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Crossing the Germline Barrier: The Three Genome Baby

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ABSTRACT: The moral boundary separating somatic cell genetic modification and germline genetic modification has been in effect for over 30 years. Recently, with the introduction of nuclear DNA transplantation and gene editing, and with the rising interest in preventing the transfer of certain deleterious genes to newborns, that moral boundary has been called into question. The paper discusses the origins of the somatic cell and germline moral distinction and explores new initiatives to undertake genetic modification of gametes or embryos.

KEY WORDS: gene editing; germline; somatic cell genetic modification; Asilomar; CRISPR; ooplasmic transfer; mitochondrial disease; nuclear genome transplantation

I. INTRODUCTION

A. History of the Ethics of Germ-Line Gene Therapy/Enhancement

In June of 1980, three leading American clergy wrote a letter to US President Jimmy Carter requesting that he authorize a broad review of genetic experimentation on humans. Dr. Claire Randall, General Secretary of the National Council of Churches; Rabbi Bernard Mandelbaum, General Secretary of the Synagogue Council of America; and Bishop Thomas Kelly, General Secretary of the United States Catholic Conference issued their concern to the President: "Who shall control genetic experimentation and its results which could have untold implications for human survival. Who shall benefit and who shall bear any adverse consequences directly or indirectly?"[1]

It was seven years after Stanley Cohen and Herbert Boyer published the seminal paper that transformed molecular genetics and jump-started the era of gene splicing.[2] In addition, it was only five years after the Asilomar Conference, where scientists worked out a preliminary plan for establishing laboratory practices for the newly discovered recombinant DNA technology.

As a response to this influential letter, President Jimmy Carter established the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. He appointed Morris Abram as its chair. Mr. Abram was the son of a Romanian immigrant and German mother who grew up in a small town in Georgia. He attended the University of Georgia, received a law degree at University of Chicago, and received a master's degree from Oxford University. Among his many appointments, he served on the staff of prosecutors at the International Military Tribunal at Nuremberg

and was President of Brandeis University. When his report was completed on November 16, 1982, he noted in his letter to President George Bush, "The Commission believes that it would be wise to have engaged in careful prior thought about steps such as treatments that can lead to heritable changes in human beings or those intended to enhance human abilities rather than simply correct deficiencies caused by well-defined genetic disorders."[1]

The report distinguished between genetic engineering of somatic cells and genetic engineering of zygotes (fertilized eggs) or germ cells. In its findings, the commission wrote, "especially close scrutiny is appropriate for any procedures that would create inheritable genetic changes; such interventions differ from prior medical interventions that have not altered the genes passed onto patients' offspring."[1]

This document set the stage for over 30 years of policy on human genetic engineering. During that period there was an implicit consensus within the scientific and regulatory communities that somatic cell gene therapy was acceptable medical experimentation once it was approved by federal authorities and ethics committees, but that genetically modifying the germ cells was out of bounds. It was a kind of Maginot Line that was widely publicized in science magazines and the media to reduce public concerns about eugenics. And by and large it worked.

Science magazine carried a news story in June 1983 with the headline, "Clerics Urge Ban on Altering Germline Cells." A resolution against germline genetic modification was circulated to religious leaders by activist Jeremy Rifkin. *Science* reported, "The leaders of virtually every major church group in the United States have signed a resolution calling for a ban on genetic engineering of human reproductive cells."[2]

Although human germline investigations were first considered *verboten*, human somatic cell gene therapy was launched as a new subfield of clinical medicine. The first human experiments were performed outside the United States in Israel and Italy by an American scientist.

Martin Cline, a professor of medicine at UCLA with postdoctoral training in hematology-oncology, did not wait until the United States established regulations on somatic cell gene therapy. During the summer of 1980 he oversaw the transplantation of genetically engineered cells into the bone marrow of two women, a 16-year-old Italian and 21-year-old Israeli. The experiments were approved by hospitals in their respective countries. Both women had a genetic blood disease called beta thalassemia major. The experiment involved extracting their bone marrow cells that were then treated and corrected with a normal hemoglobin gene. Their genetically modified cells were then transplanted back into the women.

After an investigation by the National Institutes of Health (NIH) into Dr. Cline's experiment, it was found that he violated NIH rules, because he altered an approved protocol without permission.[4] Dr. Cline was cited as failing "to disclose to the IRB [Institutional Review Board] at Hadassah Hospital in Israel, or to the patients in Israel and Italy, that the bone marrow transplants would contain recombinant DNA material, despite the fact that the review board at Hadassah went to considerable lengths to verify that the procedure would not involve recombinant DNA."[5] According to the Department of Health and Human Services'

report, Dr. Cline used a procedure that was submitted to the IRB at University of California at Los Angeles (UCLA) in May 1979 and disapproved on July 16, 1980. The reviewers asked for more animal studies before using the procedures on humans.[5] Although no NIH funds were used to perform the human experiments in Israel and Italy, the materials used were prepared at UCLA under support from NIH. An ad hoc committee of the NIH found that Dr. Cline's activities violated both the NIH guidelines on the use of recombinant DNA and DHHS's regulations for the protection of human subjects. Dr. Cline was censured by UCLA and resigned his position as Chief of Hematology and Oncology.[5] The NIH issued sanctions against Dr. Cline that included terminating or not reviewing some of his grants and placing him under special oversight for future research.

Human gene therapy using viruses was already tried to cure genetic diseases before the discovery of rDNA. It began with the work of Renato Dulbecco, who discovered that viruses can transfer foreign genetic information into cells that they infect, that the viral genes get integrated into the genome of the cells and can be functional by expressing their proteins. Dulbecco's work was picked up by Stanfield Rogers at Oak Ridge National Laboratory. Rogers worked on modifying a viral genome and using it as a vector to carry new genes into a tobacco plant. The next step was to find a human application. Rogers teamed up with a German scientist H.G. Terheggen, who identified three children in a West German family who were afflicted with a life-threatening genetic disease called hyperargininemia.[6] The children were unable to make the enzyme arginase, which the body uses to break down the amino acid arginine. The accumulation of arginine in the blood results in mental retardation, developmental delays, and seizure disorders. Two of the three children, a 2-year-old girl and a 7-year-old girl became experimental subjects. The Shope papilloma virus was found to contain the blueprint for synthesizing the missing enzyme. The virus was purified by Rogers at Oak Ridge and injected into the girls. One review noted, "Shope papilloma virus was referred to by the investigators as a "wart" virus, a terminology that may have been reasonably appropriate for the rabbit skin but that may have disguised its potential adverse effects in human, despite the reported benign effects on inadvertently infected laboratory workers. Even so, occasional rabbit skin papillomas were known to undergo malignant transformation and the use of a known tumorigenic virus in any human study had a strong sense of prematurity."[7] In 1975, Terheggen et al. authored a paper describing the failure of the trial to repair arginase deficiency in the children.[7]

The first US-authorized human gene therapy clinical trial was approved in 1990 to treat a young girl with adenosine deaminase (ADA) deficiency, an inherited gene defect resulting in severe combined immune deficiency. Hereafter, the field grew rapidly. In 1990 there were 175 articles cited in MEDLINE that mentioned "human gene therapy." By 2000 that number rose to 1,500. The total number of articles mentioning "human gene therapy" in MEDLINE from 1990 to 2004 was 12,576. In 2003 there were 918 gene therapy trials conducted worldwide. By 2004 the United States had approved 613 gene therapy trials. From 1998 to 2003 between 1,500 and 1,900 NIH grants per year with the term "gene therapy" in the titles or abstracts were awarded.[8] There were nine journals dedicated to human gene therapy in 2005, the first being *Human Gene Therapy*, founded in 1990.

