future techniques designed to prevent transmission of mtDNA disease.

PGD is a technique performed in the setting of IVF to test genetically for a known inherited genetic disease and to allow selection of embryos for transfer to the uterus of the woman who will carry the pregnancy, with the goal of establishing a viable pregnancy and preventing transmission of that disease. While PGD is a powerful technique for preventing transmission of nuclear genetic diseases, there are limitations as to its reliability in effectively preventing the transmission of mtDNA disease in some at-risk females. The potential uses and limitations of PGD for preventing transmission of mtDNA disease are discussed in Chapter 2.

Heteroplasmy shift is an investigational technique that selectively targets and degrades mtDNA containing pathogenic mutations, allowing for repopulation of affected cells with resident, nonpathogenic mtDNA. It has recently has been shown to effectively reduce heteroplasmy levels and prevent transmission of pathogenic mtDNA in mouse and mammalian oocytes and one-cell embryos. As a result, heteroplasmy shift has been proposed as an alternative to MRT for preventing maternal transmission of pathogenic mtDNA mutations that precludes the need for the contribution of a second woman's genetic material (Reddy et al., 2015). Unlike MRT, however, heteroplasmy shift would not be applicable for oocytes or embryos that were homoplasmic or had high heteroplasmy levels for a pathogenic mtDNA, because retaining a certain baseline level of nonpathogenic mtDNA molecules in the cell is essential to enabling repopulation of the mtDNA pool and normal mitochondrial function after degradation of pathogenic mtDNA.

Policy Landscape

As the primary regulatory authority in this area, FDA will decide whether MRT can move forward into clinical investigations, and perhaps eventually into clinical use. While FDA does not have jurisdiction over the practice of medicine in general, it can regulate certain treatments or procedures, including the use of "human cells or tissues that are intended for implantation . . . into a human" (21 CFR 1271). FDA considers standard assisted reproductive technology (ART) procedures such as IVF to be "minimal manipulation" and thus subject only to regulations aimed at the prevention of communicable disease (FDA, 2009). However, FDA considers procedures such as MST, PNT, and PBT that entail "human cells used in therapy involving the transfer of genetic material" to be more than "minimal manipulation," and thus subject to regulation as drugs and/or biologics (FDA, 2009). In 2001, FDA advised researchers that such use of cells would require an IND, which is the first step toward clinical investigations and requires the submission of preclinical data and information on product safety, details about the technique, and proposed clinical investigation protocols.

In addition to FDA oversight, MRT research is subject to the limitations of the federal Dickey-Wicker amendment. Dickey-Wicker, included each year as a rider on the U.S. Department of Health and Human Services (HHS) appropriation bill, prohibits the use of HHS funding for research in which embryos are created for research purposes or destroyed, discarded, or subjected to risks with no prospect of medical benefit for the embryo. However, Dickey-Wicker prohibits the use of HHS funding, not the research itself, so MRT research could still be carried out with private funds, provided the technique was not prohibited or otherwise regulated by state law.

In addition, the U.S. National Academy of Sciences and the U.S. National Academy of Medicine have announced an initiative to guide decision making on gene-editing technologies. A 3-day international summit was held in December 2015, in collaboration with the Chinese Academy of Sciences and the United Kingdom's Royal Society, and an Academies consensus study has been launched to examine the scientific underpinnings of human gene-editing technologies—including potential human germline editing—and the clinical, ethical, legal, and social implications. This new Academies' effort will consider issues related to gene editing more broadly speaking, encompassing gene-editing techniques targeting nDNA and not limited to MRT or mtDNA. The consensus report of the committee conducting that study, to be released in 2016, will include findings and recommendations on the responsible use of human gene-editing research.[3]

Outside of the United States, the United Kingdom approved regulations to permit MRT in early 2015. The first preclinical research license for PNT was granted in the United Kingdom in 2005, and in the ensuing years, the United Kingdom's Human Fertilisation and Embryology Authority (HFEA) conducted extensive reviews of the preclinical evidence and solicited public opinion on MRT. The HFEA performed three scientific reviews to examine the safety and efficacy of MRT, looking at specific techniques and such alternatives as PGD. The HFEA's Ethics and Law Advisory Committee considered the ethical issues surrounding MRT, and the HFEA consulted and engaged in dialogue with the public through public workshops, surveys, and focus groups. In early 2014, the UK Department of Health released draft regulations for public review, and in early 2015, Parliament considered and approved revised regulations. These regulations require that MRT practitioners obtain a license from the HFEA, which will consider the specific context and techniques proposed for each license. As of late 2015, the United Kingdom was the first and only country in the world to have approved regulations to permit MRT.

[3] For more information, visit http://www.nationalacademies.org/gene-editing.

STUDY APPROACH

To address the study charge (see Box 1-1), the National Academies of Sciences, Engineering, and Medicine formed an ad hoc committee composed of experts from a range of disciplines, including bioethics, philosophy, law, public policy, religion, clinical investigations, reproductive medicine, mitochondrial medicine, mitochondrial biology, and patient advocacy. The committee deliberated from January to September 2015, holding five 2-day meetings, one 2-day public workshop, and public comment sessions. The committee also solicited and considered written statements from stakeholders and members of the public; in total, the committee received 32 comments submitted via the study website.

To the extent possible, the committee gathered empirical evidence by means of systematic literature reviews to inform its consideration of the ethical, social, and policy issues it was tasked with addressing. In areas in which empirical evidence is not available, however, many of the conclusions and recommendations offered in this report are based on the committee's expertise and informed judgment. To supplement its own expertise, the committee invited input from experts in the fields of mitochondrial and evolutionary biology, the ethics of reproductive and genetic technologies, and religious studies, as well as mtDNA patients through its public workshop and opportunities for public comment (see Appendix A).

ORGANIZATION OF THE REPORT

The remainder of this report is organized into three chapters. Chapter 2, "Science and Policy Context," presents an overview of reproductive medicine, mitochondrial biology, and mtDNA diseases; a review of the MRT research conducted to date; and discussion of the potential risks associated with MRT, as well as the policy context surrounding potential human clinical investigations in and clinical applications of MRT. Chapter 3, "Do Ethical, Social, and Policy Considerations Preclude MRT?," presents the results of the committee's deliberations and its findings on such issues as heritable genetic modification, implications for identity and parenthood, potential social effects, downstream implications, and alternatives to MRT. Chapter 4, "Regulation and Oversight of MRT in Humans," presents the committee's recommendations for key principles to guide clinical investigations of MRT, taking into consideration benefits and risks, informed consent, and practical challenges.

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Science and Policy Context

Evaluation of the ethical, social, and policy issues associated with mitochondrial replacement techniques (MRT) requires a comprehensive understanding of the state of the science surrounding reproductive biology and medicine, mitochondrial biology and genetics, mitochondrial DNA (mtDNA) disease, and MRT itself. In acknowledging the need for this scientific understanding, the committee also recognizes that it is the purview of the U.S. Food and Drug Administration (FDA) to thoroughly review the safety and efficacy of MRT and to determine whether the preclinical data package is sufficient for the agency to move forward with evaluation of applications for clinical investigations of MRT. Therefore, this chapter should be viewed as a nonexhaustive review of the literature surrounding MRT for the purposes of providing scientific context to inform the committee's analysis in succeeding chapters, not a judgment of the adequacy of the state of the science. To this end, the following topics are reviewed in this chapter: (1) reproductive biology and medicine, (2) mitochondrial biology and genetics, (3) mtDNA disease, (4) MRT research to date, and (5) potential risks related to MRT. The final section of the chapter describes the policy context for this study.

INTRODUCTION TO REPRODUCTIVE BIOLOGY AND MEDICINE

A prefatory summary of concepts in reproductive biology and medicine is provided here to inform subsequent discussions of mitochondrial biology and genetics, mtDNA disease, and MRT in this chapter and the ethical

BOX 2-1
Terminology in Reproductive Biology and Medicine

Blastocyst: The stage in early embryonic development, typically 4-5 days following fertilization in vitro, when the embryo comprises approximately 200-300 cells and a hollow cavity termed the blastocoel.

Embryo: Following dissolution of the pronuclear membranes and fusion of the male and female genetic material, the zygote divides to form the two-cell embryo, each cell containing equal complements of genetic and cytoplasmic material.

Gamete: An egg (oocyte) or sperm (spermatozoa) cell. In the process of fertilization, the fusion of male and female gametes gives rise to the zygote.

Germ cells: Gametes and those cells that give rise to gametes, originating with the primordial germ cells, the common precursor of both oocytes and spermatozoa.

Germline: Collectively, germ cells make up the germline.

Pronucleus: The membrane-bound nuclear genetic material derived from the oocyte or spermatozoa following fertilization.

Somatic cells: All cells of the human body that are not germ cells.

Zygote: A single cell formed following fertilization, containing separate male and female pronuclei that replicate before fusing. The zygote is sometimes referred to as a one-cell embryo, although this report does not adopt this terminology.

analysis presented in Chapters 3 and 4. The terminology of reproductive biology and medicine used throughout the report is summarized in Box 2-1.

Formation of Embryos, Germ Cells, and Gametes

Gametes are the fundamental cells involved in human reproduction and development. The initial step in human reproduction involves the fusion of an egg (oocyte) and sperm (spermatozoa) cell (*fertilization*), resulting in the formation of a *zygote*. At this stage, the zygote contains both the male and female *pronuclei* and is therefore termed di-pronucleate, or 2-PN. The pronuclear genetic material first replicates before the respective nuclear membranes dissolve, followed by fusion of the male and female genetic material and equivalent division of genetic and cellular material to form the two-cell *embryo*. The resultant embryo will undergo rapid cell division and differentiation, acting as the fundamental precursor cells for all the cells of the human body (see Figure 2-1). Derived from the embryo—specifically, from embryonic stem cells of the inner cell mass—are two distinct cell lineages: *somatic cells* and *germ cells*. Somatic cells differentiate from embryonic stem cells to form all of the cell and tissue types of the human body; primordial germ cells differentiate from embryonic stem cells along

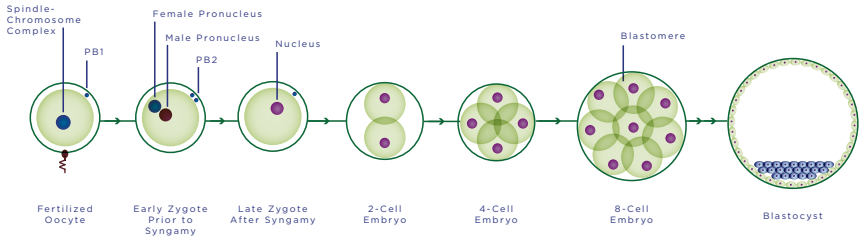

FIGURE 2-1 Embryogenesis.
NOTES: The initial step in human reproduction involves fertilization, the fusion of an oocyte and sperm cell, resulting in the formation of an early zygote. At this stage, the early zygote contains both the male and female pronuclei and is therefore termed di-pronucleate, or 2-PN. The pronuclear genetic material first replicates before the respective nuclear membranes dissolve, followed by fusion of the male and female genetic material in the late zygote, and equivalent division of genetic and cellular material to form the two-cell embryo. The resultant embryo undergoes rapid cell division, forming the 4- and 8-cell embryo and after many additional divisions, the blastocyst.
SOURCE: Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Genetics, copyright 2005.

distinct pathways to develop into either spermatozoa in the case of males or oocytes in the case of females. The cells that make up these germ cell lineages are referred to collectively as the *germline*.

Changes to the genetic material of germline cells are heritable in the case of nuclear DNA (nDNA) and maternal mtDNA. Paternal mtDNA is not transmitted to offspring, and thus changes made to mtDNA in the male germline are not heritable.[1] Mitochondrial and nuclear genetics and inheritance patterns are discussed later in this chapter in the section on mitochondrial biology and genetics.

In Vitro Fertilization

In vitro fertilization (IVF) is an assisted reproductive technology (ART) traditionally used to aid a woman in becoming pregnant when unassisted sexual reproduction and other ARTs, such as fertility medications and artificial insemination, fail to produce a pregnancy.

[1] Although paternal transmission of mtDNA in humans was noted in a high-profile case report (Schwartz and Vissing, 2002), studies of children born following intracytoplasmic sperm injection (ICSI) have failed to detect transmission of paternal mtDNA (Houshmand et al., 1997; Marchington et al., 2002). At present, therefore, it is believed that maternal transmission is the rule in humans.

In general, IVF comprises five steps: (1) stimulation, or super ovulation, to produce a larger than normally released number of oocytes; (2) oocyte retrieval, on the order of 5-30 oocytes per stimulation cycle, requiring sedation of the woman undergoing the biopsy procedure; (3) mixing of sperm with preselected, high-quality oocytes (insemination) or, more commonly, direct injection of sperm into each oocyte, termed intracytoplasmic sperm injection (ICSI); (4) culture of the embryo to day 3 or 5 (blastocyst stage); and (5) transfer of the cultured embryo to the woman who will carry the pregnancy (i.e., the intended mother or a gestational carrier). MRT is a collective set of modified IVF techniques (see the description of MRT methodology later in this chapter).

Preimplantation Genetic Diagnosis (PGD)

PGD is a technique performed in the setting of IVF to test for a known inherited genetic disease and to allow selection of embryos for transfer to the uterus of the woman who will carry the pregnancy, with the goal of establishing a viable pregnancy and preventing transmission of that disease.[2] Once a viable pregnancy has been achieved, additional prenatal diagnostic testing is essential to confirm the genetic information obtained by PGD, entailing chorionic villus sampling of fetal placental tissue, amniocentesis of discarded fetal cells, or cell-free DNA screening. The use of PGD for preventing transmission of mtDNA disease is discussed later in this chapter.

INTRODUCTION TO MITOCHONDRIAL BIOLOGY AND GENETICS

Mitochondria are microscopic organelles found in nearly all cell types of the human body,[3] best known for their role in regulating cellular energy balance. They are among the most complex cellular organelles, consisting of more than 1,100 proteins that collectively support the mitochondria's

[2] Briefly, one to several single blastomeres of a post-IVF day 3 or day 5 embryo are tested in the laboratory for the known genetic condition for which the embryo is at risk. If the blastomere biopsy is performed on day 3, the embryo can remain freshly cultured in the laboratory until genetic test results are returned; the desired embryo(s) can then be transferred to the uterus on day 5. However, if the blastomere biopsy is performed on day 5—as is now more common given that embryos generally have greater viability on day 5 than on day 3—all of the embryos in that cycle are frozen until genetic testing on each is complete. At any point in the future, as soon as the following month or up to years later, the woman who will carry the pregnancy then undergoes an additional hormone preparation cycle, and the desired frozen embryo(s) are thawed and implanted into her uterus.

[3] With the exception of mammalian red blood cells (erythrocytes) and mature ocular lens cells, which do not contain organelles and thus do not contain mtDNA.

myriad roles, including production of cellular energy, regulation of cellular metabolism, and assistance in control of programmed cell death (apoptosis).

According to the widely accepted endosymbiotic hypothesis, these organelles once were free-swimming bacteria, adept at harvesting energy by burning oxygen, that took up permanent residence within another cell (Vafai and Mootha, 2012). Several features of mitochondria serve as reminders of this unique ancestry. Mitochondria measure 500 nm-1 μm (approximately 1/50 the width of a human hair), have a double membrane,[4] and constantly "swim" within the cells of the body—very much resembling intracellular bacteria. They have retained their own genome[5] (mtDNA), another vestige of their bacterial origin. Over billions of years of evolution, virtually all of the genes once encoded by this primordial bacterial genome have been either lost or transferred to the nuclear genome. Today, human mtDNA, which is mutated in mtDNA disease, encodes 13 proteins that must operate functionally with more than 1,100 nuclear encoded proteins that are imported into mitochondria to shape the organelle's function.

Biological Functions of Mitochondria

Cellular metabolism refers to the set of biochemical processes within a cell that generate, store, or utilize energy through the making (anabolism) or breaking (catabolism) of chemical bonds between molecules. A primary function of mitochondria is to produce the majority of energy that is needed to fuel cellular processes; thus these organelles are often referred to as "the powerhouses of the cell." The nutrients people eat, such as carbohydrates, fats, and proteins, are broken down within the cell to form intermediate byproducts that are sent to the mitochondria, where they are processed further to produce energy in the form of adenosine triphosphate (ATP), the predominant molecule for storing and providing energy for cellular processes. This process by which ATP is produced, termed oxidative phosphorylation

[4] This unique inner and outer double membrane structure allows mitochondria to compartmentalize cellular components. The space between the inner and outer membrane is termed the intermembrane space. Oxidative phosphorylation takes place by pumping protons across the inner membrane into the intermembrane space, forming the electromotive force used to drive adenosine triphosphate (ATP) synthesis. The space enclosed by the inner membrane is termed the mitochondrial matrix and is home to mtDNA, as well as the majority of mitochondrial components required for the mitochondrion to carry out its various functions. The double membrane is reflective of the ancestral bacterium from which the mitochondria derived, namely a gram negative bacterium, which also contained a double membrane.

[5] The genome is the collective genetic material found within an organism. In humans, the cellular genome comprises the nuclear and mitochondrial genomes.

(OXPHOS),[6] occurs at the respiratory chain[7] and ATP synthase, located within the mitochondrial inner membrane. For this reason, cells with higher energy demands, such as muscle and brain cells, contain higher numbers of mitochondria so they can meet these energy requirements. In addition to this critical function, the mitochondria are principal regulators of a variety of cellular metabolic functions, play an important role in maintaining the proper intracellular environment, and are an integral component of apoptosis. The role of mitochondria in these various biological processes underscores the critical importance of proper mitochondrial function for sustaining human life.

The Respiratory Chain and Oxidative Phosphorylation

OXPHOS involves 5 protein complexes comprising a total of 90 proteins, 13 of which are encoded by mtDNA. The principal function of OXPHOS, discussed above, is to generate energy in the form of ATP. In mtDNA disease, mutations in mtDNA result in a lack or defective production of one or more mtDNA-encoded gene products, leading to varying degrees of dysfunction in respiratory chain activity and energy production.

Other Metabolic Pathways Within Mitochondria

As a result of electrons being driven through the respiratory chain in the process of OXPHOS, other metabolic processes can move forward as well, a process known as metabolic coupling. In this way, OXPHOS is coupled with other metabolic pathways within and external to the mitochondria. For example, mitochondria contain the machinery necessary to convert the fats, proteins, and carbohydrates people eat into intermediates that feed directly into the respiratory chain. Breakdown intermediates from these metabolic processes within the mitochondria can be exported back into the cytosol, where they are used as precursors for other molecules, such as sex hormones, fatty acids, DNA, and proteins. In mtDNA disease, these coupled reactions—in addition to OXPHOS—are also disrupted and can contribute to the observed disease clinical phenotypes.

[6] OXPHOS is the process by which the respiratory chain generates a proton gradient across the mitochondrial inner membrane via transfer of electrons from a higher-energy donor to lower-energy cellular intermediates, terminating with formation of the terminal electron acceptor, oxygen. The electromotive force generated by this proton gradient is utilized by a protein complex, ATP synthase (complex V), to produce ATP.

[7] Also known as the electron transport chain (ETC).

TABLE 2-1 Comparison of Human Nuclear and Mitochondrial Genomes

Characteristic	Mitochondrial	Nuclear
Genome Structure	Circular	Linear
Copies of the genome per cell	100-10,000 (more than 100,000 in mature oocytes)	2
Number of DNA base pairs	16,569	3.3 billion
Number of coding genes	37	Approximately 20,000-30,000
Function of gene-encoded products	OXPHOS function; mtDNA-encoded protein translation	All remaining intra- and extracellular functions required for cellular, tissue organ, and bodily functions; phenotypic traits, such as physical appearance
Mode of inheritance	Maternal	Biparental

NOTE: mtDNA = mitochondrial DNA; OXPHOS = oxidative phosphorylation.
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mtDNA Genetics and Inheritance

Mitochondria are unique in that they house mtDNA, the only extranuclear source of DNA within animal cells. While mtDNA has some commonalities with nDNA, such as comprising double-stranded DNA and containing protein-encoding genes, the two differ in many ways (as summarized in Table 2-1). These differences have important implications for mtDNA disease and MRT, expanded on throughout this and subsequent sections within this chapter.

Genome Structure and Function

The mitochondrial genome contains 37 genes, 13 of which encode for proteins that are core components of the respiratory chain and OXPHOS system, with the remaining 24 assisting in the translation of OXPHOS proteins. By comparison, nDNA encodes for an estimated 20,000-30,000 protein-encoding genes. Compared with the only 2 copies of the 23 nuclear chromosomes in almost all somatic cells, mtDNA is found in high copy number,[8] ranging from 2 to 10 copies per mitochondrion and 100 to

[8] The copy number is the number of mtDNA molecules per cell.

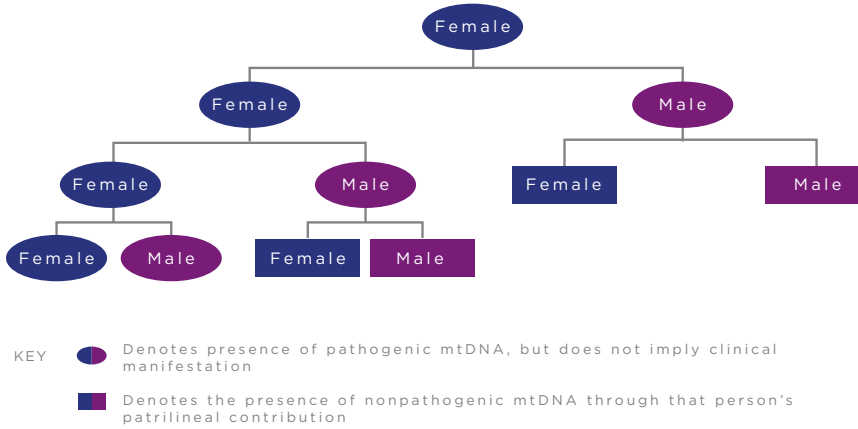

FIGURE 2-2 Inheritance of pathogenic mtDNA mutations.
NOTES: For simplicity, reproductive partners are not shown and are assumed not to carry pathogenic mtDNA mutations. mtDNA = mitochondrial DNA.

10,000 copies per cell, depending on cell type, with up to 500,000 copies in oocytes. Replication of mtDNA occurs continuously throughout the cell cycle and autonomously from nDNA, which is replicated once per cell cycle; the resulting mtDNA molecules are partitioned randomly into the daughter cells during cell division.[9] While mtDNA encodes for products that are essential for the production of cellular energy, it is generally agreed that nDNA plays the predominant role in determining characteristics of anatomy, physiology, personality, and the like.

Mode of Inheritance

As noted previously, mtDNA is solely maternally inherited in humans (see Figure 2-2). Thus, only females pass their mtDNA on to offspring, both male and female; male mtDNA is not transmitted to future generations.[10]

[9] Cell division, or mitosis, is the stage of the cell cycle that results in division of the "parent" cell into two "daughter" cells, each containing the same number of chromosomes as the parent cell.

[10] Several mechanisms help ensure maternal transmission of mtDNA. First, the unfertilized oocyte has up to an estimated 500,000 copies of mtDNA, compared with approximately 100 copies of mtDNA in sperm cells, so simple dilution makes it statistically unlikely for paternal mtDNA to be transmitted. Furthermore, the mitochondria of sperm cells are "tagged" by the oocyte for degradation following fertilization (Sutovsky et al., 1999).

In contrast, nDNA is inherited both maternally and paternally, following what is known as Mendelian or biparental inheritance.[11]

Heteroplasmy

An additional, notable feature of mitochondrial genetics is the concept of heteroplasmy. Heteroplasmy is the state in which a cell, tissue, or person contains more than one mtDNA genotype, as opposed to the state in which all copies of the mitochondrial genome are identical, termed homoplasmy. For example, a cell whose mtDNA consists of 70 percent mutant mtDNA and 30 percent wild-type[12] mtDNA is termed heteroplasmic, whereas a cell with 100 percent mutant mtDNA is termed homoplasmic. The concept of heteroplasmy and its relation to mtDNA disease and MRT is explored further in the section on complexities related to mitochondrial genetics later in this chapter.

Genetic Interactions Between nDNA and mtDNA

The mitochondrion requires extensive contributions from nDNA to perform all of its critical functions, including those encoded for by mtDNA. Eighty proteins necessary for OXPHOS function and more than 1,000 others required for mitochondrial activity and structure are encoded by the nuclear genome and imported into the mitochondria. Maintenance of this nuclear-mitochondrial cross-talk is essential for establishing and maintaining proper mitochondrial function (Lee et al., 2008). The cross-talk between the nuclear and mitochondrial genomes is an important consideration in evaluations of MRT, as its disruption could have potentially deleterious effects on overall mitochondrial and cellular health (see the section on complexities related to mitochondrial genetics later in this chapter).

mtDNA Genetic Variance in Human Populations

mtDNA molecules acquire novel mutations at a rate at least 10 times greater than that of nDNA molecules. If such mutations are acquired within oocytes, they are transmitted to any offspring conceived from those

[11] This is true for the 22 autosomal, or non-sex-determining, chromosomes. The X and Y chromosomes are responsible for determining the sex of an organism—in humans, XX for females and XY for males—and can display slightly different inheritance patterns. A comprehensive overview of DNA inheritance patterns can be found at http://ghr.nlm.nih.gov/handbook/inheritance/inheritancepatterns (accessed January 15, 2016).

[12] Wild-type is the most common DNA sequence found within a population, often referred to as the "normal" variant of a DNA sequence or gene.

oocytes. Lack of recombination between mtDNA molecules[13] and sole matrilineal inheritance of mtDNA means that acquired mtDNA mutations can be passed down via radiating maternal lineages. From an evolutionary standpoint, the persistence of certain maternally transmitted homoplasmic mtDNA mutations has resulted in the formation of stable population subgroups, known as haplogroups, sharing the same collection of fixed mtDNA variants, or haplotypes. As the females who migrated out of Africa helped colonize the globe and novel mtDNA mutations were acquired, new haplogroups branched out from the original "macrohaplogroups" (Wallace and Chalkia, 2013). The retention of novel mtDNA mutations in evolution may have been a result of random genetic drift, in the case of neutral mtDNA mutations, or of selective pressures, in the case of mtDNA mutations that conferred advantageous traits or characteristics to individuals in novel geographic regions, wherein those haplotypes became enriched (Wallace, 1994). Continents and geographic regions are therefore associated with specific mtDNA haplogroups, which might confer certain physiological advantages to individuals who live there (Wallace and Chalkia, 2013).

A few high-profile studies have provided evidence substantiating the hypothesis that certain mtDNA haplogroups underwent positive selection as an adaptive mechanism for populations that migrated to colder climates (Mishmar et al., 2003; Ruiz-Pesini et al., 2004). These studies indicate that certain mtDNA variants result in inefficient energy production by mitochondria and concurrent generation of heat. Accordingly, theory suggests that increased heat generation conferred a selective advantage to individuals living in colder climates. Thus such variants became enriched and eventually fixed in these populations, at the expense of less efficient energy production. A complementary hypothesis is that certain mtDNA haplogroups confer an energetic advantage, such as enhanced exercise capacity, to individuals through more efficient energy production and less heat generation by mitochondria. Indeed, some studies have shown a correlation between certain mtDNA variants and relative exercise performance or aerobic capacity (see Eynon et al. [2011] for a review of the evidence).

Although intriguing, haplogroup/haplotype association studies are by nature correlative given the lack of experimental systems with sufficient sensitivity to validate the causal effect of mtDNA haplotypes on human physiology and cognition. Moreover, most of these studies to date have involved very small cohorts, have been statistically underpowered, and po-

[13] During meiosis—the reductive replication and division of gametes—nDNA recombines to form new combinations of traits; however, this process does not specifically alter the *sequence* of nDNA through the introduction of novel mutations, but rather the combination of genetic variants. On the other hand, mtDNA does not undergo recombination, but is more prone to acquiring mutations; this allows the tracking of mtDNA variants through generations and among population subgroups.

tentially have been confounded by population stratification.[14] Finally, such association studies have not found that specific mtDNA variants may confer a certain functional benefit, as a specific variation in nDNA confers a certain blood type. Rather, these studies suggest that a set of mtDNA variants are inherited together, make up a specific haplogroup, and are associated with certain functional characteristics in the context of certain populations.

Conclusion: *The present state of scientific knowledge indicates that it is difficult or impossible to identify mtDNA haplogroups/haplotypes that would confer on an individual potentially advantageous traits or capacities such as enhanced exercise performance or aerobic capacity.*

mtDNA DISEASE

Mitochondrial diseases are highly heterogeneous, characterized fundamentally by a dysfunction in respiratory chain activity and corresponding reduced cellular energy production. In turn, the hallmark deleterious phenotypes of mitochondrial diseases tend to manifest in those organs with the highest energy demand, such as the brain, muscles, heart, gastrointestinal tract, and liver. At present, no FDA-approved treatment or cure exists for these diseases, and management approaches are primarily supportive and palliative. Mitochondrial disease can arise as a result of defects in nDNA or mtDNA (see the section on genetic origins of mitochondrial disease below).

Etiology, Clinical Manifestation, and Diagnosis

Genetic Origins

The respiratory chain is under dual genomic control,[15] and thus mitochondrial diseases can be of nDNA or mtDNA origin. More than 275 disease-causing mtDNA mutations have been reported across every mtDNA gene since the first pathogenic mtDNA mutation was identified in 1988 (Saneto and Sedensky, 2013). Mutations in mtDNA can be categorized according to the gene-encoded products they disrupt: (1) mutations affecting OXPHOS proteins and (2) mutations affecting the translation machinery of OXPHOS proteins. Furthermore, pathogenic mtDNA mutations can either arise sporadically (de novo), originating most commonly in early development, or be inherited. Table 2-2 lists the most common maternally inherited mtDNA diseases and their associated mtDNA mutations.

[14] Population stratification is differences in nuclear allele frequencies between research subjects due to systematic differences in ancestry (Price et al., 2006).

[15] Control by both the nuclear and mitochondrial genomes.

TABLE 2-2 Maternally Inherited mtDNA Diseases

mtDNA Disease	Clinical Presentation	mtDNA Gene/ Genotype*
Leigh Syndrome	Psychomotor delay, dystonia, seizures, abnormal eye movements, recurrent vomiting, respiratory abnormalities	*ATPase6*: m.8993 T>G ND1, ND2, ND3, ND4, ND5, ND6, COXIII, others: multiple
MELAS	Myopathy, encephalopathy, lactic acidosis, stroke-like episodes	*TRNL1*: m.3243A>G; m.3271T>C *ND1 and ND5*: individual mutations
MERRF	Myoclonic epilepsy, myopathy	*TRNK*: m.8344A>G; m.8356T>C
NARP	Neuropathy, ataxia, retinitis pigmentosa	*ATP6*: m.8993T>G
MILS	A progressive brain-stem disorder	*ATP6*: m8993T>C
MIDD	Diabetes, deafness	*TRNL1*: m.3243A>G *MT-RNR1*: m.155A>G
Nonsyndromic hearing loss and deafness	Nonprogressive, moderate to profound hearing loss associated with aminoglycoside antibiotic use	*MT-TS1*: m.7445A>G
LHON	Optic neuropathy	*ND1*: m.3460G>A *ND4*: m.11778G>A *ND6*: m.14484T>C

NOTES: * The most common pathological mtDNA point mutations are listed. LHON = Leber's hereditary optic neuropathy; MELAS = mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF = myoclonic epilepsy with ragged-red fibers; MIDD = maternally inherited diabetes and deafness; MILS = maternally inherited Leigh syndrome; NARP = neuropathy, ataxia, and retinitis pigmentosa.
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Clinical Presentation and Diagnosis

mtDNA diseases can range in severity from mild to severely debilitating or fatal, and their onset can occur in early life or adulthood. In general, mtDNA diseases tend to have later onset and to be associated with relatively milder symptoms relative to nDNA-based mitochondrial diseases, whose onset is typically earlier (often in infancy or childhood) and which

are associated with more severe phenotypes. However, at least 15 percent of pediatric-onset mitochondrial diseases are estimated to be caused by mtDNA mutations (DiMauro and Davidzon, 2005; Saneto and Sedensky, 2013), and early-onset, severe mtDNA diseases have been well documented in the clinical setting (Saneto and Sedensky, 2013). It is for this subset of mtDNA diseases that MRT would be applicable.

The principal effect of defective mtDNA is disruption of respiratory chain activity; consequent depletion of ATP levels and energy production; and eventual dysfunction and failure of cellular, tissue, and organ function. Age of onset, clinical presentation, natural history, and penetrance[16] of mtDNA diseases are extremely variable, both within and across mtDNA mutations. Nonetheless, the most common disease types, such as Leigh syndrome and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), do share certain features, aiding in their clinical diagnosis. Figure 2-3 shows common clinical manifestations of adult and pediatric mtDNA diseases.

Diagnosis

Clinical diagnosis of mtDNA diseases is a complex task. However, classic diagnostic features do exist to aid physicians in making a differential diagnosis of patients with suspected mtDNA disease. These features include (1) maternal inheritance, (2) recognition of established syndromes such as MELAS, (3) recognition of characteristic clinical symptoms (e.g., biventricular cardiac hypertrophy), (4) involvement of multiple organ systems (e.g., diabetes and deafness), (5) specific combinations of symptoms (e.g., strokes, migraines, seizures, and ataxia), and (6) certain patterns of abnormal clinical and laboratory testing results (Taylor and Turnbull, 2005). Furthermore, differential diagnosis to confirm or exclude mtDNA disease may become easier with increasingly accurate and affordable sequencing technologies.

Effect of Pregnancy on Women with mtDNA Disease

The effect of pregnancy on women with mitochondrial disease in general and mtDNA disease in particular is poorly understood. As a result of the deleterious effects of mtDNA disease on cellular respiration and energy production and the concurrent increase in respiratory and energy demands

[16] Penetrance is "the proportion of individuals with a mutation causing a particular disorder who exhibit clinical symptoms of that disorder; a condition is said to have complete penetrance if clinical symptoms are present in all individuals who have the disease-causing mutation, and to have reduced or incomplete penetrance if clinical symptoms are not always present in individuals who have the disease-causing mutation" (http://ghr.nlm.nih.gov [accessed January 15, 2016]).

