Chapter One

What is Science-based Bioethics?

Introduction

Each year, biotechnology bravely ventures into unexplored scientific territory. The year 2016 was no exception: The number of scientific breakthroughs that emerged during this year is overwhelming with gene editing (CRISPR) technologies, gene drives to eliminate harmful mosquitos, and synthetic DNA topping the list. As will be discussed in Chapter Eight, scientists have developed ingenious methods to edit the DNA code of the human genome in cells, embryos, and human beings. Equally astonishing are the reports of two new synthetic DNA bases that have been synthesized. Applying this synthetic biology technology, scientists have expanded the DNA code from 4 to 6 base pairs (Malyshev et al., 2014). Yet, the real dangers of gene editing, synthetic biology, and the creation of a synthetic human genome remain unknown, raising the question whether humankind is dramatically overstepping innate ethical boundaries.

In May of 2016, a closed door meeting convened to discuss the issue of constructing an entire human genome in a cell line, a project prospectively titled 'HGP-Write: Testing Large Synthetic Genomes in Cells'. As the New York Times reports, the meeting was invite-only and "The nearly 150 attendees were told not to contact the news media or to post on Twitter during the meeting."

In the past year, neuroscience research has led to countless innovations as well. Selected examples include: a) stem cell and genetic technologies to enhance the cognition and learning potential of mice, b) brain rejuvenation of older mice to their youthful plasticity with stem cell technologies, c) artificial intelligence in human-like robots (see https://www.youtube.com/watch?v=W0_DPi0PmF0 for a dramatic video about human-like robots), and d) genetically modified bacteria that can function as biological circuits. Even in the area of human life span, research into telomeres has generated protocols that could increase the human life span by decades. All of these advances in science raise complex bioethical dilemmas that must be addressed by legal, scientific, and ethical scholars.

Four scientific breakthroughs have paved the path for many of the above mentioned biotechnologies. The first of which, reported in 1997 (Wilmut et al., 1997), was cloning a sheep called Dolly. The groundbreaking method utilized nuclear transfer technology to produce a mammal cloned from an adult cell obtained from the mammary gland. Within a year after Dolly was cloned, scientists reported an innovative method to isolate human embryonic stem cells from discarded embryos and to maintain them indefinitely in culture (Thomson et al.,

1998). Induced pluripotent cell (iPS) technologies was the third milestone that allowed the transformation of adult fibroblasts into embryonic stem cells without using embryos as a cellular source (Takahashi et al., 2007). The leap in our understanding of the regulation of genetics is the final breakthrough. Together with the mapping of the human genome and our increased awareness of epigenetics and capacity to edit our genes, these technological discoveries have ushered in a new era of human therapeutic and research cloning. If ethically developed, these technologies will allow us to control our own biological and genetic destinies in ways never before imagined.

Every new scientific advancement and discovery generates a plethora of ethical questions and dilemmas. This book is based on the principle that bioethics itself is an amalgam of many different disciplines and skills that must include the underlying science. Once the scientific principles are understood then other bioethical approaches incorporating philosophy, social values, culture, and religion can be integrated with the scientific facts to attempt to resolve these complex, and often contentious, moral issues.

Aims of this Textbook

This book has multiple aims. It presents advancing perspectives on how scientific discoveries elicit bioethical concerns and challenges to all students interested in the future of scientific progress. Readers interested in enhancing the sciences and allied fields or pursuing careers in these fields will be pushing the boundaries of scientific discovery, and will need to deliberate bioethical issues that often arise from scientific experiments. As their professional careers in science and medicine develop, their innovative research and ability to communicate science to the public will stimulate bioethical debate. The cardinal rule in ethics is that good ethics begins with a factual understanding of the underlying science. This book thus provides the essential scientific background and bioethical information that should allow basic scientists, healthcare professionals, clinical researchers, and indeed students, to better comprehend, appreciate, and address the complex bioethical dilemmas that our society confronts now and will confront in the future.

It is important to predict what bioethical issues will emerge from new biotechnologies. The emphasis of this book highlights how understanding the underlying science can assist in resolving bioethical dilemmas. Wherever possible this book also emphasizes the key role that philosophy, cultural values, and religious approaches to bioethics can play and influence how bioethical challenges are resolved. Only then can there be a practical analysis of how to resolve, manage, or defuse the bioethical dilemmas. Rather than simply presenting hypothetical resolutions to bioethical dilemmas, this book discusses current as well

as futuristic cases to better enable students to formulate their own practical strategies for identifying and resolving emerging bioethical dilemmas.

Four Specific Objectives of this Book

To promote these aims, this book outlines four specific objectives: (1) to present the scientific basis for new biotechnologies and discuss how these technologies trigger bioethical dilemmas; (2) to highlight situations where bioethical concerns in research may differ from classical concerns of medical ethics; (3) to demonstrate when a historical analysis of ethical controversies arising from earlier biotechnological advances can, at times, provide insights into resolving current bioethical debates; and (4) to present appropriate scientific strategies that can be implemented to resolve, defuse, or manage bioethical disputes.

The first objective of this book assumes an appropriate scientific and ethical mindset to understanding both the potential and the limitations of a new technology. It is important to also recognize that bioethical dilemmas can sometimes arise from factual misinformation. Misconceptions about the underlying science may lead to misunderstandings of the emerging ethical issues that ultimately can generate bioethical shockwaves that reverberate through the government and media, distracting society from the more salient, factual issues. Thus, it is critical to grasp the underlying facts related to the bioethical dilemmas to ensure that discussions are not tainted by imprecise knowledge or scientific bias. In other words, the ability to address bioethical challenges begins with obtaining the most accurate scientific information. As senator Moynihan stated, “Individuals are entitled to their own opinions, but they are not entitled to their own facts”.

There are many misconceptions concerning the sovereignty of genetics in shaping human personality and abilities. Equally important, many students are unfamiliar with the emerging insights that can be obtained from epigenetics. In natural twinning, as one example, each twin experiences his/her individual environment simultaneously. In contrast, if someone is cloned using donor cells from a professional athlete such as Lebron James, there is a preconceived notion of how the clone’s genetic endowment will influence his life’s development. Will the cloned Lebron James also develop into a professional basketball superstar? What impact will the woman’s uterine and hormonal environment have on the clone during fetal development? How much self-motivation and what other environmental contributions will be required to develop this cloned child into a skilled athlete? These questions raise broader bioethical questions such as: Will reproductive cloning challenge human individuality or autonomy? Is it ethical to subject this cloned child to the psychological, physical, or financial pressures arising from knowledge of the successes and failures of his genetic donor, the

original Lebron James? Moreover, what other social pressures will shape his environment in order to nurture his presumed athletic ability or future as a superstar basketball player?

The general public tends to underestimate the complexity of the nature and nurture interaction in determining one's biological destiny. In particular, it is now increasingly evident that DNA, although inherited, still responds to environmental pressures (Robinson, 2004). Epigenetic research addresses these issues in understanding how "software" in programming gene regulation is influenced by chemical modifications of DNA base pairs and their associated proteins without altering the base sequences of the genome. Through epigenetic research, we are unraveling how environmental and genetic factors do not necessarily work in opposition; rather, a synergistic and continuous interaction of these factors orchestrates human behavior, aging, and disease (Goldman, 2012; Marx, 2012).

Epigenetics changes of identical twins during their youth generate dramatic changes in their athletic skills or in the diseases that they developed as adults (Aaltonen et al., 2014; Castillo-Fernandez et al., 2014; Rottensteiner et al., 2015). Studies of identical twins in Finland showed that those twins who shared the same sports and other physical activities as youngsters but different exercise habits as adults soon developed quite different bodies and brains. This study highlights the extent to which exercise shapes our health via epigenetics, even in people who have identical genes and nurturing.

In 2016, many scientific reports claimed to have developed reliable blood based assays to predict the onset of diseases such as Alzheimer's disease. This diagnostic blood test identifies men that are more likely to develop Alzheimer's disease when they has been lose their Y sex chromosome. The public often accepts these reports as absolutely accurate even though it will take decades to establish the scientific validity of these technologies.

Despite the stated goal of this book's first objective - to present the scientific basis for new biotechnologies and discuss how these technologies trigger bioethical dilemmas - there is the realization that scientific discoveries are developing and changing at such a rapid rate that it is impossible to write a comprehensive book that will remain up to date with all of the given emergent observations and discoveries. Chapter Nine has been completely re-written to focus on CRISPR and synthetic biology rather than classical genetics.

The second objective of this book focuses on differences between research bioethics and medical ethics. Bioethics is generally perceived as an all-encompassing discipline that includes medical ethics, neuroethics, genethics, environmental ethics, and research ethics. Research ethics is an emerging new discipline as the study of ethical practice and the dilemmas that arise with the acquisition of scientific knowledge and the development of new biotechnologies that impact biological species and the environment. A critical component of research bioethics, is the need to translate all research done in vitro or in vivo into

human applications. Practically, the often and unanswered question is as follows: When is it ethically appropriate to engage in the first human clinical trialto explore the efficacy of a new procedure or therapy? In contrast, medical ethics focuses on issues already available in the clinic, such as physician-assisted suicide and abortion that immediately and directly impact the patient or the patient-healthcare professional relationship. These conceptual differences may lead to the formulation of unique guidelines for each discipline.

While the concepts of this book focus on research-oriented bioethics, many questions and issues extend far beyond the research laboratory. Stem cell research is a good example that raises broader questions pertaining to the definition of human life, such as identifying the stage of embryological development at which human status or personhood is said to be attained. Another question is appropriate here: How does genomics confer species identity? Similarly, introducing human embryonic stem cells into laboratory animals to create chimeras enables scientists to better investigate how cells differentiate to become specialized cells. Research published in 2014-2015 has shown that introducing specific human genes into mice or reconstituting human astrocytes (non-neural supportive cells of the brain) into mice embryos dramatically improve learning behaviors and intelligence of these animals. Is it ethical to transplant human stem cells into mouse or chimp embryo in an attempt to reconstitute a human brain into an animal? In this way, the capacity to transplant human stem cells into animals and possibility animal genes into humans challenge the classical definition of species. Moreover, the unique status of personhood that was historically limited only to human beings is being applied to other non-human primates. Research showing human-like behaviors in non-human primates have triggered new laws that grant certain monkeys the status of personhood.

The third objective of this book is to demonstrate how the historical analysis of ethical controversies arising from earlier biotechnological advances can, at times, provide insights useful for resolving current bioethical debates. As an example, bioethical concerns about when human personhood begins in fetal development were raised in 1978 after Louise Brown became the first of more than five million “test tube” babies produced by in vitro fertilization (IVF). The success IVF has dampened the original ethical debates first raised in 1978. In contrast, the current bioethical concerns in defining human life in stem cell research often neglects IVF as a historical precedent. One could predict that if stem cell technologies prove to emerge as a successful treatment of diseases, such as diabetes or Alzheimer’s disease, the ethical concerns surrounding this technology may also become less relevant.

One historical lesson from IVF is that once a technology is shown to be effective in treating a medical condition (infertility), the public becomes less concerned about possible bioethical questions inherent in these technologies. This historical example also illustrates that as the technology is enhanced, what the public deems unacceptable shifts over time. This is a subtle societal process

which also may dull awareness of serious ethical pitfalls, particularly if the new technology confers high benefits and value to society**.**

In addition, there are times when history can offer insights into conflict resolution and management. We have seen that the original motivation for biotechnological development often differs from its eventual application. The history of cloning Dolly is an excellent example. A biotechnology company, PLL, in collaboration with the Roslin Institute, cloned Dolly for commercial purposes — to develop technology for the production of biological pharmaceuticals in animal milk at a cost significantly lower than conventional production methods. This application required the development of a procedure in the laboratory to genetically modify mammary epithelial cells to encode the production of a specific drug. Once these cells were appropriately modified in the laboratory, a procedure had to be developed to generate an animal that expressed these genetically modified mammary epithelial cells. Nuclear transfer technology using adult cells offered a viable solution to generate these types of genetically modified animals, and is the primary reason why Dolly was cloned. It was no coincidence that the term cloning never was found in their original report that appeared in Nature (Wilmut et al., 1997). Nonetheless, this publication triggered an intense bioethical debate regarding the use of cloning for human reproduction and for embryonic stem cell research. Applying historical analysis to this example, one might conclude that animal cloning may be ethical for commercial use including the development of cheaper and more efficient drugs; applying this limited technology to today's human reproduction, however, remains unethical since reproductive cloning is currently not allowed in most societies today.

However, the tide against human reproductive cloning is changing. In 1997, Gallop poll surveys showed that less than 5% of those surveyed in the United States favored cloning technologies. In 2015, the number of people who find cloning technologies ethical has risen to greater than 15%, presumably because new medical applications of human cloning (i.e. somatic cell nuclear transfer) have been implemented to treat a variety of conditions in reproductive medicine, such as mitochondrial replacement therapy.

Historical analysis also reveals that the rapid pace of biomedical research has seriously challenged society's ability to make informed and reasoned choices about whether and how to proceed with its development and use (Frankel and Chapman, 2001). Traditionally we have proceeded in a "catch-up" or "reactive" mode, scrambling to match our moral values and social and legal policies to scientific advances. Potential breakthrough technologies such as gene transfer take decades to develop, yet choices must be made immediately regarding research directions to take and treatments to investigate.

Any historical analysis should include the role of government policy and regulation in biomedical research. The United States government policy on bioethical issues is often shaped by the moral beliefs of both those in power and

the public. The belief that conception is the beginning of human life led to restrictions on the use of Federal funds to support human embryonic stem cell research, initiated by President William Clinton in 1995**.** While many criticize this federal policy, there may be a silver lining in how our government has attempted to deal with the contentious bioethical issues associated with human embryonic stem cell technology. Surprisingly and generally unappreciated is that president Bush's ban on the use of federal funds to support human embryonic stem cell research created a void that stimulated many non-federally funded research efforts that ultimately helped extend and deepen the partnership between the fields of bioethics and biomedical research. New funding streams were created with private and state funds leading to important advances (such as iPS and transdifferention technologies) that spawned new ethical debate. In 2009, President Obama instructed the National Institutes of Health to issue new guidelines for federally supported human embryonic stem cell research to better coincide with the public's belief that stem cell research has the promise to yield dramatic new therapies (Daley, 2012).

While biomedical scientists are primarily driven by the challenge to understand biological processes or the need to create new cures and treatments for major diseases, bioethical issues have begun to play a greater role in defining the landscape of biomedical research, especially in stem cell science. This is but one example that highlights the role of government in shaping the direction of biomedical research.

The book's fourth objective is to introduce science-based strategies as a method for resolving, defusing, or managing bioethical concerns. Bioethical management is a three-step process. First, the facts must be determined. Then the issues and the stakeholders must be identified. Finally proposed strategies for resolution must be created. Determining the facts implies understanding the relevant science and identifying the underlying religious, cultural, legal, or political concerns related to the dilemma. The stakeholders could be patients, companies, or governments. Finally, developing strategies to help manage or resolve bioethical dilemmas involves an integrated approach.

Classically, philosophical paradigms and traditional ethical approaches have been useful in many situations. Ethical values, however, may be relative, never absolute, and often evolving. Today, we are witnessing a paradigm shift in applied bioethics where science-based strategies have begun to offer new integrated approaches to augment the classical philosophically-based strategies. To illustrate this point, if someone believes that an embryo attains human status at conception, no amount of scientific, philosophical, or ethical discourse can sway that individual to support embryonic stem cell research because stem cells are currently derived from a conceived embryo that must be destroyed in the process of deriving stem cells. However, as scientists develop novel methods to generate stem cells, such as reprogramming a normal adult-differentiated cell into a stem cell (Wilmut et al., 2007), research utilizing these stem cells should be less ethically

charged than research using the cells of donated embryos. This book will highlight several traditional ethical approaches to help resolve issues and will illuminate how new scientific research approaches offer technological alternatives that could alleviate ethical aporias.

Political and financial considerations are also important factors in managing or resolving bioethical concerns. If new biotechnologies are restricted or banned by the federal government, there is a risk that persons with medical needs may be deprived of the future medical discoveries that could emerge from the prohibited research attempts. On the other hand, there are the doomsday scenarios, be they real or imagined, which create pressures to restrict or block basic biomedical research. As a case in point, the technology for creating synthetic biological organisms has the possibility of creating safer vectors for gene transfer in therapeutic protocols, but with “dual use” could also be applied to generate new pathogens that might trigger massive epidemics or serve as blueprints for future weapons of bioterror (Hunter, 2012; Keim, 2012). Risk-benefit analysis, treatment alternatives, and financial resource management all therefore are important considerations when deciding to fund or pursue a new direction in biomedical research.

The public, as taxpayers funding the scientific research community, has a right, perhaps even an obligation, to help shape the course of scientific research and could be playing a larger role in deciding which research is funded. While some within the scientific community fear that engaging the public in research funding decisions could be ineffective, lay leaders are, nonetheless, taking a more empowered role in funding biomedical research. Many foundations in the research-charity sector engage lay leaders (trustees) who are non-scientists to help shape and direct the research funded by these charitable organizations without hindering scientific advancement.

It is critical that scientists, physicians, and the professional scientific research community take responsibility to ensure that the science behind any technology is accurately presented and that the ethical concerns are identified and mapped. With that in mind, this book is designed so that each new technology will span two sections and sometimes two chapters. The first section focuses on a comprehensive survey of the science underlying a new biotechnology. The second section examines the ethical, religious, legal, and social challenges that are precipitated from the technology. In addition, the second section will attempt to explore various ethical approaches to try to resolve the resultant bioethical dilemmas. This integrated format is designed to help the readers of this book explore, express, and formulate their own ideas. Each section will include case studies for students to think about creatively and to allow them to formulate concrete and practical ways to resolve these controversial bioethical concerns.

In the supplementary section of this book, we include a brief description of how to write an op ed bioethical article. It is important that scientists present

complex biotechnologies and bioethics to the general public as part of their social responsibility to educate the public about the benefits and risks of new biotechnologies. We encourage our readers who may be or become experts in various scientific disciplines to express their views to the public.

Several important areas (such as animal experimentation, environmental concerns, evolution, and religion) will not be addressed in detail, as they are beyond the scope of this book. Other topics such as research freedom, research responsibility and accountability, conflicts of interest, and scarcity of financial resources will be incorporated, appropriately, into several of the chapter topics.

Conclusions

In summary, bioethics and science intersect and interact at various levels. The potential to understand basic principles in biology as well as the clinical impact of many of these biotechnologies often remains to be established as the resultant bioethical issues are further identified and debated. The resolution of bioethical dilemmas is a complicated process for several reasons. First, simple solutions to bioethical issues may be difficult to obtain because critical facts are not always available at the time when there is a need for practical decisions. Second, decisions in both science and bioethics have to be acted upon immediately in order to forge ahead in a timely fashion even when the facts are incomplete. Third, sometimes issues arise that generate a clash of ethical principles such as beneficence and autonomy. Grappling which bioethical principles will take precedence and must be addressed. These compounding factors related to bioethics may restrict one's capacity to resolve a dilemma but may allow one to develop ways to manage a bioethical conundrum.

This book proposes that both bioethics and science should exist in a mutually beneficial and symbiotic relationship motivated by a common goal to acquire knowledge purely for its own sake and for its potential for needed therapeutic applications. This is the new mission in bioethics: to provide an integrated, multidisciplinary analysis to enable our future scientists, health care providers, lawyers, and politicians to manage and resolve the many significant emerging bioethical issues.

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Chapter Ten

Ethics in Genetics

Introduction

In the previous chapter, various biotechnologies were presented that allow individuals to screen themselves or their embryos for genetic diseases. These DNA sequencing technologies can be divided into two categories: whole-genome sequencing (WGS) and whole-exome sequencing (WES). WGS is a procedure that determines the complete DNA sequence of an individual's genome at a single moment in his or her lifetime. WES technologies only allow the sequencing of the protein-coding genes in a genome (known as the exome). In addition, gene editing technologies can be applied to individuals or embryos to alter the DNA sequence of their genetic codes.

From an ethical perspective, the use of these technologies with a view to improve the health of a human being follows the bioethical principles of beneficence and human dignity (see chapter 2). Nonetheless, there are still many contentious issues that bioethicists raise regarding these technologies. In response to the press hype of gene editing technologies, a December of 2015 summit was convened in Washington, DC to explore the science and ethics of germline gene editing. The organizers concluded with the following words of caution:

> "It would be irresponsible to proceed with any clinical use of germline editing unless and until (i) the relevant safety and efficacy issues have been resolved … and (ii) there is broad societal consensus about the appropriateness of the proposed application."

This chapter will address the ethics of gene screening and gene editing. In order to assess the various bioethical dilemmas associated with these technologies, the reader must recognize two important caveats: a) these technologies are too recent and more time is needed to fully identify and explore ethical concerns, and b) there is no consensus on identifying what ethical guidelines these technologies may violate.

Genetic Sequencing and Screening in Adults and Newborns

Genetic screening is more than simply sequencing the base pairs of the human genome because it offers a window into personalized treatment. Both genomic and epigenetic genetic screenings in cell free blood DNA can be used to

diagnose disease states. In addition, exosome diagnosis in another method that is generating a great deal of excitement. Exosomes are lipid nanovesicles, on the order of 30–200 nm, secreted from cells and found in all bodily fluids such as plasma, urine, and cerebrospinal fluid (CSF). Although exosomes were discovered over 30 years ago, they were originally thought to be nothing more than a garbage disposal system for cellular debris and proteins. More recently, interest in exosomes has increased with better understanding of their capabilities to utilize exosomes in the development of biofluid-based, real-time molecular diagnostics, as drug delivery vehicles, and as tools for biomedical research. Exosomes contain not only proteins, but also different types of RNA transcripts, such as messenger RNA (mRNA), microRNA (miRNA), other noncoding RNA, ribosomal RNA (rRNA), and transfer RNA (tRNA). These differences in exosome-derived RNA profiles could be harnessed to distinguish healthy vs. disease states.

The ability to detect the nucleic acid profile of a tumor for example, in a noninvasive way, via a blood draw or urine sample, without the need for a potentially invasive tissue biopsy is a significant advance, especially when sample tissue is difficult to access. Prostate cancer is a good example of how exosomes could improve patient management. It is estimated that 30% of men age 50 or older will have some form of prostate cancer (although only about 15% of men will be diagnosed during his lifetime); however, many of these men have low-risk prostate cancer that will not likely progress to a life-threatening stage. Exosome analysis might help differentiate between low-risk and high risk prostate cancer. Exosome diagnosis is also being assessed to diagnose Alzheimer's disease and assess a patient's immunological compatibility for organ donations.

Screening Adults. As the cost of classical genomic sequencing or epigenetic sequencing dramatically decreases to under $1,000, its applications will impact many individuals. It is important to highlight several features of genomic sequencing. First, it differs from obtaining genetic information from a family history. A family history of a patient may reveal very little about an individual's biological propensity to disease, which could be easily gleaned from genetic testing. Yet, taking a family history will still reveal a great deal of information about the personality and environmental background of the patient within his or her family. Second, in genomic sequencing, tools are available to distinguish genetic factors from environmental and life course contributions to disease. Third, some outcomes of DNA analysis may reveal unsolicited (often referred to as incidental) findings that the patients do not expect and may not want (Rigter et al., 2014).