The distinction between somatic cell gene therapy (SCGT) and germline gene therapy (GLGT) was solidly set—at least initially. Alexander Capron, a member of the "splicing life" commission gave a justification for the somatic-germline boundary. "The major reason for drawing a line between somatic-cell and germ-line interventions…are that germ-line changes not only run the risk perpetuating any errors made into future generations of nonconsenting 'subjects' but also go beyond ordinary medicine and interfere with human evolution." Capron acknowledged that somatic medicine also interfered with "natural evolution," but unlike germline genetic engineering, which is intentional, the effect of somatic genetic modification on natural evolution is inadvertent.[9]

A background paper on gene therapy prepared by the congressional Office of Technology Assessment (OTA) in 1984 also emphasized the ethical differences between somatic and germline genetic modifications. "The different social and ethical considerations that arise from somatic versus germ cell manipulations are elaborated further in the sections below on medical and social aspects of gene therapy…The consensus about the propriety of somatic cell therapy does not extend to treatment for traits that do not constitute severe genetic diseases, and does not encompass germ line gene therapy in humans. The question of whether germ line gene therapy should ever begin is now highly controversial. The risk to progeny, relative unreliability of the techniques for clinical use, and ethical questions about when to apply it remain unresolved. The question of whether and when to begin germ line gene therapy must therefore be decided in public debate informed by technological developments."[10]

The NIH gave its Recombinant DNA Advisory Committee (RAC) first line authority to review any proposals involving human genetic modification. The RAC established "Points to Consider" for setting its internal guidelines that would define the proposals it would accept. In its Points to Consider the RAC and its working group on human gene therapy stated it would not, at the time, entertain proposals for germline alterations but agreed to review proposals for somatic cell gene therapy.[11] Moreover, the RAC reported that it would not consider germline therapy protocols "until somatic cell therapy has progressed and public discussion of the implications of germ line work has broadened."[12] The boundary was supported by a number of public interest groups including the Committee for Responsible Genetics (hereafter Council for Responsible Genetics), which formed in 1983 and had an advisory board of distinguished scientists and public health advocates.

The term *human germline genetic modification*, also known as *germline genetic therapy* (GLGT), has been defined as "techniques that would attempt to create a permanent inheritable (i.e., passed from one generation to the next) genetic change in offspring and future descendants by altering the genetic makeup of the human germline, meaning eggs, sperm, the cells that give rise to eggs and sperm, or early human embryos."[13]

The distinction made between SCGT and GLGT was well received in the scientific community as well as the public arena. It gave scientists a morally justifiable path to proceed with human clinical trials for SCGT distancing it from eugenics, while keeping GLGT in abeyance unless or until a public consensus could be reached. However, some scientists were not satisfied with a total ban on GLGT. So they began parsing the term by

distinguishing therapy from enhancement. Eliminating a disease gene from the human zygote was considered a part of the beneficence objective of medicine, namely curing or preventing disease. Enhancement was seen as a form of medical hubris, outside the traditional ethical imperatives of medicine. Although the distinction between SCGT and GLGT is reasonably sound, that is not the case with the distinction between genetic enhancement and genetic therapy. Consider, for example, the possibility of using germline modification to prevent disease as in a gene that immunizes someone from a viral illness—a kind of genetic vaccine. Is it therapy (disease prevention) or enhancement (disease resilience)?

Initially, GLGT for enhancement only had support from a small minority of biologists. One of these was Princeton University geneticist Lee Silver, who was quoted as saying, "all of the reasons people have given for saying [germline therapy] is wrong are either irrational or religious-based."[14]

Most considered it politically incorrect to advocate the use of GLGT, even if it could be done safely. As SCGT became well grounded in the scientific and regulatory communities, the growth of interest in GLGT, where eliminating a disease allele in the germline so it would not appear in the DNA of a newborn, had gained attention. Articles published in major journals on the ethics and science of GLGT questioned the logic of maintaining a sharp and inflexible boundary between somatic and germline interventions. Ironically, one of the principal arguments for reinterpreting the boundary and removing the prohibition against GLGT was the eugenics example—a term many scientists did not wish to be associated with.

In the case of recessive genetic disorders, for a child to acquire the illness, such as cystic fibrosis, Tay-Sachs disease, or sickle cell anemia, he or she would need to inherit a mutated allele from each parent. If the parents were both carriers of the trait, then a child has a 25 percent chance of acquiring both alleles and thus developing the disease. If GLGT could repair the mutation, then neither the traits nor the disease would be passed on. As one report notes, "In theory, successful [human germline genetic modification] could eradicate a genetic disease in a family by permanently replacing a gene containing a mutation with a normal copy of that gene."[14] By most accounts, this is a form of eugenics or cleansing the genome of "bad genes."

LeRoy Walters, who for many years directed the Center for Bioethics at the Kennedy Institute of Ethics at Georgetown University, served on the RAC and helped to develop the "Points to Consider" document. As early as 1986, Walters discussed germline therapy as a technical and public perception problem, which might be more efficient at eliminating genetic diseases than SCGT.[15] Seven years later, in an article in *Science*, Walters provided arguments for and against germline gene modification, reviewed its successes in animals, and discussed the merit of keeping it on the public agenda. From the standpoint of ridding diseased genes from the genome Walters wrote, "Germ-line gene modification is more efficient than the repeated use of somatic cell gene therapy over successive generations."[16]

The first journal published that specialized in gene therapy began in 1990. With the title *Human Gene Therapy*, the journal ran a number of articles challenging the moral boundary between somatic and germ line genetic modification. Other journals such as the

Kennedy Institute of Ethics Journal also ran articles challenging the germline prohibition. In 1992, Munson and Davis wrote, "Somatic cell gene therapy has yielded promising results. If germ cell gene therapy can be developed, the promise is even greater: hundreds of genetic diseases might be virtually eliminated." They go on to say the claims that it would be morally unacceptable are inadequate and that there is no moral reason "not to develop and employ germ-line gene therapy…that medicine has a prima facie obligation to do so."[17] Berger and Gert, setting the moral boundary between positive and negative eugenics, wrote in the *Journal of Medicine and Philosophy* in 1991, "In distinguishing between positive and negative eugenics, the concept of malady is applied as a definitional criterion for identifying genetic disorders that could qualify for germ-line therapy."[18]

Clearly, the moral boundary initially set between somatic and germline was being eroded in the bioethical community. Schichor et al. wrote that the 1998 UCLA Symposium entitled "Engineering the Human Germline" may well have been "a watershed moment for the pro-germline engineering forces because it gathered many pro–germline engineering academics and endowed their individual voices with the strength of academia and the scientific institution."[19] Nevertheless, there were some global forces seeking to maintain the sharp division.