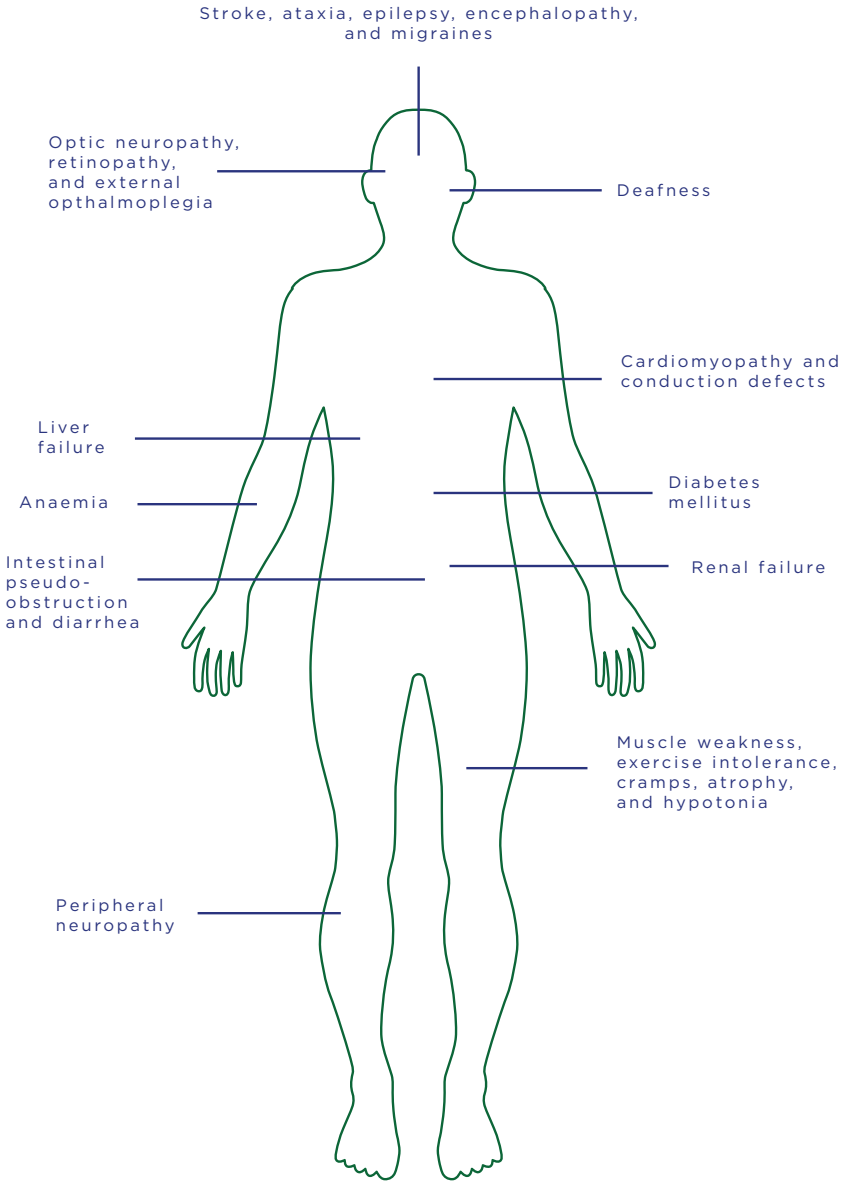

FIGURE 2-3 Potential manifestations of mtDNA diseases.
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in pregnancy, women who are at risk for or have clinically manifested mtDNA disease may develop or experience a worsening of symptoms or other obstetric complications. Given the clinical heterogeneity of mtDNA disease, the clinical course of afflicted women during pregnancy likely varies. Indeed, a review of 10 case reports of pregnancies in women with mitochondrial disease by Say et al. (2011) revealed varying levels of pregnancy complications, ranging from asymptomatic, to mild symptoms such as exercise intolerance and muscle weakness that resolved postnatally, to more serious and in some instances persistent symptoms such as kidney and nerve damage. The most commonly observed complications in this retrospective review were preterm labor and preeclampsia. To date, no cohort studies have been published on the effect of pregnancy in women with mitochondrial disease. However, an observational study currently being conducted by Robert McFarland at the University of Newcastle on Tyne is examining the incidence of pregnancy complications in patients who have mitochondrial disease or are carrying an mtDNA mutation (Feeney and McFarland, 2014). In addition, the Newcastle Mitochondrial Centre has published guidance best practices for antenatal care for women with mitochondrial disease (National Commissioning Group (NCG) for Rare Mitochondrial Diseases of Adults and Children (UK), 2013). Similarly, no studies have been published to date on the potential health effects in children gestated by women with symptomatic mtDNA disease.

Prevalence of mtDNA Disease and Pathogenic mtDNA Mutations

Determining the prevalence of mtDNA disease and prevalence of asymptomatic carriers of pathogenic mtDNA mutations has been challenging given the extensive clinical and genetic heterogeneity involved. A recent study evaluating adults (aged 16-65) referred to a mitochondrial clinic in northeast England from 1990 to 2011 estimated that at least 1 in 5,000 people harbor a pathogenic mtDNA mutation, with approximately 1 in 10,000 adults presenting with clinically manifested mtDNA disease (Gorman et al., 2015b) and 1.65 in 10,000 children and adults estimated to be at risk for development of mtDNA disease (Gorman et al., 2015b; Schaefer et al., 2008). A prospective study that evaluated the prevalence of the 10 most common pathogenic mtDNA point mutations in infants found that 0.54 percent of offspring carried at least 1 of these 10 mutations (excluding de novo mutations), suggesting that at least 1 in 200 asymptomatic people harbor a pathogenic mtDNA mutation (Elliott et al., 2008). A follow-up report to the study conducted by Gorman et al. (2015b) extrapolated from the point prevalence of pathogenic mtDNA mutations to estimate how many women may be at risk of transmitting mtDNA disease and thus could potentially benefit from MRT. Extrapolating previously ascertained

prevalence data to women of childbearing age and using fertility rates, the authors estimated that the average number of children born per year from women at risk for transmitting mtDNA disease is 152 and 778 in the United Kingdom and the United States, respectively (Gorman et al., 2015a). Such estimates are naturally tempered, however, by the fact that not all women who are at risk of transmitting mtDNA disease will decide or will be able to pursue MRT and that those who do pursue MRT may not obtain a successful pregnancy through the requisite IVF procedure.

Treatment and Prevention of Transmission of mtDNA Disease

As noted earlier, there are currently no cures or proven effective treatments for mtDNA disease (Parikh et al., 2009). Current therapeutic options for mtDNA disease focus on palliative management of an individual's organ-specific disease symptoms as they emerge over time, rather than on targeting and correcting precise biochemical pathways (Parikh et al., 2013, 2014). This approach stems from two factors: (1) the heterogeneity of mtDNA diseases, even with respect to the same causative mtDNA mutation, which makes mutation- and patient-specific treatments highly challenging; and (2) the current lack of success in effectively delivering treatments into mitochondria with pathogenic mtDNA. Furthermore, for many women at risk of transmitting pathogenic mtDNA mutations, diagnostic techniques aimed at reliably preventing transmission of pathogenic mtDNA to future offspring (e.g., PGD or prenatal diagnosis) are not viable options, as discussed below.

Management of Symptoms

Exercise—both isotonic and aerobic, as tolerated—has been demonstrated to provide significant benefit in mtDNA disease, likely as the result of a combination of inducing the formation of new mitochondria—thereby increasing the percentage of nonpathogenic mtDNA—and preferential shifting of heteroplasmy loads toward nonpathogenic mtDNA (Tarnopolsky, 2014). A range of pharmaceuticals and nutritional supplements also are commonly prescribed to support overall mitochondrial function, despite a lack of rigorous clinical investigations validating their efficacy (Parikh et al., 2009, 2014; Pfeffer et al., 2013). Other medications have been shown to have benefit for disease-specific symptoms; examples include L-arginine to mitigate or prevent metabolic stroke (Koga et al., 2005) and folinic acid to treat changes in nervous system tissue secondary to folate deficiency (Quijada-Fraile et al., 2014). Several clinical investigations currently under way are assessing the effects of existing medications approved for other indications or of novel therapeutics developed for mtDNA disease as the

primary indication. To date, none of these therapies have been shown to have clinical efficacy or have gained FDA approval for treatment of mtDNA disease (Pfeffer et al., 2013).

Gene Editing of Somatic Cells

As with nuclear genetic diseases, gene editing of somatic cells, also sometimes known as gene transfer or gene therapy, for treatment of mtDNA disease appears to hold great promise for the clinical treatment, and potential cure, of existing mtDNA disease. In those mtDNA diseases for which the causative pathogenic mutation has been identified, gene editing would allow for precise correction of or compensation for the product of the mutated gene, thus bypassing the difficulties inherent in targeting the aberrant biochemical pathways that result from each genetic disorder. Gene-editing approaches for mtDNA disease have shown initial promise in in vitro and animal studies (Viscomi et al., 2015). However, these approaches have in general shown limited success in humans because of difficulties in delivering the therapy efficiently to the desired tissues, and in the case of mtDNA disease, in transporting the corrective/compensative material efficiently into the mitochondria containing pathogenic mtDNA.

Heteroplasmy Shift

Heteroplasmy shift is an investigational technique that selectively targets and degrades mtDNA containing pathogenic mutations, allowing for repopulation of affected cells with resident, nonpathogenic mtDNA. Cell and animal models of mtDNA disease have demonstrated its preliminary efficacy (Bayona-Bafaluy et al., 2005; Srivastava and Moraes, 2001), and more recent work has shown that it can effectively reduce heteroplasmy levels and prevent transmission of pathogenic mtDNA in mouse and mammalian oocytes and one-cell embryos. As a result, heteroplasmy shift has been proposed as an alternative to MRT for preventing maternal transmission of pathogenic mtDNA mutations that would preclude the need for the contribution of a second woman's genetic material (Reddy et al., 2015). Unlike MRT, however, heteroplasmy shift would not be applicable for oocytes or embryos that are homoplasmic or have high heteroplasmy levels of pathogenic mtDNA, because retaining a certain baseline level of nonpathogenic mtDNA molecules in the cell is essential to enabling repopulation of the mtDNA pool and normal mitochondrial function after degradation of pathogenic mtDNA.

Preimplantation Genetic Diagnosis

PGD is a powerful technique for preventing the transmission of inherited nDNA diseases. However, only a handful of studies have evaluated PGD for selection and transfer of embryos in females at risk of transmitting known pathogenic mtDNA mutations. With at least one exception (Mitalipov et al., 2014), live-born children born following PGD generally have exhibited no adverse health outcomes, although there has been little long-term follow-up of these children beyond birth or infancy (Heindryckx et al., 2014; Monnot et al., 2011; Sallevelt et al., 2013; Steffann et al., 2006; Treff et al., 2012).

A limitation of the use of PGD to prevent transmission of mtDNA disease is that the technique involves selection of an embryo with the lowest detected heteroplasmy level; therefore, it may reduce but does not definitively eliminate the risk of transmitting mtDNA disease to offspring. Although no formal guidelines exist regarding an acceptable heteroplasmy threshold for embryo selection and transfer, Samuels et al. (2013) recently reported a model of mtDNA heteroplasmy inheritance predicting that transfer of an embryo with a heteroplasmic mutation level above 5 percent may result in a significant chance of mtDNA disease in offspring. Therefore, many families considering PGD to prevent transmission of mtDNA disease are now advised to transfer embryos with a heteroplasmic mutation level of 5 percent or less (Sallevelt et al., 2013). It is possible, however, that women at risk for transmitting mtDNA disease may not produce oocytes, and hence embryos, with low enough levels of pathogenic mtDNA molecules to be deemed acceptable for transfer. This is always the case in women who are homoplasmic for a pathogenic mtDNA mutation, all of whose oocytes will be homoplasmic for the mutation, and occurs with elevated probability in women with high heteroplasmy levels for a pathogenic mtDNA mutation, all of whose oocytes may carry the mutation to a degree that would preclude their selection and intrauterine transfer.

An additional limitation of PGD for mtDNA disease is the potential occurrence of random and rapid changes in mtDNA heteroplasmy levels following embryo implantation, a phenomenon caused by random segregation of mtDNA and the mtDNA bottleneck (see the section on complexities related to mitochondrial genetics later in this chapter), which could result in higher than expected heteroplasmy levels of pathogenic mtDNA in critical tissues of offspring born following PGD. Relatedly, while PGD may reliably reduce heteroplasmy levels of pathogenic mtDNA and prevent manifestation of mtDNA disease in offspring, females born as a result of PGD may still be at risk of transmitting mtDNA disease to offspring because of higher than expected heteroplasmy levels in their oocytes.

Given the above uncertainties, embryo selection via PGD may not

represent an effective method for reliably preventing the transmission of mtDNA disease in women who are at known risk. Recent data in human embryos suggest that refined MRT protocols would be able to produce embryos with heteroplasmy levels below recommended thresholds (see the discussion of MRT research to date below) and thus might more reliably prevent maternal transmission of pathogenic mtDNA mutations in immediate offspring and future generations.

MRT RESEARCH TO DATE

As discussed above, PGD has limitations with respect to its efficacy for reliably preventing maternal transmission of mtDNA disease, and PGD is not a preventive option for women who are homoplasmic, and may not be an option for women who are heteroplasmic, for pathogenic mtDNA mutations. Prospective mothers who are at risk for transmitting mtDNA disease to their offspring and wish to pursue reproductive options that mitigate the risk of this transmission thus must choose among options that allow for varying degrees of nuclear genetic connection between the child and the prospective parents: using the assistance of a woman who provides an oocyte or embryo, adoption, or childlessness. Therefore, current preventive and alternative reproductive options do not fulfill the desire of prospective mothers to have an nDNA-related child at sharply reduced risk for developing mtDNA disease. MRT is being investigated as a way of providing these benefits.

Two such proposed techniques—maternal spindle transfer (MST)[17] and pronuclear transfer (PNT)—involve, in principal, the formation of a reconstructed oocyte or zygote, respectively, in which the intended mother's mutated mtDNA would effectively be replaced with an oocyte provider's nonpathogenic mtDNA (see Figure 2-4).[18] The reconstructed oocyte or zygote would contain parentally derived nDNA and would theoretically be devoid, or have very low levels, of maternally derived pathogenic mtDNA. The reconstructed embryo would then be tested by PGD to determine

[17] Also known as metaphase II spindle transfer (MII-ST), spindle-chromosomal complex transfer, or spindle transfer (ST).

[18] This report uses the term "nonpathogenic mtDNA" to describe mtDNA contributed from the female oocyte provider, with the understanding that following genetic testing of provided oocytes for known pathogenic mutations, any provided mtDNA would be presumed—but given the rapidly expanding and shifting knowledge of mitochondrial biology and genetics, could not be assumed—to be free of pathogenic mtDNA mutations.

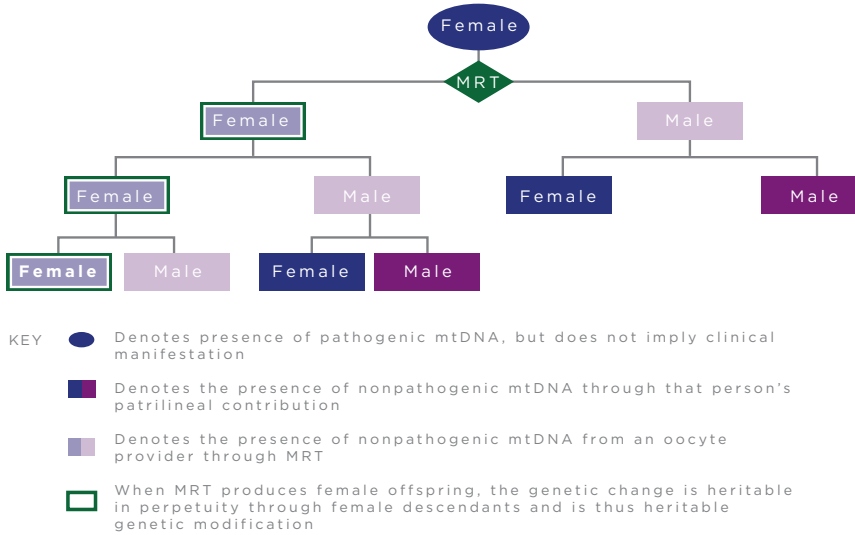

FIGURE 2-4 Heritable genetic modification via MRT.
NOTES: MRT = mitochondrial replacement techniques; mtDNA = mitochondrial DNA. MRT replaces pathogenic mtDNA from the intended mother with nonpathogenic mtDNA from an oocyte provider. For simplicity, reproductive partners are not shown and are assumed not to carry pathogenic mtDNA mutations.

heteroplasmy levels,[19] as well as undergo other genetic testing for chromosomal abnormalities and sex selection (if utilized). The sections that follow describe the methodology of these techniques in more detail.

Demonstrating the safety and efficacy of MRT entails evidence of minimal pathogenic mtDNA carryover[20] (and subsequent heteroplasmy), as well as normal health and growth in offspring born as a result of MRT. The high-level summary of MRT research that follows is therefore focused on those human in vitro and animal studies that were designed as proof-of-principle to demonstrate the feasibility of MRT for preventing mtDNA disease transmission and is structured to emphasize review of these outcome

[19] As previously described, PGD may not be a reliable method for preventing transmission of mtDNA disease in women who are at known risk of transmitting mtDNA disease because of limitations related to complexities of mitochondrial genetics. With the advent of increasingly sensitive and accurate sequencing technologies, however, PGD is expected to be a reliable technique for determining the efficacy of MRT prior to embryo transfer.

[20] As described previously, current standards of care for preventing mtDNA transmission stipulate that heteroplasmy levels in embryos should be less than 5 percent to mitigate the chance of mtDNA disease in offspring.

measures. A more detailed review of these and other studies of MRT can be found in Appendix B.

A third technique—polar body transfer (PBT)—has recently been proposed as an alternative or complement to MST and PNT. Compared with these latter two techniques, PBT has been less thoroughly investigated with respect to prevention of mtDNA disease transmission. PBT is discussed briefly in this chapter for general background purposes but is not included in the committee's analysis of ethical, social, and policy issues associated with MRT.

Other methods involving oocyte and embryo cell modification for preventing the transmission of mtDNA disease—namely cytoplasm (ooplasm) transfer, somatic cell nuclear transfer (SCNT), embryo cell nuclear transfer, and germinal vesical transfer—have been raised in various contexts in other forums. To the committee's knowledge, FDA currently is not considering these techniques for preventing transmission of mtDNA disease, however, so they are not discussed here.

Maternal Spindle Transfer

MST would entail removal of the nDNA (specifically, the metaphase II spindle-chromosome complex,[21] or MII-SCC) from the intended mother's oocyte and its subsequent fusion to an oocyte provided by another woman that contained nonpathogenic mtDNA and from which the nDNA had been removed.[22] The reconstructed oocyte would then be fertilized with the intended father's, or another man's, sperm and cultured in vitro to the blastocyst stage. At this point, the blastocyst would undergo genetic testing to determine mtDNA heteroplasmy levels, chromosome abnormalities, and sex (if utilized). Embryos that met established criteria for these parameters would be transferred into the uterus of the woman intended to carry the pregnancy (see Figure 2-5). As in PNT, a small amount of cytoplasm would be carried over in the karyoplast[23] removed from the intended mother's oocyte, and thus there would be a nonzero chance for carryover of the intended mother's pathogenic mtDNA. This and other potential risks associated with MRT are discussed later in this chapter.

[21] During metaphase II, the chromosomes are attached at their centromeres to microtubules that connect to the spindle apparatus, which aids in aligning the chromosomes at the equator of the cell (the metaphase plate) in preparation for separation of the sister chromatids during anaphase II.

[22] The term "enucleation" is sometimes used to describe the removal of nuclear genetic material from the metaphase II oocyte; at this meiotic stage, however, the chromosomes are not encompassed by a nuclear membrane and thus do not constitute a true nucleus.

[23] Karyoplast is nuclear genetic material and cytoplasm encapsulated by a plasma membrane.

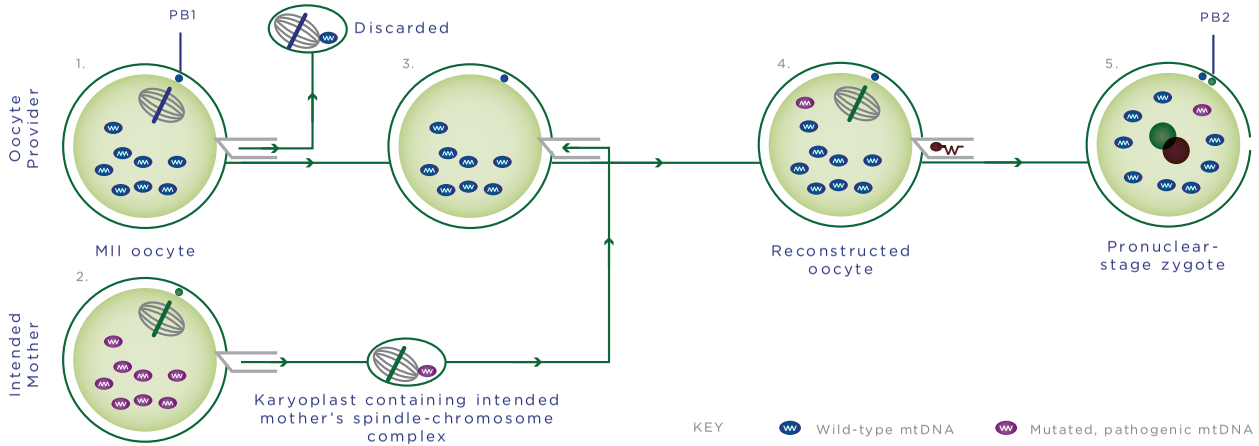

FIGURE 2-5 Maternal spindle transfer.
NOTES:
1. The spindle-chromosome complex is removed as a karyoplast from the provider oocyte and discarded.
2. The spindle-chromosome complex is removed as a karyoplast from the intended mother's oocyte and fused to the provider oocyte from which the nuclear DNA (nDNA) material has been removed; the intended mother's oocyte is discarded.
3. The reconstructed oocyte contains the intended mother's nDNA and oocyte provider's nonpathogenic mtDNA.
4. The reconstructed oocyte is fertilized by intracytoplasmic sperm injection (ICSI) with the sperm provider's sperm.
5. The fertilized oocyte is cultured in vitro and transferred at the blastocyst stage to the woman who will carry the pregnancy.
Cells and cellular contents not drawn to scale; MII oocyte = metaphase II oocyte; mtDNA = mitochondrial DNA; PB1 and PB2 = 1st and 2nd polar body.

SOURCE: Modified figure based on those appearing originally in: Richardson, J., L. Irving, L. A. Hyslop, M. Choudhary, A. Murdoch, D. M. Turnbull, and M. Herbert. 2015. Concise reviews: Assisted reproductive technologies to prevent transmission of mitochondrial DNA disease. *Stem Cells* 33(3):639-645. License information available at: http://creativecommons.org/licenses/by/4.0.

MST in Animal Models

Wang et al. (2001) first reported MRT to be compatible with full-term mammalian development in a mouse model, wherein transfer of the MII-SCC was performed between oocytes of two genetically distinct mouse substrains. Of note is that the average body weight of the offspring at 10 days of age was within normal range for the oocyte donor substrain, which the authors suggest could indicate that factors in the oocyte donor's cytoplasm could have an effect on the transferred nDNA. More recently, researchers at Oregon Health & Science University (OHSU), led by Shoukrat Mitalipov et al. (the OHSU Group), pioneered MST in rhesus macaque, a nonhuman primate model (Lee et al., 2012; Tachibana et al., 2009, 2013). Initial work by the OHSU group demonstrated the feasibility of MST for producing oocytes capable of fertilization and embryonic development (Tachibana et al., 2009). This study also showed that MST was capable of producing live-birth macaque offspring whose body weight was comparable to that of controls and that presented with nondetectable mtDNA carryover. A 3-year follow-up study found that these offspring were healthy, displayed no mitochondrial dysfunction, and presented with no significant change in mtDNA heteroplasmy levels in blood and skin samples over time (Tachibana et al., 2013). The OHSU group informed the United Kingdom's Human Fertilisation and Embryology Authority (HFEA) during its most recent review of MRT that it intends to enter the macaque offspring into a breeding program to assess their fertility status, as well as to conduct more detailed investigations into the potential physiological effects of MRT (HFEA, 2014b).

Additional work by the OHSU group in macaques indicated that oocytes from females born as a result of MRT may have higher than expected levels of mtDNA carryover (Lee et al., 2012). In two female fetuses conceived by MST that were recovered preterm for analysis, mtDNA carryover was less than 0.5 percent in somatic tissues and organs. While 11 of 12 oocytes from each fetus contained less than 5.5 percent of carried-over mtDNA; 1 oocyte from each fetus contained a more substantial level of mtDNA carryover (16.2 percent and 14.1 percent). These data confirm that, while MRT would likely prevent significant mtDNA carryover and heteroplasmy in somatic tissues and organs of offspring born as a result of MRT, oocytes of females born as a result of MRT could harbor significant and clinically relevant levels of carried-over mtDNA.

MST in Human Oocytes

The OHSU group demonstrated the feasibility of MST for producing human oocytes capable of fertilization and normal embryo development in oocytes provided by healthy female volunteers (Tachibana et al., 2013).

Compared with macaque oocytes subjected to MST, whose rates of normal fertilization were comparable to those of controls, a significant proportion of human oocytes subjected to MST showed abnormal fertilization, as evidenced by an irregular number of pronuclei in the MST zygote. Of those zygotes that were normally fertilized, development to the blastocyst stage was comparable to that of controls. An average mtDNA carryover of 0.5 percent was observed in MST embryos, confirming the ability of MST to reliably limit mtDNA carryover.

A study conducted by Paull et al. (2013) at the New York Stem Cell Foundation confirmed the feasibility of MST in human oocytes, although metaphase II oocytes were parthenogenetically activated to avoid formation and destruction of potentially developmentally competent embryos. Following MST and artificial activation, an average of 0.36 percent mtDNA carryover was observed in MST zygotes. Finally, researchers at the Wellcome Trust Centre for Mitochondrial Research at Newcastle University (the Newcastle Group) have begun work on MST in human oocytes alongside PNT in zygotes to facilitate comparison of the two techniques (HFEA, 2014b). This work is still in progress.

Pronuclear Transfer

Compared with MST, wherein the transfer of genetic material would take place between metaphase II oocytes prior to fertilization, PNT would entail the transfer of nDNA between fertilized oocytes, or zygotes, prior to fusion of the pronuclei (syngamy). Specifically, the male and female pronuclei would be removed in a karyoplast from the zygote of the intended parents and fused to an enucleated zygote of the sperm provider's sperm and the oocyte provided by a woman other than the intended mother. The reconstructed zygote would then be cultured in vitro to the blastocyst stage. At this point, the blastocyst would undergo genetic testing to determine mtDNA heteroplasmy levels, chromosome abnormalities, and sex (if utilized). Embryos that met established criteria for these parameters would be transferred into the uterus of the woman intended to carry the pregnancy (see Figure 2-6). As in MST, a small amount of cytoplasm would be transferred within the extracted karyoplast containing the pronuclei and would likely contain a variable, nonzero amount of the intended mother's pathogenic mtDNA. This and other risks associated with PNT are discussed later in this chapter.

PNT in Animal Models

The availability of proof-of-principle studies in animal models to demonstrate the safety and efficacy of PNT is limited. Using a mouse model of mtDNA disease ("mito-mouse") harboring a large-scale mtDNA dele-

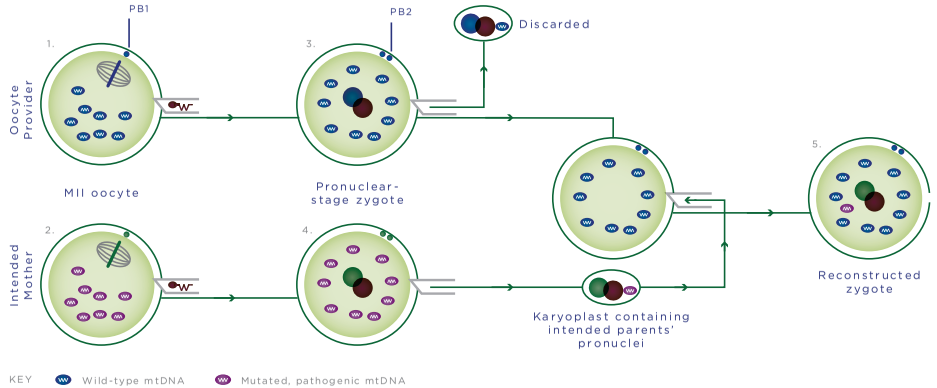

FIGURE 2-6 Pronuclear transfer.

NOTES:

1. The provider oocyte is fertilized by intracytoplasmic sperm injection (ICSI) with the sperm provider's sperm.
2. The intended mother's oocyte is fertilized by ICSI with the sperm provider's sperm.
3. The male and female pronuclei are removed from the provider zygote and discarded.
4. The male and female pronuclei are removed from the intended mother's zygote and fused to the enucleated provider zygote. The enucleated zygote of the intended mother is discarded.
5. The reconstructed zygote contains male and female nuclear DNA from the intended mother and sperm provider and nonpathogenic mtDNA from the oocyte provider. The zygote is cultured in vitro and transferred at the blastocyst stage to the woman who would carry the pregnancy.

Cells and cellular contents not drawn to scale; MII oocyte = metaphase II oocyte; mtDNA = mitochondrial DNA; PB1 and PB2 = 1st and 2nd polar body.

SOURCE: Modified figure based on those appearing originally in: Richardson, J., L. Irving, L. A. Hyslop, M. Choudhary, A. Murdoch, D. M. Turnbull, and M. Herbert. 2015. Concise reviews: Assisted reproductive technologies to prevent transmission of mitochondrial DNA disease. *Stem Cells* 33(3):639-645. License information available at: http://creativecommons.org/licenses/by/4.0.

tion (ΔmtDNA), Sato et al. (2005) determined that PNT was effective in preventing the expected mtDNA disease phenotype in ΔmtDNA mito-mice offspring. Corresponding measurement of ΔmtDNA levels showed that the proportion of ΔmtDNA molecules increased significantly over time. As noted by the authors, however, mtDNA molecules with large-scale deletions exhibit a replicative advantage over normal mtDNA molecules, and ΔmtDNA levels therefore might be expected to increase over time. Furthermore, the authors note the limited ability to translate the findings of this study to humans given that maternal transmission of mtDNA deletions in humans is not commonly observed.

A recent study by Neupane et al. (2014) compared mtDNA carryover and developmental competence in mouse oocytes and zygotes subjected to MST and PNT, respectively. The authors found no significant difference in mtDNA carryover in MST oocytes (<2.15 percent) and PNT zygotes (<2.6 percent). In further assessment of mtDNA carryover in PNT-derived blastomeres, one blastomere contained 4.9 percent karyoplast-derived mtDNA, while the remaining seven blastomeres showed no detectable mtDNA carryover. In parthenogenetically activated MST oocytes, development to the blastocyst stage was statistically similar to that of controls. Neither cleavage rate nor blastocyst formation differed significantly between parthenogenetically activated MST and PNT embryos.

PNT in Human Zygotes

The Newcastle Group, led by Douglass Turnbull et al., pioneered PNT for the prevention of transmission of mtDNA disease. They performed initial work in fertilized zygotes,[24] which are typically discarded during the course of fertility treatments (Craven et al., 2010). They found the developmental potential of reconstructed zygotes to be approximately 50 percent that of nonmanipulated abnormally fertilized control zygotes, a difference they attribute to the possibility that the reconstructed zygotes lacked the requisite complement of maternal and paternal pronuclei. Optimization of the procedure significantly minimized mtDNA carryover, which ranged from nondetectable to 11.4 percent. In response to the HFEA's most recent scientific review, the Newcastle Group reported that they have begun to assess the efficacy of PNT in normally fertilized zygotes, and have seen reproducibly high rates of blastocyst development from PNT zygotes. The group also reported that mtDNA carryover levels were nondetectable or less than 2 percent. The researchers identified "subtle differences in embryo development" in PNT zygotes, which they are investigating (HFEA, 2014b). There

[24] Zygotes that contain an abnormal number of pronuclei: one pronucleus (1N) or three pronuclei (3N), as compared with the normal complement of two pronuclei (2N).

are no published reports of PNT performed in human zygotes with the intent of preventing transmission of mtDNA disease in live-born children.[25]

Polar Body Transfer

A set of techniques for preventing mtDNA disease transmission related methodologically to MST and PNT—polar body 1 transfer (PB1T) and polar body 2 transfer (PB2T)—was recently documented as a potential alternative or complementary technique for preventing transmission of mtDNA disease (Wang et al., 2014). PB1T and PB2T entail the transfer of the first or second polar body to an enucleated or hemi-enucleated mature oocyte or zygote, respectively. Compared with MST and PNT, PBT has been less rigorously researched and reviewed with respect to the prevention of transmission of mtDNA disease. Furthermore, there is some reservation as to its potential future applicability given the lack of successful replication in mammals (Wolf et al., 2015). The HFEA conducted a comprehensive review of PBT for prevention of the transmission of mtDNA disease and the surrounding research landscape (HFEA, 2014a). In this review, the HFEA found that, while this research is still in its infancy as a potential MRT, PBT could potentially have advantages over MST and PNT, such as reduced mtDNA carryover, the absence of cytoskeletal inhibitors, and less invasive manipulations. More extensive preclinical research is needed in human oocytes and zygotes, however, to determine the feasibility, efficacy, and safety of PBT and whether these potential advantages would in fact be realized.

RISKS RELATED TO MRT: SCIENTIFIC COMPLEXITIES AND TECHNICAL UNKNOWNS AND UNCERTAINTIES

The clear benefit of successful implementation of MRT would be to give women who carry pathogenic mtDNA mutations the option of hav-

[25] One case report documents PNT attempted in human zygotes with the intent of producing viable human offspring (Zhang et al., 2003) in a patient with a history of failed IVF treatments. Briefly, patient and provider oocytes were fertilized by ICSI, and the pronuclei from the patient's zygotes were fused to enucleated provider zygotes via electrofusion. Five of seven successfully reconstructed zygotes were transferred to the patient's uterus. A triplet pregnancy was achieved in the patient, but all three fetuses were lost during the pregnancy. The researchers report that all three fetuses presented with normal karyotypes, contained nDNA solely from the intended parents, and contained no detectable mtDNA from the intended mother (Zhang et al., 2003). There is some debate, however, as to whether these findings are relevant to current safety considerations for MRT. While some have suggested that the observed adverse outcome might be related to the PNT technique, others have argued that it was a result of technical error (UK Parliament House of Lords, 2015). Inclusion of this experiment in this report is not intended to convey validation or support of this case report by the committee, but to provide a more complete overview of the published literature on PNT.

ing genetically related offspring at greatly diminished risk of mtDNA disease (the potential social and ethical benefits of MRT are discussed more thoroughly in Chapters 3 and 4). This section provides a nonexhaustive overview of the risks, unknowns, and uncertainties associated with MRT.

Complexities Related to Mitochondrial Genetics

Because the mitochondrial genome is maternally inherited, exists in high copy number, and exhibits evolutionary genetics distinct from those of nDNA, several inherent complexities are associated with mitochondrial genetics that do not arise with nuclear Mendelian genetics. Three concepts of mitochondrial genetics are important considerations in MRT: heteroplasmy, mtDNA bottleneck, and mtDNA evolutionary theory (Carelli and Chan, 2014; DiMauro and Schon, 2003; DiMauro et al., 2013; Reinhardt et al., 2013). Overall, these complexities underscore the relatively unpredictable nature of mitochondrial genetics, which could complicate the ability of preclinical studies to predict with certainty the safety and efficacy of MRT in humans.