Genetic analysis can reveal diseases that are life-long, as well as predicting those that are late-onset. Yet, the accuracy of DNA sequencing analysis in predicting late-onset diseases is not as accurate, in part because the individual has not yet presented with any symptoms. Until science uncovers the role of each gene within the human genome and how all genes interact with one another, interpreting the DNA data related to late-onset diseases will remain a challenge. The sheer amount of information afforded by genome sequencing also raises ethical issues related but not limited to: informed consent, privacy, data ownership

and sharing, and the regulation of this technology. Despite these ethical concerns, there is a need for many volunteers to have their DNA sequenced and analyzed, even though they themselves may not gain any useful medical information for decades to come.

Children. What about the rights of parents to genetically analyze their newborn children? Newborn screening can benefit newborn children if there is a family history or other signs of a disease such as congenital hypothyroidism and phenylketonuria. Most bioethicists support genetic screening for diseases that manifest at birth or during childhood. However, genetic screening of newborns or young children, for late-onset diseases such as Hungtinton's disease, breast cancers, or Alzheimer's disease, presents serious bioethical challenges (Anderson et al., 2014). Specifically, the question is whether such testing or screening violates the autonomous right of the child to decide whether he or she wants to know if he or she is carrying the gene for Hungtinton's disease?

Incidental Genetic Findings

In 2013, the publication of the controversial "American College of Medical Genetics and Genomics (ACMG) Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing" created a huge debate regarding whether or not the recommendations in this report were ethical. These recommendations (Green et al., 2013) are summarized as follows:

1. Mutations found in the genes on the minimum list should be reported by the laboratory to the ordering clinician, regardless of the indication for which the clinical sequencing was ordered.

    a) Additional genes may be analyzed for incidental variants, as deemed appropriate by the laboratory.
    b) Incidental variants should be reported regardless of the age of the patient.
    c) Incidental variants should be reported for any clinical sequencing conducted on constitutional (but not tumor) tissue. This includes the normal sample of a tumor-normal sequenced dyad and unaffected members of a family member.

2. The Working Group recommends that laboratories seek and report only the types of variants within these genes that have delineated as causing diseases.

    a) For most genes, only variants that have been previously reported and are a recognized cause of the disorder or variants that are previously unreported, but are of the type that is expected to

cause the disorder, as defined by prior ACMG guidelines should be reported.
b) For some genes, predicted loss-of-function variants are not relevant (e.g., COL3A1 and most hypertrophic cardiomyopathy genes).

3. It is the responsibility of the ordering clinician/team to provide comprehensive pre-test and post-test counseling to the patient.

    a) Clinicians should be familiar with the basic attributes and limitations of clinical genetic sequencing.
    b) Clinicians should alert patients to the possibility that clinical sequencing may generate incidental findings that could require further evaluation or information that the patient may not want to know.
    c) Given the complexity of genomic information, the clinical geneticist should be consulted at the appropriate time, which may include ordering, interpreting, and communicating genomic testing.

There are three key bioethical issues that emerge from these recommendations: a) the long-standing inconsistencies between consensus guidelines and clinical practice regarding risk assessment, for adult-onset genetic disorders in children, obtained using family history and molecular analysis; b) the disparate assumptions regarding the nature of whole genome and exome sequencing and how they affect arguments for and against reporting; and c) the implicit differences in how to reveal genetic information to maintain the best interests of the child.

This working group defended their recommendations in stating: 1) the potential benefits of revealing incidental genetic findings outweigh any harm, and 2) in other areas of diagnosis such as radiology, incidental findings should be reported without ethical concerns. Those who criticize these recommendations claim that in fact, the potential harm of revealing information outweighs the benefits. In addition, they reject the analogies drawn between genetic sequencing and other areas of medicine. Finally, they maintain that these guidelines violate the longstanding consensus against testing children for adult-onset conditions. In many countries (USA, as well as Great Britain and other European countries) the practice is to not disclose to families the genetic information about a child, unless it is immediately relevant to their health care (Clarke, 2014).

Genetic Screening in Pre-Implanted Embryos

The use of genetic screening technologies (including PGD) to select for embryos that do not carry specific mutations seems at first to be uncontroversial.

It allows for the selection of healthy embryos. However, several highly contentious ethical concerns arise from PGD. What should be done with those pre-implanted embryos that are not selected for implantation into a woman's uterus? Is discarding an embryo that is a carrier for a genetic disease ever justified? Are there conditions when prospective parents can justifiably discard an embryos? Is it ethical to create savior siblings by selecting embryos that, after gestation and birth, might serve as an organ donor for an existing, sick sibling? These types of ethical questions are rooted in cultural and religious beliefs (see Chapters 5 and 7) rendering them difficult to resolve.

Thought Question: Under what circumstances should incidental genetic findings be revealed to the patient and/or the parents? In the 1990's diagnosis of Duchenne's muscular dystrophy (DMD) involved obtaining tissue or blood samples from the pregnant woman, her husband and the embryo that she was carrying to ascertain whether the embryo inherited the X-linked mutation responsible for DMD. In about 5-10% of the cases it was clear that the husband was not the biological father of the child. Should the physician present this information to the couple?

As mentioned in Chapter 7, several religions such as Catholicism believe that discarding a pre-implanted embryo is akin to murder because this embryo if implanted into a woman's uterus will generate a child. Some ethicists believe that allowing embryo selection will lead to a slippery slope situation in which embryos will be selected for non-medical reasons such as gender, increased athleticism or increased intelligence. Arthur Caplan, the director of the division of medical ethics at NYU Langone Medical Center in New York City states, "I believe that the future of PGD is in both looking for traits that parents do not want in their children and in selecting for traits that they do very much want to try to pass on. The morality of eugenics, both negative—eliminating unwanted traits—and positive—selecting for desired traits—will surely loom very large as the key moral question facing those offering PGD and those seeking to utilize it."[1]

Another ethical issue that is debated is the use of these technologies to screen for late-onset diseases. From a predictive perspective, Huntington's disease is a disease for which genetic analysis can accurately predict disease onset. In contrast, for other diseases such as cancer, genetic analysis only predicts the risk of developing a disease within the lifetime of the individual. For example, a woman who is a carrier for the BRCA mutation possesses a significant higher risk of developing ovarian and breast cancer during her lifetime than a woman lacking this mutation. But having this mutation does not mean that she will definitely develop breast or ovarian cancer, nor does it mean that a woman who does not have the mutation will not. As mentioned earlier, until the functions of all

[1] http://www.aacc.org/publications/cln/2014/january/Pages/Preimplantation.aspx

genes and their interactions are known, it will be difficult to make precise predictions from genetic sequencing technologies.

The question of whether a person wants to know his/her genetic predisposition for a specific disease is a final ethical concern that needs to be addressed. Some individuals who have a family history of Hungtinton's disease do not want to be genetically tested and know their fate. Yet, they do want to have a healthy, disease-free child. Several individuals, in particular, women, will undergo PGD and instruct the physician **not to tell her whether she is carrying the fatal Hungtinton's disease mutation**, but rather the physician should only implant a selected embryo that only inherited the normal but not Hungtinton's disease causing mutation.[2] In this case, her autonomous choice is to deny obtaining her genetic information regarding Hungtinton's disease and yet have a child that is free of the gene mutation that would cause Hungtinton's disease.

Legal Issues Related to Genomic Screening

From a legal standpoint, privacy concerns and the accuracy of genetic diagnosis are major issues regarding DNA sequencing and PGD. There have been several legal cases and judgments brought against commercial DNA testing laboratories due to incorrect PGD results. Currently genetic screening is not 100% accurate and raises the following question: under what circumstances is malpractice justified? Is there a legal justification to sue a physician if genetic screening is not offered to the couple or if the selected embryo implanted actually developed the disease that the DNA screen did not successfully target?

http://www.sciencemag.org/site/products/lst_20110304.xhtml

*Privacy Issues***:** Another legal issue relates to protecting the individual from having his genetic background revealed to unwanted recipients such as employers or maybe even health insurance companies. In light of these concerns, the US Government signed into law the Genetic Information Nondiscrimination Act (GINA) in 2008. This law bans U.S. employers from using genetic information in hiring, firing, promotion and compensation decisions, as well as from collecting genetic information from employees. Furthermore, GINA prevents health plans and insurers from denying coverage or boosting premium prices based on a person's genetic information, including his or her family history. It also forbids these organizations from requesting or requiring people to undergo genetic testing. GINA provides greater protection than the 2003 enacted law called the Privacy Rule, implemented as part of the Health Insurance Portability and Accountability Act (HIPAA) that established

[2] http://www.cnn.com/2014/02/22/opinion/klitzman-genetic-testing/

federal regulations for the use and disclosure of protected health information. What is frustrating to clinicians and researchers is the absence of evidence that such federal regulations are making patient records more secure.

Another unanswered question is whether federal legislation protecting genetic information might inadvertently foster the public's apprehension of genetic testing. GINA was initially designed to provide sufficient privacy protection so that the public feels safe to participate in genetic research, to pursue genetic testing for themselves and to share the findings with family members who might also be at risk, as well as with health care providers who can help affected individuals treat or manage their conditions. Is GINA meeting these expectations (Prince, 2014)?

Forensic Science: Interestingly, the capacity of law enforcement officials to use genetic information to identify a criminal is becoming quite sophisticated (Kayser and de Knijff, 2011). Over half of the States obtain DNA samples from arrestees, currently totaling over 10 million DNA samples collected and retained in the USA forensic bank.[3] Most often this data bank is used to identify an exact match with DNA obtained from crime scenes. If, however, the DNA obtained at a crime scene does not match the FBI data bank, the FBI could still use that DNA sample for DNA profiling. DNA profiling refers to the use of DNA sequencing technologies to predict physical characteristics (hair color, eye color, facial geometry, and height) and diseases that the criminal may possess. DNA profiling also can identify whether the DNA sample obtained at the crime scene is related to a person in the FBI data bank. As scientists learn more about the role of genetics and behavior, DNA profiling will eventually be used to predict violent behavior or anger management disorders from the suspect's DNA.

The following case presents an interesting ethical conundrum related to forensic DNA analysis. In a small town in Virginia, DNA obtained from a crime scene revealed that the criminal had four mutations commonly associated with Gaucher's disease. Most people with Gaucher's disease require biweekly treatments administered within a hospital setting. The law enforcement officers investigating this crime went to the only local hospital that treats patients with Gaucher's disease and demanded that the hospital administrators provide them with a list of all individuals being treated for this genetic disease. With this information, the police would be able to generate a list of suspects to interrogate. Did the hospital administration have the right to refuse to release this medical information because it violates private GINA laws? Some argue that the police have the right to this information because they have the right to contact hospitals and seek medical information on whether someone was admitted and/or treated for gunshot wounds. Others would argue that, in fact, the two situations are ethically different. The alleged criminal who was shot by the police is entering the hospital's ER in full view of the present public, and by default relinquishes their right to privacy. In the situation where the criminal has Gaucher's disease, the right

[3] http://www.pbs.org/wgbh/nova/next/body/dna-databases/

of privacy is legally protected when the patient comes in for treatments. **What do you think?**

One of the most famous legal cases involving the use of biological testing was the Charlie Chaplin paternity law suit. In 1943, the starlet Joan Barry accused actor Charlie Chaplin of fathering her child. At that time, research had begun to identify the ABO blood group classification of people. Although blood tests definitively excluded Chaplin as the father, the court did not allow this evidence to be admitted as evidence, and Chaplin was ordered to pay child support to Barry. It is unclear exactly why the court did not accept the blood type tests as evidence in this case. The media claimed that the blood tests were not scientifically accurate or that Chaplin had ingested some chemical to change his own blood type. Chaplin's second wife, Lita Grey (who was divorced from Chaplin in a bitter, proceeding), asserted that Chaplin had paid corrupt government officials to tamper with the blood test results. While the media and even the court did not understand the science of blood typing, this case did spur the passage of new laws regarding the use of biological data as forensic evidence.

A difficult obstacle in forensic medicine is establishing that the actual DNA obtained at the crime scene was from the alleged criminal who committed the crime. Sometimes, it is difficult to separate the perpetrator's tissue (e.g., sperm) from tissue belonging to the victim. Moreover, there are documented cases in which the alleged criminal DNA actually was contaminated with DNA from the law enforcers investigating the crime scene or from individuals working in the forensics laboratory.

Ethics of Gene Editing and Synthetic Biology

The potential power of using gene-editing systems (see Chapter 9) to treat a wide variety of genetic diseases does not deter bioethicists from raising bioethical concerns. Many of these concerns have been raised in regards to other biotechnologies such as human cloning and stem cell technologies. These ethical concerns include:

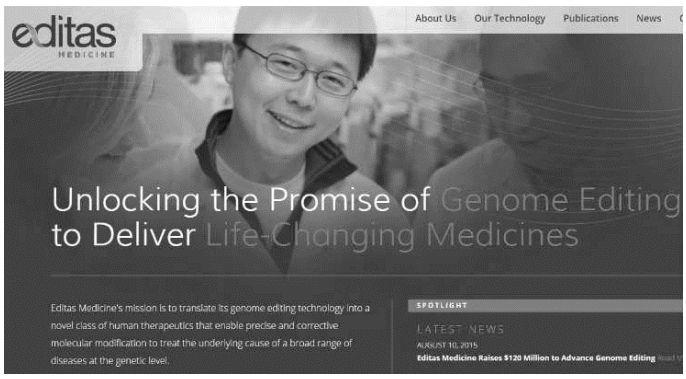
1. Playing God, (see Textbox 1)
2. Violating the principle of justice as the high cost of gene-editing will only benefit the rich,

Textbox I: Playing God

The argument that human beings should not "play God" has been used to claim that specific technologies, such as gene editing, are unethical. In fact, many of the technologies discovered have biological precedents. Gene editing, for example, is based on the discovery of an enzyme called CAS in bacteria that functions as a defense against foreign DNA, either viral or plasmid.

3. Negatively tampering with our genetic integrity by editing the "Book of Life",
4. Introducing technology to create more potent bioterror weapons,
5. Genetic engineering of human IVF embryos,
6. Engaging in germline therapies,
7. Parents who alter the genetics of their fertilized eggs or children violate the autonomous rights of their children,
8. Applications of these technologies for non-medical purposes, such as increasing EPO levels in athletes, or for non-medical enhancements (intelligence, looks (blond hair), athleticism, personality traits).

While these bioethical concerns need to be addressed, one must remember that if and when gene-editing systems[4] will be successfully used to treat diseases, many of these concerns will fade into the background. This is precisely the lesson we learnt from IVF technologies. As it became clear that IVF was an effective method for infertile couples to have healthy children, the ethical outcries (related to designer babies and discarding embryos) heard in the early nineties faded in the 21st century. Even the Catholic Church has lowered its noise in opposing IVF.



In 2015, Editas, a biotechnology company, was founded in part by Jennifer Doudna and Feng Zhang, two of the first developers of the CRISPR technology. One of the company's objectives is to initiate the first clinical trials using CRISPR to correct a rare eye disorder called Leber congenital amaurosis (LCA). The condition mainly affects the retina, resulting patients having a difficult time seeing anything other than large, bright shapes. Why does Editas want to try CRISPR for this condition? First, it's an easy disease to target. The treatment (which involves injecting people with modified viruses carrying the CRISPR technology that will go in and repair the faulty DNA) can be

[4] Gene-repair systems may convey a more ethical semantics then "gene-editing systems" see Loike, 2015.

injected directly into the retina and, in this case, used to delete the portion of the CEP290 gene that's responsible for the disease. Second, because this disease affects vision, it will be easy to assess the clinical effectiveness of the therapy. However, if CRISPR is effective, it may prove to be very expensive since there are only about 600 people who have the type of LCA that could be treated. From a bioethical perspective, we have a situation where the technology is expensive, rendering it inaccessible to many patients and violating the bioethical guideline of "justice". What remains unclear is whether the clinical success of using CRISPR to treat LCA patients will accelerate the use of CRISPR to treat other diseases and, in turn, significantly lower its costs thus making the technology more accessible to all patients. The fact that more than six companies are employing gene editing technologies for clinical applications highlights its great clinical potential.

In addition, technologies are being developed to reverse some of the gene editing systems. As discussed in Chapter 9, RNA-guided gene drives are capable of spreading genomic alterations made in laboratory organisms through wild populations to address environmental and public health problems. However, society must be aware of the possibility that unintended genome editing might occur through the escape of strains from laboratories, leading to the prospect of unanticipated and possibly harmful ecological changes. In 2015, scientists examined the efficacy of CRISPR-Cas9 gene drive systems in wild and laboratory strains of the yeast Saccharomyces cerevisiae (DiCarlo et al., 2015). The researchers designed two molecular confinement approaches capable of overwriting any changes introduced by an earlier gene drive. The first, called a split drive, involves separating Cas9 and guide RNAs so they are not encoded in the same organism. Cas9 was encoded on an unlinked episomal plasmid and the gene drive element contained only the guide RNA. Because the gene encoding Cas9 is required and is unlinked from the drive, and since wild yeast populations do not encode Cas9, the [guide] RNA-only drive is unable to spread in wild organisms lacking Cas9. In the second containment strategy, Cas9 is designed to target genes in which a DNA sequence not found in wild-type organisms has been inserted. As expected, gene drive-containing yeast was unable to affect yeast lacking the synthetic target sequence. These molecular safeguards should enable the development of safe CRISPR gene drives for diverse organisms and minimize the risk of unwanted genome editing. Lastly, these scientists showed that a trait imposed on yeast using a gene drive could be reversed by using another gene drive to overwrite the initial change. In doing so, the gene drive machinery remained in place, but rendered the genetic change inactive. **Once again, we have an example in which a scientific discovery can override a potential bioethical dilemma in which a technology could inflict unwanted harm (maleficence).**

In August of 2016 a survey carried out by Pew Research Centre[5] found that a majority of adults (~70%) in the USA are worried about the potential use of genome-editing technologies to give children a reduced risk of disease. Respondents who said they were familiar with genome editing were more likely to want it for their own child, and there was more acceptance of genome editing if people were allowed to choose which diseases would be affected. Yet, fifty-four percent of adults surveyed felt that genome editing to prevent serious disease in a baby and give it the average level of health would be appropriate. However, the same amount of people felt that genome editing to make someone healthier than any existing human was crossing a bioethical line. The survey also found that religious people are less likely to support such interventions, and that the more committed to religion someone is, the more likely they are to think that enhancement technologies are meddling with nature and 'playing god'. Interestingly, many respondents also said they had mixed views about current enhancements such as cosmetic surgery.

Equally important is the fact that ethical concerns related to synthetic biology technologies are equally as complex as gene editing, with some differences. Yet, there is a fundamental question that needs to be addressed regarding synthetic biology technologies. What are the actual benefits of synthetic biology? As mentioned in the previous chapter, the capacity to expand our genetic base pairs will eventually allow scientists to create a wide variety of new types of proteins. They hope that these proteins could be used to generate better vaccines for diseases. How valid is this scientific claim? Secondly, could synthetic biology technologies be applied to create more virulent bioterror weapons (see Chapter 14 on “Dual Use”)? Finally, as financial resources for biomedical research become more difficult to obtain, should this area of science be a top priority for governmental funding? The answers to these questions remain elusive at this time and questions remain regarding what clinical applications will be developed from research in synthetic biology. While it is quite difficult to regulate technological advancements, research in this area will most likely proceed because history has shown that human beings are often mesmerized by new technologies.

There are other ethical challenges in genetic engineering that need to be addressed. In August of 2015, Dr. Smolke and her team at Stanford University reported in the Journal Science the complete synthesis of opioids in genetically modified yeast. They created one form of yeast that converts sugar into hydrocodone, the active ingredient in Vicodin. Another yeast strain makes a compound called thebaine — which can easily be turned into many opioids, including oxycontin, codeine and morphine. Her goal was to open the door to the quick development of better medications of all sorts and to make morphine more available in developing countries, where there's a shortage of painkillers. Currently the opioid yields from these yeast strains are small. But once the process has been optimized, these modified yeast strains should make it much easier and cheaper

[5] http://www.pewresearch.org/fact-tank/2016/07/29/the-religious-divide-on-views-of-technologies-that-would-enhance-human-beings/

to manufacture new painkilling medicine. In addition, scientists will be able to leverage this technology to reduce some of the narcotics' side effects, and/or make medications that are less addictive.

The genetically modified yeast strains have triggered an ethical debate about how to regulate these organisms to prevent "home-brewing morphine." These genetically modified yeast could, one day, be grown at home and used to turn sugar into heroin — which is easily made from morphine or thebaine, and to put more inexpensive addicting drugs on the street. The DEA shares concerns about using yeast for home-brewing. But the agency is also worried about large drug cartels. These cartels could find a way to increase production and increase their profits — all on the backs of people who are addicted to opiates.

Thought Question: From an ethical perspective it is important to assess whether the harm emanating from the illegal opiof market is more important than the fact that over 5 billion individuals around the globe do not have sufficient access to pain killers because of their high cost. In this case should beneficence trump over maleficence?

What are your thoughts?

Conclusions

In this chapter, many ethical challenges were presented related to three types of genetic technologies: genetic screening, gene editing and synthetic biology. There is no doubt that ethicists have used and will continue to consider the classical ethical guidelines to limit the application or delay in engaging human trials of these technologies. Nonetheless, one must recognize the allure of basic scientific innovation and technology, even if the health benefits are not clearly defined (as in expanding our repertoire base pairs from 4-6) or and even if the health risks may be higher than society is normally comfortable with (i.e., gene editing). Coupled with the allure of these new technologies is the fact that patients with untreatable and fatal diseases are desperate and will engage in unproven therapies with the small hope that if may attenuate their disease. Thus, despite any bans or fund restriction, these technologies will develop at a rapid pace.

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Companies employing gene editing technologies

Editas: Editas, a company that we profiled before which was founded by 5 of the world's leaders in gene editing. Editas has exclusive rights to the one issued patent for CRISPR granted to the Broad Institute and Harvard University.

Caribou Biosciences: Backed by Atlas Venture, Caribou was founded by Jennifer Doudna who was also one of the cofounders of Editas. When the key CRISPR patent was granted to another Editas founder, Feng Zhang, Jennifer broke off from Editas taking her intellectual property in the form of her own pending patent for CRISPR. It is with this patent that she is hoping to stake her claim.

Intellia: Founded in 2014 by Caribou Biosciences and Atlas Venture, Intellia was funded by both Atlas Venture and Novartis Institutes for Biomedical Research (NIBR). Novartis has exclusive rights to use Intellia's CRISPR platforms to develop CAR-T therapies.

CRISPR Therapeutics: CRISPR Therapeutics was founded in April of 2014 by Emmanuelle Charpentier, also one of the co-inventors of the CRISPR/Cas9 technology.

Cellectis: Cellectis is a French company that is involved in both gene editing and cancer immunotherapy. The Company has worldwide rights to a patent family titled "Engineering Plant Genomes Using CRISPR/Cas Systems" upon which they have developed a platform to improve the quality of crops for the food and agriculture industries.

Precision Biosciences: Precision's Directed Nuclease Editor™ (DNE) technology enables the production of genome editing enzymes. Precision controls a growing patent estate consisting of over fifteen allowed genome engineering patents in the U.S., Europe and Australia.