B. International Initiatives on Banning Germline Genetic Modification

The European states approached germline genetic modification with skepticism, cultural angst, or simply outrage. Elias and Annas capture the sentiments of one researcher during an international scientific meeting. "At a workshop on International Cooperation for the Human Genome Project in Valencia in October 1988, French researcher Jean Dausset suggested that the human genome project posed such great potential hazards that it could open the door to Nazi-like atrocities."[20] Dausset proposed a moratorium on the manipulation of gametes and embryos.

Some European states enacted legislation that proscribed scientists from making heritable alterations to human reproductive cells. Others didn't quite go so far and left open a window for treating or eliminating genetic diseases in embryos and gametes. Mauron and Thévoz reported on their survey to gauge European opinion. "The majority express more or less severe reservations about any interventions on the human germ-line, including therapeutic ones."[21]

During the summer of 1990, the Council for International Organizations of Medical Sciences held its 34th Round Table Conference in Tokyo and in Inuyama City, Japan that included 102 participants from 24 countries. The subject of the Round Table was "Genetics, Ethics and Human Values: Human Genome Mapping, Genetic Screening and Therapy." The Council issued "The Declaration of Inuyama," which stated, "The modification of human germ cells for therapeutic or preventive purposes would be technically much more difficult than that of somatic cells and is not at present a prospect. Such therapy might, however, be the only means of treating certain conditions, so continued discussion of both its technical and its ethical aspects is essential. Before germ-line therapy

is undertaken, its safety must be very well established, for changes in germ cells would affect the descendants of patients."[22]

In the Convention of Human Rights and Biomedicine, the Council of Europe passed what some believe is the most comprehensive and authoritative agreement on germline genetic modification of humans. Article 13 of the Convention states, "An intervention seeking to modify the human genome may only be undertaken for preventative, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants."[23] With five ratifications including four member states, the convention came into force on January 12, 1999. The principle behind the council's decision is that germline gene modification violates an implied right to inherit a genome that has not been artificially changed. The Convention was signed by 31 of the 41 member states of the Council of Europe. It was ratified by 28 members as of March 11, 2014.[24]

In 1988 *The Lancet* published the recommendations of the European Medical Research Councils with representation from Austria, Denmark, Finland, France, the Netherlands, Norway, Spain, Sweden, Switzerland, the United Kingdom, and West Germany. The councils agreed to disallow germline gene therapy.[25]

A 1994 report on human gene therapy by the International Bioethics Committee of the United National Educational, Scientific and Cultural Organization (UNESCO) stated, "All major statements about germline intervention condemn its present use. That position is clearly correct…The use of germline interventions for enhancement purposes should be categorically prohibited."[26] UNESCO adopted a nonbinding Universal Declaration on the Human Genome and Human Rights on November 11, 1997, signed by 186 nations. The document gave to its International Bioethics Committee a mandate to study "practices that could be contrary to human dignity, such as germline interventions." Section 24 of the UNESCO declaration states the following:

> *The International Bioethics Committee of UNESCO should contribute to the dissemination of the principles set out in this Declaration and to the further examination of issues raised by their applications and by the evolution of the technologies in question. It should organize appropriate consultations with parties concerned, such as vulnerable groups. It should make recommendations, in accordance with UNESCO's statutory procedures, addressed to the General Conference and give advice concerning the follow-up of this Declaration, in particular regarding the identification of practices that could be contrary to human dignity, such as germ-line interventions.*[28]

In 2003 the UNESCO IBC issued a follow-up report in which it discussed germline intervention. "On ethical grounds most national and international institutions have strongly discouraged or prohibited germ-line intervention…a distinction between 'therapeutic' purposes and 'enhancement of normal characteristics' is far from clear…Because of the many technical problems and uncertainties about possible harmful effects on future generations, germ-line intervention has been strongly discouraged or legally banned."[28]

C. Ooplasm Transplantation

In 1997, Jacques Cohen and his colleagues at the Gamete and Embryo Research Laboratory, of the Institute for Reproductive Medicine and Science at Saint Barnabas Medical Center in West Orange, New Jersey, reported the first human pregnancy following the administration of a procedure called cytoplasm transfer. The technique, which can be done by electrofusion or injection, involves the transfer of donor ooplasm from a healthy egg, between 5 and 15 percent by volume, to the egg of a woman who has had difficulty conceiving.

Cohen[30] describe the first woman to receive the ooplasm transfer. The patient had a history of poor embryo development and recurrent implantation failure. She was 39 years old with a six and a half year period of infertility. She failed to conceive on four previous attempts. The woman gave her consent to the ooplasm transfer from a donor egg of a 27-year-old woman. Both had undergone ovarian stimulation to retrieve the eggs. The assisted reproduction group at St. Barnabas retrieved 20 mature eggs that were considered poor quality because of mitochondrial mutations. Fourteen of the patient eggs were infused with cytoplasm of seven donor eggs. Six of the patient eggs were not infused with ooplasm, but were fertilized with the husband's sperm. These served as controls. Nine of the 14 treated eggs were successfully fertilized and activated, ready for implantation in the uterus. Brown[29] describe the process as follows: "In this technique, normal mitochondria (in cytoplasts) would be transferred into the oocyte, and thus dilute the effect of any mitochondrial DNA defect. Cytoplasmic transfer between human oocytes has been done to try and improve the outcome of assisted reproduction methods."[29]

At Saint Barnabas Medical Center, a baby girl was born at term weighing about 9.5 pounds. The Assisted Reproductive Technology team noted that little is known about the pathophysiology of the human oocyte used in the pregnancy.[30]

New York Times science journalist Gina Kolata covered the story on August 19, 1997, as follows:

> *Dr. Cohen selected four patients who had repeatedly failed to become pregnant because their embryos kept dying and matched them with egg donors, healthy young women whose eggs are easily fertilized and whose embryos develop normally. Each woman and her egg donor took fertility drugs that controlled ovulation so they would ovulate in synchrony.*
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> *Then Dr. Cohen, working with Dr. Steen Willadsen, a Danish scientist who discovered how to clone sheep and cows from early embryo cells, removed eggs from each woman and her egg donor and, using a pipette, sucked about 5 percent of the cytoplasm from the donor's egg, an amount equivalent to the cytoplasm in five red blood cells, Dr. Cohen said. They injected this cytoplasm, along with a single sperm cell, directly into the egg of the infertile woman. The sperm would fertilize the egg while the cytoplasm, they hoped, would allow the embryo to develop. For comparison, they left other eggs from the women untouched and simply injected them with sperm to see if they would be fertilized.*