Heteroplasmy: Threshold Effect and Mitotic Segregation

As previously described, heteroplasmy is the state in which a cell, tissue, or individual contains more than one type of mtDNA genotype. In most cases, cells containing pathogenic mtDNA mutations manifest cellular dysfunction only when the levels of pathogenic mtDNA molecules accumulate to a certain threshold level at which clinical symptoms of mtDNA disease develop (*threshold effect*). Depending on the particular mutation, the threshold level is typically 60-90 percent mutant mtDNA. The level of heteroplasmy can also increase or decrease in different tissues of an individual at different rates as a result of shifts in the proportion of pathogenic mtDNA transmission occurring randomly during cell division, a concept known as *mitotic segregation*. During cell division, pathogenic mtDNA molecules can be partitioned unequally into daughter cells, shifting the level of heteroplasmy in resulting daughter cells. If this happens to a great enough extent, the level of pathogenic mtDNA molecules within a tissue can reach the threshold level for manifesting as mtDNA disease. This phenomenon underscores the difficulty of extrapolating heteroplasmy levels measured in blood to those in all potentially symptomatic tissues.

mtDNA Bottleneck

During oocyte development in the developing fetus, a phenomenon known as the *prenatal mtDNA bottleneck* occurs, in which only a frac-

tion of the founding pool of mtDNA molecules are partitioned to daughter oocytes (Stewart et al., 2008). It is estimated that the number of mtDNA molecules is reduced from more than approximately 100,000 in the mature oocyte to as few as 10 copies in primordial germ cells (Shoubridge and Wai, 2007). As a consequence of this mtDNA bottleneck, rapid changes in the level of mtDNA mutations from one generation to the next can occur. For example, a mother may have low-level heteroplasmy of a pathogenic mtDNA (e.g., 10 percent) but bear a child who has high levels of heteroplasmy or is homoplasmic for that pathogenic mutation. Another, less intensely studied mtDNA bottleneck is the *postnatal mtDNA bottleneck*, which can occur during embryonic and fetal development and results from unequal distribution or selective replication of mtDNA molecules in developing embryonic and fetal tissues.

These issues result in complexities in evaluating the risks associated with MRT. In model systems, MRT has resulted in variable levels of carryover, with the most successful experiments documented to have resulted in less than 1-2 percent carryover of mtDNA molecules from the affected female's oocyte. This low-level carryover is expected to be compatible with clinically unaffected offspring. Because of poorly understood bottleneck effects, however, some offspring may have higher-than-expected levels of pathogenic mtDNA molecules in some tissues that could exceed the threshold level required to manifest disease. This phenomenon is exemplified by cases of cytoplasm transfer,[26] a procedure used for treatment of idiopathic infertility that involved injection of cytoplasm from oocytes provided by other women into the oocytes of intended mothers (Barritt et al., 2001a,b; Brenner et al., 2000, 2001, 2004; Cohen et al., 1997, 1998; Huang et al., 1999; Lanzendorf et al., 1999). Some offspring born following cytoplasm transfer were found to have surprisingly high mtDNA levels from the provided oocytes compared with the volume of oocyte cytoplasm injected (Brenner et al., 2004). This observation may be attributable to bottleneck effects during embryonic development, but it is difficult to evaluate because these procedures were not performed quantitatively and were documented loosely. With regard to MRT, female offspring born as a result of MRT could present with low-level heteroplasmy in somatic cells but produce

[26] Cytoplasm transfer was performed in the United States from 1997 to 2001 for treatment of infertility resulting from implantation failure due to poor embryo development. In a July 2001 letter to sponsors/researchers, FDA asserted jurisdiction over cytoplasm transfer on the grounds that it involved "human cells used in therapy involving the transfer of genetic material by means other than the union of gamete nuclei" (FDA, 2001a), requiring that an Investigational New Drug application be filed before clinical application of cytoplasm transfer could proceed. This effectively halted the clinical application of cytoplasm transfer, and since that time there has been no report of researchers attempting to use cytoplasm transfer for the treatment of infertility or other indications.

offspring with high levels of mtDNA mutations as a result of a potential bottleneck effect occurring in the development of their oocytes.

Evolutionary Theory: mtDNA and nDNA

Another relevant complexity is the potential for incompatibility ("haplogroup incompatibility") between artificially combined nuclear and mitochondrial genomes from two genetically distinct individuals, as in MRT. Ample evidence in model organisms indicates that such evolutionary divergence could lead to incompatibilities between certain mtDNA and nDNA genomes. Studies of outbred strains of model organisms, for example, have identified specific mtDNA variants that are "compatible" only with certain nuclear genome backgrounds (see Reinhardt et al. [2013] and Wolff et al. [2014] for a review). Relatedly, some have suggested that co-adapted mtDNA-nDNA pairings that are advantageous to the organism are likely to be preserved, while incompatible mtDNA-nDNA pairings are likely to be selected against (Morrow et al., 2015; Reinhardt et al., 2013). Accordingly, the artificial combination of a mitochondrial genome that has not co-evolved with a provided, "foreign" nuclear genome, as in MRT, could theoretically result in disruption, and possible failure, of critical mitochondrial processes. Experts in the field of mitochondrial genetics, however, disagree as to whether these incompatibilities would manifest in humans as phenotypically relevant adverse effects. An opposing argument is the anecdotal observation that humans across vastly divergent mtDNA haplogroups have reproduced with no apparent untoward effects on human health (IOM, 2015).

Another potential impact of mtDNA-nDNA mismatch is the manifestation of male-specific deleterious phenotypes. Evolutionary theory holds that, because mtDNA is solely maternally transmitted, it could accumulate mutations that are advantageous to females but detrimental to males. In fruit flies, for example, strains containing mtDNA that is "foreign" to the nuclear genome show dramatically altered expression of genes specifically in males but not in females—particularly those genes related to male reproductive organs (Innocenti et al., 2011). Hence, evolutionary theory and model organism studies indicate that if MRT led to a mismatch between mtDNA and nDNA, male infertility would be a theoretical possibility.

A proposed solution to mitigate the uncertainty of haplogroup incompatibility is "haplogroup matching," wherein the mtDNA of oocyte providers would be sequenced to select for those providers that were of the same haplogroup as the intended mother. The counterargument to this proposition is that haplogroup matching would not entirely mitigate the risk of mtDNA-nDNA mismatch because the genetic variants of putative

incompatibilities are poorly understood and thus may not be captured in haplogroup matching (Morrow et al., 2015).

Uncertainties and Unknowns Related to MRT Research

Certain aspects of MRT present an additional set of uncertainties and unknowns with regard to the potential safety and efficacy of first-in-human clinical investigations of the proposed techniques. These aspects include (1) limitations of current animal and in vitro models, as well as the available data, for purposes of predicting the safety and efficacy of MRT in humans; (2) the uncertainty of techniques such as PGD, amniocentesis, and chorionic villus sampling (CVS) for validating efficacy of MRT—namely for quantifying pathogenic mtDNA carryover and heteroplasmy load; and (3) the potential for yet unknown adverse effects of reagents and manipulations employed in MRT on the resulting embryo, fetus, or future child.

Limitations of the Current State of MRT Science

Research to date has provided data to support the feasibility and efficacy of MRT, although the translatability of such data is limited. The briefing document for FDA's Cellular, Tissue and Gene Therapies (CTGT) Advisory Committee states: "These studies provide preliminary evidence that PNT and [MST] methods may be feasible. However, these data cannot be seen as traditional POC [proof-of-concept] studies. . . . Because most of these studies were not done with models of mitochondrial disease, it is not clear whether these data provide any support for the potential effectiveness of these methods in humans" (FDA Cellular Tissue and Gene Therapies Advisory Committee, 2014b). The HFEA echoes this observation in its most recent scientific review, noting that "some consulted experts recommend that as a 'gold standard' they would like to see experiments conducted using oocytes from women affected by mitochondrial disease to see if pathogenic mutations behave differently" (HFEA, 2014b). The HFEA also notes caveats on the implementation of this recommendation, such as the wide range of potential mtDNA mutations and the potential burden of ovarian stimulation for women with mtDNA disease.

With respect to both fundamental basic and translational science, the CTGT Advisory Committee "generally agreed that there is not sufficient animal data (particularly with regard to follow-up of offspring) to support the use of the mitochondrial manipulation technologies in first-in-human clinical trials" (FDA Cellular Tissue and Gene Therapies Advisory Committee, 2014a). This discerned lack of evidence in support of the safety and efficacy of MRT has implications for the assessment of benefits and risks inherent in the ethics of recommendations to proceed with MRT.

Efficacy: Validation of MRT

As discussed earlier in this chapter, PGD is not at present a reliable method for preventing transmission of mtDNA disease given the improbability of procuring an embryo with sufficiently low levels of heteroplasmy for transfer, as well as the potential for postnatal bottleneck amplification of pathogenic mtDNA molecules following embryo transfer. Experiments with cytoplasm transfer discussed earlier in this chapter highlighted the latter concern. Similar concerns arise regarding the ability of PGD, and correspondingly amniocentesis and CVS, to predict accurately the expected level of heteroplasmy in the tissues of offspring born as a result of MRT. As discussed earlier, current standards of care for the use of PGD to prevent transmission of mtDNA disease stipulate that heteroplasmy levels must be less than 5 percent to mitigate the chance of mtDNA disease in offspring. At present, the estimated amount of mtDNA carryover with MRT techniques is less than 1-2 percent; however, the potential for postnatal bottleneck amplification remains a concern in analyses of efficacy.

Safety: Manipulations and Reagents Used in MRT

Inadvertent physical damage or epigenetic changes to the reconstructed oocyte or zygote are a potential risk stemming from the manipulations inherent in and reagents used for MRT. Visualization of the MII-SCC in MST, for example, would require polarized light birefringence, whose safety is currently unknown. While the pronuclei in PNT would be visualized more easily than the MII-SCC, they would be larger and more difficult to manipulate, potentially resulting in greater cellular trauma (Craven et al., 2010). There could also be an increased risk for aneuploidy or chromosomal abnormalities—particularly potential loss of chromosome(s) during nuclear transfer—as a result of MRT. This risk could be augmented in MST given that the MII-SCC is not enclosed by a nuclear membrane.

Sendai virus would be used in MST and PNT for fusion of the karyoplast to the recipient oocyte or zygote. Unlike the reagents used in manufacturing processes upstream of MRT, which would be washed away or diluted in subsequent steps, Sendai virus would be injected directly into the cell, which would develop into the embryo that would subsequently be transferred into the woman who would carry the pregnancy. There could be unknown risks associated with the immunogenicity of the virus that could adversely affect the embryo or offspring. The cytoskeletal inhibitors used to aid removal of the karyoplast from the oocyte or zygote (e.g., nocodazole and cytochalasin B) could also pose an unknown risk to the oocyte or zygote. Of note, cytochalasin B would be used in both MST and PNT, and nocodazole would additionally be used in PNT.

Vitrification for Stage Matching

Matching the developmental stage of the intended mother's oocyte or zygote and the oocyte or zygote provided by another woman is critically important, as noted by the Newcastle Group in evidence submitted to the HFEA (HFEA, 2014b). Given the potential difficulty of synchronizing oocyte retrievals for both MST and PNT, oocyte or zygote vitrification could be necessary. Work by Tachibana et al. (2013) revealed that the cytoplast may be more sensitive than the nDNA to vitrification-induced damage, at least in the macaque model, while Paull et al. (2013) provided evidence for the feasibility of using cryopreserved karyoplasts containing the MII-SCC in MST. These findings suggest an experimental design wherein the oocyte providing the nDNA of the intended mother would be cryopreserved, if necessary, to ensure that it matched the developmental stage of the provided oocyte.

Conclusion: *The field of mitochondrial genetics is characterized by complexities that make predicting the behavior of mtDNA—at the cellular, tissue, and systemic levels—difficult and uncertain. Collectively, these complexities can be viewed as an unknown variable in predicting the efficacy and safety of MRT in humans. The current state of MRT science and unknown physiological impact(s) of reagents and procedures implemented in MRT present an additional set of uncertainties and unknowns. A thorough understanding of the state of the science related to the unknowns of mtDNA genetics and MRT is important for informing the benefit and risk assessment entailed in potential regulatory decisions regarding if, when, and how to proceed with MRT in first-in-human clinical investigations.*

POLICY CONTEXT

In the United States, MRT would be subject to a complex landscape of state and federal laws and regulations. The legality of the research on MRT—and perhaps even the clinical application—would vary from state to state as a result of differing laws on fetal and embryo research, including cloning. Federal funding for MRT research would likely be unavailable because of current legislative restrictions against funding research on human embryos. In the event that MRT were to move into clinical investigations, FDA has asserted regulatory jurisdiction, and a careful stepwise process, which would include FDA oversight and institutional review board (IRB) review, would be required before any form of MRT would be approved for marketing. If it were approved, there would be some potential mechanisms for oversight in the postapproval context. Potential oversight of both the

research on and clinical use of MRT would be complex, with uncertainty over the precise interpretation of how laws and regulations would apply.

Regulation of Related Technologies

Although MRT is relatively new, policies on similar technologies could apply to MRT and illustrate some of the ways in which these techniques could be regulated. Oversight of MRT would likely involve the same statutes and regulations that apply to IVF, PGD, preimplantation genetic screening (PGS), and cloning. Not only is MRT similar in some ways to IVF, PGD, and PGS, but these technologies would also be performed in conjunction with MRT.

In Vitro Fertilization

Since the 1978 birth of Louise Brown, the first baby conceived by IVF, it is estimated that more than 5 million babies have been born as a result of IVF (ESHRE, 2012). This technology, in which embryos are created outside the body and then implanted, was developed and disseminated with minimal federal oversight. In the mid-1970s, the U.S. Department of Health, Education and Welfare (DHEW) appointed an ethics advisory board (EAB) to study IVF and review proposals for federal funding for IVF research. The EAB concluded that IVF was ethically acceptable; however, the EAB no longer functioned as of 1980. Because DHEW regulations required that a federal ethics board review funding proposals, and the EAB no longer functioned, this created a de facto moratorium on federal funding for IVF research. As a result, IVF was developed with private funds and with minimal federal regulation or oversight (Knowles and Kaebnick, 2007).

FDA did not clarify its jurisdiction over IVF until 1998, when it released a proposed rule for oversight of human cellular and tissue-based products (HCT/Ps) (FDA, 1998a). The final rule, released in 2001, defines HCT/Ps as "articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient." The rule divides HCT/Ps into two categories with corresponding levels of regulation: minimally manipulated HCT/Ps are lightly regulated, and more-than-minimally manipulated HCT/Ps are regulated as drugs and/or biologics. Minimally manipulated HCT/Ps, which include semen, oocytes, and embryos, must be screened for communicable diseases (unless provided by an intimate partner), and manufacturers of these HCT/Ps must register with FDA (FDA, 2001a).

ART programs are subject to these HCT/Ps regulations, so they must screen gametes for communicable diseases, register with FDA, and follow guidelines for handling tissues. In addition, FDA regulates the drugs and

devices that are used in conjunction with IVF, such as drugs that stimulate production of oocytes for retrieval. According to a different law, clinics also must report their pregnancy success rates to the U.S. Centers for Disease Control and Prevention (CDC), which collects and publishes data for certain procedures performed by clinical programs conducting "treatments or procedures which include the handling of human oocytes or embryos" (42 U.S.C. 263a-1 et seq.). Clinics that do not report these data to CDC are identified as having failed to report in CDC's publication of data and face expulsion from the Society of Assisted Reproductive Technologies (SART) for failure to report (Knowles and Kaebnick, 2007; SART, 2016).[27]

Preimplantation Genetic Diagnosis (PGD) and Screening (PGS)

PGD and PGS are techniques used in conjunction with IVF to test embryos for genetic disorders before intrauterine transfer. PGD involves the performance of diagnostic genetic tests to determine whether specific gene or chromosome disorders—such as a mutation that causes cystic fibrosis or an array that would determine a precise chromosomal abnormality—are present or absent in an embryo. In contrast, PGS uses biomarkers to screen for an increased risk that an embryo will harbor any chromosomal abnormality, such as trisomy 21, which causes Down syndrome; a positive biomarker screen would then need to be followed up with a definitive diagnostic test. The first successful clinical application of PGD was reported in 1990 by Handyside et al. (1990) for prevention of transmission of X-linked disorders.

The regulation of PGD and PGS is essentially identical to the regulation of IVF. Although PGD and PGS entail laboratory testing, they are not subject to the Clinical Laboratory Improvement Amendments (CLIA), which ensure the quality of laboratory testing through such requirements as specific levels of education, training, and experience for laboratory personnel (42 CFR § 493.17). Normally, laboratories that perform diagnostic tests must be compliant with CLIA to receive Medicare or Medicaid reimbursement. However, laboratories are subject to the regulations only if they perform tests on "materials derived from the human body for the purpose of providing information for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of, human beings" (42 CFR § 493.2). Thus far, the Centers for Medicare & Medicaid Services (CMS) has not interpreted CLIA as applying to laboratories that perform PGD or PGS, either because an embryo is not "derived from the human

[27] The *2013 Assisted Reproductive Technology Fertility Clinic Success Rates Report* estimates that ART surveillance covered 98 percent of ART cycles performed in the United States in 2013 (CDC et al., 2015).

body" but is a new and unique entity (Nagy et al., 2012) or because the tests are diagnosing embryos, not "human beings" (Hudson, 2006).

Cloning

After Dolly, a sheep that was the first animal to be cloned using the nucleus from an adult somatic cell, was born in 1996, federal and state governments rushed to regulate this somatic cell nuclear transfer (SCNT) technology. Although no federal law was enacted, California passed a statute that banned reproductive cloning in 1997, and more than a dozen states followed suit with statutes banning either reproductive cloning or all SCNT, even for nonreproductive research. FDA asserted jurisdiction over cloning in 1998 with a letter to IRBs (FDA, 1998b). FDA informed IRBs that clinical research on human cloning is subject to FDA regulation, and would require the submission of an Investigational New Drug (IND) application. This letter did not analyze the specific statutory basis for FDA's authority, but a subsequent letter in July 2001 (FDA, 2001b) pointed to the 2001 final rule on regulation of HCT/Ps (21 CFR § 1271), as well as a 1993 *Federal Register* notice that clarified FDA's authority over human somatic cell therapy and gene therapy products (58 FR § 53248).

Germline Modification

Modification of the human germline—that is, modification of gametes or embryos that results in heritable genetic modification—is legal in the United States. However, several regulatory barriers have effectively prevented it from being carried out in many settings. First, the National Institutes of Health's (NIH's) Recombinant DNA Advisory Committee (RAC), which oversees and reviews proposals for research funded by NIH or conducted at institutions funded by NIH for similar projects that involve recombinant or synthetic DNA, has stated in guidelines since 1985 that it "will not at present entertain proposals for germ line alterations" (NIH Recombinant DNA Advisory Committee, 1985). Second, FDA, which, as discussed earlier, has regulatory authority over cell and gene therapy products, has never approved a proposal to modify the germline. Finally, the Dickey-Wicker amendment, a rider on each year's U.S. Department of Health and Human Services (HHS) appropriations bill, prohibits the use of HHS funding for research that creates embryos for research purposes or destroys, discards, or subjects an embryo to risks with no prospect of medical benefit for the embryo. Therefore, federal funding for preclinical research on germline modification has long been unavailable. More recently, Francis Collins, director of NIH, stated that NIH "will not fund any use of gene-editing technologies in human embryos." He noted that the

"concept of altering the human germline in embryos for clinical purposes has been debated over many years from many different perspectives, and has been viewed almost universally as a line that should not be crossed" (NIH, 2015).

This idea of "a line that should not be crossed" is reflected in the laws and regulations of many nations. Twenty-nine countries prohibit germline modification; the salient laws or regulations of 10 more countries, including the United States, are either ambiguous or would restrict but not fully prohibit it. This opposition to germline modification exists even in countries that allow other types of research involving human embryos: 13 of the countries that ban germline modification permit human embryonic stem cell research, and the United Kingdom permits MRT but prohibits all other types of germline modification (Araki and Ishii, 2014). In 2015, the International Bioethics Committee of the United Nations Educational, Scientific and Cultural Organization (UNESCO) called for a temporary ban on editing of the germline, stating that "interventions on the human genome should be admitted only for preventive, diagnostic or therapeutic reasons and without enacting modifications" that would be passed on to future generations (IBC, 2015). The U.S. National Academy of Sciences (NAS), U.S. National Academy of Medicine (NAM), Chinese Academy of Sciences, and the United Kingdom's Royal Society convened an international summit on human gene editing in December 2015, and a committee formed by the NAS and the NAM will issue a report in 2016 on the clinical, ethical, legal, and social ramifications of both somatic and germline human gene editing.

Potential Federal Regulation of MRT

If MRT moved from preclinical to clinical research, various federal prohibitions and regulatory schemes, including those reviewed above, could apply to the techniques.

Dickey-Wicker and Federal Funding

> The Dickey-Wicker Amendment states:
> (a) None of the funds made available in this Act may be used for—
>
> > (1) the creation of a human embryo or embryos for research purposes; or
> > (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).
>
> (b) For purposes of this section, the term "human embryo or embryos" includes any organism, not protected as a human subject under 45 CFR 46

> as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

This statute prohibits the use of HHS funding for such research; however, it does not prohibit the research itself. MRT research that involved destroying embryos or manipulating embryos with no medical benefit to the embryos (i.e., if the embryos were not implanted) would be ineligible for HHS funding. Conversely, it might be the case that MRT research that involved transfer for gestation could be funded.

U.S. Food and Drug Administration Regulatory Authority

FDA does not regulate the practice of medicine itself, but instead has the authority to approve the introduction of a new drug, device, or biologic into interstate commerce[28] (e.g., 21 U.S.C. 355(a)). The agency's authority to regulate drugs and devices is found in the Federal Food, Drug, and Cosmetic (FD&C) Act, and its authority to regulate biologics is in Section 351 of the Public Health Services (PHS) Act.

In a 2001 letter to researchers, FDA asserted regulatory authority over "human cells used in therapy involving the transfer of genetic material by means other than the union of gamete nuclei," and noted that this genetic material includes cell nuclei, oocyte nuclei, and ooplasm containing mitochondrial genetic material. The letter stated that any clinical research involving these techniques would require submission of an IND. Current MRT technologies, such as PNT, MST, and PBT, would all likely fall under this definition, thus giving FDA authority over MRT (FDA, 2001b).

As discussed above, FDA regulates standard IVF procedures as "minimal manipulation" and requires only registration of facilities and screening for communicable diseases. However, FDA has stated that the manipulation of HCT/Ps used in MRT, including "human cells used in therapy involving the transfer of genetic material (cell nuclei, oocyte nuclei, mitochondrial genetic material in ooplasm, genetic material contained in a genetic vector)," constitutes more-than-minimal manipulation and thus the manipulated HCT/Ps would be regulated as drugs and/or biologics (FDA, 2009). Whether a particular MRT technique would trigger regulation as a drug or biologic would depend on the specific technology and materials used in the technique.

[28] The federal courts and FDA define "interstate commerce" broadly, and FDA asserts jurisdiction over products made from or with interstate components. See http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm073820.htm (accessed January 15, 2016).

U.S. Food and Drug Administration Regulatory Approval

Regardless of the product classification, the steps to FDA approval of MRT would be similar. Researchers wishing to conduct clinical investigations of any MRT technique would first be required to submit an IND. FDA does not regulate MRT as a technique per se, but rather the "product" that is considered a drug and/or biologic—in this case, the manipulated oocytes or zygotes (FDA, 2009). The IND includes preclinical data, information about the methods and products to be used, information about the investigators, and detailed protocols for the proposed clinical study. If the application is authorized, clinical investigations may begin. If the investigations are successful, a Biologic License Application (BLA) or a New Drug Application (NDA) can be submitted. If FDA determines, among other considerations, that the product is safe and effective and that its benefits outweigh its risks, the BLA or NDA can be approved and the product marketed in the United States.

Recent advances in the use of CRISPR-Cas9 and other tools for so-called gene editing (in which targeted changes are made in genes) have raised the question of whether this technique should ever be used in human gametes and embryos, a use that could result in intergenerational change in nDNA. To date CRISPR-Cas9 has been attempted in China with nonviable human embryos, as a demonstration of proof-of-principle, as well as demonstration of some of the technical challenges related to accuracy and precision of such changes (Liang et al., 2015). A number of countries have domestic law or have signed on to international instruments prohibiting such efforts if aimed at producing intergenerational changes in the germline (Council of Europe, 2015), and on December 18, 2015, the U.S. Congress passed an omnibus spending bill for fiscal year 2016[29] that would seem to forestall FDA consideration of any application to try such a technique in human clinical investigations, that is, in investigations involving transfer to a woman for gestation of the modified embryo. MRT might not result in heritable changes under all circumstances, so the applicability of the budget provision noted above to the clinical research discussed in this report is unclear, and determination of its applicability would necessarily be determined by FDA Counsel.

[29] See Sec. 749, Consolidated Appropriations Act, 2016, Public Law 113, 114th Cong. (December 18, 2015). Available at: https://www.gpo.gov/fdsys/pkg/BILLS-114hr2029enr/pdf/BILLS-114hr2029enr.pdf (accessed January 11, 2016).

Recombinant DNA Advisory Committee

NIH's RAC, which is authorized by the PHS Act,[30] provides oversight and review of basic and clinical research funded by NIH or conducted at institutions funded by NIH for similar projects involving recombinant or synthetic nucleic acid molecules, which are defined as

> (i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids;
> (ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
> (iii) molecules that result from the replication of those described in (i) or (ii) above. (NIH, 2013)

Current MRT techniques, such as MST, PNT, and PBT, do not appear to fit this definition, as they do not involve the recombination of nucleic acid molecules or the use of synthetic nucleic acid molecules. Thus, it is unlikely that these techniques would fall under the jurisdiction of the RAC.

If the RAC were to have jurisdiction over an MRT technique, NIH's Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules specify practices and requirements that would apply to research on the technique. NIH-funded research projects must comply with the NIH guidelines, and projects not funded by NIH must do so as well if they are conducted at or sponsored by an institution that receives NIH funds for similar projects. The guidelines require that before a clinical investigation begins, a project must (1) be approved by the Institutional Biosafety Committee (IBC); (2) be approved by the IRB; (3) obtain all applicable regulatory authorizations (e.g., IND approval); and (4) complete the RAC process, which includes initial RAC review upon submission, as well as public RAC review and discussion if deemed necessary. Once clinical investigations have begun, the RAC requires annual reports and safety reporting (NIH, 2013).

State Laws

Many states have laws regarding cloning, embryo research, stem cells, and other areas relevant to MRT. The language used in these statutes could affect whether MRT clinical research or clinical application would be legal in a state or whether certain MRT techniques would be prohibited. For example:

[30] 42 U.S.C. 282(b(16)).

- Arizona prohibits the creation of an embryo by any means other than the "combining of a human egg with a human sperm."[31] Under this law, both MST and PNT could potentially be illegal: MST involves fertilization of a *reconstructed* human oocyte with a human sperm, and PNT involves fertilization of two human oocytes, followed by transfer of the nuclear genetic material from the resulting intended parents' zygote to the provider zygote to form a reconstructed zygote, which is cultured in vitro to a human embryo.
- California prohibits reproductive cloning and defines cloning as the transfer of a nucleus from a human cell from "whatever source" into a human oocyte for the purpose of initiating a pregnancy that could result in the birth of a human.[32] Unlike other state cloning laws, the California law does not limit its prohibition on nucleus transfer to nuclei from somatic cells. Under this law, it appears that some versions of MRT would be permissible (PNT would involve transfer of a nucleus into a zygote, not an oocyte), but some would not (MST would involve transfer of nuclear genetic material into an oocyte).
- Louisiana prohibits the use of a fertilized ovum for any purpose other than "for the support and contribution of the complete development of human in utero implantation" and prohibits the creation of a fertilized ovum "solely for research purposes." Under this law, then, MRT clinical investigations resulting in the creation of embryos for purposes other than implantation would be illegal, and clinical practice of PNT could be prohibited as well, because one fertilized ovum would not be implanted.[33]
- Several states have prohibitions on nontherapeutic research involving embryos, which presumably would prohibit MRT research that did not result in intrauterine transfer but permit research use of MRT if it were intended to lead to gestation and birth; these states include Michigan,[34] Pennsylvania,[35] and South Dakota.[36]

Institutional Oversight

Institutions have an important role in the oversight of research. FDA requires that any human subjects research requiring its approval be reviewed

[31] Az. Rev. Stat. § 36-2311-2313.
[32] Ca. Health & Safety Code § 24185.
[33] La. Rev. Stat. tit. 9, §§ 122-129.
[34] Mich. Comp. Laws § 333.2685 (1).
[35] Pa. Cons. Stat. tit 18. § 3216 (a).
[36] S.D. Codified Laws sec. 34-14-16 through 34-14-20.

by an IRB.[37] IRBs are established or designated by the institutions conducting the human subjects research, but the federal government provides detailed guidance on IRB functions and duties. IRB considerations include risks, benefits, and informed consent. Any institution that receives federal funds for research involving human subjects must establish an IRB, and all such research performed at the institution must be reviewed by the IRB, regardless of its source of funding.[38] As applied to MRT, an investigator would be required to obtain IRB approval for clinical research performed under an IND. IRB review would not be required for research involving purely in vitro embryo manipulation unless the research on the embryos would reveal identifiable information about the people who provided the embryos.[39]

For oversight of research involving recombinant or synthetic DNA, NIH requires that institutions establish an IBC. Like IRB review, IBC review is required for research at any institution that receives federal funding, regardless of the source of funding for the research (NIH, 2013). If MRT clinical research were subject to RAC oversight (see section on potential federal regulation of MRT earlier in this chapter), IBC review would be required.

Postapproval Regulation

FDA approvals are for specific indications. But even if FDA approved MRT for a specific indication, it could be used "off-label"—that is, used for an indication for which it had not been approved (see also the discussion of off-label use below). Once MRT had been approved, the FDA regulations that would apply to its clinical use would be limited to a group of postmarket measures that would be less stringent than the premarket controls. Still, there would be a few avenues for additional oversight and control of MRT, such as FDA's Risk Evaluation and Mitigation Strategy (REMS) program or professional monitoring.

Background

As discussed above, FDA has the authority to approve MRT for a particular intended use (i.e., its labeled use), which would allow it to be marketed for that use. Marketing includes both advertising and a range of other promotional efforts. As noted, however, FDA's regulatory authority

[37] 21 CFR Part 56.

[38] 45 CFR Part 46.

[39] In practice, some institutions use the committees established to review embryonic stem cell research to review all embryo research, but this is not required by law.

wanes following approval. A clinical provider may use an approved product for an off-label purpose if, based on his or her best knowledge and clinical judgment, it is being used in the "practice of medicine" (21 CFR 312.2(d)). For example, if FDA approved MRT for the intended use of preventing the transmission of known pathogenic mtDNA mutations, a clinician could use the technique for the off-label indication of treating infertility. Although those who might receive FDA approval for mitochondrial replacement would not be permitted to market or promote a use of the product that has not been approved by FDA,[40] the agency cannot prevent clinicians from using the product in any manner they deem appropriate, based on their clinical judgment. FDA does note that physicians "have the responsibility to be well informed about the product, to base its use on firm scientific rationale and on sound medical evidence, and to maintain records of the product's use and effects" (FDA, 2014). Physicians are also subject to regulation in the form of state licensing and discipline procedures, as well as the threat of medical malpractice.

In addition to off-label clinical use, investigational use of an approved product is permitted if all of the following conditions are met:

- The new use is not intended to be submitted to FDA to support a new indication or a significant change in labeling or advertising.
- The new use does not significantly increase the risks of the product, and the investigation is conducted in compliance with IRB and informed consent protocols.
- The new use is in compliance with requirements concerning the promotion and sale of products (21 CFR 312.2(b)(1)).

As applied to MRT, these stipulations might permit a researcher to conduct clinical investigations of the use of MRT to treat such conditions as infertility without first obtaining FDA approval. Even if such use were shown to be successful, however, the product could not be marketed for that purpose without first undergoing FDA review. Notably, Shoukhrat Mitalipov, a pioneer of MRT, has announced publicly that he has submitted an IND to FDA to conduct clinical investigations of MRT for treatment of age-related infertility (Connor, 2015).

The limitations on marketing, as opposed to use, can have a significant effect on the scale of off-label use. In some areas of medicine, having marketing authority can give a sponsor, such as a pharmaceutical company, much larger market shares than would be garnered by any comparable drug

[40] See, e.g., sections 505(a), 515(a), 501(f)(1), and 301(a) and (d), of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355(a), 360e(a), 351(f)(1)) and 331(a) and (d).

without such privileges. In some areas, however, such as pediatric uses or cancer treatments, off-label use is exceedingly common.

For MRT, even off-label uses would be subject to rules concerning the safe handling of human cellular material, including oocytes, spermatozoa, and embryos. These rules are aimed at preventing communicable disease and require establishments (e.g., IVF clinics) to, for example, screen provided cells or tissues (21 CFR 1271). In addition, CDC collects and publishes pregnancy success data for ART techniques, which could include MRT because it is among the "treatments or procedures which include the handling of human oocytes or embryos" (42 U.S.C. 263a-1 et seq.).

Mechanisms for Postmarket Control

Once approved, a cell-based product, including the manipulated cells used in MRT, remains subject to controls by FDA (up to and including withdrawal of the approval), requirements for reporting to CDC, state laws governing licensing and discipline, medical malpractice suits, and the effect of insurance coverage decisions on its availability.

Risk Evaluation and Mitigation Strategy (REMS) Under the Food and Drug Administration Amendments Act of 2007, FDA has the authority to require a REMS from investigators. The REMS helps ensure that a postapproval product is used in a manner such that its benefits outweigh its risks, and applies to any use of the product, whether on- or off-label. FDA can require a REMS either as part of the approval process or after approval if new safety information emerges.

REMS programs vary significantly depending on the level of risk associated with a product. A REMS may require only that a medication guide be dispensed to patients with each prescription or it may require that the manufacturer send information on the risks of the product to health care providers and professional associations. The REMS for the acne medication isotretinoin (i.e., Accutane), for example, consists of a complex system for risk evaluation and mitigation that requires all patients, providers, and pharmacists to be registered in the iPLEDGE system in order to use, provide, or prescribe Accutane. Among other requirements, patients must demonstrate understanding of the drug's risks and agree to use two forms of contraceptives while taking Accutane; providers must counsel patients about contraceptive use, provide scheduled pregnancy testing, and prescribe only a 30-day supply; and pharmacists must dispense the drug in a safe and systematic way (FDA, 2012).

Postmarketing requirements, commitments, and warnings FDA can require sponsors to perform postmarketing studies and postmarketing clini-

cal investigations (so-called Phase 4 investigations) for approved products (FFDCA 505(o)(3)). Such requirements can be imposed at the time of approval, or after approval if FDA becomes aware of new safety concerns. FDA can require the conduct of studies or investigations to assess a known serious risk, further examine a potential serious risk, or identify an unexpected serious risk. Each year, FDA must review the status of such studies and investigations, publish a summary in the *Federal Register*, and provide a report to Congress on the findings. FDA can also highlight any new concerns by communicating directly to physicians, by adding warnings to the label, or by narrowing or even completely withdrawing the approval.

State licensing Individual states have boards that license and monitor medical professionals to ensure ethical practice that meets the standard of care. Any practitioner could be disciplined for use of MRT—whether on- or off-label—that was inappropriate for the patient (e.g., overly risky or unlikely to provide benefit) or that was provided before informed and voluntary consent had been obtained. These boards vary widely in their stringency, but exist as a possible mechanism for monitoring new therapies and watching for problems.

Professional monitoring Professional societies play an important role in maintaining a standard of care in medicine. Each of the major medical societies has programs or documents that describe and periodically update the factors most salient to good practice in their field. At times these societies also have stepped in to help maintain high standards in fields that escape some of the formal mechanisms that exist for this purpose, such as surgery (which often innovates without the formal clinical investigations that trigger IRB review) and some forms of embryo research (where the absence of federal funding means far less opportunity for federal oversight). The mechanisms used by societies can range from data collection and publication of success rates, by technique and by clinic, to detailed protocols that are deemed best practices. SART is one example in the ART area, having used guidelines and recommendations for laboratory personnel, procedures, and safety, as well as membership for clinics that follow these voluntary measures, to steer practice in the appropriate direction (SART, 2007, 2008). More than 90 percent of ART clinics in the country are SART members (SART, 2015).