Sangamo: California based Sangamo is a $1 billion company that uses a gene editing system based on zinc-finger nucleases and has quite a head start over Editas. Sangamo has entered Phase 1 clinical safety trials for their gene editing technique that is showing encouraging results as a possible functional cure for HIV.

Chapter Five

Bioethics of Reproductive Cloning: Patenting a Designer Human Being

Introduction

Somatic cell nuclear transfer technology (SCNT) can be used for either reproductive or therapeutic/research cloning. As discussed in the previous chapter, reproductive cloning involves generating an exact genetic copy using a donor cell and the enucleated oocyte obtained from the same individual (oocytes may alternatively be obtained from the donor's mother, sister, or grandmother). Since mitochondrial DNA (mtDNA) comes from the oocyte, obtaining an enucleated oocyte for nuclear transfer, from any donor other than a maternal relative, will result in progeny that is not an exact genetic clone because the embryo will possess nuclear DNA identical to the donor cell's and mtDNA identical to the oocyte's. Although mtDNA represents less than 1% of the total DNA in a cell, it contains critical information regarding the energetics of a cell. Another problem with generating an exact genetic clone using SCNT is understanding how gene expression is related to epigenetic instability.[1] This is the reason why identical twins may have the same genetic information, but are not precisely identical in behavior, health, and even physical traits.

There are various situations that elicit profound ethical debates related directly to reproductive cloning. The most obvious is whether and how cloning technologies should be applied to humans. Employing SCNT to generate embryos from multiple parental donors is one ethically challenging example of using this technology.

Academic Arguments Against Human Reproductive Cloning

Current research in animals indicates that the success rate of reproductive cloning is quite low; therefore, many fertilized zygotes or embryos will be destroyed or discarded during any attempts to clone human beings. While Chapter 4 provided some scientific reasons to support research in reproductive cloning, there several cultural, morality-based arguments, and science-based arguments to oppose reproductive cloning.

[1] Epigenetics effects refers to changes in gene expression that are not determined primarily by the underlying DNA sequence. Epigenetic regulation refers to the mechanisms, mainly DNA methylation and histone modifications that license regions of the genome for expression while shutting down others.

Cultural and Moral Arguments: The first morality-based argument against reproductive cloning is the belief that life begins at conception and that all human beings therefore possess, from the moment of conception, intrinsic and unique value. Individuals advancing this argument oppose human cloning on the grounds that human zygotes and embryos, whether generated by cloning technology or IVF, deserve "full moral respect." They support the view that, as in "natural fertilization," a cloned embryo produces a new and complete human organism whose development into a child follows a genetic-based cellular protocol. These embryos possess a unique genome and the epigenetic primordial for self-directed growth into adulthood. Since SCNT involves the destruction of many pre-implanted embryos in order to generate one viable organism, opponents of reproductive cloning believe there should be a ban on reproductive and therapeutic cloning research, because pre-implanted and implanted embryos are considered to be potentially viable human beings.

The second morality-based argument for banning reproductive cloning is that this technology is unnatural and beyond the ethical boundaries of human experimentation. There is both a theological and secularist perspective to this argument. The theological argument is that reproductive cloning is immoral because human beings should not "play God". The argument is that scientists should not tamper with nature in an inappropriate manner, e.g., genetically manipulating God's creations (Savulescu 2009). The secularist argument stems from the idea that nature should not be manipulated into potentially harmful situations. The argument focuses on the fear of the unknown. Nature has a "natural" way in which it evolves and scientists should not be creating situations that typically would not have occurred without intervention.

Scientific Arguments Banning Human Cloning: From a scientific or medical perspective, reproductive cloning is associated with a high medical risk and potential dangers inherent in the SCNT process. Opponents of reproductive cloning cite the many animal studies that associate reproductive cloning with many harmful side effects, such as spontaneous miscarriages as well as birth defects in the newborn animals. Cloning experiments in animals also document increased damage to the immune system, risk of death from pneumonia, development of tumors, and risk of liver failure. Almost half of all cloned animals suffer from a condition known as *Large Offspring Syndrome* (LOS), which can cause terminal problems including enlarged placentas, fatty livers, and underdeveloped vital organs. In addition, some cloned animals (especially mice) may appear healthy at birth, but in fact have a reduced life expectancy as compared to animals generated by natural reproductive processes. While there is no clear data on the potential medical risks of reproductive cloning in human beings, many opponents of reproductive cloning believe that the high risks in animals are a valid indicator for similar high risks in humans.

There are also reported risks to animals carrying cloned fetuses. For example, animal welfare organizations point to the fact that even the Food and Drug Administration's (FDA) report in 2007, just prior to their approval of using cloned farm animals for food, states that "weak or non-existent uterine contractions, poor mammary development and failure to lactate" were found in animals carrying cloned fetuses.[2]

Textbox I. The use of cloning technology to produce children has been described as a dangerous experimental procedure. Firstly, there is no possibility for its subjects (the children created by it) to provide informed consent. Secondly, giving adults the opportunity to have what has been called the "ultimate 'single-parent child'" may also contribute to the commodification of children, and could deny children the possibility of a relationship with both a genetic mother and father. Finally, reproductive cloning may lead to generating designer babies with specific personality traits that burden them with the expectation that they will be like the individuals from whom they were cloned.

How would you address these issues?

Emotional Arguments Banning Human Cloning: Another argument presented by opponents to human reproductive cloning is psychological and emotional in nature. Opponents argue that cloning is a threat to human individuality. Normal human reproduction is designed to combine genetic elements from two parents to form a single progeny. In contrast, reproductive cloning can generate an identical DNA-copy of one parent, which could create a great psychic burden on the cloned child. Opponents of reproductive cloning believe that children should be valued for how they develop as individuals, not according to how closely they meet their parents' genetic expectations. In other words, each child has a right to develop naturally from their unique set of genetic information and not to develop into his or her genetic progenitor. There also is a concern of the impact this will have in familial relationships. How will society view and treat cloned children? Will cloning create new family structures? Reproductive cloning technology also has the potential to allow for the design of babies to alter gender preference, appearance, athletic potential, or behavioral characteristics. Designing babies for purposes of vanity could affect the nature of the family unit and parent-child relationships. This could, in turn, affect the psychological pressures on the cloned child. Anti-reproductive cloning bioethicists supporting this argument cite studies showing that naturally conceived identical twins may exhibit increased psychological problems related to their inability to define their unique individuality (Sutcliffe and Derom, 2006).

The emotional argument, first publicized by Dr. Leon Kass states that reproductive cloning should be banned because we intuit and we feel, without

[2] http://www.fda.gov/AnimalVeterinary/SafetyHealth/AnimalCloning/ucm124840.htm.

argument, the violation of things that we rightfully hold dear (Kass 1997). In various pieces, Kass describes human cloning for reproductive purposes as revolting, grotesque, repugnant and Frankensteinian. He urges us to ban the cloning of human beings, as it is a 'clear fork in the road' where the wrong choice could lead us into a dystopian 'Brave New World'. Moreover, Leon Kass, states that reproductive cloning is "the first step toward a eugenic world in which children become objects of manipulation and products of will."[3] Cloning will destroy the idea of the "unique humanness" of human life and the meaning of our embodiment, our sexual being, and our relations to ancestors and descendants. In fact, Leon Klass employs the "yuck factor" as an ethical argument to ban cloning. Dr. Kass defined the bioethical "yuck factor" as being an unethical technology based on an intuitive negative response rather than on concrete ethical or moral values.

How significant are emotional arguments in bioethics and cloning? In 2016 several hundred participants were surveyed about their attitudes towards human reproductive cloning (May 2016). Most participants condemned human cloning as immoral and illegal giving anxiety as their most common reason. Only about a third of participants selected "disgust" or "repugnance" as the emotional reason for banning human cloning. One could therefore conclude from this one small study that the "yuck" factor reaction to cloning is not widespread.

Arguments that Promote Reproductive Cloning Research

One of the main reasons for developing reproductive cloning technology is the belief that the current proscription against reproductive cloning may not be immutable if advances in technology yield a process superior to traditional assisted reproductive techniques used to treat infertility. In addition, those who favor research in reproductive cloning believe that the science-based arguments against reproductive cloning are weak. More importantly, they are confident that, as this technology improves, the gain in scientific knowledge will outweigh most ethical concerns.

Bioethicists who favor reproductive cloning research believe, first and foremost, that a fertilized zygote or pre-implanted embryo does not constitute a human being and does not confer personhood status. They believe that SCNT resembles tissue culture technology. Any replicating cell contains the genetic information required to develop into a potential fetus, but this information is suppressed. Unless implanted into a uterus, the zygote or pre-embryo cannot develop into a human being and, therefore, does not have personhood status. Thus, the destruction of many pre-implanted zygotes and pre-implanted embryos, required for human reproductive cloning, do not present an ethical problem for these bioethicists. Indeed, most oocytes fertilized in vivo fail to generate a viable child and are subsequently discharged from the woman. Thus, sperm and oocytes

[3] http://www.bioethics.gov/transcripts/feb02/feb13session4.html

can be functionally and morally identified as any other cellular components of the male or female body. In fact, sperm or oocytes are not the only biological sources for genetic donation in cloning. Fibroblasts or blood cells can be de-differentiated into oocytes or stem cells that can serve as genetic donors for cloning.

Many scientists who support reproductive cloning research also believe there is nothing immoral in man “playing God”, especially when medical benefits are to be gained from this research. Moreover, reproductive cloning is not an unnatural event in biology, as it occurs in several species. For example, the little fire ant, *Wasmannia auropunctata*, and the lizard *Leiolepis ngovantrii*, can clonally reproduce (Schwander and Keller, 2012).

The risks of fetal defects and spontaneous miscarriages associated with cloning in animals is of concern to all parties in this debate. However, many scientists believe that further experimentation will greatly reduce these medical risks. Almost all proponents for reproductive cloning believe that human experimentation should not begin until the known side effects of cloning in animal models are more significantly reduced to minimize potential health risks. In fact, several recent studies have shown that calves and pigs cloned using SCNT are born healthy, and do not express many of the aforementioned medical problems seen in other animals (Lanza et al., 2003). Scientists who support reproductive human cloning have also suggested that many of the defects observed in animal cloning are, de facto, due to poor culture conditions, and that cultural conditions have been improving and becoming more optimized for human embryos and cells over the past 36 years of assisted reproductive technologies (Zavos 2003). Additionally, scientists have also noted that LOS (*Large Offspring Syndrome*) appears to be correlated with incorrect imprinting of the *IGF2R* gene (Young et al., 1998) and that this gene is not imprinted in humans or other primates (Killian et al., 2001), suggesting the absence of this gene in humans will render human cloning technologies safer. As of 2016, there is no consensus on the safety of human cloning because various cloning studies in animals claim minimal side effects while others report serious health concerns with this technology.

The argument that cloning challenges definitions of individuality, or that it may influence the psychology of the cloned individual, does not present a real problem to proponents of human cloning. They claim that this argument ignores the normality of naturally born identical twins. Nurture is of equal, if not greater, importance as nature in the development of human personality. Moreover, using SCNT technology for human cloning will generate offspring that will have significant differences in their mtDNA from the person providing the donor cells. However, if the oocyte is obtained from the same person as the donor cell, or from a female blood relative of the cell donor, this will not be the case. Even an exact genetic clone may not necessarily develop the same personality as the parent. Epigenetic events during embryonic stages, and environmental factors during development and growth of the child, are major impacts that shape personality and behavior. The psychological normalcy observed in many naturally born identical

twins argues against the possibility that a cloned child will experience psychological harm emanating from a diminished sense of individuality and personal autonomy.

Historical Insights of Cloning

A historical review of the medical risks associated with IVF is relevant to the debates surrounding reproductive cloning. One historical lesson from IVF is that it takes decades to assess the medical risks associated with reproductive technologies. Almost five million IVF generated babies have been born worldwide and over five hundred thousand in the United States since its inception in 1978. Yet, only in the last several years have studies examined prenatal complications associated with the procedure. In general, there are no significant medical risks to babies born via IVF technology. The major malformation rates ranged from 0% to 9.5% for IVF and 0–6.9% in the control groups (Hyrapetian et al., 2014). There are a few studies (Hansen, Kurinczuk et al., 2002) that claim that IVF technology is associated with increased birth defects, but it has been difficult to arrive at any definite conclusion as to whether the birth defects reported are due to the age of the parents or to IVF. Some of the reported risks to the mother are thought to result from the hormones taken to induce ovulation and to maintain the pregnancy, rather than the actual IVF procedure. Other risks to the mother are easily managed, such as infections and a risk of hemorrhaging. If there is a medical need to engage in reproductive cloning, then care will be taken to begin human trials only after animal studies have shown its safety.

Another question is whether reproductive cloning will lead us down the slippery-slope road to eugenics. Actual IVF outcomes weaken any slippery-slope arguments, as the universal use of IVF technology has neither created legions of less-than human children, nor contributed to a disintegration of the nuclear family. Nonetheless, whether or not these historical lessons regarding IVF can be applied to human cloning still remains controversial.

Reproductive cloning is fundamentally different from IVF in one respect. The goal of IVF is to produce a genetically unique human being that carries genetic information from two parents. In contrast, nuclear transfer technology produces offspring that may only differ in their mtDNA and possible epigenetic variation, while remaining essentially genetically identical to their donor cell. Attempting to ascribe a percent difference between the donor and genetic clone can be uninformative since human beings and chimpanzees differ in their DNA by about 1-2%. DNA homology from a human male, however, more closely resembles the DNA of a male chimpanzee than the DNA from a human female, because of the Y chromosome. In clones where only mitochondrial differences exist, genomic differences could account for less than 0.1% difference between donor and clone.

Assessment of any reproductive technology will require decades of observations on human development, from infancy into old age, to determine the medical and psychological risks of such a procedure to the individuals involved and to society. It is interesting that, on a theoretical level, one would have expected the FDA to engage in these long-term studies before approving IVF procedures, in order to ensure that there are no effects on the mother or child. Nonetheless, one could speculate that political pressure, from the >12% of couples in the United States who are infertile, have influenced FDA decisions, even though there is already an array of alternate methods for treating infertile couples.

Religious Beliefs Regarding Human Cloning - Introduction

Different religious beliefs concerning when human life begins, and whether human beings should engage in “unnatural biological processes for conception”, deter consensus on controversial issues such as cloning and stem-cell research (Frazzetto 2004). Yet, current human reproductive cloning technologies may challenge the boundaries of parenthood and social responsibility as they were described in the Bible. For example, who is the cloned child's genetic mother or father? As we understand those terms from a biblical perspective, if a woman cloned herself, would the child be that woman's daughter or her twin sister? Will the cloned child be “fatherless?”

Not surprisingly, organized religions, such as the Catholic Church, have taken a strong interest in the cloning debate. Many Catholic scholars have issued strong words of caution, or outright condemnation, of any research that creates, uses, or destroys human embryos.[4] The impact of their campaign against cloning can affect public opinion and has indeed influenced scientific policy. Many Western countries with primarily Catholic populations have banned human cloning and/or the creation of human embryonic stem-cell lines, or at the bare minimum, have issued strict regulations on such research. Aside from the issue of when an embryo attains human status, many of the major religions strongly reject reproductive cloning because it is unnatural, and they consider life to be a “gift”' from God. They also hold the belief that the creation of human life is to come from both a “unitive and procreative act of sexual intercourse” and that therefore, IVF or reproductive cloning is never permissible because it is not a unitive act between a husband and wife.

Nevertheless, religious leaders rarely speak with a unified voice. Although some faiths hold irrevocable positions against cloning, other religions have found room in their beliefs and traditions to accommodate the potentially beneficial aspects of this technology. In essence, different attitudes towards human cloning center on a few fundamental questions: Does an embryo hold the status of a

[4] Catholic doctrine according to the Vatican bans all embryonic cloning. http://www.vatican.va/roman_curia/secretariat_state/2004/documents/rc_seg-st_20040927_cloning_en.html

person? Is its destruction during research a murder? Does cloning corrupt family relationships? And, ultimately, does cloning mean tampering with God's creation and millennia of human ethical, social, and sexual arrangements?

Varying Religious Views on Reproductive Cloning

In order to prepare for the bioethical dialogue concerning cloning, one must be able to address a significant population that has a stake in the debate – the followers of various religions. Although polls have already shown that a great majority of Americans oppose cloning, this opposition is mostly representative of religious people. An ABC poll carried out in 2001 asked a random national sample of American adults whether human cloning should be legal (Bainbridge, 2003): 95 percent of evangelical Protestants wanted it to be illegal, compared with 91 percent of Catholics, 83 percent of non-evangelical Protestants, and 77 percent of non-religious respondents.

As stated above, the Catholic Church has become the leading voice against any form of human cloning, and even against the creation of human embryonic stem-cell lines from “excess” IVF embryos. Their prohibitive stance is based on a 1987 document entitled “Instruction on Respect for Human Life in its Origin and on the Dignity of Procreation (Donum Vitae),” published by the Congregation for the Doctrine of Faith. Roman Catholics believe that cloning is contrary to moral and natural law, since it is in opposition to the dignity of both human procreation and the conjugal union. Any attempts at cloning are therefore a violation of the dignity of the human embryo, which, in Catholicism, is granted the status of a person from the moment the oocyte is fertilized (also referred to as the moment of conception).

The above Catholic doctrine provides a relatively recent definition of personhood in the Christian tradition. The medieval church, in line with Aristotelian doctrine, believed that an embryo acquired a soul only when it took recognizable human form. Consequently, abortion was only considered to be a venial sin in the Middle Ages, not a mortal sin comparable to murder. A drastic change took place in 1869 when Pope Pius IX, who, most likely influenced by advances in embryological research, declared that an embryo bore full human status from the time of fertilization (Lachmann 2001). Since then, the Catholic Church has upheld the position that the destruction of an embryo after conception is a mortal sin. No distinction is made between embryos conceived naturally and those created through IVF or cloning, although many Catholic leaders strongly oppose unnatural methods of reproduction and prohibit any procreative act that is not unitive between a husband and a wife.

Buddhism,[5] by contrast, does not have the same fundamental opposition to cloning as the Catholic Church. "Many of these theological objections disappear

[5] Buddhism is divided into roughly three major branches: the Theravada, the Mahayana, and the Vajrayana. The Theravada claims to be the oldest school and has at its goal self-liberation. The Mahayana shares much with the Theravada but espouses the idea of saving other beings as the

when cloning is viewed from a Buddhist perspective," said Damien Keown, a Reader in Buddhism in the Department of History at Goldsmiths College, University of London, UK, and an authoritative voice on Buddhist responses to cloning and other biomedical issues. The Buddhist view of the world, and mankind's place in it, differs from that of monotheistic religions. In Buddhism, there is no supreme or divine creator whose plan might be distorted by human manipulation of nature. In addition, Buddhists believe that the creation of life is not a fixed or unequivocal process. "Buddhism teaches that life may come into being in a variety of ways, of which sexual reproduction is but one, so sexual reproduction has no divinely sanctioned priority over other modes of procreation," explained Keown. Life can, therefore, begin in many ways and therefore, theologically, cloning would not be seen as a problematic technology. Furthermore, in contrast to other larger religions, Buddhists regard human individuality as an illusion or mirage. Cloning, therefore, would not threaten or devalue the personality or character of an individual (Simpson et al., 2005).

Similarly, Hindu views adopt a somewhat neutral position towards cloning. Hindu views are incredibly diverse within the religion. There have been scriptural traditions that assert conception as the initiation of human existence, but there are also views focused predominantly on the compassion and “healing” of cloning research (Banchoff 2008).

Islamic law remains concerned with reproductive cloning procedures, particularly with respect to their impact on inter-human and familial relationships. "Islam regards interpersonal relationships as fundamental to human religious life," said Abdulaziz Sachedina, Professor of Islamic Studies at the University of Virginia (Charlottesville, VA) and a leading scholar of Islamic views on cloning. The preservation of the parent–child lineage is of utmost importance to Muslims, as are the spousal relationships that encourage parental love and concern for their children. Thus, Islam is concerned with moral issues related to the genetic replication and embryonic manipulation associated with these technologies. Will these technologies lead to incidental relationships between a man and a woman without a spiritual and moral connection between them?

According to the Muslim sacred text, the Koran, moral personhood is a process and is not granted at the embryonic stage. Unlike the Catholic Church, most Sunni and Shiite jurists would “have little problem” endorsing ethically regulated research on embryonic stem (ES) cells, because the fetus is accorded the status of a legal person only at the later stages of its development (Hug, 2006). Muslims would therefore endorse reproductive cloning to help infertile couples, only if it was within marital bounds, and would reject it if it were to break familial relationships. However, Islam does not support surrogate parenting or adoption. Therefore, under Islamic law, excess embryos or embryos generated via IVF could not be used by anyone other than the couple who created them**.**

highest goal. The Vajrayana is an occult Buddhism that emphasizes esoteric rituals and practices taught by a master.

However, it is sometimes unclear if all Muslims share this view. According to a 2001 poll by the Council on American-Islamic Relations (CAIR), 81 percent of 1008 Muslim respondents said they were opposed to human cloning. Furthermore, in 1983, the Islamic Organization for Medical Sciences (IOMS) convened a seminar on the Islamic view of human reproduction, and ultimately determined that human cloning was not permissible.[6] The Islamic Fiqh Academy had a unique view on the topic. After a conference in Casablanca, the academicians concluded that, although human cloning does not question Islamic belief and the Will of Allah, for "cloning is a cause and only through Allah's Will it can produce the effect," human cloning does bring forth "extremely complex and intractable social and moral problems."[7]

In Conservative and Orthodox Judaism, human status or personhood requires implantation of a fertilized zygote into a woman and for the embryo to develop for at least 40 days. However, reproductive cloning may challenge deeply held beliefs about creation and mankind's relationship with God. If God is seen as the only Creator, and creation of the world as being a completed act, then human beings have no right to tamper with it. Conversely, many Jewish thinkers regard God as the Power of Creation and view creation as a transformative process that invites human participation. In other words, human beings are viewed as partners in the creation process. Several Jewish scholars advocate the view that reproductive cloning represents a process that human beings should utilize to accomplish good. Dr. Edward Reichman, a leading Jewish bioethicist, commented that, "[t]he process or 'mechanical' aspects of human cloning present no major legal obstacles from a Jewish perspective" (Frazzetto 2004). He further stated that the low efficacy and potential adverse outcomes of human cloning are legal concerns that would lead society to reject any human cloning at this time. Prospectively, creating people of legally ambiguous lineage, who may suffer profound social and psychological complications, may preclude any future acceptance of cloning despite perfection of the procedure from a medical perspective (Frazzetto 2004). But unlike the Catholic doctrine, these Jewish thinkers do not believe that ensoulment occurs at conception.