> *Two of the infertile women became pregnant with the eggs that had received new cytoplasm. One, a 39-year-old woman who had been trying for six and a half years to become pregnant, had a baby girl in May, and the other is still pregnant. The third woman became pregnant but miscarried. In the fourth, the problem turned out to be with her husband's sperm; even the donor's eggs died when they were fertilized with those sperm.*
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> *Dr. Cohen and Dr. Willadsen also tried a different method, essentially taking a membrane-wrapped ball of cytoplasm from a donor's egg and adding it to the egg of a recipient, in much the same way that researchers add cells when they clone animals. They slipped the ball of cytoplasm under the zona pellucida and then gave the egg a jolt of electricity to make the ball of cytoplasm merge with the egg. After doing that, Dr. Cohen and Dr. Willadsen injected sperm into the egg to fertilize it. (They could not simply let sperm penetrate the egg on their own because the jolt of electricity also made the zona pellucida impermeable to sperm.)"*
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> *That method required seven different manipulations of eggs and embryos, Dr. Cohen said. He tried it with three infertile women and ended up with three healthy-looking embryos that he transferred to their uteruses. But none of the women remained pregnant. ''This by no means means that it doesn't work," Dr. Cohen said.* [31]

To describe the technique of ooplasm transfer (OT), also called cytoplasmic transfer, some commentators use the analogy of spoiled milk. If you have a quart of milk turning rancid, empty out half the container and fill it with fresh milk. The added fresh milk will overtake the rancid taste and the milk will pass the taste test. With OT, not all the mitochondria will be healthy. But the idea is that the mutated mitochondria will be sufficiently diluted to produce a healthy embryo.

Between 1997 and 2001, OT was used at Saint Barnabas in 12 pregnancies out of 28 attempts in 25 women with 16 babies born; 15 appear healthy. At least two babies were shown to have triparental inheritance, or DNA from three distinct genomes. The OT researchers acknowledged that "mixing two different maternal sources of ooplasm may generate mitochondrial DNA heteroplasmy in the offspring."[32] The investigators also acknowledged that they crossed the germline barrier, with little fanfare compared to the crossing of the sound barrier or the cloning barrier with Dolly the sheep. "These are the first reported cases of germline mitochondrial DNA genetic modification, which have led to the inheritance of two mitochondrial DNA populations in the children resulting in ooplasmic transplantation."[32] The investigators could draw no conclusions about the incidence of chromosomal and congenital abnormalities in children born from the technique.[33]

D. Is Ooplasm Transfer Germline Modification?

Has a child born through ooplasmic transplantation had its germline genetically modified? It appears to depend on how one defines germline genetic modification. One popular definition states that a modification of the germline means that new genetic material is introduced into the gametes (sperm or egg) or early embryo, and that these changes in the embryo are passed on to subsequent generations. By this definition, since donated (foreign) mitochondrial DNA is introduced into the egg, as long as the donated DNA is passed on to the infant, it would be considered germline genetic modification. It is possible that the foreign DNA could help the embryo develop without appearing in the cells of the newborn and being passed on to future generations. That is what heteroplasmy means.

On the other hand, some commentators believe that germline modification is synonymous exclusively with changes in nuclear DNA. If that is the criterion, then OT would not constitute germline genetic modification. Of course, the nuclear DNA could be modified by the interactions it has with foreign mitochondria. "Much, after all, is unknown about nucleo-mitochondrial interactions…Modifications in the mitochondrial DNA may also influence nuclear gene expression."[34]

It has been argued that the mitochondria, with a mere 37 genes representing 0.1 percent of the 20,000 to 30,000 genes in the nucleus, do not contribute to the phenotype of the individual and therefore any of the traits that make us human. The argument has been used to dismiss the idea of parental rights of the donor of the enucleated egg. According to this view, the donor would have no more right to shared parenthood than that of a blood donor. However, as the science behind mitochondria was advanced, some minds were changed. *The New Scientist* wrote, "Now it appears that we have seriously underestimated the influence that mitochondria have. Recent research suggests that they play a key role in some of the most important features of human life. This raises the ethically troubling prospect—once widely dismissed, including by this publication—that children conceived in this way will inherit vital traits from three parents."[35]

E. The US Food and Drug Administration Exerts Regulatory Oversight

The US Food and Drug Administration (FDA) promulgated regulations on assisted reproduction facilities in 1998. The agency issued three draft rules that would require (1) registration for facilities working with reproductive tissue, (2) screening for communicable disease, and (3) adherence to FDA good tissue practices for "minimally processed or manipulated" tissues transplanted from one person to another for their normal structural functions.[36] In 2001, the FDA announced it would regulate cytoplasmic transfer in assisted reproduction after it published its regulations on "Human cells, Tissues, and Cellular and Tissue-Based Products." The agency stated, "embryo culture media and other such products are regulated as medical devices by FDA, and establishments that manufacture embryo culture media are subject to the device regulations."[37] The FDA asserted that it can regulate research on human subjects that involves the use

of modified cells or tissue. Federal regulations on human subject research is distinguished from clinical practice, where physicians can treat patients with off-label drugs according to standards set within their states and professional community. In contrast to clinical practice, where physicians rely on their own clinical judgments, research entails the systematic investigation, testing, and evaluation designed to contribute to generalizable knowledge.

On July 6, 2001, the FDA sent a letter to *in vitro* fertilization clinics and ART researchers stating that human cells used in therapy involving the transfer of genetic material was within its regulatory mandate. "We want to advise you that the Food and Drug Administration has jurisdiction over human cells used in therapy involving the transfer of genetic material by means other than the union of gamete nuclei." The examples it cited included oocyte nuclei and ooplasm which contains mitochondrial genetic material. "The use of such genetically manipulated cells (and/or their derivatives) in humans constitutes a clinical investigation and requires submission of an Investigational New Drug (IND) application to FDA."[39]

The letter asserting regulatory jurisdiction directly impacted the assisted reproduction work at St. Barnabas. The clinicians there were advised that they were required to submit an IND prior to performing ooplasmic transfer on human eggs that were destined for transplantation in gestational women. They interpreted OT as a form of gene transfer research with donated mitochondrial DNA. According to a report of the President's Council on Bioethics, "This [letter] sent a shock wave through the ART community, and most, if not all practitioners halted the procedure altogether rather than submit to the IND process."[39]

A dozen years after the FDA announced it would regulate cytoplasmic transfer, the *Food and Drug Law Journal* wrote, "Cytoplasm transfer is an example of a procedure with yet unknown risks and consequences."[40] Of the approximately 1,500 mitochondrial proteins, only 13 are encoded by the mitochondrial genes. The vast majority are encoded by the nuclear DNA and exported to the mitochondria. Since the nuclear DNA and proteins come from one woman and the mitochondrial DNA and its proteins come from another, if they do not work together, it could result in problems in embryonic development.