Insurance coverage Insurance availability can have a strong influence on how often a product or procedure is used, whether on- or off-label. In general, insurance companies will cover a product or procedure only if it is "medically necessary" (Bergthold, 1995; IOM, 2012) and considered a therapy rather than an experimental procedure. This affects uptake two

ways. First, it means coverage often is not available for uses that are off-label (thus lacking FDA approval) and not yet employed widely enough to have generated the data necessary to persuade the insurer that the off-label use is a proven therapy. Second, insurers distinguish among indications, so that, for example, they might cover a procedure if used to circumvent a disease such as MELAS but not cover the same procedure if used to circumvent a natural condition such as menopause. In this way, what some have called "enhancement" applications may well lack coverage. Lack of coverage, of course, will limit the number of patients who can afford a procedure.

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3

Do Ethical, Social, and Policy Considerations Preclude MRT?

The unique combination of characteristics of mitochondrial replacement techniques (MRT) raises a novel collection of ethical, social, and policy issues. First, MRT would create embryos that if transferred would result in offspring with genetic material from two women of different maternal lineage,[1] a novel intervention never before approved by U.S. federal regulatory authorities.[2] Second, if MRT were carried out to conceive female offspring, the resulting mitochondrial DNA (mtDNA) modifications would be heritable (i.e., could be passed down through generations) in female offspring due to the matrilineal nature of the inheritance of mtDNA, and the effects of those modifications (whether beneficial or deleterious) could

[1] Every individual has genetic material from many individuals and ancestors. For instance, due to the matrilineal nature of the inheritance of mtDNA, each individual has genetic material from their mother, grandmother, great-grandmother, etc. Therefore, MRT is unique in that it would involve combining the genetic material of two women of different maternal lineage—nuclear DNA (nDNA) from the intended mother who carries a pathogenic mtDNA mutation and mtDNA provided by a woman without pathogenic mutations in her mtDNA. In the instance where some level of mtDNA from the intended mother is carried over to the embryo created by MRT, this embryo would also contain mtDNA from two women of different maternal lineage.

[2] U.S. federal regulatory authorities have never approved a cell-based product that involves genetic material from two women of different maternal lineages, as would MRT. In the case of unapproved cytoplasm transfer in the late 1990s/early 2000s, the U.S. Food and Drug Administration (FDA) halted the application of these techniques and asserted the agency's jurisdiction in reviewing and approving any clinical applications of the techniques. To the committee's knowledge, there was no application to FDA to pursue cytoplasm transfer techniques, and therefore, MRT represents a unique opportunity for U.S. regulatory review.

persist for generations. Third, the effects of the genetic modification performed on oocytes or zygotes, once carried out, would not, at this time, be reversible.[3] Fourth, the genetic modification would affect every cell type in the resulting individual, thus affecting the total organism rather than being confined to a specific organ system. This chapter explores the most prominent ethical, social, and policy issues raised by these characteristics of MRT and presents the committee's analysis of whether these issues preclude its introduction into first-in-human clinical investigations.

The chapter first examines the parental motivation to access MRT. It then turns to the central ethical, social, and policy issues related to genetic modification of germ cells and the germline; this section addresses the latter three of the four issues enumerated above: the genetic modification would be heritable, irreversible, and would affect every cell type of the resulting individual. Next is a discussion of unintended downstream implications of MRT. The chapter continues with a discussion of two other major ethical, social, and policy issues related to MRT: (1) the DNA contribution of two women of different maternal lineage (the intended mother, who would provide the nuclear DNA [nDNA], and the individual providing an oocyte or zygote, who would provide the nonpathogenic mtDNA), and (2) the creation, manipulation, and possible destruction of human gametes and embryos in MRT that would be involved in MRT research or clinical application. Following the analysis of these issues, the chapter concludes with a discussion of key differences between nDNA and mtDNA as related to the foundational question of whether it is ethically permissible for clinical investigations of MRT to proceed.

The title of this chapter—"Do Ethical, Social, and Policy Considerations Preclude MRT?"—comes from the committee's charge (see Box 1-1 in Chapter 1), which raises the core question regarding the ethics of moving forward with MRT:

> the committee's report will address the conduct of clinical investigations of these novel techniques [for prevention of the transmission of mtDNA disease], including *the foundational question of whether safeguards such as specific measures and public oversight could adequately address the social and ethical concerns, or whether those concerns preclude clinical investigations*. [italics added]

The evaluation and analysis of ethical, social, and policy issues in this chapter reflect the committee's attempt to answer this foundational question. The committee's analysis included discussion of whether the ap-

[3] Only in highly hypothetical future technologies would genetic modifications introduced by MRT be reversible. The committee refers to the irreversibility of MRT in this report as it reflects the current state of science and the ethical analysis that accompanies MRT today.

propriate approach should be (1) to begin from a permissive perspective that would support going forward unless restrictions are justified, or (2) to begin from a restrictive or precautionary perspective that would support restrictions on going forward until risks have been sufficiently managed or controlled, or prohibit going forward at all based on fundamental ethical, social, and policy concerns. The committee used an approach that recognizes important aspects of liberal democratic theory, which acknowledges the acceptability of individual interests and desires and the autonomy of parental decision making in a society capable of deliberation, transparency, and the rule of law, along with an optimism about scientific knowledge. The committee applied this approach with a healthy skepticism as to whether foundational concerns about some of the ethical, social, and policy issues raised by MRT could be addressed at all.

For the committee's analysis, this meant recognizing the importance of research for advancing medicine, in light of the ethical, social, and policy concerns raised by this technology, including respect for the interests of women who carry a risk of passing on serious disease, tempered by consideration of the risks and uncertainties of a first-in-human application of MRT. The latter include uncertainties regarding the likelihood and severity of both known and unknown risks to future children, the likelihood and consequences of intergenerational effects, and the downstream implications of introducing a new reproductive technology with a unique combination of characteristics. The following sections examine these issues in turn, presenting the committee's conclusions regarding each, as well as an overall conclusion regarding the full range of issues taken together.

DEMAND FOR MRT

MRT, if proven to be effective, would represent the only reproductive option for mitigating the risk of maternal transmission of pathogenic mtDNA to children that would also preserve the nuclear genetic relationship between the prospective mother and child. Without the prospect of MRT, families face the choice of risking mtDNA disease in offspring born as a result of unassisted sexual reproduction[4] or selecting reproductive options

[4] The probability of maternal transmission of mtDNA disease is highly variable and depends on a number of factors, including mutation type and heteroplasmy level in the intended mother. Furthermore, such factors as postnatal bottleneck and penetrance might affect the tissue distribution of mtDNA mutations and clinical manifestation in offspring born as a result of MRT. As a general principle, the higher the heteroplasmy level in the intended mother, the higher is the probability of clinically manifest mtDNA disease in offspring. (See the detailed discussion in Chapter 2.) The recurrence risk for offspring of females carrying pathogenic mtDNA mutations is estimated to be 1-4 percent if the female is asymptomatic and up to 50 percent if the female is symptomatic (Falk, 2010).

that would result in a child lacking a nuclear genetic relationship with the prospective mother. The motivation of prospective parents to pursue MRT thus is likely to fall into two overlapping categories: (1) the value to parents of bearing offspring with an nDNA connection to both parents whose risk of developing mtDNA disease would be significantly reduced, if not eliminated; and (2) eradication of mtDNA disease from future maternal descendants.

Genetic Relatedness as a Social and Emotional Value

Parents considering MRT would do so out of a desire to have children who have a nuclear genetic connection to both prospective parents (otherwise they would pursue other, less resource-intensive and more proven interventions, such as oocyte or embryo donation).[5] Although it may constitute, at least in part, a socially constructed value that differs across societies (Sault, 1996), nuclear genetic relatedness is a deeply held, significant value for some people, for a variety of reasons. Having a child genetically related to both prospective parents may be part of one's conception of traditional family formation. Physical and physiological resemblance of the child to both parents—and to their siblings and kinship network—could also be psychologically and socially meaningful. Indeed, some research has shown that this resemblance can be a powerful basis for kinship bonds across generations that can often "cement" parent-child relations (Heijkoop et al., 2009; Loomans, 2013; Plomin et al., 1997).

Nuclear genetic relatedness is not, however, a universal desire, and different women and families vary in how they understand genetic kinship and in the priority they place on genetic relatedness. In a study of women who were known or at-risk carriers of pathogenic mtDNA mutations, for example, 52 percent viewed having genetically related offspring as "very important," 43 percent as "somewhat important," and 5 percent as "not important" (Engelstad et al., submitted). Generally, social trends in the use of assisted reproductive technology (ART) support the argument that many prospective parents see value in having genetically related children, although many who pursue ART place greater importance on having children regardless of their genetic relation (Kirkman, 2008; Ravin et al., 1997; Thornton et al., 1994; van den Akker, 2000). For example, the advent and uptake of such techniques as those based on oocyte and sperm donation has seen users of ART accept some loss of genetic kinship when using a third party to aid in family formation.

Sociological evidence also suggests significant demographic variations. Studies of in vitro fertilization (IVF), for example, highlight that, because

[5] In some cases, the sperm may be provided by a man who is not the prospective father, in which case it is the prospective mother that desires the genetic connection.

of variations in insurance coverage, time demands, and costs of treatment, the benefits of the technology accrue most commonly to those with health insurance covering the costs, those with the financial ability to pay for fertility services themselves, and those with the flexibility to schedule the time-intensive procedures (Bell, 2009). Indeed, the relative importance placed on genetic relatedness may be influenced by whether a prospective parent perceives it as being an attainable goal (Bell, 2009; Thornton et al., 1994).

The popular press has recently covered the topic of adopted children's desire to connect with their parents, and the challenges they face in doing so (Neville, 2015; Pine, 2015). Studies of adolescents and adults born as a result of oocyte or sperm donation—in which half of the individual's genetic information is derived from the individual providing the oocyte or sperm—have suggested that some individuals experience confusion surrounding their identity upon disclosure of the nature of their conception due to the genetic contribution of someone not acting as their parent (Hewitt, 2002; Mahlstedt et al., 2010; Turner and Coyle, 2000).

The use of ART has allowed people to become parents through a variety of innovative methods that blur the conventional meanings of kinship, family, and genetic relatedness. In this sense, MRT is not particularly novel. The notion of genetic relatedness, however, is complicated in the case of MRT, primarily because a child born as a result of these techniques would be both genetically related, via nDNA, and genetically unrelated, via mtDNA, to the intended mother—a novel phenomenon in human reproduction. The potential ethical, social, and policy implications of the contribution of DNA from a third party are discussed later in this chapter.

Finding: The parental desire for offspring who share a nuclear genetic connection with both parents is widely held but not universal.

Finding: Although prospective offspring born as a result of MRT would lack an mtDNA connection with prospective mothers, MRT could satisfy a deeply held desire on the part of these mothers to have a child who bears an nDNA connection to them.

Inability of Current Alternatives to Achieve All Goals

For prospective parents who might consider using MRT, mitigating the risk of mtDNA disease in their children and future generations while retaining a nuclear genetic connection to their children currently represents an otherwise unachievable combination. At present, prospective mothers who are at risk for transmitting mtDNA disease to their offspring must choose among reproductive options that allow for varying degrees of nuclear genetic connection between the child and the prospective parents with

variable risk of transmitting mtDNA disease: unassisted sexual reproduction, preimplantation genetic diagnosis (PGD), oocyte or embryo donation, adoption, or childlessness.

Unassisted Sexual Reproduction

Unassisted sexual reproduction would provide for a full nuclear genetic contribution from both prospective parents. For women who are heteroplasmic for pathogenic mtDNA mutations, however, it would present a variable, unknown risk of transmitting mtDNA disease, owing to the complexities of mtDNA genetics. For women who are homoplasmic for pathogenic mtDNA mutations, the risk of transmitting mtDNA disease would be 100 percent (although penetrance of the disease across the offspring's lifetime could depend on a variety of factors).[6]

Preimplantation Genetic Diagnosis

PGD would preserve the nuclear genetic connection between the child and both prospective parents. For some women at risk of transmitting pathogenic mtDNA mutations, however, it is not a viable option for reliably preventing transmission of mtDNA disease (see the discussion of PGD in Chapter 2).[7]

Oocyte and Embryo Donation

Oocyte donation with fertilization by the intended father or a sperm provider represents a reproductive option for prospective parents that could reliably prevent transmission of pathogenic mtDNA from the prospective mother. However, it would not permit a nuclear genetic connection to the prospective mother while retaining the genetic connection to the prospective father. In the case of embryo donation, the transmission of mtDNA diseases from the prospective mother would be prevented, but the resulting child would not have a nuclear genetic connection to either the prospective mother or prospective father. Moreover, while in clinical best practice all

[6] This concept is exemplified by one of the common mtDNA homoplasmic mutations that can cause blindness—Leber's hereditary optic neuropathy—which exhibits increased penetrance in carriers who smoke or consume alcohol.

[7] As previously described, PGD may not be a reliable method for preventing transmission of mtDNA disease in women who are at known risk of transmitting such disease because of limitations related to complexities of mitochondrial genetics. With the advent of increasingly sensitive and accurate sequencing technologies, however, PGD is expected to be a reliable technique for determining the efficacy of MRT prior to embryo transfer.

efforts are made to obtain as much information as possible on the health history and status of gamete or embryo providers, including genetic risk factors, there is always a chance that the provider could carry unknown health risks that could be transmitted to the offspring.

Adoption

An additional option for preventing transmission of mtDNA disease is adoption, although this option would not result in any genetic connection between offspring and prospective parents. Like oocyte donation, adoption presents issues to be weighed by prospective parents. For instance, a range of well-known features of the adoption process are of potential consequence for the prospective parents and offspring, including the uncertain time frame for completion of the process; the potential for birth parents to claim or reclaim parental rights or custody; limited information about health risks; concern about the ability to create a cohesive family unit; preferences for (and the often limited number of) children who are young, healthy, and with a racial/ethnic and religious background similar to that of the adoptive family; and the potential for long-term psychosocial complications for the adopted child (Collishaw et al., 1998; Smyer et al., 1998). It is important to note, however, that, while challenges to adoption exist, the benefits to adopted children, adoptive families, and society can be significant.

Childlessness

If none of the above reproductive options are appealing to prospective parents for preventing transmission of pathogenic mtDNA mutations, the remaining option is to forgo having children or additional children. This option would guarantee the prevention of maternal transmission of mtDNA disease to offspring and future generations but at the cost of the parents having no (additional) offspring, nDNA-related or otherwise.

In sum, each of the above options would achieve some of the desirable attributes of MRT as a reproductive technique, but none would achieve all of them.

Finding: If a woman is at risk of transmitting mtDNA disease to her children, she currently has three alternatives to MRT that would allow her to have children with a significantly reduced risk of mtDNA disease: adoption, oocyte donation, and embryo donation. In the case of oocyte donation, children would not have a genetic relationship with the intended mother, and in the case of embryo donation or adoption, with either of the prospective parents.

Finding: In some instances, PGD is not a reliable technique for reducing transmission of mtDNA disease for women who are at risk of transmitting pathogenic mtDNA mutations to their offspring.

Procreative Liberty and Parental Desire to Pursue MRT

Procreative liberty is generally taken to mean the right of prospective parents to decide whether and when to have children, without unjustifiable restraints or restrictions (which would be a *negative right*). In some contexts, this definition has been expanded to include other choices related to reproduction, including the method by which one reproduces (i.e., unassisted sexual reproduction or ART). A more contentious aspect of reproductive rights is whether there is a *positive right* of prospective parents to avail themselves of social resources in accessing scientific advances in reproductive technologies, including entitlement to any available ART. Some have suggested that the regulatory and financial investments required for the development, evaluation, and delivery of the techniques amounts to a claim on collective resources that necessarily entails the recognition of a positive right in relation to MRT (Baylis, 2013; Bredenoord et al., 2008; Robertson, 1988).

While collective resources must be brought to bear to create and maintain the infrastructure and processes necessary to ensure oversight and safe use of goods and services—whether they be drugs, medical devices, cleaning supplies, or toys—the use of such infrastructure and processes does not invoke or suggest a positive right to claim provision of those things or services, nor is it the result of anything like a conscious trade-off between the use of resources for one purpose versus another. The pursuit of research in the United States triggers regulatory oversight, and while some may see this as a sort of claim on collective resources, the same argument could be made about a multitude of examples for which a system of evaluation and regulation exist, up to and including the resources needed to ensure good medical care for women who conceive through unassisted sexual reproduction. Therefore, if FDA were to approve MRT, its availability to a few does not create the recognition of such a positive right, any more than it would be in the case of the pursuit of any area of research, submission of licensure applications to regulatory bodies, or delivery of regulated services.

However, every reproductive choice—be it the birth of a child through unassisted sexual reproduction or the use of ARTs such as gamete donation, embryo donation, and gestational carriers—involves risk and has the potential for considerable health and social implications. MRT provides a potential opportunity to avoid a predicted health risk but with the uncertain potential to incur unknown developmental risks to the future child and unknown risks to future generations associated with the techniques.

As a general matter, parents have broad discretion to make decisions about the care, custody, and control of their children, including putting their children at some risk in the conduct of everyday family life. With regard to procreative liberty, the U.S. societal experience with the use of ART to treat infertility has revealed great tolerance for parental decisions to impart unknown risks to future children in the pursuit of relatively novel reproductive technologies. In those cases, the desire to conceive and bear children (whether genetically related or not) rather than to adopt or remain childless has effectively been given priority over concerns about risks to children born as a result of the novel technologies. To the extent that social concerns have arisen, they have not been identified or addressed through restrictions imposed by the U.S. legal and regulatory system, though this may be a function of the limits of regulation rather than a conscious decision. For example, the system has allowed the development and initial investigations and eventual clinical use of IVF, intracytoplasmic sperm injection (ICSI), and PGD with minimal FDA oversight (although regulation of medical practice at the state level does serve to regulate IVF), all of which may expose the future child to some risk. MRT would satisfy a strongly felt desire to bear genetically related offspring, coupled with the reduced risk of passing on mtDNA disease (Klitzman et al., 2015). MRT would not treat an existing person for a disease, illness, or condition, so its pursuit does not address a medical need per se. But satisfying a desire to bear genetically related offspring through use of MRT requires clinical interventions provided by professionals using manipulated materials, and thus is within the regulatory authority of FDA.

While pursuit of reproductive goals and desires deserves to be respected within the bounds of options made available through research and clinical settings, the responsibilities of professionals and the oversight process necessarily also include the protection of the health and well-being of a child created through use of these techniques. Upholding these responsibilities requires limits on initial investigations and potential eventual use(s) of MRT. The committee believes that MRT could move forward within such limits, through means noted later in the report.

Conclusion: *The desire of prospective parents to have children who are at significantly reduced risk of manifesting serious mtDNA disease and with whom they have an nDNA connection is justifiable, and clinical research on the use of MRT could be permitted within limits. These limits would be focused on protecting the health and well-being of the children who would be born as a result of MRT.*

GENETIC MODIFICATION OF GERM CELLS AND THE GERMLINE

Numerous ethical, social, and policy issues arise when one is considering techniques, such as MRT, that involve genetic modification of human germ cells or gametes. Although the term "genetic modification" could be used to encompass a variety of techniques, including gene editing, here the committee uses the term to mean changes to the genetic material within a cell. The type of genetic modification associated with MRT is the combination of mtDNA from one woman with nDNA from another woman of different maternal lineage within an oocyte or zygote. While there is no direct modification or editing of the mtDNA sequence itself,[8] the novel combination of mtDNA from one woman and nDNA from another would not occur in unassisted sexual reproduction or in other ARTs. Thus, the committee considers MRT to be "genetic modification" of the oocyte or zygote.

The statement of task provided by FDA to this committee defines "germline modification" as "human inheritable genetic modification."[9] Some have defined these terms differently. During deliberation over MRT, for example, the United Kingdom used a working definition of "genetic modification" as "the germline modification of nuclear DNA (in the chromosomes) that can be passed on to future generations."[10] This committee, in contrast, views "genetic modification" and "germline modification" as two separate concepts, the first being "changes to the genetic material within a cell" and the latter "human inheritable genetic modification." Using these definitions, the committee finds that MRT involves genetic modification, but that it constitutes heritable genetic modification (germline modification) only if used to produce female offspring because mtDNA is solely maternally inherited, and therefore any changes to mtDNA in male offspring would not be inherited by their descendants.

A clear line has been drawn in U.S. policy on genetic modification in humans between somatic cell genetic modification, which is not heritable, and germline modification.[11] Recent advances in MRT research have reignited ethical debates over long-standing prohibitions on heritable genetic modification, and require clarification of the meaning and use of these terms.

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[8] While there is not direct gene editing of the nucleotide sequence of mtDNA through MRT, the overall frequencies of mtDNA alleles within the population are altered.

[9] The committee has adopted the shorter synonym "heritable" (instead of "inheritable") in this report.

[10] See https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/332881/Consultation_response.pdf (accessed January 15, 2016).

[11] See overview of the germline modification policy context in Chapter 2.

Finding: MRT results in genetic modification of germ cells. Because mtDNA is solely maternally inherited, MRT producing *female* offspring would constitute heritable genetic modification (germline modification). Although MRT results in genetic modification of germ cells, those modifications are not heritable in males. Thus MRT producing *male* offspring would not constitute heritable genetic modification (germline modification).

The following sections review issues associated with genetic modification of human germ cells and heritable changes to future generations ("crossing the germline").

Genetic Modification of Human Germ Cells

Discussions about human genetic modification often distinguish between somatic and germ cell modification. The ethical, social, and policy issues involved differ, largely because modifications to germ cells may be heritable if and when individuals whose oocytes or sperm have been modified choose to reproduce, and whatever modifications have been introduced into the germline have effects potentially in perpetuity. By contrast, genetic modifications to somatic cells do not survive beyond the life of the affected individual. Some people oppose human genetic modification in general, whether at the germ or somatic cell level, and indeed some of the arguments presented here may be relevant to both. Taking this into account, this section provides a broad overview of issues raised by the fact that MRT results in human genetic modification at the level of both germ and somatic cells, including safety concerns, concerns surrounding interference with nature and "playing God," and concerns surrounding eugenics and attitudes toward disability.

Safety Concerns

A primary concern in considering the ethics of genetic modification is the safety of any proposed techniques. This committee's task was not to review the preclinical evidence for MRT to determine the safety of the techniques, but to address the foundational ethical question of whether it is ethically permissible for clinical investigations of MRT to proceed. Because safety considerations are central to the ethics of MRT, a major premise of the committee's deliberations was the understanding that FDA would perform a stringent analysis of the preclinical evidence for MRT to determine whether the safety of the techniques is adequate to support clinical investigations (see also the section in Chapter 4 on assessing benefits and risks).

Concerns Surrounding Interference with Nature and "Playing God"

One objection to human genetic modification is that it constitutes an inappropriate interference with nature. For some people, this objection relates to a call for limitations on the degree of control humans exercise over their biological makeup. For others, this concern focuses on risk and is based on a belief that the natural, unaltered human genetic state is to be protected for fear of poorly understood consequences of changes in the fundamental nature of humans. Similar concerns are echoed in debates over genetically engineered foods and vaccines, with "natural" forms being preferred in part because they are perceived as safer. While respect for the "natural" genetic blueprint of humans is understandable, it is unclear how to characterize such a state of nature as safer or superior given that it is the source of a large burden of human genetic disease (Cotton, 2007; McKusick, 2007; Stenson et al., 2009); thus, "unaltered" nature can be far from an ideal default. Humans have long strived to improve on their natural state for themselves as well as for their children through a variety of activities (pursuing treatments for illness and disease; seeking advantages in education; and even enhancing desirable traits, such as boosting immunity through vaccination).

In the committee's view, the need to understand the consequences of a new genetic technology is crucial, and argues for careful and incremental advances—much as has been the case in other instances, most notably gene transfer research in humans. The desire to protect what is "natural" about human genetic composition solely because it is perceived to be better is not, in this committee's judgment, a basis for maintaining a "natural" state in which individuals suffer severe, debilitating diseases.

Concerns about interventions in nature are often expressed in public debates in the language of "playing God." For instance, the committee received public comments suggesting that the use of MRT would equate to "playing God."[12] In general, this metaphor is frequently invoked, with little or no connection to specific religious traditions and beliefs, in order to dismiss some reproductive and genetic technologies—or some uses of those technologies—as illegitimate. Warnings against "playing God" decry human pride, arrogance, and the like. Those who "play God" are accused of hubris, overreaching, and defiance of limits, while those who refrain from "playing God" are deemed to have proper humility in recognition of human finitude and fallibility. Whether these characterizations of the vices and virtues of different interventions in nature are defensible depends

[12] One public comment submitted to the committee states, "This 'creation' attempt is nothing other than playing God, arrogantly assuming that we flawed humans can improve ourselves. That perfection could ever be created by the imperfect. This will never work, will assuredly backfire and many will suffer."

on whether the "natural" state should be maintained or may be modified. However, the phrase "playing God" is not always negative; it may be more neutral or even positive. Humans can even be "collaborators," "partners," or "created co-creators" with God or "agents" of God. Overall, the metaphor "playing God" itself is too vague and indeterminate to guide such judgments without additional premises and arguments.

If one turns from free-standing uses of the metaphor "playing God" to the views of particular religious traditions—for instance, the Abrahamic traditions (Judaism, Christianity, and Islam), Hinduism, and Buddhism—the literature indicates widespread concern about making heritable genetic modifications, along with widely divergent views within and across these traditions on the acceptability of exercising specific reproductive and genetic choices (see, e.g., Chapman and Frankel, 2003; Dorff and Zoloth, 2015; Evans, 2010; Lustig et al., 2008; Pfleiderer et al., 2010). It is beyond the scope of FDA's charge to the committee to delve deeply into these religious views, although it is important to recognize the depth and diversity of views among many in American society and the role of those views in their understanding of the acceptability and appropriate uses of technologies such as MRT. Because religious traditions are diverse and sometimes lead to diverging perceptions of genetic modification, selectively applying a particular religious tradition's framework to the ethical, social, and policy analysis of MRT was not an appropriate or useful grounding for the committee's analysis.[13]

Concerns Surrounding Eugenics and Attitudes Toward Disability

Some people are concerned that genetic modification of germ cells via MRT could represent a form of eugenics. The ambitions for eugenics, as well as means and policies for achieving these ambitions, have varied widely in different eras and settings. In one form, *negative eugenics*, governments have used hereditary knowledge and coercive policies such as sterilization in an effort to prevent transmission and propagation of traits believed to be hereditary. In another form, *positive eugenics*, governments and other groups have used hereditary knowledge to promote "better babies," "fitter families," and "race betterment."

Both negative and positive eugenics were practiced across the globe, including in the United States, in the early 20th century as a means of im-

[13] The committee also notes a useful initiative of the American Association for the Advancement of Science (AAAS) to convene a working group of scientists, ethicists, theologians, and policy analysts to develop a report considering the ethical, religious, and social implications of human inheritable genetic modifications (AAAS, 2000). The AAAS project also involved the development of a book of essays describing the pros and cons, and what is at stake, when society considers human heritable genetic modifications (Chapman and Frankel, 2003).

proving the gene pool of the human population (Adams, 1990; Allen, 2002; Kevles, 1985; Larson, 1995; Lombardo, 2003; Paul, 1995; Reilly, 1991; Schoen, 2005). Many commentators today, however, while recognizing that current genetic interventions aimed at preventing or treating serious disease or promoting offspring free of serious disease (sometimes referred to as the *new eugenics*) are not comparable to the negative eugenics of earlier eras, emphasize that the distinction between eugenics and recent genetic interventions depends on whether the latter are carried out in contexts where proper safeguards are in place, such as respect for and legal protection of patient and reproductive autonomy. For many observers, both social vigilance and better science are needed to ensure that old eugenic ideals promoting so-called biological fitness and devaluing the "unfit" do not reappear (Kevles, 1992; Lindee, 2005; Rapp, 2000).

Another closely related concern about genetic modification of germ cells via MRT is the potential impact on persons with disabilities. If MRT were approved in the United States, women at risk of transmitting mtDNA disease might feel pressure to use the techniques. The very existence of the techniques, coupled with any resulting pressure on families to use them, might also reinforce or result in expression of the already strong social and cultural norms that marginalize persons with disabilities.

Individuals identified with the disability rights movement have criticized prenatal genetic testing for disabilities for a number of reasons. They have suggested that this form of genetic testing (1) reinforces social discrimination against people with disabilities, (2) leads to rejection of an otherwise wanted child because parents believe the child's disability will diminish their parental experience, and (3) reflects decisions made by parents based on the misconception that a child with disabilities would not fulfill what most people seek in childrearing (Asch, 1989). On the other hand, some have suggested that individual women and families seek such testing or medical interventions not to cause negative perceptions of those with disabilities but to meet their own familial goals and to avoid imposing potential, avoidable suffering on a future child. It is also noted that many people with disabilities may still be harmed by the apparent perceptions created by prenatal genetic testing, despite the intentions of individual women and families (Parens and Asch, 1999).

In Sweden, the National Council of Medical Ethics has cited similar concerns about the effect of MRT on discrimination against people with disabilities and on society at large. In weighing the ethical and social implications of MRT, a minority of the council's members suggested that MRT is not ethically permissible, even if proven safe, in part because in the long run, it "could be a threat to the humanistic view of the individual and human dignity. . . . If this technique is permitted, we would thus risk a development towards a society that discriminates, a society that places demands

on citizens to reject and make the right choices, a society that becomes more technified and where what we consider makes us human is lost" (Swedish National Council on Medical Ethics, 2013).

Similarly, the United Nations Educational, Scientific and Cultural Organization (UNESCO) stated that "the human genome underlies the fundamental unity of all members of the human family, as well as the recognition of their inherent dignity and diversity. In a symbolic sense, it is the heritage of humanity" (UNESCO, 2005). One might thus contend that genetic modifications of future individuals are ethically unacceptable because they alter a fundamental aspect of human existence in ways that are passed on to successive generations. Along these lines, some have proposed a Genetic Bill of Rights, indicating that "all people have the right to have been conceived, gestated, and born without genetic manipulation" (Board of the Council for Responsible Genetics, 2000). Of course, reproductive technologies have an impact on genetic makeup in various ways, as do epigenetic effects of the in utero environment and experiences and exposures after a child is born. Together, these observations suggest calling the human genome "the heritage of humanity" is a vague and aspirational basis for crafting policy related to the use of MRT. Therefore, the committee is not persuaded that MRT should be prohibited based on arguments that the genome represents an inviolable "heritage of humanity" or that there is an inviolable right to be born without the aid of MRT.

The process of MRT would lead to the creation of an individual, the primary intervention occurring at the stage of an oocyte for maternal spindle transfer (MST) or a fertilized oocyte (zygote) for pronuclear transfer (PNT). Some commentators have expressed concern that because a future child cannot make an informed decision about being born as a result of MRT, nor can any descendants of that future child, the techniques are unethical (Darnovsky, 2015). Every other ART shares the similar feature that future children who are the product of such a technology cannot consent to its use in their conception, nor does any child conceived through sexual reproduction have the ability to consent to its own "natural" conception. For these reasons, the committee does not find the lack of child consent to be an insuperable ethical objection to using MRT. However, MRT represents an important new development in two respects. First, the issues around developing a potential research protocol for MRT are novel with respect to applying federal regulations (including informed consent); consent is discussed in detail in Chapter 4. Second, MRT involves the introduction of genetic modifications that could be passed on to future generations, as discussed in the next section.

Heritable Changes to Future Generations: "Crossing the Germline"

Ethical, social, and policy issues raised by MRT may vary depending on whether it works as intended. If MRT were proven to be successful, children born as a result of the techniques, and in the case of females their future offspring, would be spared from serious, life-threatening mtDNA diseases that they would otherwise have been at high risk of both inheriting and transmitting. If MRT were proven to be unsuccessful or caused significant adverse consequences, any missteps in the genetic modification and the associated health outcomes would be transmitted irreversibly to future generations via any female offspring. For some people, however, even successful interventions that result in a heritable genetic modification are unacceptable because it is impossible to say with certainty that the interventions would be safe and have the intended results into the infinitely foreseeable future (Bonnicksen, 1998). Some people might be comfortable with genetically modifying a single embryo in the interest of avoiding a life-threatening disease but would deem such modifications unacceptable if the effects became heritable and thereby unbounded in duration in terms of the number of future individuals affected (Bacchetta and Richter, 1996). Concerns about the risk of heritable change and the effects on future generations are valid and important, and both restrictions on the application of MRT and the collection of information about its effects would be crucial aspects of acceptable policies that would have to be in place for MRT investigations to proceed (see the discussion in Chapter 2 of the policy context surrounding heritable genetic modification).

Creating a heritable genetic change in humans also could, over a long time frame, skew evolutionary processes by introducing deleterious, irreversible genetic effects that would be detrimental to the human species. Some people argue that the human gene pool is a resource shared among the world's people—similar to air or water—and should not be purposefully changed without the consent of all humans (Suzuki and Knudtson, 1990). However, population genetics amply demonstrates that the scale of use of MRT required to have such an evolutionary effect would be enormous—and unlikely to occur.

As discussed in Chapter 2, while the exact prevalence of mtDNA diseases is unknown, estimated ranges indicate that these diseases are collectively rare. The limitation of MRT to women at risk of transmitting severe mtDNA disease, in combination with the likelihood that not all women potentially eligible for MRT would be interested in the techniques, indicate that the number who would potentially pursue MRT is likely quite small. One study estimates that the average number of children born each year to

women at risk of transmitting mtDNA disease in the United States could be 778 (Gorman et al., 2015).[14] Given the small number of individuals at risk for severe mtDNA disease who might qualify for and also decide to use MRT should it become available, MRT would be unlikely to have significant effects on evolutionary processes.

Conclusion: *Although a variety of ethical, social, and policy concerns have been raised about human genetic modification, whether heritable or not, through the use of MRT, these concerns warrant significant caution and the imposition of restrictions rather than a blanket prohibition on the use of MRT to prevent transmission of serious mtDNA disease.*

UNINTENDED DOWNSTREAM SOCIAL IMPLICATIONS OF MRT

Most of the issues discussed below are premised on speculation about a broad application of MRT that goes beyond pathogenic mtDNA diseases and the circumscribed conditions and applications detailed in this report. However, some of these issues and their implications apply to both circumscribed and broad applications of MRT.

Equitable Access

If MRT were approved, regulation and uptake of and access to the technologies could interact with important social values concerning equity in access to medical treatments. The ability to diagnose mtDNA diseases has improved in recent years, but recognition of potential symptoms and the knowledge and ability to seek appropriate care from a team of specialists are still most likely among individuals with higher levels of health literacy, access to health insurance, and the financial means to pay for services not covered by insurance. Indeed, it has been documented that women of low socioeconomic status are marginalized in reproductive policies and view themselves largely as outsiders with respect to the array of reproductive technologies available at IVF clinics in the United States (Bell, 2009). Given the likelihood that MRT would be available only in one or two U.S. centers, access could be further limited to women who could afford the cost of the procedure and an extended stay away from their job and home life. And if mtDNA haplogroup matching were implemented in the context of human investigations or clinical use, the scarcity of oocyte providers with particular haplogroups also could result in inequitable access to MRT.

[14] This text has been updated since this report's initial release.