Government Regulation of Human Cloning

Governments around the world have expressed a wide range of policies on human reproductive cloning. Many countries have a complete prohibition of reproductive cloning, while others have no policies on record. Over 30 countries, including France, Germany, and the Russian Federation, have banned human

[6] http://www.islamset.com/healnews/cloning/index.html

[7] http://www.albalagh.net/qa/ifa.shtml; see Vaidyanathan, Brandon, et al. "Rejecting the conflict narrative: American Jewish and Muslim views on science and religion." Social Compass 63:478-496, 2016 for a discussion on the Jewish and Muslim views whether there is a conflict between science and religion.

cloning altogether. Fifteen countries, such as Japan, the UK, and Israel, have banned human reproductive cloning, but permit therapeutic cloning. Many other countries, such as the United States, have yet to pass any official legislation (Camporesi and Bortolotti, 2008). In the United States, various congressional bills are proposing a one million dollar fine, plus a ten-year prison sentence, for any individual who engages in reproductive cloning. However, there are only a limited number of laboratories, in either academia or corporate environments, which have reported using SCNT in animals. The restrictions of government funding for research in reproductive cloning have opened the door for entrepreneurs to support the technology via private funding. As mentioned in Chapter 4, Boyalife Group in China will begin cloning cows in 2016-2017.

Does society have the right to ban or limit scientific advancement or progress (UNESCO 2009)? There are many advocates of reproductive cloning who propose that procreative liberty and reproductive freedom are intrinsic rights within the American Constitution.[8] However, most advocates of human reproductive cloning believe that society should, at least for now, refrain from human experimentation until the medical risks seen in some animals have been reduced or eliminated.

The history of science supports the assertion that new technologies often lead to valuable benefits. Supporters of reproductive cloning believe that this technology will eventually provide both valuable basic research and the possibility for spin-off technologies that will enhance our capacity to improve animal and human reproduction. Along with improving reproduction, reproductive cloning could aid in the development of new therapies in the area of reproductive medicine and other areas concerning health. As discussed in the previous chapters, cloning technologies has led to new clinical applications in the area of reproductive medicine.

Cloning Noah's Ark

From a biological perspective, cloning may challenge biological diversity or eliminate the need for the male species since the ova and donor cells could be obtained from two women or the same woman. Large-scale cloning could deplete genetic diversity, making a species susceptible to specific diseases. Many scientists believe it is diversity that drives evolution and adaptation. However, proponents of cloning argue that the high cost of cloning would limit such a large-scale use as to threaten human biodiversity. In addition, does cloning violate the bioethical guideline of equal access or "justice", where such an expensive technology would create a divide between couples who are wealthy and those who are poor?

[8] http://writ.news.findlaw.com/grossman/20011120.html

As mentioned briefly in Chapter four, several groups have successfully used SCNT technology to clone an endangered species using members of non-endangered species as surrogate mothers. For example, in 2000, a humble Iowa cow gave birth to a rare, endangered, ox-like Asian gaur. This was the first example of trans-species cloning. Incidentally, and perhaps humorously, the newborn gaur was named Noah. It was implied, then, that Trans-species cloning could help *reincarnate* some species that are already extinct.

Several other successes at cloning exotic or endangered species have been reported. Examples are the Gaur (*Bos gaurus*), Banteng (*Bos javanicus*), and Bucardo (*Capra pyrenaica pyrenaica*). In another experiment, an African wildcat was cloned using an ordinary house cat as the oocyte donor and surrogate mother. Other endangered animals that have been cloned include the Indian desert cat, a bongo antelope, a Mouflon sheep, and a rare red deer. Efforts are currently underway to use nuclear transfer technology to clone giant pandas, the Siberian Tiger, white rhinoceros, and Arabian oryx as well.

The distinguishing feature of all these examples is that they employed trans-species cloning. In these instances, the oocyte cytoplasm being used to create the embryo was derived from common domesticated species, while the cell nucleus was obtained from the endangered species of interest. Trans-species clones, inevitably, differ from both of the parental species in their nucleo-mitochondrial characteristics. At the very least, mitochondria inherited from the recipient oocyte could influence specific functions in the trans-species organism, such as muscle development. Yet, trans-species cloning offers a method for animal conservation in situations where other reproductive technologies, such as artificial insemination, have failed. In addition, animals resulting from these trans-specific cloning efforts are scientifically valuable for their insights into the functional relationships involved in nucleo-mitochondria dialogue.

The major ethical questions raised in trans-species cloning include: a) Does the creation of nuclear-mitochondrial hybrid animals interfere with natural species evolution? Is it appropriate to play God or manipulate nature and create nuclear-mitochondrial hybrid animals? b) Will this technology inevitably lead to the use of large mammals, such as cows, as artificial incubators for human embryo development? c) How will these trans-species be valuable in species conservation? Many of the ethical concerns associated with genetic modifications of species are viewed in a similar vein as issues in generating trans-species clones.

There are other concerns associated with trans-species cloning. The clone would be born to a surrogate mother, most likely from a different species, and may have to be raised partially, or even entirely, by humans. More research must be done to examine the impact of one species nurturing another species. Furthermore, for many species, successful reintroduction to the wild after human rearing is rarely achieved. Therefore, this technique would be of limited use in

terms of replenishing a viable population of the endangered species. There is, nonetheless, scientific literature that suggests that certain species, including some amphibians, may benefit from restoration efforts of reproductive cloning due to their intrinsic biological systems, which have favorable characteristics that increase the likelihood of success (Holt et al., 2004).

Cloning our Neanderthal Ancestors

Since the initial extraction of the Neanderthal DNA (See chapter four), bioethical contentions, provoked by the pursuit of the Neanderthal genome, have appeared in the public. The use of SCNT and other genetic-based technologies to clone a Neanderthal being may create a situation that would be an affront to many religious and moral beliefs.

One important consideration in cloning a Neanderthal individual is identifying the scientific objectives of such a project. Will cloning Neanderthals increase our knowledge about human development? Will such clones help scientists understand how Neanderthal genes could protect modern man and woman from specific diseases (Church et al., 2013)? Do the answers to these questions justify efforts in cloning a Neanderthal individual?

Cloning Neanderthals raises not only the ethical aspects of cloning an extinct species, but the religious and moral objections against human reproductive cloning. A central issue is whether a cloned Neanderthal would be considered human. Much opposition comes in response to the uncertain behavior and cognitive abilities of the Neanderthal clone. From anthropological evidence and genetic analysis, such as mtDNA sequencing, it is postulated that the early Neanderthals would have many rational capabilities similar to those of the modern *Homo sapiens*, hence calling into question the ethical responsibilities involved in cloning Neanderthals. In fact, from mtDNA sequence analysis, the number of differences between the human mtDNAs and the Neanderthal mtDNA varied from 201 to 234, which is less than the differences between human and its closest living species – the chimpanzee (Clark 2008). Given that a Neanderthal might express human-like cognitive abilities, would it have the same rights as a human being? And does it demand us to reconsider bringing to life an individual that may very well express individualism, intelligence and autonomy? Would we be able to provide the clone with a suitable habitat, given the potential great offense people may take at its existence? Most likely, such a creature would live its existence as a research subject.

A second objection may come forth concerning the method by which a Neanderthal is gestated. Is it possible to implant a Neanderthal embryo inside a human uterus? If so, it may challenge the bioethical principle of human dignity, as well as potentially violate other principles such as non-maleficence and justice. The use of technology to create human-like organisms that may not have the same cognitive potential as human beings might be considered as violating human dignity. Biotechnologies should be used to enhance human beings, animals, and the environment, whereas, technologies that hinder human cognition or intelligence are difficult to ethically justify. **In research and medicine, biotechnological applications should be guided not by what you can do, but rather what you should do.**

Conclusions

It is always difficult to predict which innovative biotechnology will be accepted. When IVF was first introduced in 1978 many scientists and bioethicists speculated that the technology was too dangerous and would result in too many babies born with birth defects. However, as this biotechnology gained widespread acceptance as a viable alternative for infertile couples to have children, the ethical concerns dissipated.

In 2015, according to data collected by Gallup, 15% of Americans believed human cloning to be morally acceptable. That is an 8% increase from the 7% who considered human cloning morally acceptable in 2001 (Newport, 2015).

As of 2015, there are many health and psychological concerns regarding reproductive cloning. If this biotechnology were improved to demonstrate a low risk procedure, and if the medical need for reproductive cloning became established, one could speculate that the ethical concerns related to this new technology may also become diminished.

Bioethical Challenges: Case Scenarios

1. **An unmarried 35-year-old woman desperately wants a child. She has just read that bone-marrow or body fat-derived stem cells can be triggered to differentiate into a potential "sperm-like cell" capable of fertilizing her own ova. She would serve as the gestational mother. What are the underlying bioethical issues that she should consider in making an informed decision about whether or not to differentiate her own stem cells to generate an embryo?**

2. **In 2008, the FDA stated that milk and meat from cloned cattle was safe for human consumption. What are the bioethical issues that emerge from this FDA announcement?**

3. **A hamburger made from cow muscle grown in a laboratory was fried, served and eaten in London in 2013. The cost to prepare this hamburger was about $300,000. Research in producing lab-made meat could provide high-quality protein for the world's growing population while avoiding most of the environmental and animal-welfare issues related to conventional livestock–based meat production. What bioethical guidelines are challenged by this research?**

See the following video on YouTube produced by past students.
https://www.youtube.com/watch?v=pPpZ-ILyiwo

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Chapter Nine

Gene Editing and Synthetic Biology

Introduction

In a 1989 interview, James Watson, one of the founders of the structure of DNA, said, “We used to think that our fate was in the stars. Now we know in large measure, our fate is in our genes.”[1] With that knowledge, an intrinsic human desire to control and change our fate developed through biotechnology that manipulated the human genome.

The overall objective of molecular genetics is to better understand how genes and regulatory elements of the genome function in response to various developmental and environmental cues. Rapid advances in mathematical, computational, and molecular biology, along with new technologies in DNA microarrays, will revolutionize genomics in the next few decades. Out of the almost 9 million known species, only several hundred complete genomes from different organisms have been sequenced.[2] As of 2014, about 290,000 genomes from different human volunteers have been completely sequenced[3].

Prelude to Genetics and Disease

The term genetics, coined by William Bateson in the early 1900s, comes from the Greek term “to generate” and is the science of biological heredity and variation. In 1866, Gregor Mendel, an Austrian monk, published the results of his decade-long investigations on the inheritance of "factors" in pea plants. He suggested that every cell contains pairs of “factors” and that each pair determined a specific trait. The members of each pair segregated from each other in the process of sex-cell formation so that a gamete contained one member of each pair. The segregation of one pair was independent from the segregation of all other pairs of factors. It wasn’t until 1909 that a Danish botanist, Wilhelm Johannsen, introduced the word "gene" to characterize Mendel’s "factors."

As a prelude to any discussion of genetics and disease, it is important to highlight several basic principles of genetics and its relation to disease. The first principle is that *genetics* refers to the study of specific, individual genes and their role in inheritance. Chromosomes contain the majority of a cell’s genetic

[1] Interview in L. Jaroff, “The Gene Hunt,” Time, March 20, 1989, 62-67.
[2] http://ngs-brescia.blogspot.com/2012/02/do-you-know-how-many-species-are-there.html
[3] https://www.technologyreview.com/s/531091/emtech-illumina-says-228000-human-genomes-will-be-sequenced-this-year/

information and are composed of nucleic acids and proteins. DNA sequences can also be divided into genes, where each gene encodes the information necessary to synthesize one or several proteins. The location of a gene on a chromosome region is called a locus. In humans there are about 20,000 genes. At any given gene locus, DNA sequences may differ from one individual to another in some small ways. These different DNA sequences within a gene locus are termed alleles. In most animal and plant populations, 10-20% of the genes are composed of multiple alleles. There are several processes by which different alleles develop. One process is through mutations such as a point mutation, where one nucleotide is replaced by another. Another process involves a section of DNA eliminated or translocated from one chromosome location to another on the same or different chromosome.

The second principle is that every human being has mutations in their genes and these mutations can occur at almost any location within the whole genome. This means that there are no individuals with “perfect” human genomes. Some of these changes have no major phenotypic expression in that organism. Other mutations can lead to disabling conditions, specific disease states, or death. Conventional wisdom holds that all the cells within a given organism carry the same genome and that phenotypes are due to variation in gene expression. This is not entirely true. Somatic mutations frequently occur after fertilization and get passed on with each round of mitosis, leaving a trail of base pair changes that varies from cell to cell. Each cell has a set of mutations that is unique to that cell.

The third principle relates to the heterogeneity of response to genetic mutations and the consequences of genetic changes. Cells have a remarkable ability to edit their DNA in order to ensure that mutations do not occur at a high frequency. Even when mutations do occur, these changes have no perceptible effects because the genetic code is redundant. A point mutation that has no effect at all on the expression of the protein it codes for is called a silent mutation. Changes in the DNA sequence that **do not** have profound effects on protein function occur if the changes do not dramatically change the three dimensional structure of the protein.

Genes also vary a great deal with respect to how much they can be mutated before harmful changes occur in the organism. Some genes, such as those that encode the basic components of metabolism, replication, transcription and translation machinery, are hard to mutate without harming an organism. We see very little variation in those gene sequences from one organism to another. Such genes are said to be conserved. In contrast, individuals with genes responsible for cystic fibrosis express a wide range of disease severity because there are many types of mutations in the gene encoding the transporter protein involved in the disease. Thus, **allelic heterogeneity** implies that there are many places on the gene that can be mutated and that not all mutations have the same impact on phenotypic or disease expression.

Some changes in alleles or DNA sequences can be favorable and promote a healthier life. As an example, there are several alleles that encode for the protein apoE, which is a ligand for the LDL receptor. It is a critical membrane protein in cholesterol regulation. In Italy there is a community near Milan whose residents are less likely to develop atherosclerosis because of a fortunate mutation in one of their forbearers. Their apoE isoform, referred to as apoE2, appears to protect them from developing atherosclerosis. In addition, the expression of this type of apoE also has been shown to be an important determinant of Alzheimer's disease. Individuals with the apoE4 isoform have a higher rate of atherosclerosis, heart disease, and Alzheimer's disease.

The term *genomics* refers to the study of an organism's entire genetic makeup, which is called a genome. The study of genomics includes understanding how the genome interacts with environmental or non-genetic factors, such as a person's lifestyle. This new area of science has the potential to improve our understanding of complex diseases such as diabetes, heart disease, and asthma, as well as to improve medical treatment.

The fourth principle is that epigenetics regulate genes and their functions. Epigenetics involve methylation or acetylation of either nucleotides or DNA associated proteins, such as histones. Through the processes of methylation and acetylation, the contraction and expansion of DNA can be controlled. When an acetyl group is added to the lysine region of the histone, the chromatin that is wound around the histone becomes uncondensed. This unraveling leads to the expression of the gene. When a methyl group is added to the lysine region of the histone, the DNA can either become condensed or uncondensed. This depends on which lysine on the histone the methyl group associates with. As expected the modifications of histones can either result in the expression or silencing of a gene. If you think of our DNA as an immense piano keyboard and our genes as keys -- each key symbolizing a segment of DNA responsible for a particular note, or trait, and all the keys combining to make us who we are -- then epigenetic processes determine when and how each key can be struck, changing the tune being played.

The final principle in basic genetics is that the understanding of any genetic process or phenotype will often require a complete understanding of how each region of the DNA operates within the whole human genome. For example, there are genes that increase the chances of getting lung cancer in smokers, and yet, there are many heavy smokers whose genetic makeup enables them to never come down with lung cancer.

Parental Genes Impact the Health of the Offspring

On a basic level, each parent donates one chromosome and consequently one gene to the child. Over 60% of the genes have the ability to undergo alternative splicing in order to form several protein products. This accounts for the excess of

200,000 different proteins expressed in human beings and encoded within about 20,000 genes. The nature of each contributed gene influences the health of the child in various ways. If a genetic disease is inherited in a dominant manner, such as Huntington's disease, then one parent donating this mutated gene to the fertilized egg will result in a child who will eventually be stricken with the fatal disease. Statistically, each child in such a family has a fifty percent chance of inheriting the gene for Huntington's disease.

Most genetic diseases, however, are recessive disorders. To be affected by a recessive disorder requires that an individual possess two abnormal or mutated copies of a gene. Therefore, each parent must donate one copy of the abnormal gene to the child. Cystic fibrosis and Tay-Sachs are examples of recessive disorders. A person who obtains only one abnormal copy of a gene for a recessive condition is known as a carrier. In general, a carrier of a genetic condition will not develop the disease and should not have any health-associated abnormalities due to the presence of a recessive gene.

Many human diseases, such as heart disease, Alzheimer's disease and cancer, are influenced by multiple genes in a complex fashion. It should be noted that being genetically predisposed to a disease does not necessarily mean that an individual will suffer from the disease in question. It simply means that there is an increased risk of developing the disease. Of great concern is a woman who tests positive for a genetic mutation in BRCA1. Women with this mutation may have a 55 to 85 percent chance of developing breast cancer by age 70, as well as having a 40 to 60 percent chance of developing ovarian cancer. Yet, only about 10-20 per cent of all diagnosed breast cancers have a family history in part because many diseases are regulated by other genetic and environmental factors,

Genetic Testing and Screening

There are several types of genetic tests available to the developing fetus or newborn baby, which identify genes that affect the health of the child. Pre-implantation Genetic Diagnosis (PGD) is done in pre-implanted embryos, allowing the couple to select an embryo that does not contain the gene causing the specific disease. Dr. Mark Hughes developed PGD in the mid-1980's, with Robert Winston and Alan Handyside, as a screen to test which embryos will develop cystic fibrosis. PGD is performed on an embryo, created via in vitro fertilization (IVF), which has developed to the 8th stage. Using micro-manipulation techniques, one of the cells is removed and tested for a specific mutation using PCR (polymerase chain reaction). Sometimes, chromosomal aberrations, as seen in Down's syndrome, are detected in this removed cell using fluorescence *in situ* hybridization technologies. Using PGD, embryos are selected that do not express two defective genes or even an embryo that does not express one defective gene (such an embryo will not develop into a child who will be a carrier for the recessive disorder). One or two of these embryos are implanted into the woman. As of 2014, over 1,000

healthy babies were born using PGD.[4] The list of diseases that now can be screened using PGD is over one hundred and includes cystic fibrosis, Down's syndrome, Duchenne muscular dystrophy, Huntington's disease, certain forms of early onset Alzheimer's disease, sickle-cell disease, and Tay-Sachs disease. PGD can also be used for sex selection. As of 2015, the error rate for misdiagnosis varied between 0.5-1% depending on which diseases were screened (Tiegs et al., 2015). Some of the errors result from a rare phenomenon that the cell removed from the 8 cell embryo may not be representative of the other cells. This phenomenon is called mosaicism. In other words, one cell would appear to lack the genetic defect whereas the remaining cells in the embryo would be abnormal or vice versa.

There appears to be a misnomer in calling this test Pre-implantation Genetic Diagnosis. In reality, this test is a way to screen pre-implanted embryos for specific genetic mutations. Therefore, it should be renamed Pre-implantation Genetic Screening (PGS) or Pre-implantation Genetic Testing (PGT).

Prenatal diagnostic testing is another way to assess reproductive risk. Prenatal diagnostic testing involves testing the fetus before birth to determine whether it has a certain hereditary or spontaneous genetic disorder. The most common tests used to detect abnormalities in a fetus include ultrasonography, chorionic villus sampling (CVS), amniocentesis, and percutaneous umbilical blood sampling. CVS involves removing a small amount of tissue called the chorionic villi, which is located on the outside of the fetal gestational sac and will later become the placenta. The chorion, as fetal tissue, shares its genetic makeup with the fetus, not the mother. The chorion has many small, finger-like projections on its outer surface, and a few of its cells may be carefully removed without disturbing the pregnancy. The chorionic villi cells may be used for chromosome analysis or other genetic testing, but cannot be used to test for open neural tube defects. CVS is available from 10.0 to 13.3 weeks of pregnancy. The CVS may be performed trans-abdominally by guiding a thin needle through the abdominal wall to the chorionic villi and then withdrawing a small amount of this tissue.

There are considerable efforts to test the genetics of a fetus by obtaining fetal DNA from the blood of the pregnant woman. During pregnancy, 5% to 15% of noncellular — so-called "cell-free" — DNA fragments in the maternal blood are of placental origin. While the amounts of fetal DNA is low, genetic analysis of DNA can be done on obtaining only several molecules of fetal DNA. Employing this type of prenatal testing could reduce costs by as much as 90%. In addition, cell-free fetal DNA testing has a very low false-negative rate (0.5%), which means that only women confirmed to be at high risk for fetal abnormality need to subsequently undergo amniocentesis.[5]

[4] http://www.ivf-infertility.com/ivf/pgd.php
[5] http://www.medscape.com/viewarticle/871296

Most genetic tests are offered primarily to couples with an increased risk of having a baby with a genetic abnormality (such as Down's syndrome) or a chromosomal abnormality (particularly when the woman is aged 35 or older). In Sardinia, for instance, where beta thalassemia is a relatively common genetic condition, prenatal genetic screening programs have produced striking results. Following fetal diagnosis of homozygous beta thalassemia, most couples decide to terminate the pregnancy. Overall, since the introduction of widespread genetic education, counseling, and screening programs in Sardinia, "the incidence of beta thalassemia major has been reduced from 1 of every 250 live births in 1975 to 1 of every 4000 in 1996, with 94% of the cases prevented" (Cao and Kan, 2013). Notable reductions in incidence due to targeted prenatal testing are reported for other disabling conditions as well, such as Tay-Sachs disease among Ashkenazi Jews, spina bifida in Britain, and Down's syndrome in the United States (Harper and SenGupta, 2012).

Newborn genetic screening is aimed at identifying infants who have genetic conditions that can be helped by early intervention. In many cases, this early intervention means the elimination or reduction of symptoms that would have left an unscreened individual with a lifetime of disability. Historically, this type of screening was strongly influenced by a genetic disease called Phenylketonuria (PKU). PKU is a genetic metabolic disorder that is easily treated by restricting certain foods from the diet; if left untreated, however, the disorder causes severe mental retardation. PCR is a common method for screening newborn babies for PKU**.**

Carrier screening is usually carried out in adults and involves identifying unaffected individuals who carry one copy of a gene for a recessive disease condition. The most common tests in carrier screening are cystic fibrosis, Tay-Sachs, and sickle cell trait. As a case in point, since carrier screening has begun for Tay-Sachs, the incidence of babies born with this disease has decreased dramatically in New York City alone. It is unusual to see a baby with this condition after 2000. Individuals can also undergo pre-symptomatic testing for predicting adult-onset disorders such as Huntington's disease or for estimating the risk of developing adult-onset diseases which have multifactorial etiologies like cancers, ischemic heart disease, asthma, diabetes, and Alzheimer's disease.

Epigenetics

No chapter in the genetics of disease can omit discussing epigenetics. Epigenetics is a hereditable process but differs from Mendelian genetics. In Mendelian genetics, changes in the base pair sequence of a gene can be a critical determinant of its activity. Epigenetics is the study of changes in gene activity that are caused by chemical modifications of specific base pairs or proteins that govern gene expression. It can be viewed as the software of the genome. What scientists have learned over the past several decades is that these changes can be passed

down at least one successive generation. Epigenetics regulates gene expression by orchestrating a set of chemical reactions that switch parts of the genome off and on at strategic times and at specific DNA locations. The epigenetic changes include DNA methylation and histone modification, which regulate high-order DNA structure and gene expression. Epigenetic regulation of gene activity involves a structure called an epigenome that sits on top of the genome, just outside it (hence the prefix epi-, which means above). The epigenome consists of chromatin, a protein-based structure, around which the DNA is wrapped, whose activity can be regulated by post-translational modifications and methylation of specific bases such as cytosines. In general, chromatin and DNA methylation results in gene silencing. On the other hand, the addition of acetyl groups unwinds the DNA around the histone spool and makes it easier for the RNA to transcribe a particular gene.[6] It is through epigenetic marks that environmental factors like diet, stress and prenatal nutrition can make an imprint on genes that is passed from one generation to the next. As James Watson said in 2003 “you can inherit something beyond the DNA sequence. That's where the real excitement of genetics is now."