F. Studies of Ooplasmic Transfer

Research on ooplasmic transfer in animals began to emerge within a few years after the FDA put a halt to its use on human pregnancies. The guiding question was whether OT was safe and effective for the offspring. An important study was undertaken by Mark Sharpley and colleagues. Sharpley is at the Center of Mitochondrial and Epigenomic Medicine at the University of Pennsylvania. His group was investigating a fundamental biological problem on the uniparental inheritance of mitochondrial DNA. The contribution of the male mitochondria is disabled during fertilization and only the female mitochondria are functional. Sharpley[41] created heteroplasmic mitochondria in mice by producing admixtures of mtDNA. They observed that heteroplasmic mice were less fit than their homoplasmic counterparts. The heteroplasmic mice had reduced physical activity, and exhibited behavioral abnormalities and impaired learning. "[O]ur data indicate that the

differences between mtDNAs within a mammalian species may not be neutral and that intraspecific heteroplasmy can be sufficiently deleterious as to favor the evolution of uniparental inheritance."[41] Nick Lane, who authored a book on mitochondria titled *Power, Sex, Suicide: Mitochondria and the Meaning of Life*,[43] referred to the work of Sharpley et al. in an article in *Cell* discussing the problems associated with mixing mitochondria in an embryo. Although ooplasmic transfer was used in the mice experiments, neither author discusses its implications for ooplasmic transfer in human oocytes. Lane, a professor in the Department of Genetics, Evolution and Environment at University College London, discusses human oocyte ooplasmic transfer in his book. After referring to the reproductive procedures at St. Barnabas, Lane says, "The profound disquiet felt about mixing two mitochondrial populations, which nature strives so hard to avoid, combined with the suspiciously high rate of developmental abnormalities leading to miscarriage, has led to the technique being placed on hold in the United States. Even so, to an open-minded sceptic, perhaps the most surprising finding is that it works at all."[42]

Levy[43] raise the dangers of ooplasmic transfer in human oocytes. They note that the technique was applied to humans with "astonishing speed" and without the benefits of "extensive research to evaluate the efficiency and the possible risks of the method."[43] These researchers concluded in 2004, "At present, in the absence of validation by proper cell culture experiments or detailed animal research, the application of [ooplasmic transplantation] in humans is difficult to justify."[43]

In a twist of irony, the person who led the program at St. Barnabas in using ooplasmic transfer co-authored an article that questioned the long term safety of the technique. The authors noted that more than the mitochondria were transplanted by the donor egg. In the article by Barritt[44], the authors stated, "transfer of small amounts of cytoplasm probably involves mRNAs, proteins and mitochondria, as well as other factors and organelles. Even though the use of cytoplasmic transfer has been employed in several IVF clinics—and pregnancies have resulted—it is not known definitively whether the physiology of the early embryo is affected."[44] Among the risks discussed were chromosomal abnormalities, heteroplasmy of offspring with mtDNA from the mother and the donor, the transfer from the donor of other organelles and proteins, the creation of mismatches in the interactions between the nuclear and mitochondrial DNA, and finally, continuation of heteroplasmy through the germline across generations.

Thus, while many questions were raised about ooplasmic transfer, which was discontinued in the United States, another research program was in full gear to prevent the transfer of mitochondrial disease to a mother's offspring. In contrast to ooplasmic transfer, which did not prevent all the mutated mitochondria from being transferred from mother to child, the new procedures are designed to eliminate all the gestational mother's mitochondria in the fertilized embryo.

G. Mitochondrial Disease

There have been many articles and books written in support of germline genetic modification. Yet serious attention was not given to gaining federal and public support for

crossing the germline until mitochondrial disease was introduced as a sympathetic case in assisted reproduction with its universally accepted goal of preventing a child born with such an affliction. What is mitochondrial disease? The mitochondria are semiautonomous organelles (components in the cytoplasm of eukaryotic cells—cells with a nucleus) with their own genomes including transcriptional machinery. Mitochondrial cells contain 37 genes that encode 13 proteins, 22 transfer RNAs, and 2 ribosomal RNAs. In contrast, the nuclear genome consists of about 20,000 genes.

Mitochondria are the powerhouses of cells—they store and transmit chemical energy, which is why they are often referred to as the cell's batteries. The mitochondria can multiply when the energy needs of the cell increases. The primary function of the mitochondria is the generation of the molecule ATP (adenosine triphosphate) from food sources. ATP is a molecule that transports chemical energy to the cells and is therefore essential for cell metabolism.

Mitochondrial diseases are a group of disorders that can cause debilitating, chronic illness. They are the result of either inherited or spontaneous mutations in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA), which lead to altered functions of the proteins or RNA molecules that normally reside in the mitochondrial cells. These mutations can be present at birth or develop later in life and cause mild to severe physical, developmental, and mental disabilities. When mitochondria aren't working properly, they can disrupt function in almost any of the body's organs. Depending on which cells are affected, symptoms may include loss of motor control, muscle weakness and pain, gastrointestinal disorders and swallowing difficulties, poor growth, cardiac disease, liver disease, diabetes, respiratory complications, seizures, visual/hearing problems, lactic acidosis, developmental delays, and susceptibility to infection.

Mitochondrial diseases can be difficult to diagnose. At least 1 in 8,500 of the population carries a pathogenic mtDNA mutation, and it is estimated that up to 4,000 children per year in the United States are born with a type of mitochondrial disease. They are progressive and incurable, though some treatments are available depending on the case.

H. British Preparation for Germline Modification

One nation in Europe that has chosen to follow its own path in navigating between somatic and germline modification is England. The British have developed a highly regulated system for Assisted Reproductive Technology (ART). It supported the first use of *in vitro* fertilization on humans. Louise Brown became the poster child of the new technology of reproduction and a scientific result of great national pride. Research into human reproduction in the United Kingdom has not been thwarted by religious or right-to-life movements as they have been in the United States. British scientists studying embryos and reproduction work in a regulated environment, heavily monitored by a national ethics commission and parliamentary oversight. When the issue of germline modification for preventing the transmission of mitochondrial disease arose, the British put into play nationwide surveys, public consultations, and a study by its leading ethics commission.

In the United Kingdom, two pieces of legislation regulate the use of embryos in reproduction and research. The Human Fertilisation and Embryology Act of 1990 (HFEAct), amended in 2008, covers assisted reproduction,[45] whereas the Human Fertilisation and Embryology Regulations of 2001 address the use of embryos in research.[46] These acts prohibit the alteration of the nuclear or mitochondrial DNA of any cell in the human embryo and the implantation of an egg or embryo that has its DNA altered. In the 2008 amendment of HFEAct, there is a provision, subject to parliamentary approval, that permits altered germline DNA "to prevent the transmission of serious mitochondrial disease, under a new section 3ZA (subsection 5)."[47]

The agency with authority to regulate under the legislation is aptly named the Human Fertilisation & Embryology Authority (HFEA). The agency undertook years of review and public consultation on modifying the germline for preventing the transfer of mitochondrial disease before issuing its recommendations to the government. It sponsored two scientific reviews on the safety and efficacy of the methods in 2011 and 2013, and undertook consultations to gauge public responses to nuclear transfer. The consultations included public workshops in various cities, 1,000 face-to-face interviews in 175 random locations, questionnaires given to stakeholder groups and members of the general public, public meetings, and patient focus groups. The HFEA found that there was general support for "mitochondrial donation" in the United Kingdom. In its scientific reviews, the HFEA found that studies on safety were done mostly on animals and that more research was needed on human embryos, because they questioned whether the findings on animals were definitive for humans. The agency also indicated that reliable tests were needed to find mitochondrial disease caused by nuclear DNA mutations. It recommended that any child born from the nuclear transfer techniques should be followed over an extended period.