This reality is a microcosm of the overall U.S. health care system, in which many cutting-edge technologies are more readily available to individuals of high socioeconomic status. Yet the likelihood that MRT for the prevention of maternal transmission of mtDNA disease would first be available to individuals of high socioeconomic status is not a reason to abandon the development of these techniques. Because women of low socioeconomic status have traditionally been excluded from reproductive technologies, it would be important for the multidisciplinary teams that would conduct potential human clinical investigations on MRT and eventually apply it in patient populations to pay particular attention to the challenge of reaching individuals in their community who might benefit from these techniques. Such efforts could entail working to identify family members of current mtDNA disease patients who might also be at risk of or suffering from these diseases, and could be carried out in conjunction with partnerships with mitochondrial disease advocacy groups, MRT researchers, and clinicians.

Expanded Applications of MRT and Enhancement

Once MRT had been approved, FDA could find it challenging to control applications of the techniques because the agency's authority is greater during the research stage than during the postapproval marketing stage, when off-label uses are permitted (see the discussion of the policy context in Chapter 2). Because the U.S. system regulates the products used in medicine, not medical practice, the greater regulatory oversight and control of use that exist in some other countries, such as the United Kingdom, are not exercised here. In theory, and based on observations of past practice, MRT has the potential to be applied beyond any approved use.

Indeed, a chief concern surrounding MRT is the potential for its application for purposes beyond preventing the transmission of serious mtDNA diseases. One area of expanded application that raises particular concern for the public is "enhancement." For instance, several genetic studies have identified statistical associations between mtDNA haplogroups (fixed sets of variants that make up population-defining haplotypes; see Chapter 2) and such traits as exercise performance and aerobic capacity. Most of these studies to date remain controversial because of the small sample sizes, issues of population stratification, and the lack of robust experimental systems in which to demonstrate causality. Traits such as athleticism and aerobic capacity are classically highly polygenic (influenced by more than one gene), so contributions to these traits from mtDNA are expected to be small. Yet while at present it is very challenging to identify mtDNA variants that would confer on offspring a marked improvement in physical performance or aerobic capacity, the expanded use of MRT for such "energetic" enhancement purposes is a theoretical possibility.

A long and significant debate in the ethics literature is focused on the distinctions among prevention, treatment, and enhancement. Efforts to establish a clear definitional boundary between treatment and enhancement confront examples that defy simple classification, such as vaccination to enhance an individual's immunity against infectious disease. For MRT, similar gray areas might include its use to avoid a common mtDNA variant that confers a small statistical risk for developing a disease with limited morbidity, or a rare mtDNA mutation whose pathogenicity for a severe disease remains controversial. Another might be the possible use of MRT for some forms of female age-related or idiopathic infertility; the experiments with cytoplasmic injection of the 1990s suggest there might be some interest in this application. At the far and hypothetical end of this spectrum, and at present lacking an evidentiary base, would be enhancement applications such as seeking oocyte providers whose mtDNA might convey some advantage—for example, the capacity for greater aerobic capacity or physical performance. For individuals with serious mtDNA disease who had already decided to use MRT, an attempt to identify a "best" mtDNA provider, regardless of how unrealistic, could occupy a gray area with respect to such enhancement of the future child. This possibility appears remote, however, given the limited expectation of such benefit and the significant additional time, effort, and potential expense that would be entailed beyond those already associated with MRT.

In Chapter 4, where the committee presents its recommendations, Recommendation 1 outlines the conditions under which the committee believes FDA should consider approving clinical investigations of MRT. One of these conditions is that FDA review the scientific evidence on the utility of haplogroup matching and if compelling, consider it as a means of mitigating the risk of mtDNA-nDNA mismatch. The committee believes this would likely be a primary criterion for the selection of oocyte providers for MRT, which could in practice preclude the option of selecting a haplotype for enhancement purposes. At the most basic level, as long as the underlying motive for prospective parents pursuing MRT remained having a child unaffected by mtDNA disease, there is no reason to believe that enhancement would be seriously considered by those parents. Nonetheless, this sort of scenario has led to discussions about treating children as "products" to be designed according to parental desires, similar to the discussions that once took place with sperm donation, oocyte donation, IVF, and PGD. Thus, clinicians, investigators, regulators, and policy makers will need to be cognizant of these hypothetical concerns as the field evolves.

Further complicating these discussions is the distinction between curing a disease and circumventing it through preventive measures. MRT would not reduce the risk of a mother's mtDNA mutation developing into disease, nor would it cure a mother's preexisting mtDNA disease; rather, it would

be used to prevent that disease in her offspring. Circumvention or prevention does not in itself transform a medical intervention into an enhancement, although as noted above, prevention can in some cases occupy a gray area in this regard. But this goal of MRT does speak to the availability of alternatives, such as oocyte donation and adoption, that provide some of the benefits of reproduction via MRT, although not the nDNA connection that comes with use of these techniques. This point is relevant in deciding whether the risks of such an intervention are reasonable in relation to its possible benefits.

In the committee's view, the differences between mtDNA and nDNA, discussed in more detail below, and the fact that, as opposed to gene editing, MRT procedures lack the precision and flexibility to target particular phenotypes helps circumscribe MRT's applications and places some natural limitations on the potential for its misuse. Thus, it may not be necessary or useful to draw strict lines among prevention, treatment, and enhancement for purposes of developing an ethical boundary for MRT. As discussed in the remainder of this report, including Recommendation 6 in Chapter 4, the use of MRT would need to be appropriately controlled in the U.S. market to limit off-label applications beyond its intended use. If postmarket controls were not implemented and enforced, off-label use could allow physicians to perform MRT for a wider range of purposes than those for which it had been tested and approved (see also Chapter 2 for discussion of the policy context for MRT).

Female idiopathic or age-related infertility is a likely candidate for expanded use of MRT, one that would significantly enlarge the pool of possible patients (Connor, 2015). As noted above, experience with IVF in the 1980s and 1990s demonstrated that a technique developed to circumvent a specific problem (in that case, blocked fallopian tubes) can, under some circumstances, be expanded to much broader patient populations than originally intended. While IVF was not itself the subject of FDA regulation at that time, this experience demonstrates the potential expansion of indications for MRT, whether in the form of off-label use or research aimed at obtaining an additional, approved indication.

In addition to concerns that MRT could be used off-label in embryos not at risk for mtDNA disease, the developing science around the role of mitochondria and mtDNA in other chronic conditions may signal additional potential applications for MRT (see Chapter 2). As the science in this area develops, potential applications of MRT could include a wider array of diseases (such as diabetes and cancer) in which it is suspected that mtDNA may play a lesser but still significant role. Any effort to expand MRT to such "suspected or secondary mtDNA diseases" would need to be undertaken only after careful professional consideration and regulatory deliberation.

In sum, special attention needs to be paid to any potential expansion of MRT as a means of treating idiopathic or age-related infertility or preventing transmission of mtDNA that might be linked to diseases or conditions with tangential connections to mtDNA. The committee does not suggest an absolute limit on any eventual applicability of MRT to other conditions or diseases, but rather believes FDA and relevant professional societies need to take a cautious approach, with deliberate attention to ethical, social, and policy issues, in considering any uses of MRT beyond the primary indication of preventing transmission of serious mtDNA disease.

Conclusion: *Federal regulation would be needed and principled professional society guidelines that interpret the regulations would be helpful to limit the use of MRT to the prevention of transmission of serious, life-threatening mtDNA diseases and to prevent slippage into applications that raise other serious and unresolved ethical issues.*

DNA CONTRIBUTION OF TWO WOMEN

This section focuses on how MRT would introduce genetic material from two women of different maternal lineage—the intended mother's nDNA and mtDNA from the woman providing an oocyte or zygote. In so doing, MRT would result in a novel combination of, and interaction between, mtDNA and nDNA different from that which would otherwise be the case, with potential implications for identity, kinship, and ancestry.

Identity

As some other reviews have suggested, introducing the mtDNA of a second woman could cause the child born as a result of MRT to have a confused or conflicted self-perception (see Nuffield Council on Bioethics, 2012, pp. 70-72). Such effects on self-perception could arise as a function of the desire for knowledge about the meaning of the oocyte provider's mtDNA for the child's identity or for information about the identity of the oocyte provider. Some popular media characterizations go so far as to suggest that children born as a result of MRT would have two mothers (Tingley, 2014), capturing the concern that the replacement of a population of mtDNA could mean that the child's identity was determined by contributions from two different women, giving the child some shared identity with both. Some scholars also defend the claim that MRT would result in three genetic parents on the basis that the issue of relevance is the "presence or absence of identifiable genetic material from someone other than the two individuals identified as genetic parents" (Baylis, 2013).

A desire for knowledge about the evolutionary origin of the oocyte pro-

vider's mtDNA or to know the identity of the provider, although legitimate and potentially of interest to some children born as a result of MRT, could be mitigated or fully addressed, for example, through systems for documenting, tracking, and possibly facilitating receipt of information from the oocyte provider. With respect to the concern that children born as a result of MRT could experience confusion about whether their identity had been fundamentally altered as compared with what would have been the case without mtDNA from an oocyte provider, this is a metaphysical issue that will not be solved through empirical study. In Chapter 4, the committee discusses its conclusion that MRT would result in a new child who would not have existed but for the conduct of the technique. If children born as a result of MRT accepted this formulation, their understanding of their existence should be no different from that of children born as a result of other ART procedures. Indeed, every child who is the result of unassisted sexual reproduction after a period of contraception is a different child from the one who would have been born had the intended parents not sought to prevent earlier pregnancies. Offspring who did not accept this formulation would likely perceive that MRT had prevented a likely condition of having mtDNA disease, and thus that they had personally benefited medically from the procedure, not that their identity had been altered in any confusing manner.

In the committee's view, experience with MRT births and the collection of information about MRT offspring would be necessary before factors relevant to conceptions of identity could be applied to assessments of the benefits and risks of MRT over time. There is no direct precedent on which to base conclusions about whether the unusual configuration of genomes of a child born as a result of MRT would yield a confused or conflicted self-perception of sufficient concern to render proceeding with MRT investigations unacceptable. Systematic studies in children born after cytoplasm transfer in the late 1990s have not yet been reported. However, there are some analogies that could be informative with regard to the influence of donated genetic material on identity formation in recipient individuals. Studies of adolescents and adults born as a result of oocyte or sperm provision—in which half of the individual's genetic information is derived from a gamete provider—have suggested that some individuals experience confusion surrounding their identity upon disclosure of the nature of their conception due to the genetic contribution of someone not acting as their parent (Hewitt, 2002; Mahlstedt et al., 2010; Turner and Coyle, 2000). However, it has been found that timely and appropriate disclosure of the conditions of the child's conception, as well as access to identifying information about the gamete provider, is critical to healthy identity formation, the development of positive self-conception, and psy-

chological development (Hewitt, 2002; Kirkman, 2003; Mahlstedt et al., 2010; Turner and Coyle, 2000).

Organ or tissue donation shares with MRT the transfer of biologic (and hence genetic) material from a provider to alleviate disease manifestation in the recipient. In this area, retrospective studies have found conflicting results with regard to the influence of the provided material on the recipient's perceived self-identity: while some recipients view provided tissue merely as part of a "machine" and thus do not perceive a significant impact on their identity (Sanner, 2001, 2003; Sharp, 1995), others believe their identity was altered or at least affected by the receipt of someone else's biologic/genetic material, in some cases even perceiving that they have taken on the mental, physical, or social traits of the provider (Sanner, 2001, 2003; Sharp, 1995).

The experiences of individuals born from oocyte or sperm providers, as well as recipients of organ or tissue donation, are interesting but provide limited insight into the potential identity-related issues facing any children born as a result of MRT. Children born from third-party nDNA providers (oocytes or sperm) are often deeply curious about whether they share similar characteristics (physical or behavioral) with their genetic mother or father because, from a social perspective, these traits are carried in nDNA. By contrast, mtDNA is not typically associated with the complex behavioral and physical traits attributed to nDNA, and therefore it is less clear how or whether obstacles to healthy identity formation would arise as a result of MRT. In the committee's view, MRT, if safe and effective, could have a significantly positive impact on individuals born as a result of the techniques primarily because of the physical health benefits realized. In addition, family and social support for any child born as a result of MRT would likely play an important role in facilitating healthy, positive self-perception that would acknowledge the novel genetic combination that contributed to the child's existence.

Kinship

Regardless of whether the sense of self and perception of his or her identity of a child born as a result of MRT were affected, it appears likely that the child, and his or her family, could have different perceptions of the relevance of the unusual combination of genetic relatedness resulting from MRT. For example, questions could arise about whether MRT had altered kinship and if so, whether it had done so to an extent that was troubling with respect to its impact on the child. The concept of kinship is fluid, and families in U.S. society have many different combinations of genetic, birth, and social parents. Whether adopted or born as a result of the use of provider gametes or gestational carriers, some children find it important to seek

information about their biological origins, and the same could be the case for children whose mtDNA came from an oocyte provider.

Ancestry

An interesting aspect of MRT is that, although it is valued specifically for its potential ability to preserve a genetic connection between the resulting child and his or her mother, in the process it would alter the child's mtDNA, which is a primary means of ascertaining one's maternal ancestry. Recent decades have seen a growing popular interest in what genetic analysis can reveal about an individual's ancestral origins. Genetic ancestry has become linked to important social and political debates over citizenship, social group boundaries, race, immigration policy, and exclusion. Much of the focus of interest in genetic ancestry revolves around analyzing mtDNA due to its matrilineal inheritance. If women with mtDNA disease used MRT for conception, their sons and daughters (as well as all future offspring with the new maternal mtDNA) would carry the mtDNA of the provider, not of the mother whose nDNA they had inherited. It is not possible to predict how mtDNA ancestry will develop in the future or how genetic ancestry information would be used.

An mtDNA provider's contribution would connect her to the resulting child through the sharing of an aspect of their lineage or ancestry. The novel combination of mtDNA and nDNA that would result from MRT blurs traditional notions of relatedness in ways that may undermine intergenerational connections and lineage as measured by mtDNA.

Conclusion: *An individual born as a result of MRT would have genetic contributions from two women of different maternal lineage, which would introduce complexities that might affect how the individual experiences his or her identity, kinship, and ancestry. These complexities could also affect future descendants of any females born as a result of MRT. These complexities do not form a basis for prohibiting initial investigation of MRT; rather, they are a matter for reflection by families considering undertaking MRT and for societal discussions related to conceptions of identity, kinship, and ancestry.*

MANIPULATION OF EMBRYOS

MRT necessarily would involve the manipulation of human gametes and embryos, a topic of significant ethical, social, and policy debate. These manipulations might include fertilization via ICSI, biopsy of embryonic cells for testing, and removal of genetic material from one oocyte or zygote and its transfer to another oocyte or zygote. In addition to manipulation, MRT

would involve the creation and possible destruction of embryos, both in the research phase and in clinical use. The ethical, social, and policy concerns surrounding the creation and destruction of embryos are long-standing, and not unique to MRT. For example, IVF involves the creation of embryos and usually results in a number of unused embryos that are destroyed, frozen and stored for potential future use, or donated to others for their use in reproduction or for research; embryonic stem cell research requires the destruction of embryos for derivation of the stem cells.

The manipulation, creation, and destruction of embryos are opposed by a range of groups, and federal funding for research involving these processes is severely restricted (the Dickey-Wicker amendment prohibits federal funding from the U.S. Department of Health and Human Services [HHS] for embryo research that destroys, discards, or offers no prospect of medical benefit to the embryo (45 CFR § 46.204(d); see also Chapter 2). Other technologies that involve creation or manipulation of embryos, such as IVF and PGD, were developed outside of the federal regulatory scheme, so the examination and potential regulation of the manipulation, creation, and destruction of gametes and embryos for the purpose of clinical investigation and as part of an IND are novel.

The "moral status" of the embryo is central to the debate over the manipulation, creation, and destruction of embryos. Some scholars argue and many others believe that morally significant life begins at conception, that legally significant personhood should begin at conception, and that human embryos are indeed human beings (Noonan, 1970). A report by the UK Department of Health & Social Security's Committee of Inquiry into Human Fertilisation and Embryology (1984, p. 61) ("The Warnock Report") observes that in this view, "the human embryo is seen as having the same status as a child or an adult, by virtue of its potential for human life." In a more recent publication, George and Lee (2009, p. 301) argue that "the embryo has the same nature—in other words, it is the same kind of entity—from fertilization onward; there is only a difference in degree of maturation, not in kind." This argument generally relies on the notion that an embryo has "all of the internal information needed … and the active disposition to develop itself to the mature stage of a human organism" (George and Lee, 2009, p. 301). Some who argue for the equal moral status of the embryo evoke religious views; for example, the Catholic Church holds that the embryo "must be treated from conception as a person" (Catholic Church, 2003, para. 2274).

Others contend that the moral status of an embryo is not equivalent to that of a person, arguing that this status is conveyed at some later point in development. Sandel (2004, p. 208) states that "the fact that every person began life as an embryo does not prove that embryos are persons," and notes that "although every oak tree was once an acorn, it does not follow

that acorns are oak trees." Among proponents of this view, ideas on the point at which personhood begins vary, ranging from the beginning of sentience, to the onset of brain activity, to the development of cognitive abilities such as reasoning (Department of Health Education and Welfare Ethics Advisory Board, 1979; NIH Human Embryo Research Panel, 1994). Others do not speculate on the point at which an embryo becomes a person, but contend that it is at least sometime after successful implantation. The British Royal College of Obstetricians and Gynaecologists, noting that around 60 percent of embryos are spontaneously aborted within the first days and weeks after fertilization, observes, "It is morally unconvincing to claim absolute inviolability for an organism with which nature itself is so prodigal" (Royal College of Obstetricians and Gynaecologists Ethics Committee, 1983, p. 13).

A third view falls somewhere between the two described above, denying full moral status to the embryo but nonetheless according it a "measure of respect" (Department of Health & Social Security (United Kingdom), 1984, p. 62). According to this view, the embryo is not a "full human being," but neither is it "a mere thing, open to any use we desire" (Sandel, 2004, p. 208). The moral status of an embryo increases as it accrues qualities that make it more similar to a person, such as genetic uniqueness, the potential for full development, sentience, brain activity, a degree of cognitive ability, human form, and the capacity for survival outside the womb (NIH Human Embryo Research Panel, 1994). A preimplantation embryo possesses some of these qualities—genetic uniqueness and potential for full development—so by this view it deserves a measure of respect that is not due to the sperm or the oocyte. This third view holds that the absence of all other qualities, however, "makes it unreasonable to think of personhood as beginning here and places limits on the degree of respect accorded" (NIH Human Embryo Research Panel, 1994, p. 39).

The creation, manipulation, and possible destruction of embryos would occur both in the preclinical research phase of MRT and in clinical investigations or clinical use of MRT. Because MRT is still in development, preclinical research could involve the creation and destruction of many embryos in efforts to improve the techniques to the point at which clinical investigations could safely proceed. Any preclinical data required by regulators for consideration in advance of first-in-human investigations could increase the numbers of embryos created, many of which would likely not be transferred for implantation. The creation of embryos solely for research purposes is controversial. Those opposed argue that fertilization is the first step in bringing a human being into existence, and that creation of embryos for research purposes is "inherently disrespectful of human life" and open to significant abuses (NIH Human Embryo Research Panel, 1994, p. 42). Even those who do not accord full moral status to an embryo might be

wary of creating embryos for research. The National Institutes of Health's (NIH's) Human Embryo Research Panel concluded that the embryo "does not have the same moral status as an infant or child" but recommended minimizing the creation of embryos by allowing such research only when "the research by its very nature cannot otherwise be validly conducted," or when it is necessary for the validity of a study that is "potentially of outstanding scientific and therapeutic value" (NIH Human Embryo Research Panel, 1994, pp. x, 44, and 45).

In addition to the research phase, embryos might be created and destroyed in clinical research on or the regular clinical use of MRT. Even at its most efficient and successful, PNT would require the destruction of one zygote because it would involve the transfer of nDNA from one zygote to another, resulting in the destruction of the first zygote. On the other hand, the efficient and successful performance of MST would in theory involve only the destruction of one unfertilized oocyte in the usual course of the procedure. In recent preclinical research on MST, however, an unexpected number of MST embryos developed abnormally (Tachibana et al., 2013); therefore, the procedure could require the creation of many extra embryos to produce a sufficient number viable for intrauterine transfer. For those who consider embryos to have moral status, destruction of a potentially viable embryo in the regular practice of MRT—not just in the research phase—might be unacceptable. It is possible that some research could be conducted on poor-quality embryos that were nonviable, although this possibility could depend on the specific preclinical research conducted or on clinical diagnostic needs (Baylis, 1990; Gavrilov et al., 2009).

Finally, clinical use of MRT would likely produce unused embryos, much as has been the case with IVF. Although there are no official numbers, a conservative estimate indicates that more than a million embryos, most of them excess from IVF, remain in storage across the United States (Lomax and Trounson, 2013),[15] with many more being stored around the world. Families that created the embryos face the choice of what to do with them; options include freezing and storage for potential future transfer, destruction, donation to research, or donation to another individual or couple for their reproductive purposes. The surplus of embryos created by MRT, although on a much smaller scale relative to IVF, could result in effects similar to those seen in IVF: emotional reactions and financial concerns due to fees required for storing embryos. Clinical phases of MRT also could introduce emotional hardship in that very few MRT embryos might be produced for each woman. A male-only restriction on clinical investigation, as proposed

[15] In response, Snow et al. (2015) argue that there were significant methodological inaccuracies in the calculation of this estimate and suggest that the number of stored embryos is actually significantly higher.

by the committee in Chapter 4, could impose an additional emotional burden by further limiting the number of usable embryos.

Conclusion: *Religious, ethical, social, and policy issues are associated with the creation, manipulation, and destruction of human embryos. However, the responsible use of human embryos in research on and clinical use of MRT would give women at risk of transmitting mtDNA diseases the opportunity to have genetically related children who would be at significantly reduced risk of having these diseases. Useful ethical frameworks have already been developed that could inform appropriate bounding of embryo manipulation in the conduct of preclinical and clinical investigations of MRT.*

CONCLUDING DISCUSSION

There is no question about the importance of mtDNA to the health and development of humans. Any focus on the difference in size of mtDNA and nDNA, as well as the substantially larger number of genes encoded by nDNA, masks the critical contributions of mtDNA to health and normal function. Quantification of the relative amount of DNA or number of genes in the two genomes is likely to distract from the fact that relatively small changes in mtDNA lead to devastating health effects for affected individuals, and it is this fact that motivates the development and proposed use of MRT. It also is clear that genetic ancestry is closely linked to mtDNA. In fact, mtDNA is crucial for tracking and charting notions of ancestry.

The potential use of MRT entails a unique combination of characteristics not seen in other proposed techniques for preventing inherited disease. In contrast with inherited nDNA diseases, there currently are no adequate alternatives for achieving the goals of prospective parents who face a high risk of transmitting mtDNA disease, which are to have a child who shares an nDNA connection with them and who is at significantly reduced risk of developing mtDNA disease. For most nDNA disorders with Mendelian inheritance, PGD offers an effective means of embryo selection to avoid transfer of an affected embryo. This is not a highly reliable option for mtDNA disease for reasons articulated in Chapter 2. In addition, MRT would offer a heretofore unavailable approach for replacing pathogenic mtDNA prior to transfer.

In light of the relative and important, albeit different, scientific, medical, and social contributions of mtDNA and nDNA to health, well-being, and conceptions of identity, as well as the unique combination of characteristics of MRT as an approach, a central question for the committee was whether the sort of heritable genetic change resulting from MRT raises

ethical, social, and policy issues comparable to those raised by heritable modification of the nuclear genome.

Finding: There are significant and important distinctions between modification of mtDNA and nDNA that matter for an analysis of the ethical, social, and policy issues of genetic modification of germ cells and the germline:

- MRT is different from any technology that could be applied to the nuclear genome in that it would entail replacement of pathogenic mtDNA with unaffected mtDNA, as opposed to targeted genomic editing of either mtDNA or nDNA. The replacement of whole, intact, and naturally occurring mitochondrial genomes represents a qualitatively different form of heritable genetic change from that resulting from any approach for modifying nDNA, which would likely involve editing rather than en bloc replacement of chromosomes—the closest parallel to MRT.
- While mtDNA plays a central role in genetic ancestry, traits that are carried in nDNA are those that in the public understanding constitute the core of genetic relatedness in terms of physical and behavioral characteristics as well as most forms of disease.
- While some forms of energetic "enhancement" (such as selecting for mtDNA to increase aerobic capacity) might hypothetically be possible through MRT, they appear to be far fewer and more speculative relative to what might be possible in modifications of nDNA.

None of these distinctions are meant to imply that mtDNA is unimportant from the perspective of health, genetic relatedness, or potential energetic enhancement, but that its modification is meaningfully different from that of nDNA.

Conclusion: *The significant and important distinctions between modification of mtDNA to prevent transmission of mtDNA disease through MRT and modification of nDNA (1) have implications for the ethical, social, and policy issues associated with MRT, and (2) could allow justification of MRT independent of decisions about heritable genetic modification of nDNA.*

The ethical, social, and policy issues associated with MRT need to be considered in light of the interests of women desiring to prevent transmission of mtDNA disease while preserving an nDNA connection with their future offspring. In the committee's judgment, none of these ethical, social, and policy considerations individually or in combination warrant a prohi-

bition on proceeding with initial investigations of MRT in humans. In the case of each area examined for this consensus study, the ethical, social, and policy considerations fall into one or more of three categories: (1) considerations similar to those experienced and successfully addressed in the use of other forms of assisted reproduction, (2) those that could be addressed in policy or practice, and (3) those that do not rise to the level of a prohibitive concern. Any pursuit of the reproductive interests of individuals can be limited by interests in protecting the health and well-being of children, both those who would be born as a result of MRT and any future generations, and the need for precautions regarding possible deleterious effects of heritable genetic modifications. By limiting initial MRT research to cases in which there could be no intergenerational effects, the first uses of MRT could be assessed for safety in a highly circumscribed context. Only through such a slow, cautious approach can the appropriate balance be struck between women's pursuit of their reproductive interests and the protection of the health and well-being of children.

Conclusion: *While significant ethical, social, and policy considerations are associated with MRT, the most germane of these issues can be avoided through limitations on the use of MRT or are blunted by meaningful differences between the heritable genetic modification introduced by MRT and heritable genetic modification of nDNA. Therefore, the committee concludes that it is ethically permissible to conduct clinical investigations of MRT. To ensure that clinical investigations of MRT were performed ethically, however, certain conditions and principles would need to govern the conduct of clinical investigations and potential future implementation of MRT.*

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4

Regulation and Oversight of MRT in Humans

As discussed in Chapter 3, the committee believes that the constellation of ethical, social, and policy issues surrounding mitochondrial replacement techniques (MRT) does not preclude clinical investigations of these novel techniques, but does warrant proceeding with caution while adhering to a circumscribed set of conditions. Within the realm of the U.S. Food and Drug Administration (FDA) regulatory review and oversight, MRT entails challenges that would demand tailored regulatory review and oversight strategies. These challenges include (1) establishing a sufficient level of preclinical evidence, which would entail creating, manipulating, and possibly destroying human embryos for research purposes; and (2) drawing conclusions from very small sample sizes for first-in-human research that would challenge even well-established rare disease research methodologies, introduce challenges for protecting participant privacy, require long-term follow-up of potential offspring beyond traditional clinical investigation evaluation stages, and challenge decisions about when it would be appropriate to expand MRT.

This chapter reviews the trajectory of any potential preclinical and clinical investigations of MRT and the ethical, social, and policy considerations that would need to guide MRT investigations throughout the phases of research, the regulatory approval process, and postapproval. Addressed in turn are assessment of benefits and risks, submission of preclinical evidence prior to authorization of clinical investigations of MRT in humans, conditions for clinical investigations, principles and practices that should guide clinical investigations, extension of MRT research to female embryos, informed consent, and guiding principles for oversight.

ASSESSMENT OF BENEFITS AND RISKS

In making regulatory determinations, FDA must form a conclusion that a treatment or technology is safe, that it is effective for its intended use, and that its benefits outweigh its risks. In assessing effectiveness, FDA applies standards to ensure the highest possible scientific validity and integrity. FDA's judgment about benefits and risks takes into account (but is not limited to) the subjective perspective and experience of the expected beneficiaries of the treatment or technology.

Those affected by mitochondrial DNA (mtDNA) diseases and at risk of transmitting them to their children are uniquely positioned to inform FDA's understanding of the clinical and personal context of these diseases. Proponents of MRT sometimes describe the use of the techniques as either a preventive measure or a therapy for children with mtDNA disease. Because in vitro fertilization (IVF) techniques are required as part of the MRT process to create an embryo, MRT would not treat a preexisting person or prevent a likely medical condition in an already existing individual. With this in mind, MRT has at least two potential benefits.

The first potential benefit of MRT is subjective, and depends on how important it is to individuals at risk of transmitting mtDNA disease to have children who are genetically related by nuclear DNA (nDNA) (but not mtDNA) and thereby at significantly reduced risk of manifesting mtDNA disease. Another suggested benefit is the reduction in the number of children who would be born with serious mtDNA disease as a result of access to this reproductive technology. At present, women at known risk of passing on serious mtDNA diseases choose childlessness, adoption, or the use of donor oocytes or embryos (preimplantation genetic diagnosis [PGD] is not offered by most U.S. academic medical clinics due in part to the fact that it is not a reliable means of avoiding transmission of mtDNA disease, as discussed earlier). But others choose to have genetically related children through unassisted sexual reproduction, and some of the resulting children are likely to be affected by mtDNA disease of varying severity at some point in their lives. If MRT were available, some women would have a reproductive option that could allow them to reduce the likelihood of passing on the disease mutation, and if MRT were successful for them, the overall number of children born with a risk of serious mtDNA disease would be somewhat reduced.

Both of the above benefits are relevant in determining whether the risks of MRT are reasonable in relation to its potential benefits. However, one of those benefits would accrue primarily to the prospective parents and the other at a population level; neither would accrue to the children who would be born as a result of MRT and thus would not have existed (either with or without mtDNA disease) but for the performance of MRT. This complicates

assessment of the benefits and risks of MRT. Typically, assessment of benefits and risks in clinical medicine is performed in contexts in which the same individual both realizes benefits and bears risks. In biomedical research, by contrast, some individuals are asked to consent to bear risks voluntarily to enable potential benefits that would be enjoyed largely or even exclusively by others. MRT challenges both paradigms in that the child born would not exist but for the MRT procedure, and so could not be asked for or give consent to participating in the research that led to his or her existence. This scenario raises important challenges related to both consent (discussed later in this chapter) and assessment of benefits and risks. These challenges argue for an approach that entails weighing, first and foremost, the probability of significant adverse outcomes borne by the children born as a result of MRT against the benefits accruing to families desiring children who are related to them through their nDNA.

The irreversibility of MRT introduces additional complexity to assessment of the appropriate balance of benefits and risks, and supports the imposition of conditions that could minimize risk until there was a high degree of confidence in the success of the techniques. The irreversibility of the "intervention" also creates challenges relative to respecting the right of research subjects to withdraw from research. Similar issues of irreversible risk and long-term assessment of benefits and risks have been navigated in somatic cell gene transfer, as well as in cell-based interventions and some surgical therapies. Research investigations with these techniques must deal with the tension among subjects' right to withdraw from research, the irreversible nature of the intervention, and the research goal of long-term collection of information (with some investigations following subjects until they die and seeking permission for autopsy as part of research participation). MRT shares many similarities with these other research areas, with the addition that a child—who did not and could not consent to long-term follow-up—would be born if the research were successful. As in other examples, children who become research subjects once they are born would retain the right to withdraw from further participation in the research protocol, and hence discontinue their participation in systematic evidence collection, even if they could not effectively "withdraw" from the intervention.

Broader challenges affecting the assessment and balancing of benefits and risks of MRT stem from the fact that there are five potential parties with interests affected by MRT: (1) individuals who provide gametes (oocytes or sperm) that are used to construct embryos, (2) the intended parents, (3) the gestational carrier (if needed), (4) the child born as a result of MRT, and (5) the potential future offspring of female children born as a result of MRT. Each of those parties could be exposed to risk directly or indirectly at some point in the preparation for and application and outcomes of MRT. Indeed, some of these risks are no different from those encountered in tech-

niques commonly used in clinical applications of assisted reproduction. In the research context, risks to all parties involved in the research process need to be considered, even if they technically do not qualify as "research subjects" under federal regulations. In the case of MRT, attempts to minimize risk and burden for one of the above parties could interact with risk for another. However, the health and well-being, and minimizing risk of harm to any future children born as a result of MRT warrants priority in balancing benefits and risks in the design of clinical investigations.

As a general matter, parents put their children at some risk, even when it entails no direct benefit to the child. For example, parents take children with them in a car when running errands, which increases the risk of injury with no benefit to the child. In the context of human reproduction, this issue has arisen repeatedly with the introduction of new technological advances. Notably, it was the subject of discussion when infertile couples sought to use IVF and its variants (e.g., intracytoplasmic sperm injection [ICSI]). Protecting the health and well-being of future children demands that their safety be the primary value in any assessments of the benefits and risks of MRT.

Conclusion: *In assessing the ethics of the balance of benefits and risks in MRT clinical investigations, minimizing the risk of harm to the child born as a result of MRT is the primary value to be considered.*

PRECLINICAL EVIDENCE TO SUPPORT MRT

FDA requires that sponsors submit preclinical evidence before clinical investigations in humans are authorized. Preclinical studies serve several purposes, including enabling researchers to characterize risk, optimize techniques, and establish that the prospect of clinical application warrants investigation in human subjects. The goals of preclinical research on MRT would be to determine whether the techniques were reasonably safe for initial use in humans and whether they exhibited effectiveness that would justify clinical applications and commercial development.

FDA has traditionally demanded some evidence of "feasibility and rationale for clinical use" (FDA, 2015) for gene and cell therapies, in addition to evidence of safety, before approving the move from preclinical research to clinical research in humans. In the case of MRT, the effect of the exposure would go beyond that of traditional gene and cell therapies in that a child would be born as a result of the procedure. As discussed in Chapter 2, proof-of-concept studies for MRT have been conducted to varying degrees in animals (see also Appendix B for more detailed information on MRT research conducted to date). The main outcome measures in animal studies of maternal spindle transfer (MST) and pronuclear transfer

(PNT), which include mtDNA carryover and resultant heteroplasmy levels, have been studied in mice, "mito mice" (which carry a large-scale mtDNA deletion), and rhesus macaques. Animal studies to date have found varying levels of mtDNA carryover, ranging from undetectable (Tachibana et al., 2009) to 39.8 percent in first-generation mice and 22.1 percent in second-generation mice in the only study that has evaluated the effects of MRT in second-generation animals (Wang et al., 2014).

FDA guidance indicates that the kind, duration, and scope of required preclinical evidence will vary with the duration and nature of the proposed clinical investigations (FDA Center for Biologics Evaluation and Research, 2013). As suggested by FDA's Cellular, Tissue and Gene Therapies (CTGT) Advisory Committee, additional preclinical evidence for MRT would likely need to include studies of a sufficient number of animals (mothers and offspring) from various species, with evaluation of safety over the long term—through all developmental stages and possibly extending to multigenerational follow-up (Liang, 2015). Multigenerational follow-up might not be necessary for animal research to support first-in-human clinical investigations in males only given that the limitation to males would avoid transmission to future generations. Before MRT was extended to female embryos, however, animal studies of second, and perhaps third, generations would need to be performed to collect data on the techniques' intergenerational safety and efficacy.

CONDITIONS FOR CLINICAL INVESTIGATIONS

Once safety had been established through preclinical research, MRT could be tested further through the transfer of embryos to intended mothers (or gestational carriers), with the intended result of the birth of children at greatly reduced risk of inheriting pathogenic mtDNA mutations known to cause severe disease. Ethically acceptable clinical investigations of MRT would depend on certain restrictions and conditions being in place for initial clinical investigations.