Drugs exist that can remove methyl groups. Such medications could have novel clinical applications – years of trauma and abuse could potentially be wiped away with a single dosing. Besides the obvious need for further safety investigations (potentially beneficial methyl groups could be erased as well), would such treatments violate an ethical obligation to not alter the human genetic code? As epigenetics have revealed, our evolutionary connection to our ancestors is more complex than simple nucleotides. It is also made up of epigenetic modifiers that have been passed down from generation to generation.[6]

At first glance, epigenetic trans-generational inheritance of acquired characteristics is reminiscent of a theory of genetics proposed by Jean-Baptiste Lamarck (e.g., a giraffe, through evolutionary processes, has a long neck because he must reach the highest branches to obtain food). In fact, the current underlying mechanisms of epigenetics provide scientific evidence describing how the environment can trigger heritable changes. There is ample evidence in animals and even in human beings that environmental factors shape health and disease via epigenetic mechanisms that mediate gene-environment interactions. According to Dr. Moshe Szyf, a leading geneticist, epigenetics is a physiological mechanism by which the genome senses the world and changes itself (Narain, 2012).

A 1974 experiment on mice (Bailey et al, 1974) may present evidence for epigenetic influences within the ovum. Two strains of mice, which we will call “A” and “B”, are relevant. The researchers discovered a gaping difference in the violence levels between two groups of male mice that were from distinct combinations of these two strains:

[6] http://discovermagazine.com/2013/may/13-grandmas-experiences-leave-epigenetic-mark-on-your-genes

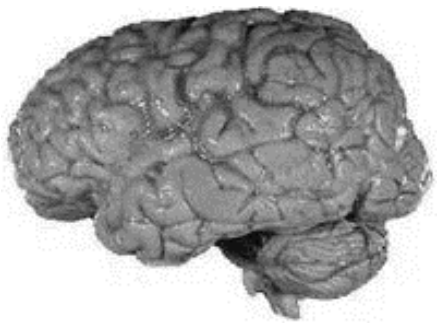
- The offspring of ("A" female mated with "B" male) female mated with (pure bred "B") male tended to be much more violent than:
- The offspring of ("B" female mated with "A" male) mated with (purebred "B") male.

There should be no chromosomal difference between the two groups (all should have "B" Y chromosomes and within both groups there should be a relatively equal amount of "A" and "B" X chromosomes). This logic led the researchers to conclude that there must be a cytoplasmic difference in the ova of strain "A" and strain "B" that affects the mouse pup's inclinations towards violence (the researchers suggested that perhaps it was simply a "mitochondrial protein or enzyme which interacts either with other cytoplasmic or nuclear factors"). It is also possible that epigenetic factors during pregnancy may affect behavior.

Genetics and Human Behavior

Unlike genes that are directly responsible for diseases like Hungtinton's Disease, Tay Sachs or Alzheimer's disease there are genes that, in combination with environmental factors, influence human behavior. For example, it has been known for a long time that certain human behavioral characteristics are rooted in our genetic background and mimic behaviors observed in other members of the animal kingdom. However, studying genes that affect behavior creates a unique set of scientific problems. The majority of behavioral genetics studies have focused on genes that influence criminal tendency, cognitive ability, novelty seeking, mental disorders, addiction to drugs or alcohol, and sexual behavior. Most geneticists interpret current scientific data to show that these behavioral traits are complex in their pattern of inheritance and involve a combination of many genes interacting with environmental factors. The following section will briefly summarize genes that affect intelligence, sexuality, violence and other behaviors.

Intelligence. Genes that influence intelligence have been a keen interest in major research centers around the world. A variety of methodologies have been employed to examine the genetic contribution to cognitive ability (intelligence or I.Q.). Yet, there are problems inherent in studying the genetics of intelligence. These studies require the investigators to define intelligence in a measurable and definitive fashion. For example, is IQ a sufficient measure of intelligence? The problems of cognitive assessment may in part be responsible for the scarcity of well-designed studies to characterize specific genes that contribute to the development of intelligence. Furthermore, intelligence is often seen as a highly complex trait, with many possible influential genetic factors. Therefore, the precise genetic and epigenetic polymorphisms underlying normal-range intelligence differences remain mysterious and vastly undefined (Haggarty et al., 2010).



Studying the genetic role of intelligence also highlights how environmental factors may account for the difficulties in identifying specific genes. Specifically, it is difficult to sort out environmental versus genetic or epigenetic factors that influence behavior, in part because genes that regulate aspects of behavior appear to be highly responsive to environment stimuli. Traditional research strategies, which include studies of twins and adopted children, are often used to distinguish between biological and environmental influences on specific behaviors (nature vs. nurture). Many of these studies have not yielded sufficient results to dramatically expand our understanding of how environment and genetics interact to affect behavioral characteristics.

The inability to positively identify intelligence genes via genome-wide scans or state-of-the art technologies is leading some scientists to propose that genes do not play a major role in determining intelligence. Rather, environment and maternal effects may be the critical parameters that account for intellectual abilities.

Sexuality. There has been a great deal of effort to examine the role of genes in sexual behavior. Such studies have been going on for decades and usually involve trying to identify sexual patterns among monozygotic twins, dizygotic twins, or adoptive siblings. Many studies have focused on homosexual behaviors and several papers utilize two lines of evidence that homosexuality is influenced by polymorphic genes: (i) twin studies indicate that there are both genetic and environmental factors that contribute to the expression of the homosexual phenotype (Ramagopalan et al., 2010), and (ii) male homosexuality appears to be inherited more frequently from the matrilineal lineage. These studies suggest the existence of polymorphic, heritable maternal effects and/or polymorphic X-linked genes influencing male homosexuality. In some studies the researchers found that 52% (29/56) of monozygotic twins, 22% (12/54) of dizygotic twins, and 11% (6/57) of adoptive brothers were homosexual. Thus, heritability of homosexuality was considered to be substantial under a wide range of assumptions about the population base rate of homosexuality and the ascertainment bias. However, the rate of homosexuality among non-twin biologic siblings was significantly lower than would be predicted by a simple genetic hypothesis and by other published reports. From the rates of homosexuality observed in monozygotic and dizygotic twins, ordinary siblings, and adoptive brothers and sisters of homosexual men and women, overall heritabilities of 31 to 74% for males and 27 to 76% for females were estimated. The observation that male homosexuals usually have more gay brothers than gay sisters, whereas lesbians have more gay sisters than gay brothers, suggested that the factors responsible for familial aggregation are at least partially distinct in men compared to women.

Hamer and his colleagues (Mustanski et al., 2005) performed one of the most complete and largest studies in an attempt to identify a gene for homosexuality. In 1993, he studied pedigree and linkage analyses of 110 families of homosexual men. Increased rates of same-sex orientation were found in the maternal uncles and maternal male cousins of these subjects, but not in their fathers or paternal relatives, suggesting X-linked transmission. Linkage analysis using DNA markers in a selected group of 40 families, in which there were 2 gay brothers and no indication of non-maternal transmission, demonstrated a correlation between homosexual orientation and the inheritance of polymorphic markers on the X chromosome in approximately 64% of the sibling pairs tested. The linkage to markers on Xq28 (on the tip of the long arm) indicated a statistical confidence level of more than 99% that at least 1 subtype of male sexual orientation is genetically influenced. Hamer (LeVay and Hamer, 1994) emphasized that the findings of his study should not be interpreted as 'medicalizing' homosexuality because sexual preference should be viewed, he insisted, as a behavioral variable. His studies were consistent with the observation that homosexuality seems to run in the female line.

What motivated Hamer's research in the genetics of homosexuality? Hamer hoped that scientific research would help dispel some of the myths about homosexuality that have clouded the gay and lesbian community in the past years. Hamer also recognized that educating the public about genetics and behavior would eventually improve our understanding of the individuals' natural rights and human diversity.

In 2014, a new report probed a genome-wide linkage scan on 409 independent pairs of homosexual brothers and confirmed Hamer's results that there are genes that influence the sexual orientation of males (Sanders et al., 2014). First, they found a region in chromosome 8 that influences male sexual orientation. Second, they confirmed Hamer's earlier studies that the Xq28 region on the X chromosome also influences male sexual orientation. However other reports suggest genetic linkage of homosexuality to other chromosomes. Yu et al., (2015) report a linkage to chromosome 22. Clearly much more work is required to elucidate the role of genetics to sexual orientation.

In 2015, a new study reported that epigenetic effects influence sexual orientation (Balter, 2015). Researchers found five genome regions where the methylation pattern appears very closely linked to sexual orientation. A model that predicted sexual orientation based on these patterns was almost 70% accurate within this group. However, analysis of these epigenetic regions did not predict sexual orientation in the general population.

If there are genes that influence gay behavior, then it will be important to understand how this trait provided an evolutionary advantage since, by its intrinsic nature, gay couples do not procreate. Genes that regulate sexuality may be part of the Darwinian "paradox". Evolutionary models have proposed suggesting that

polymorphic genes that influence homosexuality confer a reproductive benefit to heterosexual carriers, thus offsetting the fitness costs associated with persistent homosexuality. Genes that confer gay tendencies may in fact offer evolutionary advantages in heterosexual individuals such as making them more loyal, considerate or empathic. Genes that promote same-sex bonding may also reduce aggression within social communities and encourage resource sharing, which may also have provided an evolutionary benefit.

Two models have been suggested that describe the evolutionary benefits of male homosexuality: heterozygote advantage and sexually antagonistic selection. The former was discussed in the previous paragraph and proposes that the benefits of gay tendencies in heterozygous, heterosexual men were so great that they overpowered the disadvantages from lack of procreation in homosexual men. This theory is only enhanced under the knowledge that historical anti-gay attitudes may have caused gay adults to procreate regardless of their homosexuality. The heterozygote advantage model can be applied to females as well. Researchers recently demonstrated a correlation between increased masculinity in women and a larger amount of sex partners throughout life. The other model, sexually antagonistic selection, can only be applied to male homosexuality. It proposes that female fitness is increased by the presence of alleles inducing male homosexuality, although male fecundity is negatively harmed if not indifferent (Burri et al, 2015).

Emanuele et al., (2007) examined whether genes affect human romantic bonding and found a significant association between a certain neurotransmitter gene (the dopamine D2 receptor gene) and a specific style of love characterized as EROS (a loving style characterized by a tendency to develop intense emotional experiences based on the physical attraction to the partner). These associations were present in both sexes. Some studies link gene patterns to the number of sexual partners a person has (Burri et al., 2015). They show that genetic factors responsible for nonheterosexuality are shared with genetic factors responsible for the number of lifetime sexual partners via a latent sex typicality phenotype in human females. Another area that is ripe for exploration is the genetics of transgenderism. As of 2016 there were no significant papers published on this topic. Yet, one can expect that more researchers will examine the genetics of transgenderism in the future.

Violence. Behavioral genetic research has analyzed thousands of sibling pairs and has pointed to the “inescapable conclusion” that genetic factors do contribute, to a certain degree, to the etiology and cause of violence (Ferguson, 2010). Some conclude from these studies that approximately 50% of the variance in antisocial phenotypes is the result of genetic factors (Ferguson and Beaver, 2016). Examining genes that regulate violent behavior has been supported by both academic centers as well as governmental agencies that monitor terrorism and the crime rates within a specific society or country. Violent behavior is affected by social and possibly genetic factors (Tuvbald and Baker, 2011). This research

points to the importance of a nurturing social environment in childhood, good early education, and success in academic areas. Peer influence is also of critical importance in predicting violent behavior. Many twin and adoption studies indicate that child and adolescent antisocial behavior is influenced by both genetic and environmental factors, suggesting that genetic factors directly influence cognitive and temperamental predispositions to antisocial behavior. These predisposing factors and socializing environments, in turn, influence antisocial behavior in children. Research also suggests that for some youth with early onset behavior problems, genetic factors strongly influence temperamental predisposition, particularly oppositional temperament, which can negatively affect experiences. When antisocial behavior emerges later in adolescence, it is suspected that genetic factors contribute less. Such youths tend to engage in delinquent behavior primarily because of peer influences and/or have experienced abuse in the home.

Based on genetic analysis, several studies have suggested that genes coding for the monoamine oxidase (MAOA) and tryptophan hydroxylase (TPH) enzymes are linked to specific cases of violent behavior. These genes code for certain enzymes that are responsible for the metabolism or synthesis of three neurotransmitters (serotonin, norepinephrine, and dopamine) that have been associated with the onset of aggression or violence. Serotonin is one neurotransmitter that is responsible for moods, appetite, sexual activity, homeostasis, and sleep. Norepinephrine regulates stress and moods in the brain. Dopamine regulates emotion, the "pleasure center" of the brain, and motivation.

One problem with linking MAOA encoding-genes to behavior is that, in the literature, there are scores of behavioral characteristics that have been ascribed to this enzyme. The same polymorphisms of these genes are said to predict variation in other behavioral and physical traits. The idea that one or two genes could be responsible for so many disparate behaviors is biologically implausible. In addition, the genetics underlying violent behavior is complex. A 2014 study uncovered at least 13 genes that changed during evolution as cats morphed from displaying wild aggressive behaviors to friendly behaviors (Montague et al., 2014).

Other behaviors. There are now several studies that link genetic markers to the ability of individual to lose weight. Pathway Genomics is a company that will analyze your genes to provide clues to effective weight loss programs. A study in 2016 examined happiness in twin siblings who were separated after birth and brought up by different families with different socio-economical backgrounds. Following years of observation, the team found that twin siblings who have the same genetics report the same levels of happiness no matter how they are affected by environmental factors. Although other factors play an important role in an individual's happiness, the effect of such factors does not last for long. The study revealed that genetics accounts for 48 percent of the influence behind feelings of happiness while 40 percent is tied to other incidents that happen every day and 12 percent is linked to other elements.

Synthetic Biology

The last part of this chapter will focus on synthetic biology. The UK Royal Society has defined synthetic biology as “an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems.”[7]

Scientists have been attempting for years to expand nature's genetic four letter alphabet, consisting of the nucleotide bases cytosine, guanine, adenine and thymine (represented by the letters "C," "G," "A" and "T," respectively). In 2014, Romesberg et al., published a seminal paper culminating 14 years of NIH-funded research and reported the synthesis of two new synthetic nucleotides X (d5SICS) and Y (dNaM). Impressively, they were able to generate bacteria that could replicate DNA containing this new base pair. Romesberg hypothesized that his expanded genetic alphabet is either so foreign that the genetic framework simply doesn't recognize it as an error, or, more likely, has no way to fix or change the X and Y letters. To ensure that such bacteria never leave the laboratory, he modified the E. coli to replicate these nucleotides in the DNA, but did not use a customized clip of genetic code to build proteins, so the new letters were not expressed in any new genes. His X and Y nucleotides are hidden away on a length of DNA that essentially functions as untranslatable code (Chen et al., 2014).

The practical translational application of expanding our base pairs remains to be investigated. However, in the area of computing, the expanded DNA code may offer a significant technological advantage. Biomolecular computing using DNA offers an alternative non-microchip technology for storing information. Using our four base-pair system, a DNA-computer with one liter of fluid would contain six grams of DNA and would have a memory capacity of 3072 Exabytes (one billion gigabytes). The idea of using an expanded DNA code would increase the storage of these computers dramatically and improve the power of biomolecular computing. The development of aptamers is another application of synthetic base pairs. Aptamers are small DNA molecules capable of specifically binding proteins or other cellular targets. One could view aptamers as a chemical equivalent of antibodies and could be used to target tumor cells (Sefeh et al., 2014). Incorporating synthetic bases into aptamers could affect tissue targeting and improve specificity.

Some ethicists fear that incorporating synthetic base pairs into humans in a clinical situation would be very risky. This is because our bodies would be completely defenseless against modified base pairs without any mechanism to identify or break down such artificial material. Thus, unseen complications or

[7] http://www.synbioproject.org/topics/synbio101/definition/

mutations caused by the meddling of the natural genetic code occur would be unstoppable.

A research team at Stanford successfully created a biological transistor, cleverly named a transcriptor, within human cells. Like transistors, transcriptors are on-and-off switches, gatekeepers or “gates” of information input, storage, and output. Transcriptors give cells already programmed to store and transmit information a “brain,” a system of logic governing the way they deal with that information. It is “biological internet” that transmits genetic information between cells and a rewritable DNA data storage system. The transcriptor, similar to the way a transistor amplifies electrical signals, can allow small changes in enzyme activity to trigger much larger changes in gene expression. The transcriptor has the ability to report whether the cell has been exposed to a specific stimulus. Such technology has the potential to revolutionize disease detection.[8] The insertion of one or more transcriptors into bacteria transforms them into microscopic calculators.

Clinical samples are complex environments, in which it is difficult to detect signals. Scientists have used the transcriptor's amplification abilities to detect disease markers in the blood and digestive system, even if present in very small amounts. They also succeeded in storing the results of the test in the bacterial DNA for several months. As a proof of concept for creating intelligent bacteria, the authors connected the genetic transistor to a bacterial system that responds to glucose, and detected the abnormal presence of glucose in the urine of diabetic patients. In future, this work might also be applied to engineering the microbial flora in order to treat various diseases, especially intestinal diseases.[9]

Gene Editing

Any discussion on genetics must include new technologies in gene editing (Kaufmann et al., 2013). Currently there are at least four different systems by which the base pair of DNA can be targeted to either replace or delete the DNA. These gene editing technologies are:

1. Zinc finger nucleases,
2. TALEN From the French word “claw” (Transcription Activator-Like Effector Nucleases),
3. BuD nucleases,
4. CRISPR/Cas9 (Clustered Regularly Interspersed Short Palindromic Repeats) nuclease.

[8] http://www.popsci.com/technology/article/2013-04/stanford-researchers-build-biological-transistor-within-living-cell

[9] http://m.sciencenewsline.com/news/2015060222510039

All of these systems rely on proteins or RNA to target specific sites on the DNA, a functional element to initiate double stranded breaks in order to excise the DNA, and an element that allows the DNA to be repaired.[10] In some situations, single base pairs can be changed. In other situations, specific regions of the DNA can be excised. The major difference between these different systems is how they recognize and target specific sites on DNA.

The potential applications to correct human diseases are vast. In 2014, Sangamo Biosciences used zinc finger systems to knock out CCR5 in human T cells from HIV+ patients. The HIV virus requires this receptor to enter T cells. The researchers then safely returned those cells to the patients and raised their T cell counts. (Tebas et al., 2014; Kaminski et al., 2016).

PD-1 is a receptor present on activated T cells and regulatory T (T-reg) cells, and its ligand PD-L1 is expressed by most cell types including tumor cells and dendritic cells. Anti-PD-1 antibody produced objective responses in approximately one in four to one in five patients with non-small-cell lung cancer, melanoma, or renal - cell cancer. Su et al., (2016) successfully used CRISPR to shut down PD-1, allowing T cells to attack tumor cells more efficiently.

Gene editing is being tested as a means to cure individuals who have genetic mutations causing diseases such as cystic fibrosis, muscular dystrophy, and various forms of clotting disorders. One example is to obtain adult cells from an individual who has Hemophilia A, one of the most common genetic bleeding disorders, caused by various mutations in the blood coagulation factor VIII (F8) gene. Using TALEN technology, scientists could revert the mutated DNA segment back to its normal orientation in these stem cell to obtain a cell line with the normal gene. Then these stem cells would be used in a bone marrow transplantation procedure to enable the patient to produce normal clotting factors.

A third type of application would be to use viral technology to deliver gene-editing proteins to the liver to cure individuals with type I tyrosinemia (Yin et al., 2014). Patients (about 1 in 100,000) cannot break down the amino acid tyrosine, which accumulates and leads to liver failure. In mice, scientists were able to insert the correct gene in about one of every 250 hepatocytes — the cells that make up most of the liver. Over the next 30 days, those healthy cells began to proliferate

[10] http://www.youtube.com/watch?v=zDkUFzZoQAs

and replace diseased liver cells, eventually accounting for about one-third of all hepatocytes and curing the mice.

Finally, gene editing can be applied to embryos generated in vitro to replace a single base pair mutation. This technology has been tested in mice that have genetic-based cataracts (Wu et al., 2013). In this study, about 33 percent of the mutant zygotes that were injected with CRISPR/Cas9 grew up to be cataract-free mice. Clearly, the efficiency of success must be greatly improved before applying this technology to human beings.

The most exciting gene editing system in 2015 was CRISPR. The CRISPR system offers certain benefits over the competing technologies. First the Cas9 is a highly programmable enzyme. The use of a guide RNA has the potential to make target location very specific. Second, this system can be used to multiplex or target multiple sites simultaneously. The potential of CRISPR technology is seen in the rapid development of many companies that plan to begin clinical trials using this gene editing technology. In 2015 Bayer Corp. invested $400 million in a small company called CRISPR Therapeutics and Fidelity Investments and a fund backed by Microsoft Corp. founder Bill Gates invested $120 million in Editas Medicine.

CRISPR has been used to create mice that inevitably get liver cancer. These mice can then be used in drug trials. Researchers are looking to utilize these gene-editing tools beyond medicine, as well. These new technologies are viewed as biological “superpowers”. Their envisioned uses are incredibly widespread, including being used as a solution for hunger (through genetically editing produce) and as an end to reliance on petrochemicals (researchers are working on yeast that consumes plant matter and excretes ethanol). Other companies use CRISPR to create industrial and research materials, such as enzymes in laundry detergent. Other scientists hope to bring back the woolly mammoth by using CRISPR to insert its genes into elephant embryos.[11]

Finally, CRISPR is being used to genetically modify plants and animals. CRISPR is being used to create plants that are resistant to certain viruses (Ali et al., 2015). It has been used to delete the muscle-inhibiting gene myostatin from two beagles[12] and pigs (Wang et al., 2015), in order to produce more athletic animals with double the amount of muscle mass. These genetically modified dogs are expected to have stronger running ability, which is good for hunting and police (or military) applications. CRISPR has also been used to create pigs that can serve as human organ donors. Doctors have been slow to use pigs as organ-donor alternatives for at least two reasons: first, the pig genome has a number of endogenous retroviruses that are harmless to pigs, but that could infect humans; second, the human immune system will target pig-specific proteins in the cell membranes, trying to reject the foreign bodies. The CRISPR system can inactivate

[11] http://www.wired.com/2015/07/crispr-dna-editing-2/

[12] http://www.the-scientist.com/?articles.view/articleNo/44298/title/Genetically-Engineered-Dogs/

62 of the pig's endogenous retroviruses in embryos as well as modify genes to make their tissues immune-compatible for human transplants.

One problem with CRISPR technology is that its components, an enzyme called Cas9 and a strand of RNA to direct the enzyme to the desired sequence, are too large to stuff into the genome of the virus most commonly used in gene therapy to shuttle foreign genetic material into human cells. Recently, a mini-Cas9 was isolated from the bacterium Staphylococcus aureus (Ledford, 2016). This protein is small enough to squeeze into the virus used in one of the gene therapies currently on the market. In December of 2015, two groups used the mini-me Cas9 in mice to correct the gene responsible for Duchenne muscular dystrophy.