The United Kingdom also engaged with its preeminent independent ethics organization, the Nuffield Council on Bioethics, an organization funded by the Medical Research Council, the Nuffield Foundation, and the Wellcome Trust. The Nuffield Council has a distinguished record of publications in medical ethics, Genetically Modified Organisms, research ethics, mental health, stem cells, and genetic screening. The council report released in June 2012 is titled "Novel techniques for the prevention of mitochondrial DNA disorders: An ethical review." Its report acknowledged that the current methods of preventing mitochondrial disease are a form of germline therapy. The report also questioned whether a person born with genetic material from three people will have conflicted self-identity. With many caveats listed in the report, the Nuffield Council concluded, "if the PNT [Pronuclear Transfer] and MST [Maternal Spindle Transfer] techniques are proven to be acceptably safe and effective, on balance it would be ethical for families wishing to use them to do so."[48]

After its scientific reviews, public consultations, and the March 2013 reconvened expert panel report, the HFEA issued its recommendations to the UK government. It proposed a set of experiments to be undertaken before the techniques under considerations can be safe enough to be used in humans. The chief medical officer of the United Kingdom, Professor Dame Sally Davies, announced that the government would issue draft regulations by June 2013.[49] Between February 27 and May 21, 2014, the UK

Department of Health conducted a public consultation on the draft regulations, which it issued in conjunction with its report. The agency wrote, "The consultation reached a wide audience and received 1,857 responses from research bodies, patient bodies, professional organisations, faith organisations, parliamentarians and a large number of individuals."[50] After its consultations, the government decided to put the issues and draft regulation before the parliament for its approval.

The UK government framed the issue in two important ways that formed the basis of its communications and public consultations. First, the United Kingdom described the techniques under consideration as mitochondrial donation. This is a mistaken interpretation of what takes place. If a woman whose eggs have mitochondrial mutations that would result in a pregnancy of a child with mitochondrial disease were to have the mitochondrial cells removed from her egg and replaced with donated mitochondrial cells, then we could indeed characterize the process as mitochondrial donation. In fact, the donation is of an enucleated egg. This means that a woman donates a healthy egg with its mitochondrial cells and cytoplasm after its nucleus has been removed. The nucleus of the woman seeking to get pregnant is then placed into the enucleated egg. The reason that this is an important distinction is that the enucleated egg contains more than the mitochondrial DNA; it contains other organelles such as the endoplasmic reticulum, the Golgi apparatus, and the outer membrane of the egg contributed by the donor. Stuart Newman of New York Medical College emphasizes this point.[52] The mitochondrial donation metaphor is a misnomer. It is the prospective mother who donates her nuclear DNA to the enucleated egg cell of a donor. The procedures described by the UK regulations is nuclear not mitochondrial DNA transfer and donation, as acknowledged by the UK Department of Health by one of the respondents to the draft regulations: "Neither MST [maternal spindle transfer] nor PNT [pro-nuclear transfer] involves the 'replacement' and 'donation' of mitochondria. Bother processes require a transfer of the nucleus of an unfertilized or fertilized egg to another fertilized or unfertilized egg—it is not the mitochondria itself that is transferred from one egg to another. As such, the procedure would be more accurately described as 'nuclear donation' or 'chromosomal transplantation' not mitochondrial 'replacement.'"[52]

Second, the UK government distinguishes between genetic and germline modification, suggesting that the techniques being considered to prevent the transmission of mitochondrial disease was the former (genetic modification) and not the latter (germline modification). The implication in this distinction is that germline modification is eugenics, but genetic modification is not. The HFEA consultation report stated:

> *There is no universally agreed definition of 'genetic modification' in humans – people who have organ transplants, blood donations or even gene therapy are not generally regarded as being 'genetically modified'. While there is no universally agreed definition, the Government has decided to adopt a working definition for the purpose of taking forward these regulations. The working definition that we have adopted is that genetic modification involves the germ-line modification of nuclear DNA (in the chromosomes) that can be passed on to future generations. This will be kept under review.*[53]

The two framings are mutually reinforcing. It is argued that the mitochondria only contain a small amount of DNA compared to the nucleus, and that "the tiny amount of mitochondrial DNA contributes little or nothing to personal characteristics." The popular metaphor used in government documents is that the procedure for the transplantation of a nucleus into an enucleated egg is like a change of batteries. The mitochondrial cells are characterized as the batteries of the egg, and mitochondrial disease is like a bad battery.

I. Nuclear Germline Modification

Ooplasmic transfer was developed as a treatment for infertile women. Jacques Cohen and his colleagues at St. Barnabas devised the method of oocyte supplementation to improve the chances that a woman with repeated embryo development failure can conceive. It was never designed to prevent the transfer of mitochondrial disease from mother to child. The inability of women to conceive because of mitochondrial mutations was related directly to mitochondrial disease. Diluted mutated mitochondria were not going to prevent the transfer of some of the mutated mtDNA to the offspring. Women diagnosed with mitochondrial disease were not given many options to prevent the transmission of the disease to a newborn. They could obtain an egg with healthy mitochondria from another woman, fertilize it with their husband's sperm, and gestate it in their womb. They could also adopt a child. But for women who want a child with their nuclear DNA, there were no options.

Two procedures in development were designed to prevent the transfer of mitochondrial disease to the offspring while enabling the gestational mother to have a child with her nuclear DNA. The first procedure is called pronuclear transfer (PNT) and the second is called maternal spindle transfer (MST). In slightly different ways, both procedures involve using a donated egg with the nuclear DNA removed and replacing the enucleated portion with the nuclear DNA of the gestational mother. The PNT process begins with two eggs, one from the intending mother (with mitochondrial disease) and a second from a donor that is disease free.

Each egg is fertilized by the intending father's sperm. The fertilization process produces pronuclei (the male and female chromosomes before they have merged). The pronuclei from the egg with mitochondrial disease and the donor egg are removed. The pronuclei from the intending parents is taken from the egg with mitochondrial disease and placed in the enucleated donor egg.

The result is that the pronuclei from the intending parents are now in a new enucleated egg with healthy mitochondria. The pronuclei from the donor and intending father are discarded. The donor egg with the intending parents' pronuclei is then activated for embryonic development. The embryo is placed in the intending mother's uterus for gestation. Sometimes this process is referred to as nuclear transfer (NT) because nuclear DNA is transplanted from one egg to another.