Restriction to Women at Risk of Transmitting Serious mtDNA Disease

A traditional factor in decisions regarding the initiation and conduct of clinical investigations is optimizing the balance of benefits and risks of the intervention to be investigated. Given the novelty, complexities, and uncertainties inherent in MRT, minimizing risk in the conduct of first-in-human clinical investigations of these techniques would need to be weighted heavily in favor of the health and well-being of the child born as a result of MRT. Simultaneously, maximizing the benefit gained from undertaking MRT would be an important consideration.

As discussed in Chapter 2, mtDNA diseases are clinically heterogeneous; can have early or late onset; and can result in negative health outcomes across a wide range of severity, including early death. In the case of MRT, maximizing benefit would be inextricably linked to the expected natural history and severity of the mtDNA disease at risk of being transmitted. In this sense, benefit would be maximized in initial clinical investigations by preventing the transmission of those mtDNA diseases known to be the most severe. In addition, to appropriately manage the balance of benefits and risks in MRT clinical investigations, individuals who provided oocytes would need to be pretested to ensure that they were not carriers of known pathogenic mtDNA mutations. It remains possible that an oocyte provider's gamete could harbor a pathogenic mutation not present or detectable in easily tested tissues, such as the provider's blood, cheek swab, or urine sample. Still, such testing would minimize the likelihood that the mtDNA in the provided oocyte would introduce pathogenic mutations whose transmission to the child MRT would be designed to avoid. Once the embryo had been created via MRT, performing PGD to confirm the absence of mtDNA mutations would also help ensure that this scenario did not occur.[1]

The desire to avoid life-threatening illnesses generally comports with societal values surrounding the use of reproductive technologies for preventing the transmission of inherited genetic disease. A 2005 survey of public opinion on new reproductive technologies indicated that among this group of U.S. survey participants, there was general agreement that it is appropriate to use reproductive technologies to avoid life-threatening illnesses with an early onset (Kalfoglou et al., 2005a,b). There was less support for the use of reproductive technologies when the disease to be avoided was less severe, non-life-threatening, or characterized by adult onset of symptoms. However, survey participants were largely sensitive to individual perceptions of disease severity, quality of life, and suffering caused by a particular disease, noting that these are extremely personal concepts.

Inclusion criteria for women carrying mtDNA mutations who wanted to participate in MRT research would need to reflect the societal value of avoiding life-threatening illnesses. The research would need to be limited to women who otherwise were at risk of transmitting a serious mtDNA disease, and to cases in which the mutation's pathogenicity was not disputed, and the clinical presentation of the disease caused by the mutation was predicted to be severe and characterized by early mortality or substantial

[1] As previously described, PGD may not be a reliable method for preventing mtDNA disease transmission in women at known risk of transmitting mtDNA disease because of limitations related to the complexities of mitochondrial genetics. With the advent of increasingly sensitive and accurate sequencing technologies, however, PGD is expected to be a reliable technique for determining the efficacy of MRT prior to embryo transfer.

impairment of basic function. In addition, the primacy of the interests of the child dictates that selection of oocyte providers would need to include genetic testing to confirm that the provider's oocytes harbored no known pathogenic mtDNA mutations.

Health of the Gestational Carrier

The overall health status of the woman who would carry the pregnancy of the child born as a result of MRT (i.e., the intended mother or a gestational carrier, if needed) would need to be a key consideration in the design of inclusion criteria for potential clinical investigations of MRT. In keeping with the principle of minimizing risk to favor the health and well-being of the future child, inclusion criteria for a gestational carrier would need to be based on minimizing the risk of adverse health effects to the future child while also taking into consideration the impact of carrying the pregnancy on the health of the gestational carrier. If the intended mother planned to carry the pregnancy, her medical history and available evidence on pregnancy and mtDNA disease would make it possible to determine whether she would be able to complete the pregnancy without significant risk of adverse consequences to her health or that of the future child. If a gestational carrier were being used, she, too, would need to be healthy enough to carry the pregnancy to term and not present with any known risk factors for serious adverse conditions in the future child. The committee notes that a 2014 meeting of the FDA's CTGT Advisory Committee included significant discussion on the topic of gestational carriers in MRT clinical investigations. Any future decision about the suitability of including gestational carriers in the research phase of MRT would be informed by the agency's internal and external experts.

Initial Restriction to Males

As explained in Chapter 3, MRT in male embryos would not constitute heritable genetic modification. Because of the scientific uncertainties associated with these novel techniques and because MRT in female embryos would have the effect of creating heritable genetic modification, an appropriately cautious approach to MRT research in the United States would need to include restricting initial first-in-human clinical investigations to male embryos. This restriction would be justifiable in two regards.

First, unforeseen consequences of MRT—for example, health issues due to cellular manipulation, mtDNA-nDNA incompatibility, or failure to eliminate mtDNA disease—could become apparent in the first generation. By restricting initial investigations to males, these issues could be addressed in the first generation, without the risk of their affecting future generations.

Second, certain issues would arise only in female offspring of MRT—for example, the potential that future children could inherit a higher level of pathogenic mtDNA molecules relative to the first generation (Bredenoord et al., 2015). Although issues arising only in female offspring could not be resolved as long as MRT produced only male offspring, performing MRT initially only in males would allow preclinical research on intergenerational effects to continue while at the same time allowing families to use MRT to have male children with a significantly reduced risk of mtDNA disease.

While there is ethical debate about the acceptability of sex selection, the restriction recommended by the committee is predicated not on selection of one sex over another, but on the need to proceed slowly and to prevent potential adverse and uncertain consequences of MRT from being passed on to future generations. Bredenoord et al. (2015) observe that sex selection for medical reasons is generally accepted and relevant to the case of MRT, noting that PGD was initially introduced to select female embryos so as to avoid X-linked disorders (Handyside et al., 1990). The authors further note that sex selection for nonmedical reasons is regarded by many people as "morally problematic," but that sex selection in the context of MRT would be health-related and represents a use that even those uncomfortable with sex selection would often find compelling. Appleby (2015) also suggests that an initial restriction of the use of MRT to males would be a "worthwhile limitation" because the research could "provide additional confidence" that MRT would be safe for the creation of females and subsequent generations.

In contrast, the report of the Nuffield Council on Bioethics on MRT in the UK context argues that restriction to male embryos would be unacceptable because it would result in an "experimental" group of male children, and "the boys born would need to be monitored throughout their lives and deemed healthy before females could be conceived in this way: they would in effect be experiments" (Nuffield Council on Bioethics, 2012, pp. xvi, 86). However, the context in which the committee undertook this study was focused on the prospect of initial investigation of MRT, and such initial investigation, if successful, would result in the first offspring born through MRT. The committee does not choose to characterize these children as "experimental"; however, any such births would in fact be part of an investigational context in which the first humans were produced following use of a novel technique. This unique combination of characteristics (novel technique; unique ethical, social, and policy concerns; and first-in-human use) argues for careful and responsible initial steps that would avoid risks to the extent possible and minimize them when they could not be avoided. In this framing, the limitation to male embryos would be a matter of responsible clinical investigation focused on reducing a significant risk rather

than a matter of sex preference. Notably, in at least one reported instance, PGD for preventing the transmission of mtDNA disease utilized selection of male embryos for the purpose of "avoid[ing] inheritance of the mutation in the third generation" (Treff et al., 2012, p. 1237).

Restricting initial investigations to male embryos would admittedly limit the potential benefits of the research because the research would yield no information about the effects of heritable transmission of mtDNA. An initial restriction to males also would mean that all embryos created through MRT would need to be tested for sex chromosome determination, with female embryos being frozen, donated for research, or discarded. In hypothetical but foreseeable instances in which the only MRT embryos suitable for transfer were female, some intended mothers would be unable to complete the study protocols. In addition, families who wished to have only female offspring or who were uncomfortable with sex selection would not be eligible for initial investigations.

While there are real issues related to limiting initial investigations of MRT to male embryos, the committee believes the trade-offs involved are necessary and justifiable to effectively eliminate the risk of introducing deleterious heritable genetic modifications, and are consistent with eligibility criteria, design features, and research staging used for clinical investigations in other realms of medical innovation.

Expertise of Investigators and Centers

Most MRT approaches contemplated at present would involve highly intricate micro-manipulations of human gametes and/or embryos. Use of the techniques would therefore require operator skill, which evolves over time, varies from one individual to another, and resists specification in a protocol. The inability to standardize interventions makes it extremely challenging to evaluate them. In this respect, MRT studies would face design issues similar to those encountered in surgery. The difficulty of the MRT process was described at the meeting of FDA's CTGT Advisory Committee in February 2014. Evan Snyder, summarizing responses to a question about how FDA should control the production process for MRT, said the process "requires an enormous amount of skill," and it "should only be done by specialists who have been qualified, and in specialized centers, at least initially." He noted that "every stage in the manufacturing process needs to be monitored—the operators, the equipment, the preparations" and that ongoing quality control would be needed at each one of those stages.

In draft plans for the regulation and licensing of MRT, the United Kingdom's Human Fertility and Embryology Authority (HFEA) (HFEA, 2015) proposed that MRT be restricted to clinics licensed specifically to perform

it. The licensing application would include evidence of the competence of the staff and the appropriateness of the premises for performing MRT. Specifically, all staff would have to be "suitably qualified, trained and assessed as competent for the tasks they perform," as evidenced by information on staff experience in performing micro-manipulation on oocytes or embryos, as well as specific experience carrying out MRT and any other relevant information (HFEA, 2015, p. 4). The application also would require evidence of "suitable validation of equipment and processes" (HFEA, 2015, p. 4).

Conclusion: *Given the complexity of the techniques, the performance of MRT would require specialized technical skills. FDA would need to consider the expertise and skill of investigators before approving clinical investigations.*

mtDNA Haplogroup Matching

As discussed in Chapter 2, MRT could entail some risk of adverse health effects related to nuclear-mitochondrial genome incompatibilities arising from the artificial combination of nDNA and mtDNA from genetically distinct lineages. This risk remains a significant subject of scholarly debate, even among experts in mitochondrial biology (IOM, 2015). With regard to the design of potential MRT clinical investigations and in keeping with the principle of minimizing risk to children born as a result of MRT, should FDA's review of the preclinical data package reveal compelling evidence that mtDNA haplogroup matching between potential oocyte providers and intended mothers might mitigate the risk of mtDNA-nDNA incompatibilities resulting from MRT, such matching would be a reasonable inclusion criterion for initial investigations. Depending on the degree of match required, a decision to require haplogroup matching would most likely decrease the pool of oocytes provided by individuals for each procedure and thus potentially limit the overall probability of its success. Therefore, FDA would need to weigh requiring haplogroup matching as a means of mitigating risk against the potential effect of a decreased pool of available oocytes.

To the extent that mtDNA contributes to one's sense of identity as associated with ancestry, haplogroup matching could have the benefit of retaining these ties. Haplogroup matching could restore the ancestral link to the intended mother's lineage in a child born as a result of MRT. Yet while the notion of retaining ancestral and kinship ties might be a significant value for some people, this consideration is secondary to the principle of minimizing risk to future generations in crafting MRT clinical investigations, in order to maximize the possibility of the safest and most efficacious outcome.

Recommendation 1: Initial clinical investigations of mitochondrial replacement techniques (MRT) should be considered by the U.S. Food and Drug Administration (FDA) only if and when the following conditions can be met:

- **Initial safety is established, and risks to all parties directly involved in the proposed clinical investigations are minimized. Because attempts to minimize risk and burden for one of the parties could interact with risk for another, minimizing risk to future children should be of highest priority.**
- **Likelihood of efficacy is established by preclinical research using in vitro modeling, animal testing, and testing on human embryos as necessary.**
- **Clinical investigations are limited to women who otherwise are at risk of transmitting a serious mitochondrial DNA (mtDNA) disease, where the mutation's pathogenicity is undisputed and the clinical presentation of the disease is predicted to be severe, as characterized by early mortality or substantial impairment of basic function.**
- **If the intended mother at risk of transmitting mtDNA disease is also the woman who will carry the pregnancy, professional opinion informed by the available evidence determines that she would be able to complete a pregnancy without significant risk of serious adverse consequences to her health or the health of the fetus.**
- **Intrauterine transfer for gestation is limited to male embryos.**
- **Clinical investigations are limited to investigators and centers with demonstrated expertise in and skill with relevant techniques.**
- **FDA has reviewed mtDNA haplogroup matching and if compelling, considered it as a means of mitigating the possible risk of mtDNA-nuclear DNA (nDNA) incompatibilities.**

RESEARCH IN HUMAN OOCYTES AND EMBRYOS

In addition to animal investigations, preclinical research on MRT would likely entail extensive experimentation on human gametes and embryos with no intention of performing intrauterine transfer to establish a pregnancy in a woman. Such research might be necessary to learn about and optimize the physical manipulations of oocytes and embryos required for MRT, establish optimal timing for applying the techniques in gamete provider and intended mother gametes, and provide a better understanding of the appropriate application of reagents to achieve desired effects.

Initial published studies of the safety and efficacy of MRT in the human system have tested the techniques in human oocytes provided by healthy volunteers (Tachibana et al., 2013) or parthenogenetically activated oocytes

(Paull et al., 2013) in the case of MST, or in abnormally fertilized zygotes (Craven et al., 2010) in the case of PNT. The United Kingdom's Newcastle Group is currently engaged in research aimed at comparing MST in human oocytes with PNT in normally fertilized human zygotes.

FDA performs in-depth review of in vitro and animal studies (i.e., preclinical studies) before granting permission to commence clinical investigations with human subjects. In the case of MRT, it would be important to accumulate sufficient preclinical data on how the manipulation of gametes or embryos might affect the resulting embryos so as to reduce the risk of harm to children born as a result of MRT during clinical investigations. Preclinical research involving embryos of varying quality that would not be transferred would likely be necessary to produce the data necessary to protect future children.

Conclusion: *To minimize risk to children that would be born as a result of the investigational use of MRT, the creation of human embryos solely for research purposes would likely be a necessary step in the preclinical phase.*

MRT would involve the creation, manipulation, and possible destruction of embryos not only in the preclinical research phase but also during clinical investigations and perhaps in clinical use. As discussed in Chapter 3, clinical use of MRT, even at its most efficient and successful, might require the creation of multiple embryos to produce a viable pregnancy leading to the birth of a child. The creation, manipulation, and destruction of embryos have long been controversial in the United States. Various perspectives exist on the "moral status" of the embryo, with some considering it to be a human being and thus entitled to the same protections. The creation of embryos specifically for research is particularly controversial, as the committee heard from presenters and public commenters during its public sessions (Darnovsky, 2015; Fitzgerald, 2015; Zoloth, 2015). It was the subject of discussion by the 1994 Human Embryo Research Panel and has been the focus of debates about research on nuclear transfer of somatic cells and on embryonic stem cells. In addition to these ethical debates, federal funding for research on embryos is restricted by the Dickey-Wicker amendment (see Chapter 2). While the creation of human embryos solely for research purposes is not prohibited under federal law in the United States (although some states are more restrictive), there are significant restrictions on the federal funding of such research. Even an agency request that data from such research be submitted in support of an Investigational New Drug (IND) application to start first-in-human research may well be controversial.

For both preclinical and clinical investigations of MRT, researchers would need to procure oocytes or embryos. If preclinical research required

the procurement of oocytes containing abnormal mtDNA, women with mtDNA mutations would be exposed to the oocyte stimulation and retrieval process, but without the benefit of potentially creating a child via MRT.

In part as a result of these types of ethical concerns surrounding the procurement of oocytes or embryos and the creation, manipulation, and destruction of embryos, the National Institutes of Health (NIH) developed guidelines in 2009 that articulate detailed standards for "ethically responsible" procurement of embryos for NIH-funded human embryonic stem cell (hESC) research (NIH, 2009).[2] These guidelines require, for example, that hESCs be derived from embryos that were created for reproductive purposes and are no longer needed, that all options for the embryos were explained to the potential donor, and that no payments were offered for the donated embryos. The guidelines also require a clear separation between the decision to create embryos for reproductive purposes and the decision to donate the embryos for research, as well as a detailed informed consent process. These standards, while specific to NIH and to the hESC context, address many of the same ethical, social, and policy issues that could arise in the provision of gametes or embryos for MRT.

Recommendation 2: Ethical standards for the use of human embryos in research have been developed by the U.S. National Academies of Sciences, Engineering, and Medicine (the Academies), the U.S. National Institutes of Health (NIH), and the International Society for Stem Cell Research (ISSCR). These standards include the expectation of prospective independent review of research proposals. In light of concerns about the oocyte procurement and embryo manipulations necessary for mitochondrial replacement techniques (MRT) preclinical and clinical research, regulatory authorities should ensure the ethical provenance of preclinical or clinical data submitted to the U.S. Food and Drug Administration (FDA) in support of an Investigational New Drug (IND) application. To the extent possible, regulatory authorities should ensure that sponsors adhere to ethical standards comparable to those developed by the Academies, NIH, and ISSCR. In preclinical research, nonviable human embryos should be used when possible. When use of nonviable human embryos is not possible, viable human embryos should be used only when required in the interest of developing the science necessary to minimize risks to children born as a result of MRT, and even then only in the smallest numbers and at the earliest stages of development consistent with scientific criteria for validity.

[2] While the hESC research is federally funded, the procurement of embryos and the derivation of the stem cells are not federally funded.

PRINCIPLES AND PRACTICES TO GUIDE CLINICAL INVESTIGATIONS

A range of criteria would need to be satisfied for MRT investigations to be ethically acceptable and scientifically valid. To this end, such investigations would need to be guided by the principles and practices detailed below.

Health and Well-Being of Future Children

Given the novelty and unknown potential risks of MRT, clinical investigations would need to proceed with caution, with the health and well-being of the potential child being considered at every step. The balance of benefits and risks would fluctuate as investigations moved from initial stages into studies involving greater risk (e.g., different techniques) or less benefit (e.g., populations with less severe mtDNA disease). The conditions for initial investigations laid out in Recommendation 1—including restriction to serious mtDNA disease, a healthy gestational mother, initial restriction to male embryos, and expertise of investigators—represent an attempt to prioritize the minimization of risks to future children. As data accrued on the benefits and risks of MRT, these data would need to inform the assessment of benefits and risks for potentially less beneficial or riskier investigations. If initial investigations showed that the risk associated with MRT was low, (e.g., there were no short- or long-term detrimental effects for the resulting child), it might be appropriate to offer MRT to mothers at risk of passing on a less severe mtDNA disease, always prioritizing the health and well-being of future children in the balancing of benefits and risks. This cautious, staged approach would need to be taken in the design of initial and subsequent investigations—for example, in determining the eligibility of intended mothers, numbers of participants, and pacing of investigations.

Standardized Study Designs

Clinical investigations aim to establish three core elements: the optimal conditions for applying an intervention, its safety, and its efficacy. When studies are standardized and outcomes can be compared, establishing these elements is facilitated. However, efforts to standardize MRT studies would face a number of challenges.

Isolating the causal effects of any intervention requires standardizing treatments and populations so that outcomes can be compared. In the case of MRT, the "treatment" would be complex in that it would involve highly intricate manipulations of human gametes and/or embryos. As noted above,

treatment therefore would require operator skill, which evolves over time, varies from one individual to another, and resists specification in a protocol. Standardizing eligible patients to ensure a homogeneous and comparable population of study subjects could be difficult as well, as mtDNA diseases are a heterogeneous mix of genotypes and phenotypes—both of which can be highly unpredictable. Yet investigations might need to specify eligibility criteria based on genotypes to enable researchers to disentangle the effects of MRT from those that might otherwise arise randomly or as a result of variation in disease processes.

Outcomes might also be difficult to specify. Certain outcomes—such as the level of heteroplasmy in a defined set of tissues—might offer reasonably straightforward study endpoints. However, some potential effects of MRT—such as onset or severity of a disease or condition—might be difficult to detect without years of observation among very large populations. Because of the rarity of mtDNA disease, it might be difficult to recruit a sufficiently large sample to detect unintended effects of the intervention.

Finally, it might be challenging to use comparators in studies of MRT. Comparators enable researchers to isolate the effects of a treatment from those of other factors, such as the natural course of disease. Testing MRT using randomized designs would require some means of delivering sham interventions to a set of oocytes or zygotes. Yet, because MRT would involve many different types of manipulations, the choice of sham comparators would be far from obvious. Investigators might design a sham whereby MRT would be withheld from some women, who would instead be offered the usual standard of care (e.g., PGD). A more aggressive approach would be to perform MRT by transferring nDNA from the intended mother into another oocyte or zygote from the intended mother (rather than from an oocyte or zygote provider). Both of these options would present inferential and ethical problems. The former would (primarily) test the effects of oocyte or zygote manipulation, but would tell little about the added risk of the particular manipulations used in MRT. The latter approach would be unethical because performing MRT on oocytes or zygotes with parental (and pathogenic) mtDNA would subject future children to the risks of MRT with none of the potential benefits.

Despite these challenges, it would be essential to attempt to standardize clinical investigations of MRT to the extent possible. In addition, it might be beneficial for FDA to incorporate data from research or clinical practice outside of the United States to enhance the quality of the assessment of benefits and risks. The UK regulations allowing MRT as a clinical procedure went into effect at the end of 2015; FDA could utilize any data available from these procedures or MRT procedures performed in other countries.

Conclusion: *It could be challenging to standardize study designs for MRT. However, standardizing as many components of a study as possible would allow for the collection and pooling of high-quality and interoperable data.*

Conclusion: *Data from outside the United States could be useful in FDA's assessment of the benefits and risks of MRT.*

Identity, Kinship, and Ancestry

As discussed in Chapter 3, MRT has implications for identity, kinship, and ancestry. The genetic contribution of three individuals might give children born as a result of MRT a unique perspective on their sense of self, to whom they are related and how, and their origins and lineage. While traditional oocyte or sperm provision raises similar issues, MRT is distinct from these procedures in that resulting children would be genetically related to three individuals. Clinical investigations of MRT would therefore need to include study of the potential psychological and social effects of MRT on notions of identity, kinship, and ancestry.

Recommendation 3: If the conditions of Recommendation 1 are met, the U.S. Food and Drug Administration (FDA) should ensure that the design and conduct of initial and subsequent clinical investigations of mitochondrial replacement techniques (MRT) adhere to the following principles and practices:

- **The health and well-being of any future children born as a result of clinical investigation protocols of MRT should have priority in the balancing of benefits and risks with respect to the design of investigations, eligibility of prospective mothers, numbers of participants, and pacing of investigations.**
- **Study designs of clinical investigation protocols of MRT should be standardized to the extent possible so as to minimize the number of variables and enable valid comparisons and pooling of outcomes across groups.**
- **Data from research or clinical practices outside FDA jurisdiction should be incorporated into FDA's analysis to enhance the quality of the assessment of benefits and risks.**
- **Clinical investigations should collect long-term information regarding psychological and social effects on children born as a result of MRT, including their perceptions about their identity, ancestry, and kinship.**

EXTENSION OF MRT RESEARCH TO FEMALE EMBRYOS

As discussed above, restricting the first investigations of MRT to male embryos would initially eliminate the risk of deleterious health effects resulting from the introduction of heritable genetic modifications, allow time for evidence to be collected on safety and efficacy in the first generation of male children born as a result of MRT, and provide greater understanding of the effects of genetic modification via MRT. Regardless of how safe or efficacious MRT was found to be in clinical investigations with male embryos, however, moving to female embryos would introduce the additional ethical, social, and policy issues raised by heritable genetic modification of germ cells.

In addition to issues raised in Chapter 3 regarding heritable genetic modification, the use of MRT to create and transfer female embryos would raise novel questions in the research context. Any assessment of benefits and risks would need to take into account the risks of introducing unforeseen or unintended mtDNA mutations or unexpected effects of mtDNA and nDNA genome combinations that would be experienced not only by the immediate female offspring born as a result of MRT but also by all of their prospective progeny into the future. As recommended by the committee, these potential intergenerational risks could be avoided by limiting initial clinical investigations of MRT to male embryos. However, important information and potential benefits would be gained from eventually transferring female MRT embryos, including understanding the effects of heritable genetic modification on reproduction and the health of offspring eventually born to women who were born as a result of MRT. Significantly, transfer of female embryos would minimize the risk of passing on pathogenic mtDNA mutations that might otherwise be faced by all maternal members of a family's lineage over generations, effectively preventing mtDNA disease in future generations of families known to be at high risk. Giving families the ability to bear female children is also a value to be respected and one that could be served only by transferring female embryos. The question is when in the course of investigation of MRT it would be acceptable to move to transferring female embryos. The committee identified three general criteria that would need to be satisfied before moving forward with MRT for female offspring: (1) compelling evidence of safety and efficacy in male embryos; (2) preclinical animal research showing evidence of intergenerational safety and efficacy; and (3) the existence of a shared framework concerning the acceptability of, and moral limits on, heritable genetic modification.

Compelling Evidence of Safety and Efficacy

Moving to transferring female embryos would constitute an important additional step in MRT human investigations. In addition to sharing the characteristics of MRT with male embryos, MRT involving female embryos would introduce intergenerational effects, whether those effects were positive or negative. Among the most significant concerns regarding heritable genetic modification resulting from MRT is the inability to limit unintended deleterious genetic effects to the individual born via MRT. A female born as a result of MRT who carried pathogenic mtDNA mutations could pass them on, whereas similarly affected males could not. Therefore, the committee's view is that sufficiently robust evidence of the safety and efficacy of MRT in males would be necessary before introducing the additional risks associated with the potential intergenerational effects that would accompany transferring female embryos, regardless of how long it took to collect this evidence. Sufficiently compelling evidence that would reach the level of confidence envisioned by the committee would come from experience with numerous male children followed at least during their early childhood years. While the threshold for sufficient evidence might be difficult to gauge before first-in-human investigations began, FDA could consider establishing a minimum threshold to be met before moving to MRT in female embryos. For example, should FDA ever come to the point of granting a license for the application of MRT in male embryos, it would be on the basis of evidence suggesting that certain major risks could be excluded. This evidence appears likely to be relevant for both male and female embryos. The agency could link judgments about initiating investigations in female embryos to the grant of licensure in male embryos.

Preclinical Data on Intergenerational Effects

Clinical investigations of MRT in males would generate data on safety and efficacy only in the first generation, that is, the children born as a result of MRT. Data on the effects of MRT in subsequent generations could only be generated by transferring female embryos, allowing time for these females to reach sexual maturity and choose to reproduce, and then assessing the health and well-being of these subsequent offspring. Because these data could not be collected through MRT entailing the transfer of male embryos, sufficient preclinical evidence from animal models regarding intergenerational safety and efficacy would need to be gathered before clinical investigations of MRT involving female embryos were allowed.

Shared Framework on Heritable Genetic Modification

If and when sufficiently compelling evidence of safety and efficacy from experience with male MRT offspring and preclinical data on intergenerational effects were obtained, moving to transferring female embryos would remain a controversial step in that it would entail heritable genetic modification. As articulated elsewhere in this report, the committee views heritable genetic modification via MRT as distinct in relevant and important ways from modification of nDNA—distinctions that would inform the acceptability of going forward with female embryos if safety and efficacy criteria for MRT had been established and met. A productive public discussion and process has been initiated to establish a shared framework with respect to whether heritable genetic modification is acceptable and if so, under what circumstances and for what purposes. The committee believes its analysis can aid this ongoing discussion and that any decision about moving forward with MRT with female embryos should be informed by this discussion. Therefore, the committee recommends that if and when compelling evidence of safety and efficacy is established, a decision to move forward with transferring female embryos should be consistent with the established shared framework in effect at that time concerning the acceptability of techniques that result in heritable genetic modification of human embryos.

Recommendation 4: Following successful initial investigations of mitochondrial replacement techniques (MRT) in males, the U.S. Food and Drug Administration (FDA) could consider extending research of MRT to include the transfer of female embryos if

- **clear evidence of safety and efficacy from male cohorts, using identical MRT procedures, were available, regardless of how long it took to collect this evidence;**
- **preclinical research in animals had shown evidence of intergenerational safety and efficacy; and**
- **FDA's decisions were consistent with the outcomes of public and scientific deliberations to establish a shared framework concerning the acceptability of and moral limits on heritable genetic modification.**

INFORMED CONSENT

Informed and voluntary consent of those deemed research participants in MRT clinical investigations would be required pursuant to federal guidelines and applicable state laws and institutional practices. As noted earlier, five potential parties could have interests affected in the course of the MRT process: (1) individuals who provide gametes (oocytes or sperm) used to

construct embryos, (2) the intended parents, (3) the gestational carrier (if needed), (4) the child born as a result of MRT, and (5) any potential future offspring of the child born as a result of MRT. Each of these parties has rights and interests deserving of protection, although they all might not necessarily be recognized as research subjects from a regulatory perspective (in accordance with federal or state regulations or institutional requirements or practices). MRT necessarily would involve an oocyte provider, but depending on the family structure of the intended mother pursuing MRT, sperm might be provided by either the intended father or another individual. The consent process would need to ensure that information about the MRT process was adequately disclosed and comprehended and that any decisions to participate in the MRT process were voluntary.

The research community has debated the utility of the informed consent process for years. Nonetheless, the complexities and uncertainties associated with MRT suggest that the consent process holds significant potential to provide a thoughtfully designed structure for what is ultimately a highly valuable and critical component of research. The consent process would need to ensure that those participating in MRT research understood what their participation entailed and that it was voluntary. While MRT presents a number of challenges to the consent process, as described below, efforts to develop best practices could provide a foundation for consent processes appropriate to novel reproductive technologies (Aldoory et al., 2014).

Individuals Who Provide Gametes

For women providing their oocytes for MRT research purposes, the process, and thus the implications for consent, would vary depending on the technique used. In the case of MST, the nuclear chromosomes would be removed from the provider's oocyte and replaced with the intended mother's nuclear chromosomes. Thus, in MST, the provider's oocyte would be the focus of the MRT manipulation. This reconstructed oocyte would subsequently be fertilized by sperm from the sperm provider (either the intended father or another individual). In PNT, both the provider's oocyte and the intended mother's oocyte would be fertilized in vitro with sperm from the sperm provider to create two embryos. The pronuclei would be removed from both embryos, and the oocyte provider's pronuclei would be replaced by the pronuclei of the intended mother to create a reconstructed embryo. Thus, in PNT, the provider's fertilized egg (zygote) would be the focus of the MRT manipulation. If medically acceptable, the intended mother would likely gestate the embryo; if not, a gestational carrier could be used.

The sperm provider's sperm would be used to fertilize one oocyte in the case of MST or two oocytes in the case of PNT to create embryos (one of which would be discarded after the pronuclei had been removed in the

case of PNT). Thus, in both MST and PNT, material in which the sperm provider had an interest (his sperm or a zygote fertilized with his sperm) would be the focus of the MRT manipulation.

MRT Research Procedures and Applications

As part of any clinical investigation of MRT, consent would need to be obtained for the series of interventions necessary to stimulate and collect oocytes (including both surgical and postsurgical procedures) and create embryos (if PNT were the technique being used), as well as for the intended use and disposition of a provider's oocytes, and potentially embryos. Short- and long-term risks of oocyte retrieval and any unknowns associated with these data would need to be explained to individuals providing oocytes.[3] Sperm providers would also need to be involved in the consent process for the use of their gametes to create embryos and the use and disposition of remaining sperm or embryos.

Individuals who provided gametes (both oocytes and sperm) would also need to be given the opportunity to understand the ethical, social, and policy issues associated with MRT research and the role their tissues would play in the research process. It would be necessary to explain to gamete providers the degree to which their gametes (or embryos) would be used for research purposes and stored indefinitely or destroyed.

Incidental Findings

Depending on the diagnostic techniques used to evaluate gamete providers and their gametes in the MRT research context, the consent process would need to include consideration of the possibility that the research would yield incidental findings with clear implications for participants' reproductive or other health care decisions (for example, if the gamete provider or his or her gametes were to undergo tests that revealed a particular genetic trait or mutation that would affect such decisions). Mechanisms for delivering these findings to gamete providers would have to be determined. MRT researchers and institutions would have to be informed by existing guidance documents on how, when, and to whom such incidental findings are to be reported throughout the course of research (e.g., Presidential Commission for the Study of Bioethical Issues, 2013). For instance, if a

[3] Some scholars have suggested a new category to address individuals who provide gametes. In the example of women providing oocytes for stem cell research, Magnus and Cho (2005) recommend the term "research donor" as distinct from "research subject" to signify that the risk incurred by women providing oocytes for research comes from the procurement of materials for research and not the actual research itself.

child born as a result of MRT were found to have a novel, unpredicted mtDNA disease, it would be necessary to consider whether to inform the oocyte provider of this result, as it could affect her health or the health of her children.

Incentives and Potential Financial Gain

The appropriate compensation of women and men who provide their gametes for research has been the topic of ethical and legal analysis in other clinical contexts. Some have suggested that individuals who provide gametes should receive financial reimbursement only for out-of-pocket costs or direct expenses incurred as a result of the procedures, as determined by an institutional review board (IRB) (IOM, 2013; NRC and IOM, 2010), thereby avoiding ethical issues because the gamete provider would derive no financial gain from participating in the research and would not be vulnerable to arguably undue levels of enticement. On the other hand, those who provide their gametes in the context of infertility treatments often receive financial compensation reflective of the time, inconvenience, and discomfort associated with screening, ovarian stimulation, and oocyte retrieval (ASRM, 2007); therefore, some suggest banning payments to gamete providers in the context of research would be unfair (Lo and Parham, 2009). Indeed, the International Society of Stem Cell Research's recent recommendations regarding compensation of oocyte providers suggest it is appropriate to compensate for an oocyte provider's time, effort, and inconvenience (Haimes et al., 2013). Practically speaking, moreover, finding oocyte providers in the absence of compensation is a notable challenge (Egli et al., 2011).

Any increased demand for provider oocytes and sperm resulting from the initiation of MRT research, however small, would have the potential to put some women and men of low socioeconomic status at risk for arguably undue enticement to donate gametes. In lieu of banning payments, which might be criticized as being paternalistic, the literature suggests there are opportunities to strengthen protections for all gamete providers, including those of low socioeconomic status (IOM and NRC, 2007; Lo and Parham, 2009; Lomax et al., 2007). It would be important for MRT researchers and institutions, in consultation with local review committees or a central IRB, to consider current guidance and emerging best practices in determining appropriate compensation for gamete providers, taking into account the demands placed on a gamete provider by an MRT research protocol. It would be necessary as well to give special attention to crafting a compensation and recruitment strategy that would not place women and men of low socioeconomic status in a position of being unduly enticed to provide gametes against their better judgment.

Provider Contact

An additional set of issues that would need to be part of a consent process for MRT clinical research participation or as a separate agreement with the intended parents relates to the social involvement, if any, of a gamete provider with the future child and his or her family. It is possible that a child born as a result of MRT would have an interest later in life in contacting his or her mtDNA or sperm provider. The consent process would need to include agreement among all parties as to whether gamete providers would remain anonymous to the child, or contact in the future would be possible, subject to applicable state laws (which might, in some cases, require open adoption in which future contact would be possible).

Management of Residual Gametes and Embryos

The consent process for MRT would need to include discussion of the disposition of any remaining gametes and embryos. Multiple attempts at MRT for establishment of a pregnancy could be necessary, so it would be important for gamete providers to know how any remaining gametes or embryos would be managed. In particular, if gametes or embryos were to be cryopreserved, a clear understanding of their longer-term management would be needed. For example, who would be responsible for storage costs, and who would make decisions about the use or destruction of the oocytes or embryos or their donation to other couples or for research purposes if they were no longer needed for the MRT investigation? In some cases, state laws or precedent cases could limit the gamete providers' options, and this, too, would need to be explained to them.