CRISPR is also being applied for commercial ventures such as improving the yield to generate cashmere. Most hair on a goat is coarse and thick, unsuitable for fine clothing. Cashmere comes from a second undercoat that goats grow only in the winter, where the hairs are fine and soft and downy. Cashmere is expensive because even goats that are specially bred to produce cashmere produce only about half a pound per goat. Chinese scientist have used CRISPR to disrupt a single gene in cashmere goats to improve the nature of the hair produced and yield. As of 2016, CRISPR modified goats make hair in their undercoats longer and more numerous and boosts the yield by almost 50%.[13]

A novel application of the CRISPR system is called "gene drive". Gene drive is a technology to accelerate inheritance of particular genes and alter entire populations. By incorporating a CRISPR into the desired gene, scientists can cause a gene to be inherited at a rate faster than Mendelian principles would dictate. Gene drive technologies are being applied to change wild populations of harmful organisms, such as malaria carrying mosquitos, to be less dangerous.[14] By inserting the CRISPR system within a mosquito, it is theoretically possible to create large populations of mosquitoes that will not transmit malaria, Zika, or yellow fever to humans. Gene drives supercharge genetically modified genes so that they defy the normal rules of inheritance. Normally, genetically modified traits are quite difficult to spread within a population of wild insects unless they impart a great evolutionary advantage. But when attached to a CRISPR gene-drive DNA "cassette", practically every individual in a breeding population will eventually end up being a genetically modified organism. Using gene drive technologies, genes can copy themselves onto a corresponding location in a paired chromosome, thereby overriding typical allele inheritance patterns. Gene drives can also be applied to environmental conservation, notably in fighting invasive species, such as rats on remote islands inhabited by ground-nesting birds, which are wrecking the indigenous ecosystem.

However, the power of gene-drive technology to accelerate the spread of genetic traits also introduces immense potential hazards. It would be possible that

[13] http://www.theatlantic.com/health/archive/2016/10/cashmere-goat-crispr/505163/
[14] see https://www.youtube.com/watch?v=G1L0G00nCM8.

either a rogue state or a terrorist cell might decide to generate a gene-drive organism that could pose a threat to human health or to economically important livestock. For example, this could be done by introducing a foot and mouth virus that has the potential to seriously damage the dairy and beef industries or by genetically modifying mosquitoes so that they can deliver lethal bacterial toxins to humans.

In October of 2016 a paper appeared in Nature (Bahal et al., 2016) that reported using nanoparticles instead of CRISPR to alter DNA. FDA-approved nanoparticles were used to deliver peptide nucleic acids (PNA) into the stem cells of mice to remove the beta-thalassemia mutation. Beta-thalassemia is a blood disorder that reduces the production of hemoglobin and leads to a lack of oxygen throughout body, causing weakness, fatigue and serious complications. PNAs containing a strand of healthy donor DNA encoding the hemoglobin gene were injected into the bone-marrow stem cells of live mice. These nanoparticles targeted the mutant DNA region and corrected the mutation to correct the malfunctioning gene. Successful genome editing was achieved in seven percent of cases, with elevated levels of hemoglobin evident for 140 days after treatment. Thus, PNA molecule genome editing provides a complex but efficient alternative to CRISPR.

Conclusions

The genetic composition of an individual can have profound effects on health, behavior, and disease. In some situations, such as Huntington's disease, the nature of the defect can predict age of onset and severity of the disease. In other situations, environment, diet, and life experiences may alter disease onset and progression. Studying the role of genetics in behavior is compounded by a variety of factors including the complex interaction between genetics and environmental factors. Moreover, it can be difficult to precisely measure human behavior because there are so many variations. This chapter outlined various methods for diagnosing genetic-based diseases and behavioral characteristics including searching for specific genes that influences these diseases and behavior. In part, the overall goals of these studies are: a) to reduce the probability of a child being born with a genetic-based disease or abnormal behavior characteristics and b) to understand how genetic factors contribute to disease and behavior in order to help design new therapeutic interventions.

Thought Question: What lesson can you learn from the Supreme Court Decision about patenting genes to the current patent dispute regarding CRISPR?

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Chapter Six

Human Stem Cell Research:

The Alchemist's Dream

Introduction

Conceptually, stem cell research can be viewed as a form of modern alchemy that transforms primordial embryonic cells into specialized, differentiated cells, which can be used to replace damaged cells or organs and may revolutionize medicine. There are currently many initial clinical studies seeking to examine how stem cell technology can be applied to correct organ failure, grow new organs *in vitro* for organ transplantation or treat a variety of chronic diseases that plague humans.

The clinical applications of stem cell-based therapy are vast. The potential exists to treat some of the most disabling human diseases including diabetes, Alzheimer's disease, spinal cord injuries, macular degeneration, multiple sclerosis, heart disease, neurological diseases, and cancer. According to the statistics published online by various organizations including the CDC (Center for Disease Control and Protection), there are over 200 million people in the United States suffering from chronic diseases (about 5 million Americans with Alzheimer's disease, 27 million with some form of cardiovascular disease, 26 million with diabetes, 79 million with a pre-diabetic condition, 11 million with macular degeneration, 1 million with Parkinson's disease, 13 million with cancer, and more than 50 million with osteoporosis), which are potentially treatable with stem cell-derived therapies. Moreover, some bioethicists such as Glenn McGee predict that a billion individuals around the world may be treated with human embryonic stem cells before the decade comes to an end.[1] However, the process of applying stem cell technology to treat human diseases is much slower than predicted. As of 2016, the FDA has only approved one application of stem cell technology. Cord blood-derived hematopoietic progenitor cells have been approved for certain diseases such as blood cancer and some (inherited) metabolic and immune disorders.

Stem cell research will also lead to a better understanding of fundamental aspects of biology in the areas of cellular differentiation, trans-differentiation, epigenetics, and de-differentiation**.** In this light, stem cell research simultaneously represents a domain of fundamental discovery in human biology, and also a therapy with the potential to affect human health and quality of life.

However, embryonic stem cell research is also one of the most morally controversial scientific areas of the 21st century because, until recently, these stem cells could not be isolated without destroying the early embryo. While stem cells can also be isolated from adult tissues, the current view is that embryonic stem cells obtained either

[1] http://www.springerlink.com/content/g3h427539krqp648/fulltext.pdf

from non-implanted early embryos or from discarded embryos offers the best potential source for therapeutic application, for reasons that will be explained later. There are almost 500,000 frozen embryos, stored in IVF clinics across the US, which could be donated to stem cell research.

Rarely do democratic governments try to regulate new forms of medical research; however, governments around the world are trying to regulate and restrict basic embryonic stem cell research. Why? The prevailing cultural and religious views in many Western countries claim that once an ovum is fertilized by a sperm, even outside of the womb, the resulting zygote attains human status, making the destruction of such early embryos unethical, immoral, and possibly even a form of murder (see Chapter 5). To better appreciate the dilemmas associated with stem cell research, this chapter focuses on understanding and updating the basic biological principles of stem cell development and research. The bioethical dilemmas associated with stem cell research are examined in Chapter 7.[2]

Defining and Characterizing Stem Cells

In many organisms, life begins from a fertilized egg that divides, grows, and differentiates into all the various specialized cells—such as neurons, muscle cells, pancreatic cells, and blood cells—that an animal needs to function. Cell differentiation begins with the fertilized zygote, and is a process that regulates the functional and structural specialization of cells in all organ systems within a multicellular organism. Specifically, differentiation occurs via differential gene activity, in which each specialized cell type turns on or off selected genes specific for that cell type. Cell specialization, for over 200 histologically different cell types characterized in the human body, is thus determined by the activation and suppression of a specific subset of the ~20,000 genes in the human genome.

As the egg divides and grows, new stem cells are generated to allow for the full embryological development of the organism. Stem cells are **self–renewing,** primitive cells that can develop into functional, **differentiated** cells. Stem cells are naturally occurring in all multi-cellular complex organisms, and are found at every stage of development from conception to death. In adult tissue, stem cells can replenish the wear, damage, and disease that affect tissues during the lifespan of the organism.[2]

All stem cells exhibit two fundamental properties: **self-renewal and plasticity**. Self-renewal is the ability of stem cells to divide indefinitely, producing a population of identical offspring. Plasticity describes the capacity of stem cells to undergo an asymmetric division, on cue, to produce two dissimilar daughter cells. One daughter cell is identical to the parent and continues to contribute to the original stem cell line (Fischbach and Fischbach, 2004), while the other differentiates into one of the many specialized cell types. In general, stem cell proliferation is associated with only one, not both, of the daughter cells differentiating: the other retains its undifferentiated state to

[2] An online course in stem cells is available at http://stemcellbioethics.wikischolars.columbia.edu/.

maintain the reservoir of stem cells.

Before describing the different types of stem cells, it is important to review some basic elements of early human embryology. After fertilization, the haploid nuclei of the egg and sperm in the zygote fuse to form a single nucleus containing 46 human chromosomes. The zygote, derived from the Greek words zugōtos 'yoked, or zugoun 'to yoke', undergoes cellular proliferation to form a compact ball of cells called the morula, which has the appearance of a mulberry (the Latin term *morus* means mulberry). As the morula flows through the oviduct, the cells in the embryo continue to proliferate and the morula enlarges to form a hollow sphere called a blastocyst. Within this hollow sphere, a few specialized cells form an inner cell mass within the cavity. This cellular cluster is a primary source of embryonic stem cells. The time between fertilization and implantation of the human embryo in the uterine wall is approximately 9-14 days.[3]

There are several types of stem cells:

1. Totipotent stem cells are cells that can differentiate into any of the 200 plus specialized cells in the human body. In general, **totipotency lasts for about 3-5 cell divisions** after fertilization until the embryo implants into the uterus and has the potential to develop into a complete fetus and a placenta. As the embryo further develops, germinal totipotent cells are formed migrate into the primitive gonad, also called the genital ridge, and can differentiate into either female or male germ cell precursors.

Textbox 1. In a 2015 study scientists were able to generate totipotent murine stem cells from pluripotent embryonic stem cells by altering how chromatin and histones are formed (Ishiuchi et al., 2015). The totipotent cells resembled embryos at the 2-cell stage, and were capable of creating every cell type in the mouse. Understanding how to generate totipotency, "is essential to understanding of how a maximum degree of cellular plasticity can be achieved and maintained, thereby providing more options for efficient reprogramming and potential therapeutic avenues,". This technology can be seen as opening the door to reproductive cloning where there is strong moral and ethical opposition

2. Pluripotent stem cells have the capacity to differentiate into any other cell type, but cannot be implanted into a uterus to create a fetus because they lack the essential cells of the placenta. When the number of cells in the embryo approaches 32-64 a blastocyst is formed that creates a cell-free center within the expanding cluster of cells. Cells, called trophoplasts, in its outer cell layer differentiates and forms the placenta. Cells in the blastocyst's inner cell mass, called embryoblasts, develop into the fetus and are *pluripotent* because these cells cannot form a placenta. Isolated stem cells from the inner cell mass can be adapted to grow in a Petri dish and can be induced by biological substances or by environmental

[3] http://writ.news.findlaw.com/grossman/20011120.html

conditions to differentiate into any cell type found in the body (Figure 1).

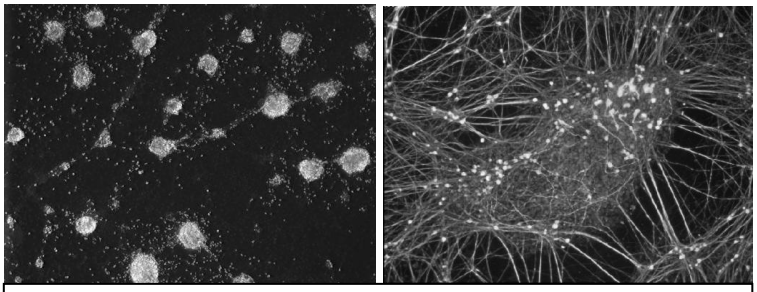


Figure 1. Stem cell differentiating into beta-insulin producing cells (left) and into neurons (right) (from NIH image gallery).

3. Multipotent stem cells are generally found in adult tissue and are technically pluripotent. They were originally thought to be responsible for the regeneration of only a very restricted set of cell lineages. However, it is becoming increasingly evident that some multipotent stem cells show considerable plasticity, and can be triggered to differentiate into a wide variety of specialized cells. Still, they cannot differentiate into as many different kinds of specialized cells as pluripotent stem cells (Wang, et al. 2009). The different underlying biological mechanisms that regulate totipotent, pluripotent, and multipotent stem cells differentiation remains an intense area of ongoing investigation.

Embryonic stem cell research aims to provide a better understanding of the underlying mechanisms of cellular differentiation. For example, once stem cells differentiate into a specific cell lineage, they do not change to other cell lineage pathways. Stem cells that begin differentiating into white blood cells will not change course and become red blood cells. In contrast, many tumor cells can jump cell lineages or de-differentiate. Therefore, there is a great need to understand the complete biology of stem cells in order to identify how physiological and non-physiological products and processes regulate gene expression and differentiation.

One of the biggest historical breakthroughs in human stem cell research occurred in 1998, when researchers led by James Thomson, isolated and grew stem cells derived from human embryos (Thomson et al., 1998). These human pluripotent embryonic stem cells were derived from fertilized embryos that were less than a week old. Five independent stem cell lines were developed that could either be perpetuated in culture for long periods of time or be frozen and recovered at a later date. Dr. Thomson used this technology to develop stem cell lines from 14 blastocysts that were obtained from donated, surplus embryos produced through *in vitro* fertilization. This was the first time human embryonic stem cells had been successfully isolated and cultured in a laboratory. Amazingly, this discovery came just seven years after the first reports of the isolation and culture of embryonic stem cells from mice (Evans and Kaufman, 1981).

At the same time researchers, led by John Gearhart, described a method to isolate and culture immature germ cells from 5 to 8 week-old fetuses that were donated anonymously by women undergoing therapeutic or spontaneous abortions (Shamblott et al., 1998). These scientists placed the stem cells, obtained from the germinal centers of the ovaries or testes, in plastic dishes and added biological factors that enabled the germ

stem cells to continue to divide while remaining in a state of suspended development, preventing differentiation. These germ cell-derived stem cells could also be frozen, recovered, and maintained as stem cells in culture. Interestingly, Gearhart's initial purpose for his research was to develop a tool for studying Down's syndrome.

The success of both Thomson's and Gearhart's research was based on their ability to retain and maintain the two fundamental properties of stem cells: self-renewal and plasticity. Both research groups showed that these cells could be repeatedly frozen and thawed while still maintaining their characteristic undifferentiated stem cell properties.

Once techniques were developed to isolate and culture human embryonic stem cells, many scientists around the world began to generate other human stem cell lines. These stem cell lines have been used as models to understand the regulation of cell differentiation and as potential sources for stem cell replacement therapy. One major clinical objective in cell replacement therapy is to use differentiated cells, such as neurons, to replace cells injured due to trauma (spinal cord injury) or neurodegenerative diseases such as Alzheimer's or Parkinson's disease. There are currently many ongoing clinical trials attempting to use cell replacement therapy for a variety of diseases. However, one major obstacle in these trials is the potential immunological rejection of the transplanted cells by the recipient patient. Ideally, stem cell therapy would be best implemented using the patient's own stem cells. The quest for generating patient-specific stem cells has led to the search for a method to utilize somatic cell nuclear transfer (SCNT- see Chapter 4) to isolate embryonic stem cells from patients. As discussed in Chapter 4, SCNT involves transferring the nuclear genetic material from a patient's own cell into an enucleated oocyte. This "fused" cell is then stimulated to develop into a pre-implanted embryo in order to harvest the embryonic stem cells from the inner mass. Stem cells isolated in this manner would be histocompatible to the patient and therefore could be used for cell replacement therapy. Reports by Noggle et al. (2011) and Tachibana et al. (2013) on the application of SCNT to human cell systems have already stimulated a great deal of research into possible methods of deriving patient-specific stem cells.[4]

In 2004, researchers in South Korea claimed to have successfully cloned a human non-implanted embryo as a source for harvesting embryonic stem cells (Kim and Park, 2013) (Hwang, Ryu et al., 2004; Hwang, Roh et al., 2005). Hwang claimed to have used extremely fresh eggs donated by South Korean volunteers. When workers in his research institute reported that they were coerced to donate their eggs, the scientific community began to learn about the scientific fraud. All of their data was falsified. Conceptually, Hwang was correct in principle, but it took another eight years until Noggle et al., (2011) and Tachibana et al., (2013) were able to apply SCNT technology to generate human embryonic stem cells.

Like SCNT, stem cell research is also susceptible to academic pressure and the risk of scientific fraud. In 2014, a group of scientists from Japan's Riken Center for Developmental Biology reported two papers in Nature. In their first paper, they reported that stem cells could be generated by exposing differentiated adult murine cells to an acid

[4] http://www.medicalnewstoday.com/medicalnews.php?newsid=70950

bath and other external environmental stresses (i.e., low pH conditions), in order to revert these cells into stimulus-triggered acquisition of pluripotency (STAP) cells. In their second paper, which appeared in the same issue of Nature, they claimed that STAP cells could also contribute to the placental tissue. This would demonstrate that STAP cells are not just pluripotent, but also totipotent, unlike embryonic and induced pluripotent stem cells. While these findings caused great excitement, it soon become clear that other laboratories across the globe could not replicate these findings. A six-person committee — three Riken scientists, two university researchers and a lawyer — found that the lead scientist, Dr. Obokata, had manipulated data in an intentionally misleading fashion. The committee branded this research as scientific misconduct.[5] Moreover, the top administrators of RIKEN, Japan's national network of research laboratories, decided to voluntarily return 1 to 3 months of their salaries in order to atone for their responsibility in the STAP stem cell fiasco (See Textbox 2).

Textbox 2: Scientific Fraud

Revelations of scientific misconduct always cause collateral damage. They taint the colleagues and co-authors of the person responsible, and can close down labs. In the case of RIKEN, a leading administrator hanged himself as a result of scientific fraud.

How common is scientific fraud? The PubMed database of biomedical research claims that only 1 in 10,000 recent papers, has been retracted. What is process of retraction? Can there be evidence of fraud but evidence not substantial so paper not retracted? However, other measures of misconduct appear to be more common. Daniele Fanelli, a senior research scientist at Stanford University, pooled data from 18 surveys and found that almost 2 percent of scientists admitted to fabricating their work or manipulating data. When asked whether they'd ever seen misconduct among peers, 14 percent said they had. Scientists have become less likely to admit misconduct," says Dr. Fanelli, "but they're no less likely to report the misbehavior of their colleagues.

Governmental fines issued for fraud are not high. But in August of 2015, the National Science Foundation ordered Northeastern University to pay back $2.7 million for nearly a decade of mishandling a grant from the agency.

Stem Cells Can Be Obtained from Various Tissues

There are six major tissue sources of stem cells: embryos, fetuses, umbilical cord blood, adult organs, amniotic fluid, and teratocarcinomas. Stem cells from the embryo or fetal tissue can either be totipotent or pluripotent, as described earlier. From an ethical perspective, it is also important to identify whether the stem cells are obtained from "spare embryos" created via IVF, cloned embryos (created for research purposes), or aborted

[5] http://www.nature.com/news/stem-cell-scientist-found-guilty-of-misconduct-1.14974

fetuses, since each tissue source of stem cells would elicit different moral perspectives (see Chapter 7). Stem cells from adults are generally *multipotent* and can be obtained from a variety of sources, including the bone marrow and most major organs. There are a few organs, such as the pancreas, from which stem cells have not been obtained. Another source of adult stem cells is human post-mortem tissue, which can be extracted up to 20 hours after death. Unlike embryonic stem cells adult-derived stem cells exhibit a more limited capacity to differentiate into various cell types.

Human amniotic fluid stem cells and umbilical cord blood may be other important sources for both basic science and regenerative medicine. These stem cells exhibit a high proliferation rate, are self-renewing, and may have a lower frequency of tumor production than embryonic stem cells (Roura et al., 2012; Cananzi et al., 2009).

Another source of stem cells is teratocarcinomas, which, historically, were first recognized as yielding pluripotent stem cells. Teratocarcinomas are gonadal tumors. These tumor cells are also one of the main components of human testicular germ cell tumors. One interesting feature of teratocarcinomas is that they contain a wide array of tissues derived from the three primary germ layers that make up an embryo: the endoderm, mesoderm, and ectoderm. Thus, they contain a large assortment of tissue types including cartilage, squamous epithelia, primitive neuroectoderm, ganglionic structures, muscle, bone, and glandular epithelia. The differentiated cells of the tumor are formed from pluripotent stem cells present in the tumor. While there is currently limited application for utilizing these cells as sources for stem cell therapy, these cells have provided great insights into the mechanisms of cell differentiation and tumorigenesis.

In 2007 and 2008, scientists claimed a major breakthrough by inducing adult fibroblasts to de-differentiate into stem cells that have pluripotent characteristics. These scientists were able to reprogram mouse fibroblasts into induced pluripotent stem cells (iPS) by genetically overexpressing four genes (oct4, sox2, klf4, and c-myc) and using subsequent drug selection for the reactivation of a marker for pluripotency (Greenbaum 2010). The process of reprogramming is slow and the frequency of developing into stem cells is low, so it could take up to 20 days to transform fibroblasts into stem cells. In addition, there are reported side effects of using iPS generated stem cells. Yamanaka et al. (Yamanaka and Blau, 2010) found that 20% of the stem cell-derived offspring developed tumors, presumably related to the activation of one of the transfected genes such as Myc (an established oncogene). iPS cells have been obtained from differentiated stomach cells, fat cells, and liver cells and can be obtained even if Myc, which can induce cancer, is omitted. The resulting stem cells do not appear to be substantially different from ES (embryonic stem cells). In 2009 and 2011, there were other improvements in iPS technology (Hong et al., 2009, Kawamura et al., 2009, Li, Collado et al., 2009, Marion et al., 2009, Utikal et al., 2009). Non-integrating adenoviral vectors or plasmids, for example, were used to achieve transient expression of reprogramming factors without disturbing the host genome. But such an approach presents two immediate problems: the requirement for prolonged expression of the pluripotency factors to achieve reprogramming, and the difficulty of repeatedly delivering the full complement of factors using different vectors.

The goal of this research was to develop viral-free systems to generate iPS (Pera 2009). A leap of faith must be taken in order to transition from proof-of-principle in mice to application in humans, and there are still scientific hurdles to overcome. If human stem cells can be generated using iPS technology, patient-specific stem cells could be made without the use of donated eggs or embryos. This technique has an obvious ethical advantage because it does not require the destruction of pre-implanted embryos. Yamanaka, who discovered iPS, received the Nobel Prize for his work in 2011.

In 2012, researchers adapted the iPS technique of Dr. Yamanaka to breed genetically engineered mice with the same cocktail of four reprogramming transcription factor genes. By having the mice drink a particular drug, these genes were turned on and embryonic stem cells appeared in multiple tissues and organs in these mice within a few weeks. The researchers extracted these cells and demonstrated through various tests that they were like those in a new embryo containing just 16 cells (Abad et al., 2013). The next step is to explore whether these *in vivo*-generated iPS stem cells are capable of efficiently generating different tissues in vital organs such as the pancreas, liver, heart, bone marrow, or kidney. Their research aims to devise methods for inducing regeneration locally, as well as in a transitory manner, for a particular damaged tissue.