The second method, MST, also involves two eggs—the intending mother's egg (with faulty mitochondria) and the donor egg (with healthy mitochondria). In each egg the

spindle of chromosomes of the originating woman was removed. There is now an enucleated or spindle-free donor egg and intending mother's egg. The latter (with mitochondrial disease) is discarded. The spindle from the intending mother's egg is placed into the donor's enucleated egg with healthy mitochondria. Finally, sperm from the intending father (or a sperm donor) fertilizes the egg with the healthy mitochondrial DNA and the intending mother's chromosomes. The fertilized egg is then transplanted to the mother's uterus for gestation.

By using the enucleated donor egg, the intending parents have removed the threat of mitochondrial disease to their newborn. As was pointed out earlier, it is a mistake to call this "mitochondrial transfer." It is closer to nuclear transfer, since it is the nuclear DNA that is moved from one egg to another.[52,54]

J. Genome Editing

One of the major limitations of human germline engineering is the lack of precision in correcting a mutation or in adding a desired gene construct to an embryo. Introducing a gene into a chromosome can end up in the wrong place. The reading frame may distort the transcription of the protein. A series of discoveries resulting in what has been called *genome editing* have been a game changer for the emerging science behind human germline modification. The new discoveries have introduced a much higher degree of precision in editing a sequence of the genome. "Present-day genome editing technology, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Cas system, have achieved far more efficient genetic engineering in higher organisms than the older techniques."[55] Twenty nucleotides can be selected and modified. The seminal work was done on bacteria with no particular reference to human germline engineering.

A team of scientists from the United States, Canada, and France published an article in *Science* in 2007 in which they described a biological system found in bacteria that protect them from bacterial viruses (bacteriophage).[56] A Copenhagen-based food company exploited the bacterial technique to protect its yogurt and cheese from phage contamination during manufacturing.[57] It is a type of bacterial immune system that works quite differently than the human immune system. The bacteria are able to cut up short segments of DNA from the invading virus and integrate those segments into an array that it carries called CRISPR. The term CRISPR is an acronym for "clustered, regularly interspaced, short palindromic repeats." When invaded by a virus, the bacteria releases an enzyme (endonuclease) that cuts up the invading DNA from the virus and intersperses short segments (called spacer DNA), within the CRISPR array. That becomes the bacterial immune system. When the virus invades the bacterium again, those DNA segments (CRISPR spacers) recognize the exogenous DNA and become templates for encoding RNA molecules (complementary to the DNA) and guide the endonuclease to break up the DNA of the invading virus.

This CRISPR technology has been generalized to editing segments of a genome in all species. It permits point-specific disabling, cutting apart, or substituting DNA sequences.

Within a period of seven years, CRISPR and Cas9 endonuclease has been successfully used in human cells to repair cystic fibrosis genes, correct genetically induced cataracts in mice. Long[58] used CRISPR/Cas 9 to correct a genetic abnormality responsible for muscular dystrophy in mice. "[O]ur results show that CRISPR/Cas9-mediated genome editing is capable of correcting the primary genetic lesion responsible for muscular dystrophy (DMD) and preventing development of characteristic features of this disease in mdx mice."[58]

Several phases of CRISPR gene editing have been described for bacterial systems. In the adaptive phase, bacteria and archaea harboring one or more of the CRISPR loci respond to a viral or plasmid challenge by integrating short DNA fragments of foreign sequences called protospacers into their host chromosome, at the end of the CRISPR array. In the expression and interference phases, transcriptions are made of the repeat spacer elements, eventually CRISPR RNAs, or crRNAs. The short crRNAs pair with complementary protospacer sequences of the invading viruses or plasmids. Finally, in the target recognition phase, the crRNAs (which bind to the complimentary sequences in the invading virus or plasmids) direct the Cas proteins (endonucleases) to silence the foreign sequences (tear them apart).

Yin[59] used a mouse model to correct a hereditary genetic disease called tyrosinemia, which is caused by a mutation in a gene that encodes an enzyme. Tyrosinemia is characterized by elevated blood levels of the amino acid tyrosine. When there is a deficiency of one of the enzymes required for the process that breaks down tyrosine, the enzyme builds up in tissues and organs, which leads to serious liver problems. After some elegant biochemistry to create the CRISPR/Cas9 construct, they injected it into adult mice through their tail veins. They reported that their data "demonstrate the potential to correct disease genes *in vivo* in adult mouse liver using a CRISPR-Cas9 system."[59] The authors believe that this therapy may be translatable to humans.

Wu[60], who had corrected cataracts in mice with CRISPR/Cas9 wrote, "we have demonstrated that the CRISPR-Cas9 system can be used to cure a genetic disease in a mouse by directly correcting the genetic defect through gene editing. In the future it would be of interest to investigate whether similar gene correction strategies could be used for mutation correction in a setting related to human diseases, such as human stem cells."[60]

Araki and Ishi[55] reported that there were two papers in 2014 "demonstrating that microinjection of Cas9 or TALENs into one cell-stage embryos led to efficient generation of targeted gene-modified non-human primates (NHPs)."[55] The successful modification of monkey genomes by CRISPR/Cas9 made alterations in human germline seem closer at hand.[61,62]

By 2013, George Church at Harvard University synthesized tens of thousands of guide RNA sequences capable of editing 90 percent of the human genes.[57] Scientists have been speaking highly of its potential to correct genetic diseases in the embryo. According to one author, "these studies suggest that the CRISPR-Cas system could be used for human gene therapy" to correct genetic defects in affected patients and their germline so that their progeny will lack the disease.[63]

K. New Companies on Gene Editing

In the 1980s, during the birth of the biotechnology industry, dozens of companies were formed to exploit the commercial applications of recombinant DNA in agricultural, medical, and industrial uses. A new generation of startups filled the high technology landscape, including names like BioGen, Genentech, Cetus, Genetic Systems, and Molecular Genetics. Similarly, new firms were formed beginning in the second decade of the 21st century. One of the earliest of these was Editas Medicine, founded in 2013. It was jump-started with $43 million in venture capital financing from Flagship Ventures, Polaris Partners, and Third Rock Ventures. Editas described its mission as translating genome editing technology "into a novel class of human therapeutics that enables precise and corrective molecular modification to treat the underlying cause of a broad range of diseases at the genetic level."[64] Among its founders are those who have made significant contributions to CRISPR technology, such as Jennifer Doudna of University of California, Berkeley and George Church at Harvard. One of the company's initial goals is to use CRISPR to correct one or more genes in blood diseases, returning the cells to the patient's marrow to produce healthy red blood cells.

L. Appeal for an Ethics Forum

As early as November 2013, scientists were beginning to speak about a wider public discussion on the applications of CRISPR to humans. One news outlet cited Nobel Laureate Craig Mello of the University of Massachusetts as saying, "the 'jaw-dropping' technique has the potential to transform the study and manipulation of genes and lowers the barrier to genetic engineering of human IVF embryos," which Mello opposes.[65] George Church also spoke emphatically about airing the social and ethical implications of CRISPR to a wider public.