Intended Mother and Intended Father (if applicable)

Consent Components Applicable Specifically to the Intended Mother

The consent process for an intended mother considering MRT in first-in-human investigations would likely be "a difficult and long-term process" (FDA Cellular Tissue and Gene Therapies Advisory Committee, 2014) requiring many conversations over time. As a participant in a first-in-human clinical investigation, the woman would assume the risk associated with the lack of prior information on the safety and efficacy of MRT in humans. The complexity of MRT also would present a psychological challenge to the intended mother in the form of overlapping uncertainties. For instance, she would have to weigh the benefits and risks, and the uncertainties, associated

with IVF,[4] with MRT itself (for which no data from a born human exist), and with the role of mtDNA in human development (evolving data), as well as any potential health risks associated with transmitting this novel genetic combination (no data available) (Bonnicksen, 1998).

In addition to the benefits, risks, and uncertainties of MRT itself, the informed consent process for an intended mother who would be gestating the embryo would need to include discussion of the aspects of (1) the procedures involved in MRT (including PGD testing in embryos and prenatal tests in fetuses, such as chorionic villus sampling, amniocentesis, or cell-free DNA screening); and (2) the genetic testing processes, and their potential limitations, that would accompany the procedures. In addition, an intended mother would need to be made aware of the potential that a child with significant disability could be born, and of the difficult decisions she might face regarding pregnancy termination if prenatal diagnostic testing revealed genetic or developmental anomalies or other adverse outcomes. Follow-up conversations as part of the informed consent process could help ensure that research participants had adequate information about the testing procedures (including information about each test's specificity, sensitivity, accuracy, risks, benefits, and limitations) used throughout the MRT process (McGowan et al., 2009).

Consent Components Relevant to Both the Intended Mother and the Intended Father (if applicable)

If intended mothers sought to avail themselves of MRT but did not have a male partner, there would not necessarily be an intended father. In those cases, an individual who provided sperm would be involved, for whom the applicable consent principles are described above. In many cases, however, there would likely be an intended father or co-parent, and although he or she might not also be a gamete provider, the legal and social role in raising the resulting child would make the following components of the consent process relevant to the intended mother and intended father or co-parent.[5]

Alternatives The consent process for MRT clinical research would need to include a thorough discussion of the alternative means of becoming a parent that would avoid the transmission of mtDNA disease, including their advantages and disadvantages. The discussion of these alternatives would

[4] IVF success rates vary from 1.2 to 23.8 percent based on the presenting indication and the treatment approach pursued (CDC et al., 2015).

[5] In the hypothetical case of a female co-parenting couple, in which one woman was the nDNA contributor (intended mother) and the other the mtDNA contributor (oocyte provider), the identification of one or both as "legal" mother would pose novel questions for the state courts.

need to be supported by a range of advisors and counselors to help inform the intended parents and answer their questions about potential participation in MRT research.

Research restrictions If protocols for first-in-human investigations required that only male embryos be transferred, it would be necessary to inform intended parents of the possibility that the oocyte retrieval and MRT process could result in only female embryos without pathogenic mtDNA and therefore otherwise suitable for transfer. Should this be the case, the intended parents would need to understand that they would be unable to have an embryo transferred as part of the research process.

Long-term follow-up The important role of long-term follow-up of any children resulting from MRT research would need to be highlighted in the consent process. It is possible that decades of observation would be necessary to detect subtle effects of such factors as epigenetic changes or variable levels of heteroplasmy in certain tissues. Lengthy observation periods also would be appropriate as a means of maximizing information gained from the small study samples that would be likely. Sponsors of clinical investigations of MRT would need to have a plan and a budget to support such long-term follow-up of any resulting offspring. Extended periods of regular, potentially invasive and intensive observation could add to the burdens of children resulting from MRT. Such follow-up could be especially burdensome if the children were otherwise healthy, because invasive monitoring would not be therapeutic for them.

Long-term follow-up would be necessary to determine whether there were issues with residual pathogenic mtDNA molecules (i.e., MRT did not effectively prevent the transmission of mtDNA disease), whether the manipulation of oocyte or zygote and the process of mtDNA replacement adversely affected the subsequent child, and whether there were any effects of possible mtDNA-nDNA mismatch. In the initial research phases, follow-up would likely include evaluations in infancy and early childhood to determine whether gross anomalies, developmental disabilities, mtDNA mutations, or signs or symptoms of mtDNA disease were present. It could be necessary to evaluate children to the point of sexual maturity to confirm that the reproductive system had not been adversely affected. Decades-long observation of children born as a result of MRT would be necessary to determine whether there were late-onset effects of MRT, and intergenerational follow-up would be necessary to track the health and well-being of subsequent generations if female embryos were transferred. While continued assent (for children) and consent (when they reach the age of consent) to this type of follow-up cannot be mandated, the intended parents would need to be well informed from the outset as to why long-term follow-up was crucial

for MRT research and to understand that it would be an important part of the child's experience as part of the research protocol.

Privacy The first individuals participating in clinical investigation of MRT could be targets of intense media scrutiny. While research centers are required to institute measures to protect the confidentiality of health information and the personal identity of research participants, participants can choose to maintain their privacy or to make themselves known to the media. Assisted reproductive technologies (ARTs) are frequently the subject of media and public interest. Therefore, although the clinical research context offers protections for patient privacy, special attention would need to be paid to preparing prospective research participants, investigators, research institutions, and their staffs and media departments for the likelihood of high-profile attention, wanted or unwanted, associated with an MRT investigation. The ethics review committee(s) charged with evaluating any initial MRT clinical investigation protocols, and the associated consent processes, would play an important role in ensuring that provisions for protecting the privacy of research participants were adequate and that the relevant parties were appropriately informed and prepared for any unintentional and inadvertent disclosures.

Gestational Carrier (if needed)

As discussed earlier in this chapter, if the intended mother were unable to carry or were at high risk for complications associated with carrying a pregnancy, a gestational carrier might be deemed appropriate or necessary. Any such gestational carrier would need to have a clear understanding of the potential benefits, risks, and uncertainties associated with participation in the MRT process. For instance, gestational carriers would need to be made aware of the potential that a child with significant disability could be born, and of the difficult decisions she might face regarding pregnancy termination if prenatal diagnostic testing revealed genetic or developmental anomalies or other adverse outcomes.

Child Born as a Result of MRT

Consent by intended parent(s) to a process that would result in the birth of a child through MRT could not fully protect the interests and welfare of future children. As mentioned elsewhere in this report, protecting the health and well-being of future children born as a result of MRT needs to be the cornerstone for all assessments of MRT and balancing of its benefits and risks, including decisions surrounding the adequacy of

preclinical studies, justification for clinical investigations, and the design of first-in-human investigations.

As noted above, once a child had been born, investigators would need to obtain parental permission for such research-related procedures as blood sampling or tissue biopsy of a newborn. To meet this need, staged parental permission could be implemented, as was planned for the National Children's Study, thereby avoiding a long and complex consent process for future interventions during enrollment in the study and allow parents to make decisions as they might arise (IOM, 2008).

The Nuffield Council on Bioethics (2012, pp. xvi, 88) argues that, in MRT clinical research, "consent to follow up would need to be included as a mandatory part of parental consent to participation in the trial." Consent to participate in research is an ongoing process, not a one-time event or a signature on a consent document. A participant's right to withdraw, without penalty, is recognized as a critical element of participation in clinical research. This right can create difficulties in the conduct of research, such as MRT, that requires long-term monitoring and follow-up. Optimal follow-up for MRT research could be decades long to make it possible to assess effects that might appear later in life or to monitor the health of offspring born to children born as a result of MRT. Parents of children born as a result of MRT would be asked to provide ongoing permission for the child's participation in follow-up evaluations, even though they would have the right to decline, just as they could withdraw from the study at any time. Similarly, children born as a result of MRT would be asked to assent or consent (at appropriate ages) to further involvement in the research, which they would be free to decline at any time.

It is ethically permissible, within limits, to try to persuade research participants, including children who have reached the age of consent, to continue to participate in research. In fact, in the committee's view, MRT research participants would have an ethical—though not legal—duty to remain involved in follow-up activities for their own benefit as well as that of other potential future users of MRT. It is reasonable for clinical researchers to use pre-enrollment consent discussions, as well as postprocedure discussions, to strongly encourage individuals to participate in follow-up. Reimbursement for costs and modest incentives, such as access to personalized medical services or general recognition and praise, are justifiable in some circumstances (Grant and Sugarman, 2004), although the individual's eventual decision must be respected. No coercion or other efforts that undermine voluntary decision making are acceptable, either to encourage initial participation in or discourage withdrawal from research.

Protection of Future Generations

MRT clinical investigations that entailed creating and transferring female embryos for gestation would raise issues related to the transmission of heritable genetic modification to future generations. Other ARTs also involve heritable genetic modification, although without the unique combination of characteristics associated with MRT. For instance, prospective parents might use the assistance of a woman who provided her oocyte to have a child genetically related to one of those parents, and that new combination of genetic material would be passed on to future generations. As described throughout this report, however, MRT is unique in that in females it would result in a potentially heritable genetic modification comprising DNA from two women of different maternal lineage. This transmission of heritable genetic modifications to future generations as a result of MRT would constitute uncharted territory for any consent process. Evaluating the risks that MRT could pose to future generations is important from ethical, social, and policy perspectives; however, clinical investigations have no mechanism for seeking consent from future generations. Thus the potential effect of MRT on future generations needs to be a key consideration in broader policy discussions and research oversight related to MRT, becasue it cannot be addressed in the consent process for MRT clinical investigations.

Conclusion: *When intended parents provided consent to the MRT process, they would be, in essence, consenting on behalf of any future children.*

- *The nature of the MRT consent process for intended parents would need to reflect a research protocol that had been crafted with the health and well-being of future children in mind.*
- *Once a child had been born as a result of MRT and reached the applicable age, it would be necessary to carry out a more traditional process of parental permission and child assent, and eventually consent by the child, for participation in ongoing research assessments.*

Recommendation 5: In addition to attention to best practices for consent in research, the U.S. Food and Drug Administration (FDA), research institutions, investigators, and institutional review boards should pay special attention to communicating the novel aspects of mitochondrial replacement techniques (MRT) research to potential research participants.

- **For individuals who provide gametes, consent processes should reflect**

  - **the range of MRT procedures contemplated for preclinical and/or clinical investigations and the general ethical, social, and policy considerations surrounding MRT;**
  - **the management of incidental findings, should they arise;**
  - **appropriate compensation, with sensitivity to socioeconomic status;**
  - **the prospect of future contact between individuals who provided their gametes and children born as a result of MRT; and**
  - **the management of residual eggs and embryos.**
- **For intended parents, consent processes should reflect**
  - **information on the MRT research protocol, with focus on the implications for the health and well-being of resulting children;**
  - **alternative ways of becoming parents that can avoid maternal transmission of mitochondrial DNA (mtDNA) disease;**
  - **the management of and potential restrictions on access to embryos created through MRT (e.g., if initial investigations are limited to male embryos);**
  - **preimplantation and prenatal genetic diagnostic tests that would be incorporated into clinical investigation protocols;**
  - **the importance of long-term follow-up and how it would be part of the experience of any child born as a result of MRT; and**
  - **the challenges of maintaining patient privacy given intense media interest in MRT.**
- **For children born as a result of MRT, consent processes should reflect assent (and eventual consent) for monitoring and research procedures to be performed after birth, up to and including seeking informed consent from the children upon their reaching the legal age of consent.**

GUIDING PRINCIPLES FOR OVERSIGHT

Although MRT is in some ways similar to other reproductive technologies, it has a unique combination of characteristics that raises a novel collection of ethical, social, and policy issues. Because of this unique combination of characteristics, MRT would require special considerations across the trajectory of regulation and oversight—from preclinical studies to authorization of an IND, potential approval for clinical use, and postmarketing surveillance. These considerations could be addressed through the following guiding principles for oversight.

Transparency

MRT would entail sensitive and controversial procedures of great interest to many people, particularly those at risk of transmitting mtDNA disease to their offspring. FDA and other regulatory authorities would need to take every opportunity to inform the public and key stakeholders about all aspects of MRT within the constraints of legal obligations regarding confidentiality. The information to be shared could include preclinical work supporting regulatory decision making and relevant emerging scientific developments, as well as decisions regarding clinical investigations, approvals, and postmarket studies. Regulatory authorities would need to promote transparency by utilizing forums that would permit an exchange of information between the agency and the public; this process could employ existing venues such as public bioethics commissions, FDA advisory committee meetings and public workshops, or meetings of the NIH Recombinant DNA Advisory Committee (RAC). In addition, FDA would need to encourage sponsors to voluntarily waive confidentiality concerning protocol design and reporting of deidentified results whenever possible, while always maintaining the privacy of the individuals participating in the research.

Public and Patient Engagement

Because MRT is currently controversial, the question arises of how the public and key stakeholders can inform regulatory decision making. FDA's decision-making process, however technical and preoccupied with assessment of benefits and risks, is ultimately informed by value judgments concerning such issues as clinical need and the availability of viable alternatives. In the case of MRT, larger debates about the ethics of reproductive interventions fall outside the agency's mandate and core competencies. In the United States, scientific and political issues in this area are for the most part addressed separately, with political issues being resolved largely by legislation and technical issues by regulatory agencies. In the United Kingdom, by contrast, both are managed by the HFEA. However, given the nature of the issues raised by MRT and the subjectivity of elements of the assessment of its benefits and risks (e.g., the importance to prospective parents of having a genetic link to their offspring), public engagement in FDA's decision-making process could be beneficial.

Other novel and controversial technologies have undergone similar public and patient engagement. Until 2014, for example, all gene-editing experiments were reviewed by the RAC, which holds public reviews and discussions when necessary. National-level bioethics commissions have been convened to address ethical, social, and policy considerations on such topics as cloning and stem cell research. And FDA's Patient-Focused Drug

Development Initiative systematically gathers patient perspectives to inform the assessment of a product's benefits and risks. The regulatory process for MRT would likewise need to incorporate the views of the public and patient populations through such mechanisms as periodic reports to the public, opportunities for public meetings, and ongoing exploration of the views of relevant stakeholders. In particular, FDA would need to encourage the participation of those affected by mtDNA diseases and at risk of transmitting them to their children, who are uniquely positioned to inform the agency's understanding of the clinical and personal context of these diseases. Mitochondrial disease patient advocacy groups and mitochondrial medicine physicians and medical societies could also play a role in informing FDA in this regard.

Partnership

FDA would need to take full advantage of partnerships with regulatory bodies in other countries where MRT research or clinical investigation is occurring so information from this research could be pooled. During prelicensure stages, the goal is to increase the quality of protocol design and regulatory decision making while reducing redundancy and risk to patients and research participants. This goal is especially important for MRT given the rarity of mtDNA diseases and the small number of patients that would be research participants. The UK regulations allowing MRT went into effect in October 2014; FDA could use any data available from these procedures, or from MRT procedures performed in other countries, to improve its assessment of benefits and risks. If MRT were approved and entered clinical use in the United States, these partnerships would need to be maintained to enhance long-term postmarket surveillance and the use of risk management tools. FDA also would need to consider partnering with other federal agencies to take advantage of their expertise, such as that in regulatory science research at NIH or that in public health monitoring at the U.S. Centers for Disease Control and Prevention (CDC).

Conclusion: *Taking advantage of expertise and data available from other U.S. agencies, as well professional societies and regulatory agencies of other countries, would likely be beneficial for FDA's regulatory decision-making process.*

Maximizing Data Quality

The importance of standardization of study designs for research on MRT was discussed earlier in the section on principles and practices to guide clinical investigations. Given the likely small numbers of MRT re-

search participants, it would be critical to standardize study designs so the highest-quality data could be collected and pooled in support of the regulatory decision-making process. Despite the challenges of standardizing MRT studies discussed earlier, FDA would need to require, to the extent possible, that sponsors have adequate resources, use appropriate designs, and plan studies that would enable cross-referencing and pooling of data.

Another aspect of data quality is the periodic review and evaluation of study data for monitoring of safety and study conduct. A data safety monitoring board (DSMB) would be needed to provide this independent review of MRT investigations. The DSMB could also play a role in reviewing prespecified stopping criteria for enrollment in and implementation of MRT clinical investigations—important for supporting the integrity of clinical investigations and the safety of research participants. Long-term follow-up would need to continue, however, even if stopping criteria were employed to prevent further enrollment in or implementation of MRT clinical investigations.

Circumscribed Use

Given the novelty of MRT, its possible intergenerational effects, and the fact that the persons most affected—future children—would lack a role in making the decision to proceed, FDA would need to restrict approval to studies involving women with mtDNA disease with a compelling clinical need. Future proposals to broaden the use of MRT for other indications (e.g., to treat idiopathic or age-related infertility) would need to be subject to fresh ethical analyses, including public discussion and debate through such mechanisms as those discussed elsewhere in this report.

If MRT were approved for clinical use in women with known pathogenic mtDNA mutations, FDA would need to use all of the tools at its disposal to control off-label uses of MRT beyond those indications and settings for which it had been tested and approved (see Chapter 3 for discussion of circumscribed use and issues regarding treatment versus enhancement applications of MRT). These tools could include mechanisms such as postapproval studies or a Risk Evaluation and Mitigation Strategy (REMS), as well as enhanced surveillance to detect adverse events (see Chapter 2).

Long-Term Follow-Up

Because the risks and benefits of MRT would make themselves known over time, FDA would need to require as a condition of approval that sponsors design, fund, and commit to long-term monitoring. In addition, FDA would need to emphasize the adverse event reporting obligations of sponsors and MRT providers, be committed to timely analysis of postmarket data, and take advantage of long-term data available from other countries.

This committee was not tasked with defining a specific period for long-term follow-up. Moreover, FDA would have to make such a determination on a case-by-case basis in close consultation with MRT researchers. However, the committee offers the following set of points to be considered by FDA in determining sufficient or optimal follow-up:

- *Ability to identify any major medical consequences*—There would have to be reasonably high confidence in ruling out procedure-related events that would be of major medical consequence to the child born as a result of MRT or would be transmissible to future generations. Such a standard would likely favor extending the period of monitoring to sexual maturity, so that gametic tissues could be studied. As used here, "major medical consequence" denotes medical events that would substantially compromise age-adjusted activities of daily living.
- *Feasibility*—Owing to the novel nature and implications of MRT, researchers would have to be expected to go to extraordinary lengths to fund and implement plans for follow-up. However, the committee also recognizes that long-term follow-up activities would be likely to present major logistical and budgetary challenges, and that it would not be desirable for the heavy demands of implementing the ideal follow-up protocol to forestall further innovative activity in this arena. Accordingly, it would be reasonable for review bodies to consider feasibility in establishing expectations for follow-up.
- *Periods of less intensive monitoring*—It might be reasonable for researchers to plan for intensive follow-up during the early years and perhaps into sexual maturity, and for less intensive follow-up to be allowed once most of the major concerns (e.g., birth defects, mtDNA disease, sterility) had been ruled out.

Recommendation 6: The U.S. Food and Drug Administration's (FDA's) overall plan for review and possible approval and subsequent marketing of mitochondrial replacement techniques (MRT) should incorporate the following elements:

- ***Transparency*: Regulatory authorities should maximize timely public sharing of information concerning the MRT activities and decisions within their jurisdiction. FDA should encourage sponsors to commit to depositing protocols and deidentified results in public databases.**
- ***Public engagement*: Regulatory authorities should incorporate ongoing exploration of the views of relevant stakeholders into the overall plan for review and possible marketing of MRT and should support opportunities for public meetings to gather these views.**

- ***Partnership*: FDA should collaborate with other regulatory authorities within and outside the United States to improve the quality of the data available for the assessment of benefits and risks.**
- ***Maximizing data quality*: FDA should require that sponsors have adequate resources, use appropriate designs, and plan studies that enable cross-referencing and pooling of data for assessments of benefits and risks.**
- ***Circumscribed use*: FDA should use the means at its disposal to limit the use of MRT to the indications, individuals, and settings for which it is approved, and should engage the public in a fresh ethical analysis of any decision to broaden the use of MRT.**
- ***Long-term follow-up*: FDA should require that sponsors design, fund, and commit to long-term monitoring of children born as a result of MRT, with a plan for periodic review of the long-term follow-up data.**

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Appendix A

Study Approach

In response to a request by the U.S. Food and Drug Administration (FDA), the Institute of Medicine (IOM) of the National Academies of Sciences, Engineering, and Medicine convened the Committee on the Ethical and Social Policy Considerations of Novel Techniques for Prevention of Maternal Transmission of Mitochondrial DNA Diseases to consider the ethical, social, and policy issues raised by the development of mitochondrial replacement techniques (MRT) and whether these issues preclude FDA from moving forward with consideration of MRT clinical investigations.

COMMITTEE EXPERTISE

The IOM formed a committee of 12 experts to deliberate on and respond to the statement of task for the study (see Box 1-1 in Chapter 1). The committee was composed of members with expertise in bioethics, philosophy, law, policy, religion, mitochondrial biology and medicine, clinical investigations, and patient advocacy. Appendix C provides biographical information for each committee member.

MEETINGS AND INFORMATION-GATHERING ACTIVITIES

The committee held five in-person meetings in 2015 (January, March-April, May, July, and September). The January, March-April, and May meetings included portions open to the public; the agendas for these open sessions are included at the end of this appendix. The committee meetings in July and September were held only in closed session.

To inform its deliberations, the committee gathered information through a variety of mechanisms: (1) the 2-day public workshop held in conjunction with the March-April meeting, which included an open public comment session; (2) the open public comment session held during its May meeting; (3) systematic reviews of the literature on the ethical, social, and policy issues associated with MRT, as well as pertinent scientific background and research (see below and Appendix B); (4) solicitation and consideration of written statements from stakeholders and members of the public through the committee's Current Projects System (CPS) website and committee email; and (5) personal communication among committee members, staff, and individuals who have been directly involved in or have special knowledge of the issues under consideration.

During the 2-day public workshop held March 31-April 1, 2015, in Washington, DC, the committee gathered input from experts in academia, policy, clinical medicine, and government on a wide range of topics related to the study charge, including (1) ethical, social, and policy implications of MRT; (2) germline modification; (3) policy analogues; and (4) mitochondrial science and medicine. As was the case during the May meeting, the committee held open public comment sessions during which it invited input from any interested parties. For those unable to travel to the meetings, a conference call number was provided. During the public workshop and May public comment session, the committee requested that commenters specifically address the following questions in their remarks:

- What ethical and social issues are raised by proposed mitochondrial replacement techniques (MRT)?
  - Should MRT be considered germline modification?
  - Is MRT different, from an ethical and social perspective, from modification of nuclear DNA?
- Should there be a distinction between genetic modification for therapeutic/prevention purposes and genetic modification for enhancement purposes? If so, how should this distinction be defined and implemented?
- What are the ethical and social issues that arise if a child is born with genetic material from three individuals?
- How should the current availability of alternative approaches—adoption and oocyte donation—factor into the assessment of allowing MRT investigations to proceed?
- What are the ethical and social ethical issues if MRT clinical investigations were to proceed?
  - Ethical and social issues in providing "consent"
  - Ethical and social issues in first-in-human investigation in women and for the creation of children

  - Other aspects of enrollment and tracking during a clinical investigation

The committee also solicited and considered written statements from stakeholders, members of the public, and other interested parties through its CPS website and email. In response, the committee received 35 written comments from a variety of individuals and organizations, including domestic and international academic researchers and scholars, mitochondrial DNA (mtDNA) disease patients, parents of children with mtDNA disease, mtDNA disease patient advocacy representatives, public advocacy groups, and others. Staff collected and compiled all comments for the committee's review, calling particular attention to recurring and cross-cutting themes and unique perspectives. All written information provided to the committee from external sources is available by request through the Academies' Public Access Records Office.

LITERATURE AND PRESS REVIEW

Academies staff conducted a systematic literature review on topics related to the ethical, social, and policy aspects of MRT, as well as foundational background research related to mitochondrial biology and genetics, mtDNA disease, and MRT research to date (see Appendix B for a selected compilation of this research). Other targeted literature reviews were conducted as novel issues arose throughout the committee's deliberations.

Science and Medicine Literature

Search parameters:

- Date range: all years
- International, English only

Databases:

- PUBMED Medline
- Scopus
- Web of Science
- Grey literature (U.S. Food and Drug Administration [FDA], National Institutes of Health [NIH], Human Fertilisation and Embryology Authority [HFEA], Nuffield Council on Bioethics)

Search strategy:

- Mitochondrial biology and genetics
  - MESH: Mitochondria/Anatomy and Histology/Chemistry/ Embryology/Genetics/ Growth and Development/Metabolism/ Physiology (sort by relevance)
  - MESH: "Cell Division/genetics" AND (with the following MESH terms searched separately) "DNA, Mitochondrial," "Genome, Mitochondrial," "Genes, Mitochondrial," "Mitochondria/genetics"
- Complexities of mitochondrial biology
  - Mitochondria* AND bottleneck
  - Mitochondria* AND haplotype OR haplogroup
  - Mitochondria* AND mismatch OR incompatibility
  - Mitochondria* AND epigenetics
- Mitochondrial/mtDNA disease
  - ("mitochondrial disease" OR "mitochondrial disorder") AND etiology
  - ("mitochondrial disease" OR "mitochondrial disorder") AND (pathophysiology OR pathology)
  - ("mitochondrial disease" OR "mitochondrial disorder") AND subtype*
  - ("mitochondrial disease" OR "mitochondrial disorder") AND diagnosis
  - ("mitochondrial disease" OR "mitochondrial disorder") AND (treatment OR therapeutics OR pharmaceutical or "gene therapy")
- Mitochondrial replacement techniques
  - MST: ("maternal spindle transfer" OR "spindle transfer" OR "spindle-chromosomal complex transfer") AND (mitochondria* OR "mitochondrial disease" OR "mitochondrial disorder")
  - PNT: (("pronuclear transfer" OR (karyotype OR karytype OR pronuclei) AND transfer)) AND (mitochondria* OR "mitochondrial disease" OR "mitochondrial disorder")
  - PBT: ("polar body" OR "polar bodies") AND (mitochondria* OR "mitochondrial disease" OR "mitochondrial disorder")
- Alternatives to MRT
  - PGD or prenatal screening: ("prenatal diagnosis" OR "prenatal screening" OR PND OR "preimplantation genetic diagnosis" OR PGD) AND ("mitochondrial disorder" OR "mitochondrial disease")
  - Adoption: (Adoption AND ("mitochondrial disorder" OR "mitochondrial disease"))

  - Surrogacy: (Surrogacy AND ("mitochondrial disorder" OR "mitochondrial disease"))

Ethics and Policy Literature

Search parameters:

- Date range: all years
- International, English only

Databases:

- Scopus
- LexisNexis
- Grey literature reports (NIH, FDA, World Health Organization [WHO], International Society for Stem Cell Research [ISSCR], United Nations Educational, Scientific and Cultural Organization [UNESCO], World Medical Association [WMA])

Search strategy:

- MRT as germline modification: ("germline modification" OR "germline manipulation" OR "inheritable genetic modification" OR germline) AND (("mitochondria replacement") OR ("mitochondria transfer") OR ("mitochondrial manipulation") OR ("mitochondrial gene replacement") OR (oocyte modification) OR ("three-person embryo") OR ("three-parent babies") OR ("nuclear genome transfer") OR ("pronuclear transfer") OR ("spindle transfer") OR ("three-person IVF") OR ("3-person IVF") OR (tri-parenthood) OR ("assisted reproductive therapy" AND "mitochondria") OR ("spindle-chromosomal complex transfer"))
- MRT/germline modification and informed consent: (consent OR "informed consent" OR "assumption of risk" OR permission) AND ("germline modification" OR "germline manipulation" OR "inheritable genetic modification" OR germline) AND (("mitochondria replacement") OR ("mitochondria transfer") OR ("mitochondrial manipulation") OR ("mitochondrial gene replacement") OR (oocyte modification) OR ("three-person embryo") OR ("three-parent babies") OR ("nuclear genome transfer") OR ("pronuclear transfer") OR ("spindle transfer") OR ("three-person IVF") OR ("3-person IVF") OR (tri-parenthood) OR ("assisted reproductive therapy" AND "mitochondria") OR ("spindle-chromosomal complex transfer")) AND (child* OR offspring OR descendant* OR progeny)
- MRT/germline modification and notions of identity/kinship: (ethic* OR "social issue" OR personhood) AND (child* OR offspring OR

progeny) AND ("germline modification" OR "germline manipulation" OR "inheritable genetic modification" OR germline) OR (("mitochondria replacement") OR ("mitochondria transfer") OR ("mitochondrial manipulation") OR ("mitochondrial gene replacement") OR (oocyte modification) OR ("three-person embryo") OR ("three-parent babies") OR ("nuclear genome transfer") OR ("pronuclear transfer") OR ("spindle transfer") OR ("three-person IVF") OR ("3-person IVF") OR (tri-parenthood) OR ("assisted reproductive therapy" AND "mitochondria") OR ("spindle-chromosomal complex transfer")))

- MRT/germline modification and notions of genetic engineering, slippery slope, designer children: ("slippery slope" OR "designer babies" OR "designer baby" OR "genetic engineering") AND (("mitochondria replacement") OR ("mitochondria transfer") OR ("mitochondrial manipulation") OR ("mitochondrial gene replacement") OR (oocyte modification) OR ("three-person embryo") OR ("three-parent babies") OR ("nuclear genome transfer") OR ("pronuclear transfer") OR ("spindle transfer") OR ("three-person IVF") OR ("3-person IVF") OR (tri-parenthood) OR ("assisted reproductive therapy" AND "mitochondria") OR ("spindle-chromosomal complex transfer") OR ("germline modification" OR "germline manipulation" OR "inheritable genetic modification" OR germline))
- Risks related to MRT: ("health problem" OR "health implication" OR harm* OR risk* OR safety OR efficacy OR epigenetic harm OR epigenetic OR carryover) and ("mitochondria replacement") OR ("mitochondria transfer") AND (("mitochondria replacement") OR ("mitochondria transfer") OR ("mitochondrial manipulation") OR ("mitochondrial gene replacement") OR (oocyte modification) OR ("three-person embryo") OR ("three-parent babies") OR ("nuclear genome transfer") OR ("pronuclear transfer") OR ("spindle transfer") OR ("three-person IVF") OR "3-person IVF") OR (tri-parenthood) OR ("assisted reproductive therapy" AND "mitochondria") OR ("spindle-chromosomal complex transfer"))

Press Search and Alerts

Database: LexisNexis

Search parameters:

- All English language news

- All available dates
- Alerts received on a daily basis

Search strategy:
((mitochondria! replacement) or (mitochondria! manipulation) or (oocyte modification) or (three-person embryos) or (three-parent babies) or (nuclear genome transfer) or (pronuclear transfer) or (maternal spindle transfer) or (mitochondria! disease and oocyte and manipulation or replacement or modification) or (mitochondria! w/p germline therapy or germ-line therapy or germ line therapy) or (assisted reproduct! and mitochondria!) or (mitochondria! and oocyte and FDA))

COMMITTEE MEETING AGENDAS

Meeting 1: January 27-28, 2015

The National Academies
Keck Center—Rooms 100 and 201
500 Fifth Street NW
Washington, DC 20001

January 27, 2015
CLOSED SESSION (8:30-11:00 AM)

January 27, 2015
OPEN SESSION (11:00 AM-12:30 PM)

11:00 AM Opening remarks to public audience
Jeffrey Kahn, Committee Chair, Johns Hopkins University

11:05 AM **Delivery of Study Charge and Q&A/Discussion with Committee**
Objectives:
- Receive study background and charge from FDA.
- Discuss task with the sponsor and determine scope of committee's work (i.e., what is in and what is out).
- Clarify issues identified by the committee and seek answers to questions.
- Discuss report audience and expected products.

Celia Witten, Director, Office of Cellular, Tissue, and Gene Therapy, Center for Biologics Evaluation and Research (CBER)/U.S. Food and Drug Administration (FDA)

FDA Panelists:
Deborah Hursh, Senior Investigator, Division of Cell and Gene Therapy, CBER
Wilson Bryan, Director, Division of Clinical Evaluation and Pharmacology/Toxicology, CBER
Lei Xu, Medical Officer, Division of Clinical Evaluation and Pharmacology/Toxicology, CBER

12:30 PM Adjourn open session

January 27, 2015
CLOSED SESSION (12:30-4:00 PM)

January 28, 2015
CLOSED SESSION (8:30 AM-5:00 PM)

Meeting 2: March 30-April 1, 2015

The National Academies
Keck Center—Room 208
500 Fifth Street NW
Washington, DC 20001

March 30, 2015
CLOSED SESSION (1:30-5:00 PM)

March 31, 2015
OPEN SESSION (8:30 AM-5:00 PM)

8:30 AM Welcome and overview of workshop
Jeffrey Kahn, Committee Chair, Johns Hopkins University

SESSION I: ETHICAL OR SOCIAL IMPLICATIONS OF MRT

Session Objectives:

- Highlight key characteristics of proposed MRT techniques raising ethical or social issues.
- Discuss the distinctive ethical or social issues that would arise with MRT techniques.

Session Co-Chairs:
R. Alta Charo, Committee Member, University of Wisconsin–Madison
Laurie Strongin, Committee Member, Hope for Henry Foundation

9:00 AM *Heather Ward*, Personal Representative
Kevin FitzGerald, Georgetown University
Thomas Murray, The Hastings Center
Laurie Zoloth, Northwestern University
Hugh Whittall, Nuffield Council on Bioethics

9:50 AM Discussion with committee and workshop participants

10:20 AM Break

SESSION II: GERMLINE MODIFICATION

Session Objectives:

- Discuss whether the manipulation of mitochondrial content raises social and ethical issues related to genetic germline modification, and whether the issues raised are similar to or different from those raised by modification of nuclear DNA.
- Discuss the historical prohibitions on germline genetic modification, the social and ethical considerations that shaped these restrictions, and whether they should be revisited.
- Consider whether it is advisable to establish controls to distinguish between genetic modification for therapeutic/prevention purposes and for enhancement purposes. What controls could be effective at maintaining this distinction?

Session Co-Chairs:
Jeffrey Kahn, Committee Chair, Johns Hopkins University
Vamsi Mootha, Committee Member, Harvard Medical School

10:35 AM *Annelien Bredenoord*, University Medical Center Utrecht
Marcy Darnovsky, Center for Genetics and Society
John Evans, University of California, San Diego
John Harris, University of Manchester

11:15 AM Discussion with committee and workshop participants

11:45 AM Lunch (Cafeteria located on third floor)

SESSION III: POLICY ANALOGUES

Session Objective:

- Discuss unique characteristics of MRT shared with similarly innovative techniques throughout history, and how the policy debates and eventual formulation of policy for those techniques can be instructive for MRT.

Session Chair:
Jeffrey Kahn, Committee Chair, Johns Hopkins University

12:30 PM	In vitro fertilization (IVF) (including donor gametes) *Nick Hopwood*, University of Cambridge (via WebEx) *Rene Almeling*, Yale University
12:50 PM	Discussion with committee and workshop participants
1:10 PM	Gene transfer in pediatric populations *Benjamin Wilfond*, Seattle Children's Hospital
1:20 PM	Discussion with committee and workshop participants
1:40 PM	Human growth hormone (hGH) use in children *Lainie Ross*, University of Chicago
1:50 PM	Discussion with committee and workshop participants
2:10 PM	Embryo and embryonic stem cell (hES) research *Patricia King*, Georgetown Law
2:20 PM	Discussion with committee and workshop participants
2:40 PM	Public comment period *David McKeon*, New York Stem Cell Foundation (3 min.)
2:43 PM	Break

SESSION IV: SCIENCE AND MEDICINE

Session Objective:

- Discuss key scientific questions regarding MRT and their ethical or social implications.