Another problem with iPS technology is that the stem cells generated do not have the same epigenetic markers as embryonic stem cells. iPS cells differed and retained residual DNA methylation patterns and the transcriptome profiles of their parental somatic cells. In contrast, embryonic stem cells generated via SCNT technology corresponded closely to similar cells generated by classical IVF technology. Thus, human somatic cells can be faithfully reprogrammed to pluripotency by SCNT and may be better suited for cell replacement therapies (Ma et al., 2014). Because of ethical concerns regarding embryonic stem cells and histocompatibility issues, research is focusing more on applying iPS cells to clinical situations (Takahashi, et al., 2016).

A new technology is developing in which one cell type is directly converted into another without going through a “stem cell” intermediate. In a 2014 paper, Dr. Yoo and his colleagues reported that co-expression of various transcription factors, enriched in the developing striatum, can guide the conversion of human postnatal and adult fibroblasts into an enriched population of neurons analogous to striatal medium spiny neurons (Matheus et al., 2014). In addition, they demonstrated that when transplanted in the mouse brain, the reprogrammed human cells persisted in situ for more than 6 months, exhibited membrane properties equivalent to native medium spiny neurons and extended projections to the anatomical targets of these cells.

Disadvantages of Stem Cells Derived from Different Sources

The major disadvantages of embryonic stem cells, apart from ethical considerations, are that they may be rejected if transplanted in an HLA incompatible

person, and that they may form tumors more easily than adult- derived stem cells. Adult tissues contain multipotent stem cells that provide another source for stem cell research. The most common organ for multipotent stem cells is the bone marrow whose stem cells can differentiate into a variety of different cell types. Moreover, the ease with which bone marrow cells can be obtained and our experience using these cells in a variety of

Textbox 3. Funding of human embryonic stem cell research.

A clear pattern has emerged over the years in states such as California (the nation's largest funder of stem cell research apart from the federal government) and Maryland to trend away from funding hESC research and provide overwhelming financial support for ethically non-contentious adult stem cells and other types of non-embryonic stem cell research. Minnesota is the most recent state to provide public money for adult stem cells and other ethically non-contentious, non-embryonic stem cell research stem cell research. In declining to fund hESC research Minnesota is echoing a trend that has been gathering momentum for years. Do you believe this is a valid approach to scientific research?

treatments (e.g., leukemia) have been a great impetus for exploring them as a source of adult stem cells. Yet, bone marrow-derived cells are not as pluripotent as embryonic stem cells. Another possible disadvantage of using stem cells from bone marrow is that about 10-20% of patients lack a sufficient number of recoverable bone marrow-derived stem cells for therapeutic transplantation because of the patients' disease.

The main advantage of using bone marrow or any adult-derived stem cells is their use in autologous therapy, which avoids the risk of tissue rejection. Adult-derived stem cells, however, have some disadvantages in therapeutic applications. One technical hurdle is that they can only be isolated in low numbers. In mouse bone marrow, stem cells represent only 1 in 10,000 cells. In addition, they are more difficult to isolate than embryonic stem cells, are notoriously slow to grow in culture, and have a restricted proliferation potential.

Another issue with adult derived stem cells is their plasticity, or ability to differentiate into other cell types. Adult derived stem cells from certain organs such as bone marrow, muscle, fat, liver, synovial membranes, and brain, express better plasticity or pluripotency than adult cells from other sources. For example, studies (Santarelli et al., 2003) showed that, even in adult rodent brains, stem cells had the capacity to generate neurons (neurogenesis). This finding may explain why patients taking antidepressants require several weeks before a therapeutic effect is seen. During this time, the antidepressants appear to stimulate the generation of new neurons in these patients. This research could lead to developing new compounds that trigger neurogenesis from endogenous adult stem cells in the brain. In fact, a San Diego-based start-up called BrainCells screens drugs that stimulate the proliferation of neural stem cells in the hope of finding new antidepressants or drugs to treat cognitive disorders, such as Alzheimer's.

Since there are several sources of embryonic and adult stem cells, it is critical to

assess which type of stem cells will generate the best therapeutic value. To date, the main disadvantages of adult stem cells are that they are: a) few in number, b) difficult to isolate and maintain in culture, c) slow to proliferate, and d) difficult to stimulate to differentiate into various other tissues types. Until we are able to test stem cells, from various sources, side-by-side in the laboratory and in a variety of experimental paradigms, the answer to whether or not adult embryonic stem cells could serve in a therapeutic mode will remain unresolved.

Stem Cell Differentiation Assessment and Targeting

There are several stages between isolating stem cells and transferring them to patients. Currently, therapeutic applications are focused on five major health problems: diabetes, blood diseases (including AIDS), neurodegenerative disease, spinal cord injuries, and cardiovascular disease. However, the critical stage in the development of these therapies is assessing the capacity of stem cells to differentiate into specialized cells.

Manipulating the extracellular environment can trigger the differentiation of stem cells into specialized cells. Differentiation into specialized cell types, for example, can be initiated by growing the stem cells at high cell growth densities, placing them on different types of non-proliferating feeder cells, adding specific growth factors, or maintaining these cells on either crude or defined extracellular matrices. Scientists are just beginning to discover the control mechanisms for generating specialized cells. A great deal of future investigation remains necessary for a complete identification of all cell culture conditions, or chemical factors, that regulate stem cell differentiation.

In the laboratory, there are several methods to assess the developmental potency of pluripotent stem cells: (1) *in vitro* differentiation in a Petri dish; (2) differentiation into teratomas or teratocarcinomas, and (3) *in vivo* differentiation when introduced into the blastocoele cavity of a pre-implantation embryo. In the first method, scientists use plasma membrane surface markers to determine whether the embryonic stem cells will differentiate into the target specialized cell. In addition to surface markers, current research also focuses on generating gene expression profiles to characterize stem cells and their differentiated progeny. In the second method, pluripotency is demonstrated when human embryonic stem cells are injected into an animal and form teratomas. The third method involves injecting the human stem cells into a developing animal embryo; pluripotency is assessed by analyzing the tissue distribution of the human cells in the animal that is born. It is important to note that testing human embryonic stem cells in this manner involves creating a human-animal chimera that may elicit bioethical concerns (see chapter 8).

In many instances, stem cell differentiation leads to a mixed population of non-differentiated cells and differentiated cell types. The differentiated cells and the non-differentiated stem cells must then be separated from one another. Separation of these two populations is possible because each cell type expresses unique surface proteins.

Clinical Applications of Stem Cell Technology

A specific lure of stem cells in cell and organ replacement therapy is based, in part, on the fact that stem cells offer an unlimited supply of potential cells to use in transplantation, in order to repair either diseased or damaged organs. In addition, stem cells obtained from the patient offer a promising method of cell or organ replacement without the risk of tissue rejection. In contrast, conventional organ transplantation involves finding a donor whose HLA antigens express the greatest compatibility with the patient's own tissue. Since it is usually difficult to find tissue-compatible donors, transplant recipients must often be placed on medications for at least a year, if not longer, to prevent their immune systems from rejecting the transplanted organs. These medications are associated with many side effects that can cause dangerous health risks (Griffith and Naughton, 2002). Although the technology that uses stem cells to generate complete organs is in its infancy, cell replacement therapy may offer a viable clinical alternative for classical organ transplantation in the future.

Other medical uses that may result from stem cell technology include: patient-specific drug development, gene therapy, and the study of underlying mechanisms of disease. As stated above, it appears that cell replacement, as opposed to organ development, is the most immediate therapeutic utilization of stem cells. In the following section, we will briefly review the current research in applying stem cell technology to treat heart disease, diabetes, and Parkinson's disease.

Heart Disease: Research on the clinical application of stem cells to heart disease is being conducted by many centers around the world. Scientists are trying to examine how stem cells can be used as a means to augment cardiac repair and regeneration (Lin and Pu, 2014). On average, an individual who experiences one myocardial infarction (MI) loses about 1 billion cardiomyocytes. Transplanting human embryonic stem cell-derived (ESC) cardiomyocytes into patients with heart disease may enhance cardiac repair and function. One fundamental medical challenge related to the use of stem cells in heart disease is the relative immaturity of current ESC-derived cardiomyocytes. Although these cells contract and generate force, their immaturity likely reduces their efficacy and host integration. In addition, these ESC is allogenic (and not from the patient's own cells) and require the patient to receive drug-mediated immunosuppression to avoid graft rejection. Finally, the safety (lack of teratoma formation or arrhythmogenesis) and longevity of ESC-based grafts will need to be carefully demonstrated.

Cardiac progenitor cells (CPS) are another cell source that might have therapeutic applications for heart disease. These cells differentiate into both vascular cells and myocardiocytes. On the basis of preclinical studies, these cells were tested in humans with ischemic heart failure who underwent coronary artery bypass graft surgery in a randomized, open-label, phase 1 study called SCIPIO. Four months after surgery, autologous CPCs, expanded from myocardial tissue harvested during surgery, were administered by intracoronary infusion. No adverse events related to CPC treatment were noted. However, the clinical outcomes were not so dramatic. CPC-treated patients had

slight, statistically significant improvement in the left ventricular ejection fraction compared to untreated controls at 4 months (36% versus 29%). Thus, there is much more work to be done before stem cell therapy can be applied to treat heart disease.

In most respects, iPSCs behave like ESCs, and thus offer their conceptual advantages. At the same time, iPSCs sidestep the ethical issues that surround ESCs. Because it is possible to generate autologous iPSCs, these cells would also circumvent the need for immunosuppression. However, production of iPSCs will require months of preparation, precluding their deployment for acute or sub-acute illnesses such as MI. Furthermore, the uniform manufacture of iPSC-derived cardiomyocytes from individual patients is a major logistical and regulatory hurdle for the clinical use of iPSC-derived cells (Li and Carlos, 2016).

Diabetes: In Type I diabetes, the beta islet cells of the pancreas, which normally produce insulin, are destroyed by an autoimmune process. The pancreas is an interesting organ because, to the best of our knowledge, it is not clear if this organ contains natural stem cells (Kopp et al., 2016). Scientists are actively differentiating embryonic stem cells into beta islet cells capable of producing insulin, in order to transplant these cells into a diabetic patient. In order for this procedure to work clinically methods must be designed that the diabetic patient's immune system will not destroy the newly transplanted islet cells in the same fashion that it destroyed its own beta cells. Even if beta cell destruction in diabetic patients were to occur, it might not occur immediately, rendering stem cell therapy a viable method to acutely treat diabetics. However, this would require periodic renewal transplantation of stem cells in order to maintain a non-diabetic state.

In a 2007 article, scientists were able to use stem cell therapy in conjunction with anti-rejection therapy to treat a small number of patients with Type I diabetes so that they did not require insulin injections (Voltarelli et al., 2007). This was the first time stem cell therapy was effective in taking diabetic patients off insulin. Since then there have been several studies examining the use of stem cells to treat diabetes (El-Badawy and El-Badri, 2016). In 2016, Doug Melton and his colleagues published a landmark paper that used encapsulated embryonic stem cell-derived islet cells to treat diabetic mice (Vegas, et al., 2016). Encapsulation prevented the recipient animal from rejecting the heterologous islet cells and still their physiological responses to produce insulin. Encapsultation protects allogenic stem cells from the host's immune system by creating a matrix barrier between the transplanted islet cells and the pancreas that allows diffusion of glucose, other nutrients, and insulin but not of larger molecules, cells, or antibodies. Moreover, even if some of the transplanted stem cell derived β cells turn tumorigenic the physical barrier limits their growth, and more importantly these tumorigenic cells cannot escape into the vascular or tissue compartments to cause system wide cancer.

Neurodegenerative Diseases: In Aug of 2014, a neurosurgery team transplanted cells from aborted human fetuses into the brain of a person with Parkinson's disease. This operation broke a decade-long international moratorium on the controversial therapy, which was imposed after many patients failed to benefit from fetal cell transplants. Parkinson's disease is characterized by degeneration of neurons in the substania nigra of the brain that produce the neuro-transmitter dopamine, which is crucial for normal

movement. Conventional treatment, such as the administration of L-dopa, replaces dopamine to treat the symptoms, but does little in slowing down the progression of the disease. These cellular therapies aim to replace the dopamine-producing (dopaminergic) cells with cells from fetal brains or with those derived from human stem cells.

Research is under way to ensure that the stem cells develop into the exact type of dopaminergic cell needed to treat Parkinson's and that they become correctly integrated into recipients' brains. Progress has been so fast that clinical trials are already on the horizon. A Japanese trial, using induced pluripotent stem cells, is planned to start in Kyoto within two years; and two trials using human embryonic stem cells are also planned – one to begin within three years in New York and the other in Europe within four to five years. In 2016, the Colorado Clinic offers stem cell therapy for back pain relief to help patients achieve relief and avoid the need for back surgery. The treatments are offered by Board Certified providers for both spinal disc and joint degeneration. The clinic offers two options. The first is giving the patients platelet rich plasma (PRP) therapy that contains many growth factors. While PRP therapy doesn't have stem cells directly, it does trigger the body's stem cells to engage in a repair process. The second option is amniotic stem cell therapy, in which amniotic fluid is harvested from consenting donors after a scheduled caesarian section. The process is FDA regulated and the fetus is safe. The third option is bone marrow derived stem cell therapy, in which the bone marrow is harvested from the patient's hip area.

Another approach to stem cell therapy is to develop medications that enhance endogenous stem cells, naturally found in many organs, to proliferate. Since most organs in the human body contain their own stem cells, specific cellular hormones or growth factors could be identified that promote differentiation *in situ*. This type of therapy would not require injection of stem cells into patients; this would allow for broad clinical applications and eliminate most bioethical and religious concerns by eliminating the need for embryos. For example, current evidence suggests that the brain contains endogenous stem cells. Thus, a drug that stimulates stem cell proliferation may one day be helpful in treating victims of strokes, Parkinson's, or Alzheimer's disease. Administering cellular hormones that summon the migration of stem cells to sites of injury presents another kind of potential therapy.

Stem cell transplantation in the brain may operate in novel ways. In the past few years, there have been reports (Lindvall and Kokaia, 2010) of stem cells used to treat spinal cord-paralyzed rats. The mechanism by which recovery from paralysis was observed remains unclear. At first, it was believed that the transplanted stem cells differentiated into new neurons that repaired damaged spinal nerves. Now, evidence suggests that the transplanted stem cells stimulate the production of specific growth factors and cytokines that promote regeneration of endogenous nerve (damaged or undamaged) and stem cells (Fernandez, Mannino et al., 2006, Cabanes et al., 2007). In a recent 2014 publication[6], scientists transplanted olfactory ensheathing cells from a paralyzed patient's own olfactory bulbs to his injured spinal cord. These offactory nerve cells are highly regenerative and offer an innovative source for nerve repair. In fact, the

[6] http://dx.doi.org/10.3727/096368914X685131

success of this trial is the first time that cell transplantation has been shown to reverse paralysis in a real-life situation in which the injury involves a combination of damage to the nerve fibre and to surrounding tissues. While the therapy did not completely restore function, it marks a very significant step towards a potential therapy. Dr. Alok Sharma, director, NeuroGen Brain and Spine Institute in India is beginning to apply stem cell therapy to treat patients with autism, cerebral palsy, and mental retardation.

Other Diseases: Another therapeutic benefit has emerged from stem cell research. Several studies (Potier et al., 2010) show that, as a result of bone marrow transplants, donor stem cells can fuse with resident host tissue cells. Therefore, injecting genetically modified stem cells might constitute a novel means of introducing new genes into the host without the use of viral vectors. The injected stem cells, which contain new genes, would fuse with endogenous cells and allow the expression of these new gene products. The use of stem cells as gene transfer vehicles may lack the clinical problems associated with conventional gene transfer using viral vectors, such as inflammatory side effects and the potential to develop certain forms of cancer.

There is a great deal of interest in applying stem cell technology to treat macular degeneration. Macular degeneration is a common eye condition and a leading cause of vision loss among people age 50 and older. It causes damage to the retinal epithelial cells in the macula, a small spot near the center of the retina and the part of the eye needed for sharp, central vision. In a 2014 study published in Lancet, Dr. Robert Lanzia from Advanced Cell Technology reported the first evidence that stem cell therapy can be used to replace the damaged retinal pigment epithelial cells (Schwartz et al., 2014). Over 70% of the transplant recipients had measurable increases in sub-retinal pigmentation, which gradually increased over time. These results are indicative of high-rate stable engraftment of the newly transplanted retinal pigment epithelial cells. Stem cell technology may therefore prove to be an effective treatment of this disease.

In July 2013, Japan's regulatory authorities gave the go-ahead for a team led by ophthalmologist Masayo Takahashi at the RIKEN Center for Developmental Biology in Kobe to collect cells to be used in a clinical iPS cell pilot study. Skin cells from a woman in her seventies with macular degeneration were reprogrammed to become retinal tissue. These cells were then transplanted into the eye, and RIKEN has reported that the patient experienced no serious side effects. This patient was the first person to receive iPS generated stem cells.

Creating human organs. The use of stem cells to generate rudimentary organs has taken off in the past five years. Using carefully timed chemical cues, researchers have produced three-dimensional structures that resemble tissue from the eye, gut, liver, kidney, pancreas, prostate, lung, stomach, breast, and brain. These bits of tissue are called organoids because they mimic some of the structure and function of real organs.

Organoids are furthering our knowledge of human development, serving as disease models and drug-screening platforms, and might eventually be used to rescue damaged organs. A key breakthrough in creating organoids has been embedding stem cells in matrigel, a soft jelly that resembles the extracellular matrix of many organs. Organoids do not function as well as human organs. Some lack key cell types; others imitate only the earliest stages of organ development or vary from batch to batch (see Willyard, 2015 for a review). Because organoids can be grown from human stem cells and from patient-derived induced pluripotent stem cells, they have the potential to model human development and disease. Furthermore, they have potential for drug testing and even future organ replacement strategies.

Ageing. The effects of aging on stem cells is an important area of research. Recent research (Goodell and Rando, 2015) has focused on the ways that genetic mutations, epigenetic changes, and the extrinsic environmental milieu influence stem cell functionality over time. One recent study reports the ways these factors interact, and how these interactions decrease stem cell health over time. The hope is to uncover potential strategies to enhance stem cell function and increase tissue resiliency into old age. Peripheral blood from young individuals, for example, is generated from around 1000 active stem cells. By the age of 70, the clonal diversity collapses, resulting in dominance of one HSC clone, such that about 20% of individuals have one clone that dominates 20 to 80% of blood cell production. Interestingly, the injection of plasma from young mice into the circulation of aged mice has recently been shown to induce a more youthful state of cells in the brain of the old animal. These findings indicate that at least some aspects of cellular aging may be reversible, perhaps through reprogramming of the epigenome.

Textbox 4. FDA Approvals of Clinical Trials

Regenerative medicine investigators at the Cedars-Sinai Medical Center have received U.S. Food and Drug Administration (FDA) approval to test a novel combination stem cell-gene therapy they've developed to stall amyotrophic lateral sclerosis (ALS) progression. Federal regulators also have told Athersys Inc. that its design of its planned Phase 3 clinical trial should proceed into clinical testing as a therapy for stroke victims using adult stem cell therapy.

Stem Cell Therapy in Sports

Several world famous sports figures, including tennis star, Rafael Nadal, NFL's Peyton Manning, NBA's Pau Gasol, and MLB's Bartolo Colon, have undergone stem cell treatment to repair injuries using either bone marrow or fat as the source. The ailing hockey legend Gordie Howe received stem cells grown by Stemedica, and his story attracted international attention. Athletes, whether playing or retired, have a special need for the regenerative abilities that stem cells might provide. They break bones, strain

ligaments, bang knees, and wear out cartilage. If their restorative capability is proven, stem cells could be considered the latest form of sports medicine.

Since Howe's treatment in late 2014, two other athletic legends have received Stemedica's cells — former quarterbacks Bart Starr and John Brodie. These cells can grow into new bone, cartilage, muscle, or connective tissue and help speed injury recovery in an athlete's knee, back, or shoulder. The NFL considers stem cell therapy a medical treatment rather than a performance-enhancing substance. As of 2014, the FDA limits stem cell therapy to the injection of the unaltered harvested cells directly to the site of the injury. Stem cell therapy is even being used in race horses; Sprinter Smoko is reported, in November 2014, to have had stem cell therapy at Murdoch Veterinary Hospital in order to repair a strained suspensory ligament in his off-foreleg. Most of these athletes had to go abroad for treatment. The alleged success in treatment has put a great deal of pressure on the FDA to initiate more clinical trials in the USA.

Stem cell therapies are extremely expensive and profitable. In fact, it is estimated that more than 600 unauthorized stem cell clinics were operating in the United States in 2016 and charge at least $30,000 per treatment. The global stem cell market is projected to grow from about $6.7 billion in 2016 to nearly $12.3 billion in 2021, registering a five-year compound annual growth rate of 13.1% for the period. In the UK, insurance cover for stem cell therapy has been offered for the first time to "democratise" a process that would ordinarily cost hundreds of thousands of pounds. A company called CellPlan is selling coverage for up to $1m (£680,000) for families who have banked their children's umbilical cord blood. Stem cells from the cord blood can be used to treat 82 diseases including leukaemia in close family members.

When Should Clinical Trials of Stem Cell Technology Begin?

While many animal studies serve as models for human diseases and have demonstrated the potential clinical applications of stem cells, translating these studies to humans is often a difficult process. There are many genetic and physiological differences between humans and mice that could account for the failure of therapeutic applications in humans.

There are several clinical trials in progress, or being planned, which aim to examine the clinical efficacy of stem cell therapies. On the commercial side, a leading regenerative medicine clinic on the West Coast, TeleHealth, is now offering multiple stem cell therapy treatments for arthritis and soft tissue injury such as tendonitis of the shoulder. The injection treatments are covered by insurance, and are offered with Board Certified doctors. This clinic claims that stem cell injection treatments possess the potential for actually repairing the cartilage damage in arthritic joints or tendon damage in an injured shoulder.

Public pressure is certainly one reason for the initiation of these clinical trials. The ethical question is whether or not the scientific basis to enter clinical trials is justified. In 2013, an expert panel of scientists had issued a report advising the Italian Government

against continuing to support a controversial stem cell therapy, deeming it 'unscientific'. The clinical protocols in question consisted of using patients' own mesenchymal stem cells, derived from bone marrow, to treat neurodegenerative conditions such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis, as well as muscle-wasting disorders. The panel found the submitted protocols incomplete. Records of preclinical studies were not included. Furthermore, there was a lack of data attesting to the quality of cellular preparation, as well as a lack of data demonstrating the expected expression of proteins in stem cells as they form new neurons. The panel felt that there was not sufficient scientific merit to approve this type of stem cell therapy.