In March 2015, the ethical concerns about CRISPR were brought to an international audience by scientists who were associated with its development. Forty-two years earlier, a group of molecular geneticists spoke publicly about the potential risks of research involving recombinant DNA molecule technology. By speaking out in journal letters and asking their colleagues to refrain from certain experiments until the risks were fully understood, they paved the way for the 1975 Asilomar Conference that brought together an international community of scientists to discuss a risk management framework. At that time, the human ethical implications of gene splicing were kept off the agenda to focus on laboratory risks of infectious agents and moving tumorigenic genes into coliform bacteria.

The first of three articles, entitled "Engineering the Perfect Baby," was published in MIT's *Technology Review*. In it the author discussed the potential use of CRISPR to modify nuclear DNA in human gametes or embryos.[66] The first gene edited monkeys were born in China in 2014. On March 19, 18 scientists signed on to a commentary in *Science* recommending an international discussion on the ethics of using CRISPR for "editing" human germ cells. Two scientists who authored the letter had been organizers

of Asilomar—David Baltimore and Paul Berg. Other signatories played a key role in developing CRISPR technology.

In a somewhat parallel case to Asilomar, it was the ease of CRISPR in making pinpoint edits to the human genome that made the issue of immanent importance. Like their colleagues of the 1970s, those concerned about CRISPR recognized that it was available to all scientists in all parts of the world. "The simplicity of the CRISPR-Cas9 system allows any researcher with knowledge of molecular biology to modify genomes, making feasible experiments that were previously difficult or impossible to conduct."[67]

The *Science* commentary made passing reference to a form of germline engineering involving the transfer of mitochondrial cells (outside the nucleus). Instead of including it under the umbrella of germline gene engineering, the authors set it aside and focused exclusively on changes in the DNA of the nucleus of the germ cells. The commentary went on to advocate national and international forums to discuss responsible uses of the technology. "Assuming the safety and efficacy of the technology can be ensured, a key point of discussion is whether the treatment or cure of severe diseases in humans would be a responsible use of genetic engineering, and if so, under what circumstances."[67]

The commentary was preceded by a meeting of interested parties in Napa, California on January 24, 2015, sponsored by the Innovative Genomics Initiative at the University of California, Berkeley and the University of California, San Francisco. The meeting was designed as "a framework for open discourse on the use of CRISPR/Cas9 technology to manipulate the human genome." The meeting's outcome was a call for a self-imposed moratorium on any attempt at germline genome modification for clinical applications on humans, while societal, environmental, and ethical implications of such activity are discussed among scientific and governmental organizations. From the website of the Innovative Genomics Initiative:

> *To be clear, our position is not a call to outright ban engineering of the germ line. Instead, we ask for a halt to experiments along these lines until a much larger meeting whose attendees represent a broad cross-section of scientific, clinical, ethical, and regulatory expertise. Whether or not individual researchers have performed human germ line editing, we must stop and ask ourselves hard questions before embarking on this path in earnest. Is it acceptable to cure genetic disease?*[68]

Within a week after the *Science* article appeared, a smaller group of scientists, five including a philosophy graduate student, signed a commentary that appeared in *Nature* titled "Don't edit the human germline."[69] One of the signatories, Fyodor Urnov of Sangamo Biosciences in Richmond, California, helped to develop the first genome-editing technology (zinc-finger nucleases [ZFNs]). This commentary was more forthright about the consequences of crossing the germline. "In our view, genome editing in human embryos using current technologies could have unpredictable effects on future generations. This makes it dangerous and ethically unacceptable."[69] This is an echo of what the citizens' committee in Cambridge, Massachusetts stated in 1976 in its report on the recombinant DNA controversy.

> *Throughout our inquiry we recognized that the controversy over recombinant DNA research involves profound philosophical issues that extend beyond the scope of our charge. The social and ethical implications of genetic research must receive the broadest possible dialogue in our society.*[70]

The Cambridge citizens questioned whether all knowledge was worth pursuing, whether any particular route to knowledge would transgress precious human liberties or values, and whether the products of this knowledge would have long-term hazards to our natural and social ecology.

The authors in the *Nature* commentary also did not see their proposal as stopping mitochondrial DNA transfer leading to the three genome baby, even though it is a form of germline modification. The British Parliament had already approved such procedures for the prevention of mitochondrial disease in newborns. The signatories make a distinction between germline editing and somatic cell genome editing, reestablishing a moral boundary that has slowly been disappearing among scientists and bioethicists. A new industry was developing, based on using CRISPR/Cas9 for curing diseases in children and adults. The investors and participants in this new sector were concerned that legislation against germline modification could also affect somatic cell gene therapy if the moral distinction was not kept clear in the public's mind.

The protection of the somatic-germline distinction was emphasized by the US National Institutes of Health director Francis Collins in late April 2015. Collins noted that editing embryos is "viewed almost universally as a line that should not be crossed."[71]

M. The Chinese and Canadian Experiments

Within a month after the *Nature* and *Science* commentaries were published calling for restraint on using CRISPR/Cas9 to genetically alter a human embryo, Chinese scientists reported the results of their experiments altering the genes of a human zygotes.[72] It was the first known case where scientists used the gene editing technology on human preimplantation embryos. The desire of Western scientists to slow down gene editing and set aside the human embryo until an international consensus could be reached failed to gain consensus from Chinese scientists who were on a fast track to explore the modification of human genes. The Chinese group was led by Janjiu Huang of Sun Yat-Sen University in Guangzhou in Guangdong Province. Gene editing was performed on embryos obtained from local fertility clinics that could not be used to produce a live birth because they were fertilized by two sperm and thus were characterized as nonviable. At least four Chinese groups are engaging in gene editing of human embryos.[73]

IVF procedures have always had a modest to low success rate, around 38 percent for women in their thirties and 18 percent for women in their forties.[75] Scientists at Saint Barnabas Medical Center believed that they could improve the success rate by infusing donated mitochondrial cells to the fertilized eggs of seeking IVF treatment. As previously discussed, alternative methods of improving IVF effectiveness include nuclear transplantation into donated eggs, a technique that has been approved for use in the United Kingdom.

A group of Canadian doctors developed another technique to improve IVF success. They used a laparoscopic procedure to remove a small sliver of ovary tissue of a woman who did not do well with IVF treatments. Scientists from the fertility company Ova Science obtained egg stem (precursor) cells from the ovary tissue. Those cells were purified, after which the mitochondrial cells were removed. They then added the mitochondria from the egg precursor cells to the mother's poor quality eggs. Using the woman's own mitochondrial precursor cells improved the pregnancy outcome of her embryos. The process was first noted by Jonathan Tilly of Northeastern University, who discovered that cells scraped from the outer surface of the ovary contain precursor cells that can rejuvenate the older eggs. This method is distinct from the method leading to the "three genome baby" because the mitochondrial precursor cells and the egg cells come from the same woman.[75] Nevertheless it does involve altering the human germ line, albeit not the nuclear DNA.

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