Session Co-Chairs:
Alan DeCherney, Committee Member, National Institutes of Health
Marni Falk, Committee Member, The Children's Hospital of Philadelphia

3:00 PM	Data on attitudes of women with mtDNA mutations toward MRT *Michio Hirano*, Columbia University Medical Center
3:15 PM	Discussion with committee and workshop participants
3:30 PM	Patient perspective on MRT *Kirah Fasano*, Personal Representative
3:40 PM	Discussion with committee and workshop participants
3:55 PM	Scientific and ethical considerations of mtDNA segregation and the bottleneck phenomenon as it applies to MRT *Eric Shoubridge*, McGill University
4:10 PM	Potential alternative to preventing transmission of mtDNA diseases: Heteroplasmy shift therapy *Carlos Moraes*, University of Miami
4:25 PM	Haplogroup compatibility and how mtDNA can influence traits beyond disease *Doug Wallace*, The Children's Hospital of Philadelphia
4:40 PM	Discussion with committee and workshop participants
5:00 PM	Adjourn day one

April 1, 2015

OPEN SESSION (8:30 AM-12:20 PM)

8:30 AM	Welcome and recap of day one *Jeffrey Kahn*, Committee Chair, Johns Hopkins University

SESSION IV: SCIENCE AND MEDICINE (CONTINUED)

Session Objective:

- Discuss key scientific questions regarding MRT.

Session Co-Chairs:
Alan DeCherney, Committee Member, National Institutes of Health
Marni Falk, Committee Member, The Children's Hospital of Philadelphia

8:45 AM Practical challenges of implementing MRT and potential effects on outcomes
Jacques Cohen, Reprogenetics, LLC

9:00 AM Discussion with committee and workshop participants

9:15 AM Consideration of potential epigenetic effects of MRT
George Daley, Boston Children's Hospital

9:30 AM Discussion with committee and workshop participants

SESSION V: CLINICAL INVESTIGATIONS

Session Objectives:

- Discuss the preclinical evidence base necessary to support first-in-human MRT research.
- Consider earlier precedents for the collection of safety and efficacy information for novel techniques (e.g., systematically collecting evidence in surgical innovation or IVF).
- Discuss what an ethical clinical investigation of MRT might look like, and consider the decision milestones that would occur across the evaluation of MRT.

Session Chair:
Jeffrey Botkin, Committee Member, University of Utah

9:45 AM Preclinical evidence base to support an IND for MRT
Wei Liang, U.S. Food and Drug Administration
John Gearhart, University of Pennsylvania
Insoo Hyun, Case Western Reserve University

10:15 AM	Discussion with committee and workshop participants
10:30 AM	Designing a systematic investigation of MRT techniques *Steven Goodman*, Stanford Medical School (via WebEx) *Doug Turnbull*, University of Newcastle upon Tyne (via WebEx)
10:50 AM	Discussion with committee and workshop participants
11:05 AM	Designing an ethically acceptable investigation of MRT in the United States *Rebecca Dresser*, Washington University in St. Louis *Robert Nelson*, U.S. Food and Drug Administration
11:25 AM	Discussion with committee and workshop participants
11:40 AM	Toleration of uncertainty for new reproductive technologies such as MRT *Aaron Kesselheim*, Harvard Medical School/Brigham and Women's Hospital *John Robertson*, University of Texas, Austin
12:00 PM	Discussion with committee and workshop participants
12:15 PM	Public comment period *Brendan Foht*, The New Atlantis (3 min.) *Rick Leach*, World Food Program USA (3 min.)
12:21 PM	Adjourn open session/committee convenes in closed session

April 1, 2015

CLOSED SESSION (12:20-5:00 PM)

Meeting 3: May 18-20, 2015

The National Academies
Keck Center—Room 103
500 Fifth Street NW
Washington, DC 20001

May 18, 2015
CLOSED SESSION (8:30 AM-5:00 PM)

May 19, 2015
CLOSED SESSION (8:30-10:00 AM)

May 19, 2015
OPEN SESSION (10:00-10:30 AM)

10:00 AM **Opening Remarks to Public Audience**
Jeffrey Kahn, Committee Chair, Johns Hopkins University

Pre-registered public commenters:
David Prentice, Charlotte Lozier Institute
Brian Niland, Personal Representative
Jaycee Hanson, International Center for Technology Assessment
Suzanne Scheller, Pope Paul VI Institute for the Study of Human Reproduction

~10:30 AM Adjourn open session

May 19, 2015
CLOSED SESSION (10:30 AM-5:00 PM)

May 20, 2015
CLOSED SESSION (8:00-11:00 AM)

Appendix B

Summary of MRT Research

The following tables are a compilation of selected maternal spindle transfer (MST) (see Table B-1) and pronuclear transfer (PNT) (see Table B-2) studies. Study endpoints, materials and methods, and results are highlighted. The data are listed as presented in the respective publications, with no further calculation or interpretation by the committee.

TABLE B-1 Summary of MST Research

Study/Model	Materials and Methods
Wang et al. (2001) Mouse (Kunming and C57BL/6J)	• Enucleation in 3 percent sucrose • Transfer of C57BL/6J spindle-chromosome complexes to Kunming enucleated oocytes • 142 oocyte-karyoplast reconstructed pairs fused by 1-3 rounds of electrofusion • 11 fused oocytes used in IVF • Transfer of eight 1-4 cell stage embryos into two foster mothers
Tachibana et al. (2009) (Oregon Health & Science University [OHSU] Group) Nonhuman primate (rhesus macaques)	• 15 MST embryos transferred into 9 females: 6 with 1-2 blastocysts, 3 with 2 cleavage stage (4-8 cell) embryos
Lee et al. (2012) (OHSU Group) Nonhuman primate (rhesus macaques)	• mtDNA copy number in karyoplasts and cytoplasts • 102 MST oocytes generated by MST • Transfer of preselected female embryos; recovery of fetuses preterm (135 days post-embryo transfer)
Tachibana et al. (2013) (OHSU Group) Nonhuman primate (rhesus macaques)	Cryo-thaw MST oocytes: • Transfer of fresh spindle to vitrified cytoplast and vice versa • Implantation of four blastocysts derived by transfer of vitrified spindles into fresh oocyte cytoplasts

Endpoints	Results
• Enucleation • Fertilization (2 pronuclei and extrusion of second polar body) • Embryonic and developmental potential • Nuclear-cytoplasmic relationship	• 100 percent enucleation • 25 pairs (17.6 percent) successfully fused • 9 fused oocytes (82 percent) successfully fertilized • One foster mother (50 percent) delivered two "transfer" pups (C57BL/6J nucleus, Kunming cytoplasm/cellular organelles) • Body weight of transfer offspring was in range for Kunming (oocyte donor) mice
• Visualization and isolation of intact MII spindle-chromosomal complexes • Karyoplast fusion • Developmental potential of embryos • F1 health, mtDNA carryover	• Karyoplasts isolated by polarized microscopy contained approximately 1.5 percent of the volume of cytoplasts • Fusion of karyoplast with SeV prevented premature activation of oocytes • Live birth of four offspring (one set of twins, Mito and Tracker; two singletons, Spindler and Spindy) • ND mtDNA carryover (using assays sensitive to detect >3 percent heteroplasmy)
• mtDNA carryover into karyoplasts • Embryonic developmental potential • Heteroplasmy in somatic tissues of preterm fetus and fetus oocytes, 135 days post-embryo transfer	• 0.6 percent carryover of mtDNA into karyoplast • 62 percent of MST oocytes developed to blastocysts after fertilization • Female MST embryos selected by TE biopsy; two female singleton pregnancies generated from selected blastocysts • mtDNA carryover (ND) or <0.5 percent (cerebrum, heart, and blood in fetus #2) in fetal somatic organs and tissues • 11/12 oocytes in each fetus displayed low (< 5.5 percent) or ND levels of mtDNA heteroplasmy; one oocyte from each fetus contained substantial mtDNA carryover (16.2 percent and 14.1 percent)
• Effect of cryo-thaw on fertilization, embryo development, and live birth following MST in oocytes	Cryo-thaw MST oocytes: • Fresh spindle to vitrified cytoplast: impaired fertilization (50 percent) and blastocyst development (0 percent), compared with 91 percent and 57 percent in controls, respectively • Vitrified spindle to fresh cytoplast: 88 percent fertilization and 68 percent blastocyst rate, similar to control (91 percent and 57 percent, respectively)

TABLE B-1 Continued

Study/Model	Materials and Methods
	2009 rhesus offspring: • Measurement of body weight, bloodwork, and mitochondrial function (birth-3 years)
Tachibana et al. (2013) (OHSU Group) Human oocyte	• 106 donated oocytes: 65 underwent MST, 33 controls • Reciprocal MST followed by ICSI
Paull et al. (2013) (New York Stem Cell Foundation [NYSCF] Group) Human oocyte	• 18 synchronized donated oocytes underwent reciprocal MST • Fusion of spindle-chromosomal complex by SeV or electrical pulse • Parthenogenetically activated
Neupane et al. (2014) Mouse (NZB/OlaHsd and B6D2/F1)	• MST and PNT: transfer of MII-SCC or pronuclei, respectively, from NZB/OlaHsd to B6D2/F1 oocytes and zygotes, respectively

Endpoints	Results
• Overall health and postnatal development of rhesus macaque offspring from 2009 study	• Live birth of a female offspring (Crysta) 2009 rhesus offspring: • Normal development • No change in heteroplasmy in blood and skin samples
• Developmental potential • Establishment and pluripotency of embryonic stem cell (ESC) lines • mtDNA carryover in oocytes and ESCs • Cytogenetic analyses • Efficacy of MST in cryopreserved oocytes	• Significant proportion of MST oocytes (52 percent) showed abnormal fertilization compared with controls (13 percent) • Normally fertilized MST oocytes had statistically significant level of blastocyst development (62 percent) similar to that of controls (76 percent) • Mean mtDNA carryover in MST embryos 0.5 percent • Mean carryover in derived ESC lines 0.6 percent • No structural or numerical chromosomal abnormalities in ESC lines
• Developmental potential of MST embryos • mtDNA copy number and volume in karyoplasts • mtDNA carryover in preimplantation embryos	• Efficient development to blastocyst stage (39 percent), statistically similar to controls (33 percent) • mtDNA copy number in karyoplasts was 0.36 percent of total mtDNA in MII oocytes; corresponded to volume of karyoplasts (0.89 percent of intact MII oocytes) • Mean mtDNA carryover 0.31 percent in preimplantation embryos • Depolimerization via cooling or cryo-thaw prevents premature oocyte activation following fusion by electrical pulse
• mtDNA carryover • Embryonic developmental competence	• Parthenogenesis: NS difference in fusion, reconstruction, two-cell and blastocyst formation rate between activated MII and control groups; blastocyst quality similar to that of controls • ICSI: NS difference in ICSI survival, 8-16 cell embryo formation between fertilized MII and control groups; no blastocyst formation in either group

TABLE B-1 Continued

Study/Model	Materials and Methods
Wang et al. (2014) Mouse	• Developmental potential (in vitro and in vivo) • mtDNA carryover (F1 and F2 generations)
Newcastle group (unpublished)[a] Human oocyte	*[In progress]*

[a] Human Fertilisation and Embryology Authority (HFEA). 2014. Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2014 update.

NOTE: ESC = embryonic stem cell; F1 = first generation; F2 = second generation; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; MII = metaphase II; MST = maternal spindle transfer; mtDNA = mitochondrial DNA; ND = non-detectable; NS = non-significant; PNT = pronuclear transfer; SeV = Sendai virus; TE = trophoectoderm.

Endpoints	Results
	MST versus PNT • NS difference in successful fusion, cleavage rate, and blastocyst formation rate between MST (parthenogenetic) and PNT • NS difference in mean mtDNA carryover: – MST oocytes: 0.29 percent (ND in 17/21, <2.15 percent in 4/21) – PNT zygotes: 0.29 percent (ND in 21/25, <2.6 percent in 4/25)
• 27 metaphase II spindle-chromosomal complexes (MII-SCCs) transferred • 18 MST embryos transferred to pseudopregnant females • mtDNA carryover: tail tip/brain tissue and internal organs (F1) and toe tips (F2)	• 85.7 percent developed to blastocyst • 44.4 percent live, healthy births • 5.5 percent mtDNA carryover (F1 tail tip/brain) • ND-6.88 percent mtDNA carryover (F1 internal organs) • ND-7.1 percent mtDNA carryover (F2 tail tip)
[In progress]	*[In progress]*

TABLE B-2 Summary of PNT Research

Study/Model	Materials and Methods
McGrath and Solter (1983) Mouse • C3H/HeJ • C57BL/6J • (ICR)	• Transfer of male and female pronuclei in karyoplast from zygote to enucleated zygote of genetically distinct substrain • Development of reconstructed zygote in vitro to day 5 morula of blastocyst • Transfer of reconstructed (64) and control (34) zygotes to pseudopregnant females
Sato et al. (2005) Mito-mouse (ΔmtDNA): C57BL/6J (B6) with 4696-bp deletion Normal control: C57BL/6J (B6)	• Transplantation of both pronuclei in karyoplast from mito-mouse zygote to enucleated normal zygotes • Avg. ΔmtDNA levels in zygotes estimated by second polar body biopsy • 39 PNT zygotes, ΔmtDNA/total mtDNA estimated to be 17-53 percent (average 25 percent), transferred into two pseudo-pregnant females • 34 non-PNT zygotes (est. 11-47 percent ΔmtDNA; avg. 32 percent) implanted in two pseudo pregnant females
Craven et al. (2010) (Newcastle Group) Human zygotes (abnormally fertilized [unipronuclear/ tripronuclear])	• 1 or 2 pronuclei transferred from abnormally fertilized zygote to enucleated recipient zygote • Monitored 6-8 days in vitro for embryonic developmental potential • Optimized procedure to minimize cytoplasm carried in karyoplast • mtDNA carryover measured in blastomeres • Total mtDNA copy number in oocytes

Endpoints	Results
• Embryonic development • Live birth of offspring	• Overall efficacy of enucleation and fusion of the pronuclei to enucleated zygote: 91 percent • 96 percent of PNT zygotes developed to morula or blastocyst at day 5 (versus 100 percent in nonmanipulated controls) • Live birth of 10 PNT offspring (16 percent) compared with 5 control offspring (15 percent); 7/10 PNT offspring survived to adulthood compared with 3/5 control offspring • Coat color phenotype of PNT offspring was that of the nuclear donor • 5/7 PNT offspring surviving to adulthood were fertile (no control value given)
• Rescue from disease phenotype • mtDNA carryover • Change in ΔmtDNA levels during embryogenesis, postnatal development and aging	• 11 mice born following transfer of PNT embryos (compared to 9 in controls) • Avg. carryover of ΔmtDNA in PNT mice: 11 percent at weaning (range 6-21 percent), increased to 33 percent +300 days from weaning (range 5-44 percent); estimated to be 43 percent at 800 days • Avg. ΔmtDNA levels in non-PNT mice: 66 percent at weaning (range 51-73 percent), 80 percent at +170 days • PNT offspring rescued from disease phenotypes: all (11) PNT mice survived >300 days after birth; comparable weight gain, no observed renal abnormalities, steady blood lactate and urea levels compared with normal controls • Non-PNT mice died at 218-277 days; exhibited renal abnormalities, elevated blood lactate and urea levels, decreased weight gain compared with normal control mice
• mtDNA carryover • Embryonic developmental potential	• 22.7 percent and 22.2 percent of 1- and 2-pronuclei transfer PNT zygotes, respectively, developed past 8-cell stage; 8.3 percent to blastocyst stage (50 percent of nonmanipulated, abnormally fertilized controls) • Optimized PNT to reduce cytoplasm volume: mtDNA carryover ND in 4 of 9 embryos; remaining five embryos: average <2 percent mtDNA carryover (range in blastomeres: ND-11.4 percent) • Range of mtDNA copy number in oocytes: approx. 100,000-850,000

TABLE B-2 Continued

Study/Model	Materials and Methods
Neupane et al. (2014) Mouse (NZB/OlaHsd & B6D2/F1)	• MST and PNT: transfer of MII-SCC and pronuclei, respectively, from NZB/OlaHsd to B6D2/F1 oocytes and zygotes, respectively
Wang et al. (2014) Mouse	• 38 PNT zygotes reconstructed • 13 PNT embryos transferred to pseudopregnant females • mtDNA carryover: examined in tail tip/brain tissue and internal organs (F1) and toe tips (F2)
Newcastle group, (unpublished)[a]	[Unavailable]

[a] Human Fertilisation and Embryology Authority (HFEA). 2014. Third scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2014 update.

NOTE: ΔmtDNA = mitochondrial DNA deletion; F1 = first generation; F2 = second generation; MII-SCC = metaphase II-spindle chromosome complex; MST = maternal spindle transfer; mtDNA = mitochondrial DNA; ND = non-detectable; NS = non-significant; PNT = pronuclear transfer.

Endpoints	Results
• mtDNA carryover • Embryonic developmental competence	• Blastocyst quality similar to that of controls • 1/8 blastomeres from cleavage stage embryos presented with 4.9 percent mtDNA carryover (7/8 ND); sensitivity of assay not disclosed MST versus PNT • NS difference in successful fusion, cleavage rate, and blastocyst formation rate between MST (parthenogenetic) and PNT • NS difference in mean mtDNA carryover: – MST oocytes: 0.29 percent (ND in 17/21, <2.15 percent in 4/21) – PNT zygotes: 0.29 percent (ND in 21/25, <2.6 percent in 4/25)
• Developmental potential • mtDNA carryover (F1 and F2 generations)	• 81.3 percent developed to blastocyst • 53.8 percent live, healthy births • 23.7 percent mtDNA carryover (F1 tail tip/brain) • 5.5-39.8 percent mtDNA carryover (F1 internal organs) • 22.1 percent mtDNA carryover (F2 toe tip)
• mtDNA carryover • Chromosomal makeup	• High rates of development to blastocyst stage • Subtle differences in embryo development

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Appendix C

Biosketches of Committee Members

Jeffrey P. Kahn, Ph.D., M.P.H. (*Chair*), is Robert Henry Levi and Ryda Hecht Levi professor of bioethics and public policy at the Johns Hopkins Berman Institute of Bioethics. He works in a variety of areas of bioethics, exploring the intersection of ethics and health and science policy, including human and animal research ethics, ethics and public health, and ethical issues in emerging biomedical technologies. Professor Kahn is co–principal investigator on a National Institutes of Health (NIH) Center of Excellence project studying the ethical, legal, and social implications of genomic research in the context of infectious disease. He was founding president of the Association of Bioethics Program Directors, a position he held from 2006 to 2010; is an elected fellow of The Hastings Center; and is currently chair of the National Academies of Sciences, Engineering, and Medicine's Board on Health Sciences Policy. Professor Kahn has published 3 books and more than 115 articles, and speaks frequently across the United States and around the world on a range of bioethics topics. Prior to joining the faculty at Johns Hopkins, he was director of the Center for Bioethics at the University of Minnesota. His education includes a B.A. in microbiology from the University of California, Los Angeles (UCLA), a Ph.D. in philosophy from Georgetown University, and an M.P.H. from the Johns Hopkins Bloomberg School of Public Health.

Jeffrey R. Botkin, M.D., M.P.H., is professor of pediatrics at the University of Utah and adjunct professor of human genetics. He is chief of the Division of Medical Ethics and Humanities and serves as associate vice president for research integrity, with oversight responsibilities for the human

subjects protection program. Dr. Botkin received his undergraduate degree from Princeton University, his M.D. from the University of Pittsburgh, and his M.P.H. from Johns Hopkins University. His research is focused on the ethical, legal, and social implications of genetic technology, with a particular emphasis on research ethics, genetic testing for cancer susceptibility, biobanking, newborn screening, and prenatal diagnosis. Dr. Botkin is currently chair of the U.S. Department of Health and Human Services (HHS) Secretary's Advisory Committee on Human Research Protections. He also is a member of the Secretary's Advisory Committee on Heritable Diseases in Newborns and Children and a former chair of the Committee on Bioethics for the American Academy of Pediatrics. He chairs NIH's Embryonic Stem Cell Eligibility Working Group and serves on the U.S. Food and Drug Administration's (FDA's) Pediatric Ethics Subcommittee. Dr. Botkin is an elected Fellow of The Hastings Center.

David C. Chan, M.D., Ph.D., is professor of biology at the California Institute of Technology. The primary focus of Dr. Chan's research is on understanding the role of mitochondrial dynamics in normal cellular function and human disease, particularly neurological disorders. Stemming from this work, he is a member of NIH's Membrane Biology and Protein Processing Study Section. He has also served on NIH's Structure and Function Study Section, on the Scientific and Medical Advisory Board of the United Mitochondrial Disease Foundation, and as co-chair of the Keystone meeting "Mitochondrial Dynamics and Physiology." Dr. Chan has received numerous awards in his academic career, including Howard Hughes Medical Institute Investigator, Mitochondrial Research Society Young Investigator, Ellison Medical Foundation Senior Scholar, Beckman Young Investigator, Rita Allen Foundation Scholar, and Bren Scholar. He was awarded the Burroughs Wellcome Fund Career Award in the Biomedical Sciences. Dr. Chan received his M.D./Ph.D. at Harvard Medical School under Philip Leder. After Harvard, he went on to perform postgraduate work with Peter Kim at the Whitehead Institute for Biomedical Research, work that clarified how the HIV1 virus enters human cells.

R. Alta Charo, J.D., is Warren P. Knowles professor of law and bioethics at the University of Wisconsin–Madison, where she is on the faculty of the law and medical schools. Professor Charo teaches in the areas of bioethics, public health law, and biotechnology policy, and has been a member of the university's institutional review board (IRB) and clinical ethics committee. She served on President Obama's transition team, focused particularly on transition issues related to NIH and FDA, and from 2009 to 2011 was on leave to serve as a senior policy adviser on emerging technology issues in the Office of the Commissioner at FDA. Ms. Charo's advisory committee

service for the federal government includes the 1994 NIH Human Embryo Research Panel and President Clinton's National Bioethics Advisory Commission (1996 to 2001). In 2006 she was elected to membership in the National Academy of Medicine, where she now serves on its Council. At the National Academies of Sciences, Engineering, and Medicine, she has been a member of the Board on Life Sciences, Board on Population Health and Public Health Practices, and the Board on Health Sciences Policy. Professor Charo co-chaired the committee on guidelines for embryonic stem cell research, and is now co-chair of the committee on human gene editing. She received her B.A. in biology from Harvard University in 1979 and her J.D. from Columbia University in 1982.

James Childress, Ph.D., is university professor and John Allen Hollingsworth professor of ethics at the University of Virginia, where he directs the Institute for Practical Ethics and Public Life. At the University of Virginia, he is also professor of religious studies in the College and Graduate School of Arts and Sciences and professor of research in medical education in the School of Medicine. His research interests include theory and method in biomedical ethics and the role of biomedical ethics in public policy. He is a member of the National Academy of Medicine, where, among other activities, he previously chaired the Health Sciences Policy Board, the Institute of Medicine (IOM) Committee on Increasing the Rates of Organ Donation (2006), and the IOM Planning Committee for a Workshop on Military Medical Ethics: Issues Regarding Dual Loyalties (2008). Dr. Childress was vice chair of the National Task Force on Organ Transplantation, and also served on the Board of Directors of the United Network for Organ Sharing (UNOS), the UNOS Ethics Committee, the Biomedical Ethics Advisory Committee, the NIH Recombinant DNA Advisory Committee, and several data and safety monitoring boards for NIH clinical trials. In 1996, President Clinton appointed him to the National Bioethics Advisory Commission. Dr. Childress is also a fellow of the American Academy of Arts and Sciences, as well as of The Hastings Center, and he has been Joseph P. Kennedy Sr. professor of Christian ethics at the Kennedy Institute of Ethics at Georgetown University. He received his B.A. from Guilford College, his B.D. from Yale Divinity School, and his M.A. and Ph.D. from Yale University.

Alan DeCherney, M.D., received his bachelor's degree in natural sciences from Muhlenberg College in Allentown, Pennsylvania, where he served on the Board of Trustees from 2006 to 2010. He received his M.D. from Temple University School of Medicine. He also holds an honorary master of arts degree from Yale University. Dr. DeCherney performed a research fellowship in immunology at the Lister Institute in London, England, as well as an internship in medicine at the University of Pittsburgh, followed

by a residency in obstetrics and gynecology at the University of Pennsylvania. He is currently associate clinical director and branch chief for reproductive endocrinology and gynecology in the intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) at NIH. Previously, he was John Slate Ely professor of obstetrics and gynecology at Yale and division director of reproductive endocrinology and infertility and women's health services and Phaneuf professor and chair of obstetrics and gynecology at Tufts University School of Medicine from 1991 to 1996. He was director of the Division of Reproductive Endocrinology at the David Geffen School of Medicine at UCLA from 1996 to 2006 and was chair of the Department of Obstetrics and Gynecology from 1996 to 2002. Dr. DeCherney is a fellow of the American College of Obstetricians and Gynecologists; past president of the American Society for Reproductive Medicine; and past president of the Society for Reproductive Endocrinology and Infertility, the Society of Reproductive Surgeons, and the Society of Assisted Reproductive Technology. He is a member of the American Gynecological and Obstetrical Society and past president of the Society for Gynecologic Investigation. He is the recipient of the President's Achievement Award of the Society of Gynecologic Investigation. Dr. DeCherney was former editor-in-chief of the journal *Fertility and Sterility*, an associate editor and editorial board member of the *New England Journal of Medicine*, and a member of the Editorial Board of *Obstetrics and Gynecology*. He served as a member of the American Board of Obstetrics and Gynecology and the Division of Reproductive Endocrinology and Infertility and is a fellow ad eundem of the Royal College of Obstetrics and Gynecology. He has been a National Academy of Medicine member since 2004, and he served as chair of the IOM Interest Group on Maternal & Child & Human Development.

Marni J. Falk, M.D., received her B.S. degree in biology and her medical degree in a combined 7-year program from the George Washington University School of Medicine. She then completed dual specialty training in a combined 5-year pediatrics and clinical genetics residency program at Case Western Reserve University and University Hospital of Cleveland in Cleveland, Ohio. She has been assistant professor since 2006 in the Division of Human Genetics in the Department of Pediatrics at The Children's Hospital of Philadelphia (CHOP) and University of Pennsylvania (UPenn) Perelman School of Medicine. Board certified in clinical genetics and pediatrics, Dr. Falk established and directs the CHOP Mitochondrial-Genetics Diagnostic Clinic to aid in the evaluation and management of individuals of all ages with suspected mitochondrial disease. She is actively involved in developing improved diagnostic approaches and resources for mitochondrial disease, including organization of a global Mitochondrial Disease Sequence

Data Resource (MSeqDR) consortium. Dr. Falk is principal investigator for an NIH-funded translational research laboratory at CHOP that investigates the causes and global metabolic consequences of mitochondrial disease, as well as targeted pharmacologic therapies, in *C. elegans*, zebrafish, mouse, and human tissue models of genetic and pharmacologic-based respiratory chain dysfunction. She has authored more than 70 publications in the areas of human genetics and mitochondrial disease. Dr. Falk also organized and directs the CHOP/Penn Mitochondria Research Affinity Group. She is a member and former chair of the Scientific and Medical Advisory Board and former Board of Trustees member of the United Mitochondrial Disease Foundation; a member of the Scientific Advisory Board of the nonprofit Genesis Project; a founding member of the CHOP Center for Mitochondrial and Epigenomic Medicine; CHOP-site principal investigator for the North American Mitochondrial Disease Consortium; a member of the Mitochondrial Congressional Caucus, Mitochondrial Medicine Society, Mitochondrial Research Society, Society for Pediatric Research, Society of Inherited Metabolic Disease, and American Society of Human Genetics; and a fellow of the American College of Medical Genetics and Genomics.

Jonathan Kimmelman, Ph.D., holds a doctorate in molecular biophysics and biochemistry from Yale University and is associate professor in biomedical ethics at McGill University, with a cross-appointment in experimental medicine. His research centers on the ethics of translational clinical research. He leads several funded projects investigating risk-benefit across the research trajectory, and directs the Studies for Translation, Ethics, and Medicine (STREAM) Group. Major publications have appeared in *Science*, *Lancet*, *British Medical Journal*, *PLoS Medicine*, and *Hastings Center Report*. His book *Gene Transfer and the Ethics of First-in-Human Experiments* (Cambridge Press, 2010) is the first full-length analysis of the ethics of translational clinical research and has been described as "set[ting] a new standard for bioethical scholarship that is at once scientifically well-grounded, politically astute, philosophically original, and a pleasure to read." Dr. Kimmelman was the winner of the 2006 Maud Menten New Investigator Prize (Institute of Genetics), received a Canadian Institutes of Health Research New Investigator Salary Award in 2008, and was a Humboldt-Bessel Award Winner in 2014. He has served in numerous advisory capacities, including as ethics committee chair for the American Society of Gene and Cell Therapy (2008-2010) and the International Society of Stem Cell Research (since 2013). He is a member of the National Heart, Lung, and Blood Institute Gene and Cell Therapy data safety monitoring board.

Anna C. Mastroianni, J.D., M.P.H., is professor of law at the University of Washington School of Law. She holds additional faculty appointments in

the university's School of Public Health and School of Medicine and at the Treuman Katz Center for Pediatric Bioethics at Seattle Children's Hospital. Prior to her academic career, she held a number of legal and federal policy positions in Washington, DC, including staff leadership positions with a presidential commission and the IOM, and served as a practicing attorney with law firms specializing in health law. She has served on a number of committees that advise the U.S. government and other entities, including the National Academies of Sciences, Engineering, and Medicine. She also served on the NIH Recombinant DNA Advisory Committee. She has been nationally recognized for her contributions to health policy, law, and bioethics as a fellow of the American Association for the Advancement of Science. Her publications include six books and numerous peer-reviewed articles on law, medicine, and bioethics, with a special emphasis on the legal and ethical challenges in public health, research with human subjects, and assisted reproductive technologies. Professor Mastroianni is a graduate of the University of Pennsylvania's School of Law (J.D.), The Wharton School (B.S), the College of Arts and Sciences (B.A.), and the University of Washington School of Public Health (M.P.H.).

Vamsi K. Mootha, M.D., is an investigator for the Howard Hughes Medical Institute, professor of systems biology and medicine at Harvard Medical School, and institute member of the Broad Institute. He runs a research laboratory located dually at Massachusetts General Hospital and the Broad Institute. Dr. Mootha's research is focused primarily on the mitochondrion, the "powerhouse of the cell," and its role in human disease. During the past decade, his research team has applied the new tools of genomics and systems biology to dissect the organelle's physiology in health and in disease. His team has characterized the mitochondrial proteome, identified transcriptional circuits controlling the organelle's biogenesis, discovered the molecular identity of the mitochondrial calcium uniporter, and identified more than one dozen Mendelian disease genes. Dr. Mootha received his undergraduate degree in mathematical and computational science at Stanford University, where he graduated Phi Beta Kappa with highest honors. He received his M.D. in 1998 from Harvard Medical School in the Harvard-Massachusetts Institute of Technology (MIT) Division of Health Sciences and Technology, where his thesis work was focused on mitochondrial bioenergetics. He subsequently completed his internship and residency in internal medicine at Brigham and Women's Hospital in 2001, after which he completed postdoctoral fellowship training at the Whitehead Institute/MIT Center for Genome Research from 2001 to 2004. He has received numerous honors, including a MacArthur Foundation Fellowship, election to the U.S. National Academy of Sciences, and a 2014 Padma Shri from the Government of India.

Laurie Strongin is founder and executive director of the Washington, DC-based Hope for Henry Foundation, which improves the quality of life of children with cancer and other serious illnesses at hospitals in Washington, DC, and around the country. Since 2003, Hope for Henry has served more than 14,000 children. Ms. Strongin also acts as a family advocate in the national discussion of ethics and genetics. For 25 years, she has helped draw attention and resources to issues of emerging national significance. In 1996, she became drawn through personal experience into the frontlines of a breakthrough medical procedure that held the promise of saving her son, among countless other children. Turning her advocacy and media skills to that issue, she participated in national medical policy panels; worked with then-House Democratic Leader Nancy Pelosi and Congresswoman Diana DeGette to urge Senate passage of the Stem Cell Research Enhancement Act; secured coverage of the issue on ABC's *Nightline* and in a Sunday *New York Times Magazine* cover story; authored "Vetoing Henry," a *Washington Post* op-ed criticizing President Bush's 2006 veto of federal funding for human embryonic stem cell research; and advocated for parental perspectives on NBC, the CBS *Early Show*, and MSNBC. Ms. Strongin has also participated as a panelist and guest lecturer in forums hosted by the Johns Hopkins Genetics and Public Policy Center. In 2009, when President Obama lifted the ban on federal funding for stem cell research, she was one of a few dozen honored guests. Her subsequent memoir, *Saving Henry* (Hyperion 2010), has been featured on a number of television programs and in *USA Today* and *The Washington Post*. Since the book's publication, Ms. Strongin has headlined more than 60 speaking engagements across the United States.

Keith A. Wailoo, Ph.D., is Townsend Martin professor of history and public affairs at Princeton University. He is appointed in history and in the Woodrow Wilson School of Public and International Affairs, where he also served as vice dean from 2013 to 2015. He is a historian of medicine and the biomedical sciences and is the author of many books, including *Pain: A Political History*; *How Cancer Crossed the Color Line*; *The Troubled Dream of Genetic Medicine: Ethnicity and Innovation in Tay-Sachs, Cystic Fibrosis, and Sickle Cell Disease*; *Dying in the City of the Blues: Sickle Cell Anemia and the Politics of Race and Health*; and *Drawing Blood: Technology and Disease Identity in Twentieth Century America*. Dr. Wailoo has also organized and edited numerous interdisciplinary studies on contemporary health and public policy, including *Medicare and Medicaid at 50: America's Entitlement Programs in the Age of Affordable Care*; *Three Shots at Prevention: The HPV Vaccine and the Politics of Medicine's Simple Solutions*; and *Genetics and the Unsettled Past: The Collision of DNA, Race, and History*. His writings have also appeared in *Lancet*; *The New*

York Times; *American Prospect; The Journal of Health Politics, Policy, and Law*; and the *Bulletin for the History of Medicine*. In 2007, he was elected to the National Academy of Medicine, where he was also a member of the Health Sciences Policy Board and served on the Committee on Increasing Rates of Organ Donation. His research has been supported by the Robert Wood Johnson Foundation, NIH, the National Science Foundation, the James S. McDonnell Foundation, and the Burroughs-Wellcome Fund. At Princeton, Dr. Wailoo teaches on the history of race, drugs and drug policy, modern genetics and public policy, and a range of other topics in history and health policy. Before joining the Princeton faculty, he taught in history and in social medicine (in the medical school) at the University of North Carolina at Chapel Hill and at Rutgers University, where he was founding director of the Center for Race and Ethnicity. Dr. Wailoo holds a Ph.D. in the history and sociology of science from the University of Pennsylvania and a bachelor's degree in chemical engineering from Yale University.