There is a fascinating report, published in October of 2014, stating that a man who was paralyzed from the chest down in a knife attack in 2010 could walk, using a frame support, after receiving stem cells obtained from his olfactory bulb (Tabakow et al., 2014). The treatment used olfactory ensheathing cells which are specialized cells that form part of the sense of smell. These cells enable nerve fibers in the olfactory system to be continually renewed.[7]

All of these stories highlight the potential gains in medicine that people believe will arise from stem cell therapy. Yet, the public must recognize that translational applications of research into clinical trials develop slowly. The Food and Drug Administration (FDA) is concerned that the hopes patients have for stem cell based cures may leave them vulnerable to unscrupulous providers of stem cell treatments that are illegal and potentially harmful. The FDA cautions consumers to make sure that any stem cell treatment they are considering is approved by the FDA or is being studied under a clinical investigation that has been submitted and allowed to proceed by the FDA. As of 2016, the FDA has approved only one stem cell product, Hemacord, a cord blood-derived product manufactured by the New York Blood Center that is used for specified indications in patients with disorders affecting the body’s blood-forming system.

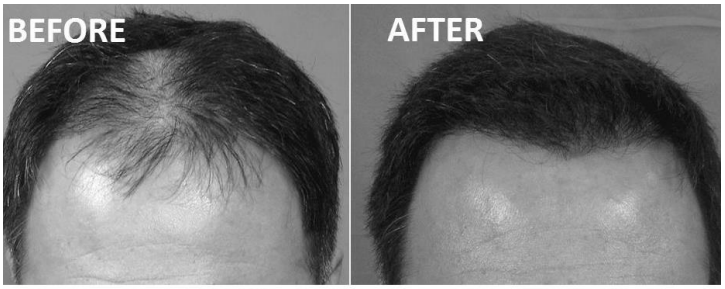
Non-Medical Applications of Stem cells

Stem cells are being studied as a potential treatment modality for a variety of non-medical conditions. Stem cells are being used for a not-quite-surgical procedure that can recontour human faces using a mixture of the patient's own fat and stem cells. This procedure is reported to enable the implanted fat cells to better "take hold" in their new location and become part of the face. In addition, these added stem cells appear to increase the blood supply to the skin to enhance its appearance.

Stem cell technology also enabled scientists at Columbia University to develop a technique to grow human dermal papilla cells, in 3-D culture, to grow de novo hair follicles in human skin, paving the way for a new approach to treating baldness (Higgins et al., 2013). In addition, in a 2014 Nature paper (Yang et al., 2014), scientists describe the method by which they were able to convert adult cells into epithelial stem cells (EpSCs) that formed hair shafts. How did the team produce these cells? The researchers

[7] A video of this report is available at https://www.youtube.com/watch?v=rhFHQMrrz4E.

converted the human skin cells into induced pluripotent stem cells by adding three genes. These iPS cells are able to change into any cell type, so the researchers converted them into epithelial stem cells, which are normally found in a part of hair follicles.



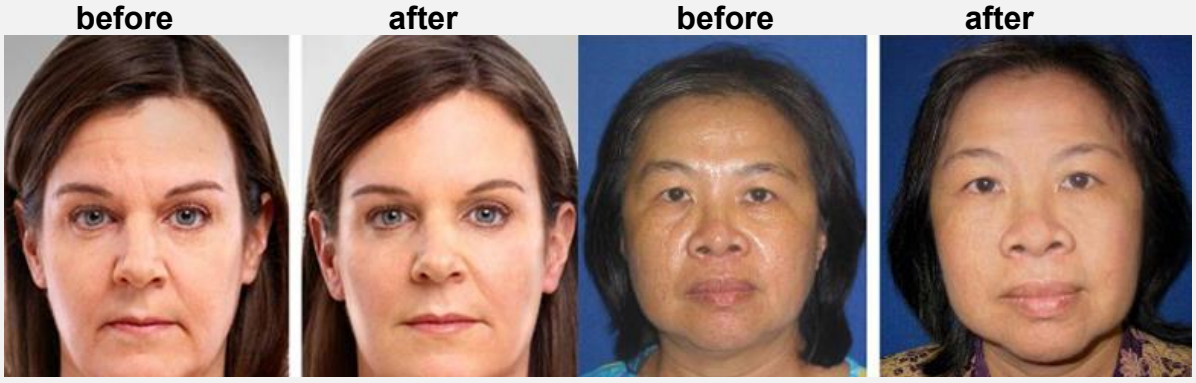
Obtained from http://www.stemrx.in/hair.html

Commercial companies recognize the potential profits of hair restoration. Histogen, Inc., a company whose focus includes hair restoration, presented clinical evidence, at the International Society of Hair Restoration Surgeons (ISHRS) Annual Scientific Meeting in Amsterdam from July 22-26, 2009, that stem cell technology can stimulate hair growth. According to Histogen, HSC is a solution containing naturally secreted embryonic proteins – growth factors that induce new hair follicle formation, hair growth, and hair thickness when injected into the scalp (Meyer-Blazejewska et al., 2011).

Dr. Daniel McGrath is an Associate of the American Academy of Cosmetic Surgery. He runs a clinic that specializes in hair restoration. He removes a small amount of an individual's blood, from which the platelet-rich plasma is obtained and mixed with a wound-healing powder called "a-cell", and injected back into the scalp. Finally, the doctor uses some massage and small needles to create tiny wounds, which trigger a healing hair-restoring response. Dr. McGrath claims that 80 percent hair re-growth or regeneration across the board is observed in his patients. One treatment costs about $3,500.

Textbox 5: Anti-aging Stem Cell Therapy

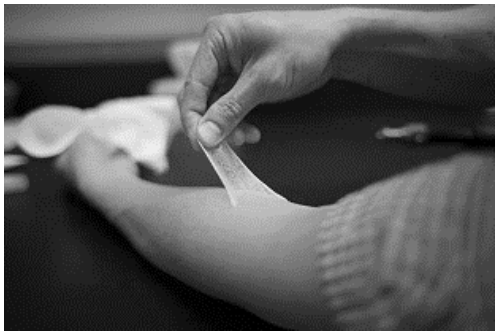
Swiss Medica Clinic provides the latest Stem Cells treatments and procedures to rejuvenate your face, body, organs and increase the feeling of well-being.



Many companies now offer stem cell therapy as its new treatment for Anti-aging (see Textbox 3). Their therapy is based on the theory that aging results from the progressive depletion of stem cells, so the introduction of new stem cells and adjunctive treatments has the potential of slowing down or reversing this process. Another serum product, marketed by Lifeline Skin Care, is based on the unproven concept that human non-embryonic stem cell extracts containing ingredients derived from unfertilized human eggs donated to the ISCO, can renew your skin to a youthful complexion. These anti-aging stem cell serums are marketed to stimulate the skin's abilities to repair itself.

An unusual application of stem cell technology comes from a California company called Ageless Derma.[8] Their skin care product, Swiss Apple Stem Cell Mask, is derived from apple stem cells and incorporates the cells of a long-living rare apple with other natural revitalizing ingredients, resulting in a gentle mask that effectively returns youthful life to the complexion. The cost of this mask is under $40, as compared to a $10,000 product sold by Angle and Weightman, whose face cream contains stem cell extracts that refinishes and re-hydrates human skin.

In June, 2011, the FDA approved a therapy that uses a person's own skin cells to help improve the appearance of smile lines that can extend from the bottom of the nose to the sides of the mouth. The treatment, called laViv, was developed by Fibrocell Science and involves taking a sample of skin cells called fibroblasts, which make collagen, from behind the person's ear. The sample is sent to the company's laboratory, where the fibroblasts are multiplied in cell culture, a process that takes 11 to 22 weeks. The cells are then sent back to the doctor, who injects them into the smile lines (or frown lines), which are technically known as nasolabial folds. The treatment was evaluated in two clinical trials, with a total of 421 patients, in which participants received either three treatments with laViv or three treatments with an injection that did not contain the cells. Six months after the third treatment, both the patients and their doctors, neither of whom knew whether the treatment or control was given, assessed the results.



One consequence of stem cell research is the development of other technologies that are less expensive and are not as ethically challenging. In May of 2016, a report appeared that describes a new and innovative technology called "second skin" (Yu, Kang et al. 2016). While the research was done to help patients who suffer from a variety of skin conditions, the application to the general public might be enormous. Second skin is made of silicon and oxygen compounds called siloxanes that link to form polymers in a thin, skin-like layer which, while removable, can stay intact for at least 24 hours. The ingredients of this product are made from common chemicals that have been deemed safe by the U.S. Food and Drug Administration. This product will help patients with eczema and psoriasis. However, for the aging public that spends billions of dollars on anti-aging creams this revolutionary product that can take some of the signs of aging

[8] http://www.agelessderma.com/contact-us.aspx.

away — at least temporarily. The layer is formed by applying two creams in succession: first, a cream containing the siloxanes; second, a cream containing a platinum catalyst that causes polymer cross-linking and consequently hardening of the material. The research was funded by a small, privately owned biotechnology company in Cambridge, Mass., Living Proof, and the product is being developed by another small, privately owned Olivo Laboratories, which owns the patents.

The use of stem cells in bone restoration is also emerging as a potential therapy for several diseases. Research has shown that mesenchymal stem cells, which reside in bone marrow, are rich sources of adult stem cells that can be used in tooth regeneration and repair (Huang et al., 2009, Mantesso and Sharpe, 2009). Dental pulp stem cells form vascularized pulp-like tissue surrounded by a layer of odontoblast-like cells expressing dentin proteins similar to those found in natural dentin. When seeded onto human dentin surfaces and implanted into immunocompromised mice, dental pulp stem cells create dentin-like structures deposited on the dentin surface.

In 2013, Google founder Sergey Brin funded a project to generate test tube or cloned beef hamburgers created from stem cells extracted from the muscle of three cows.[9] This technology to generate laboratory-cloned beef meat for human consumption is based on stem cell research. Producing laboratory–cloned beef hamburgers involves harmlessly obtaining a small sample of muscle tissue from a living animal and isolating individual muscle stem cells called myosatellites. Myosatellites can reproduce fairly quickly in the laboratory and, when cultured under the appropriate in vitro culture conditions, fuse to form muscle fibers. Layered together, these strands of muscle cells and fibers form the essential components necessary to produce cultured edible meat.

In 2013, Professor Mark Post of Maastricht University created the world's first lab-grown cloned beef hamburger. Culinary experts tasted this hamburger and concluded that it had the taste and texture of real meat, although it was a little dry. The dryness was probably due to the lack of fat cells in the meat, since it is difficult to culture adipose cells together with muscle cells. This first beef hamburger cost $350,000. Currently, the cost of the cloned beef has been reduced by 80% to $70,000. The ultimate goal is to produce a five-ounce burger, referred to as a googleburger, for only $10. Recent scientific innovations, such as the creation of artificial veins in synthetic organs, can increase the fat content and improve the taste of the burger while continuing to lower the expenses.

Cloned animal-derived hamburgers present a more sustainable option for meat production then classical hamburgers. Firstly, cows are very inefficient requiring 100g of vegetable protein to produce only 15 grams of edible animal protein. Second, cloned beef hamburgers will reduce animal wastes, a significant source of land and water pollution, and reduce the emission of methane – a gas responsible for global warming. Third, cloned beef can be genetically modified to produce healthier meat that is low in saturated fats and high in omega 3 fatty acids. Finally, cloned beef doesn't require massive animal killings and therefore minimizes the threat of animal cruelty**.**

[9] http://www.theguardian.com/science/2013/aug/05/synthetic-meat-burger-stem-cells

Medical Risks of Stem Cell Therapy

Critical safety issues must be considered in stem cell-based therapies (Heslop et al., 2015). Currently, most federally-funded programs related to stem cell technology generate embryonic stem cells that are derived from existing or newly established cell lines and are not tissue compatible to the patients. Therefore, patients receiving these transplanted embryonic stem cells will require immuno-suppressive drugs to prevent tissue rejection.

There have been reports suggesting that certain stem cell therapies involving hematopoietic stem cell transplantation have more inherent health risks than ordinary bone marrow transplantation. In addition, the time required for stem cell therapy to reconstitute the immune system may take several months after autologous transplantation and up to a year or longer after allogeneic transplantation (Wingard et al., 2010).

Tissue rejection can be avoided if patients' own stem cells are used as a source of therapy. As mentioned above, there are several ways in which patients can provide their own stem cells. In addition to pluripotent stem cells obtained from bone marrow, therapeutic cloning offers another way to generate histocompatible stem cells that would not require patients to receive immuno-suppressive drugs. Somatic cell nuclear transfer utilizes technology to transfer a nucleus from a specific cell of a patient and then fuse it with an enucleated oocyte. The resulting zygote would then be allowed to differentiate into a blastocyst *in vitro* and would serve as the source for isolating stem cells from the inner mass.

In addition to tissue compatibility, transplanted pluripotent stem cells can form tumors in animals. Researchers have identified what they call cancer stem cells in blood cancers such as leukemia, breast, and brain cancers (Zhang and Rosen, 2006). In other words, the mutations that drive certain cancers to develop in the body may originate in the body's small supply of naturally occurring stem cells. There is also evidence that cancer stem cells, which only form a small portion of the total tumor, are, in fact, the primary cells responsible for maintaining tumor growth (Spillane and Henderson, 2007). Many tumor cells have been shown to exhibit stem cell-like properties, such as reverting back to a less differentiated state and exhibiting the ability to rapidly proliferate. While it remains unclear why a small percentage of implanted stem cells form tumors, it may be related to differentiation processes that have gone unregulated.

Scientists are trying different approaches to overcome the cancer problems associated with the use of stem cells. One method is to utilize adult-derived stem cells for therapy, as these cells are considered less tumorigenic than embryonic stem cells. Another approach is to transform embryonic stem cells into specialized differentiated cells before transplantation into the patient. The hope is that once the stem cell has completed differentiation, its potential to proliferate uncontrollably will be significantly reduced**.**

If the stem cell-cancer problem is not overcome in the near future, patients may

have to accept that a side effect of potentially life-saving stem cell therapies is that a proportion of transplanted stem cells may turn into tumors within 10-20 years. Long development times have been observed in several types of cancers, including colon and prostate; these cancers take more than a decade to fully develop from the time that the earliest cancer nodule is detected. Furthermore, if stem cells are used to treat a 65-year-old patient who has Parkinson's or Alzheimer's disease, then the risk that the patient might develop cancer within 10-20 years may be one that this patient is willing to take. In contrast, given that stem cell therapies may lead to the development of cancers, their use may not be warranted in a child or young adult candidate.

As mentioned above, encapsulation is an innovative technology that may provide a solution to all of the obstacles noted above. In this manner, tissue rejection and tumorigenesis are avoided, but the release of appropriate cytokines and growth factors, which could regulate tissue repair or endogenous stem cell regeneration, is maintained.

A recent study showed that human mesenchymal stromal cells remain viable within alginate for at least 2 months (Barminko et al., 2011). They also demonstrated the benefits of transplanting immobilized stem cells as an immunomodulatory vehicle within rats that had experienced spinal cord injury. The immobilized human mesenchymal stromal cells were able to promote pro-inflammatory macrophage attenuation at the site of injury (Barminko et al, 2011). Another study revealed transplanted encapsulated mesenchymal stem cells are protected from MHC-mediated attack by activated T cells (Ansari et al, 2015). One concern with encapsulation is the looming threat that the pores of the encapsulating biomaterial will become clogged by clotting factors or large biological, thereby restricting the entry and exit of biologicals.

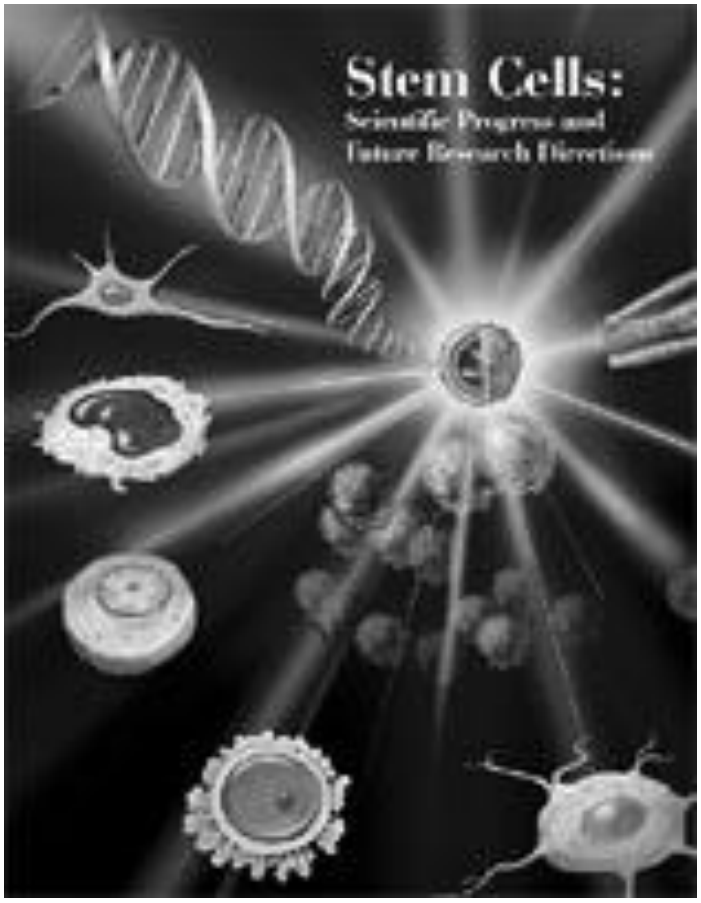
Another medical risk associated with the use of undifferentiated stem cells is called epigenetic instability (Benayoun et al., 2015). The long-term maintenance and continual passing of stem cells that is needed to preserve embryonic stem cell lines can result in aberrant methylation (or silencing) of gene promoter regions. A final safety issue is that there may be infectious agents present in the cell-feeder layers used to maintain stem cells. Currently, stem cells are most easily maintained in culture by growing them in chambers where other transformed cells, such as fibroblasts (obtained from other species), serve as feeder layers. The feeder cells supply essential nutrients required for the stem cells to maintain their state of self-renewal. In addition, these feeder layers prevent the stem cells from differentiating by secreting a variety of extracellular matrix proteins or cytokines. The human stem cells are physically separated from the cellular feeder layer by semi-permeable membranes. Embryonic feeder cells provide convenient growth and efficient study of embryonic stem cells in the laboratory but raise the risk of interspecies virus transfer. There is ample evidence that some polio vaccines used during the mass vaccination campaigns of the 1950s and 1960s may have been contaminated with the simian virus SV-40, which has been reported to be associated with a variety of human tumors. SV-40 contamination may have occurred because the vaccine was developed using monkey kidney cell lines (Petricciani et al., 2014). These types of reports suggest that feeder-cell-independent culture conditions, or serum free conditions, have to be developed to prevent infectious agents from contaminating the stem cell preparations. Thus, one goal of embryonic stem cell research is to find a way to derive

and culture cell lines without the use of feeder layers or animal serum (Crocoo et al., 2013). In fact, several groups have reported the benefits of maintaining human stem cells on hydrogel (hyaluronic acid) in the absence of any feeder layers (Liu, et al., 2012).

Conclusions

As of 2015, undifferentiated human stem cells have not cured any disease. To cure diseases, stem cells must be differentiated into more specialized cells that can be transferred to patients. Much more work is needed to understand how or whether stem cell transplants will benefit patients. One critical unknown is whether the stem cells infused into the animal or patient proliferate to replace the damaged tissue or whether these stem cells merely fuse with existing endogenous cells to affect a therapeutic response. Cell fusion has been observed between adult stem cells obtained from bone marrow and nerves from the central nervous system, in animals with spinal cord injuries that were given stem cells. Are the fused cells dead-end products that disappear with time, or are they intermediate steps in the normal process of tissue repair? The capacity of cells to fuse with one another is not unique to stem cells. Fused cells are normally found in several organ systems including the liver, intestine, placenta, skeletal muscle, cardiac smooth muscle, and bone marrow (megakaryocytes).

In summary, stem cell therapy holds exciting promise because it may greatly impact the treatment of a variety of diseases. Stem cell research offers more than just the potential to create new cell transplant protocols or cure disease; in the short term, research into stem cell differentiation will facilitate a better understanding of normal and abnormal cell differentiation, gene regulation, and embryological development from a single cell into a complete organism. The potential for a better understanding of basic biology and for the development of new biotechnologies from stem cell research appears quite promising and justifies the investment of money, time, and effort in stem cell research.



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Chapter Three

Defining Research Bioethics

Introduction

Ethics has traditionally been applied to both health care and scientific research. Over the past forty years, ethics in medicine and science has branched out in numerous directions (genetic ethics, neuroethics, animal ethics, research ethics, legal bioethics, environmental ethics, and life science ethics). While the general term "bioethics" is used to include all these areas, current analyses reveal that each of these areas of study can be viewed as intrinsically different. The first part of this chapter focuses on the need to define research bioethics and when it should be distinguished from medical ethics. The second part of this chapter outlines specific ethical guidelines that address some of the unique characteristics of research bioethics that may differ from classical medical ethics. The conceptualization of research bioethics is designed to link various avenues of science-based research into one ethical discipline that emerges from biotechnology and life-science discoveries.

Bioethics in the Context of Medical Ethics

From the time of Hippocrates until the present day, discussions relating to medical ethics have generally focused on health care professional-patient relationships. Thus, scientific discoveries that directly have an impact on the rights of the patient, the rights and obligations of the physician, and the operations of health care facilities, fall within the domain of medical ethics. Traditional issues in medical ethics include contraception, *in vitro* fertilization (IVF), assisted reproductive technologies, abortion, informed consent, organ transplantation, and end of life issues. In addition, ethical guidelines have been formulated to protect the rights of volunteers participating in clinical or research studies that may lead to new FDA-approved therapies.

Dr. Van Rensselaer Potter (Potter, 1972) was one of the first to define bioethics as "biology combined with diverse humanistic knowledge forging a science that sets a system of medical and environmental priorities for acceptable survival". In this vein, the *Encyclopedia of Bioethics* (1970) defined bioethics as, "the interdisciplinary examination of the moral and ethical dimensions of human conduct in the areas of life sciences and health care. The discipline encompasses the study of medical, legal, scientific, religious, philosophical, moral and ethical issues of life sciences."(Post, 2004).

As discussed in Chapter 2, medical ethicists have developed four general principles or guidelines to provide a framework for discussions and/or resolution of medical ethical dilemmas. They are: 1) *autonomy/respect for persons,* 2)

beneficence, 3) *non-maleficence,* and 4) *justice.* Resolving medical ethics dilemmas often requires balancing conflicting guidelines such as the rights and autonomy of the individual versus the rights of society, the potential benefit versus the risk to the individual, the short-term suffering and pain versus the long term benefits, and the moral versus medical obligations to the patients.

Historically, guidelines in medical ethics were developed in part due to atrocities in ethical conduct of research. Research ethics arose from the ashes of the Holocaust where the Nazi doctors conducted notorious and sadistic medical experiments, characterized by a total lack of voluntary consent and ethical practice as well as a pervasive pseudo-science. These lethal and murderous experiments were intended only to help the German race and German soldiers. In response to these Nazi atrocities, the Nuremberg Code (1948) was drafted by the judges who adjudicated in the Nuremberg Trial of Nazi physicians who were charged with crimes against humanity. The Nuremberg Code outlined some of the fundamental legal guidelines involving voluntary informed consent that subsequently have influenced U.S. regulations governing informed consent.[1]

The next major bioethical document was the National Research Act passed in 1974 in response to the egregious Tuskegee Syphilis Study and the Willowbrook study where mentally retarded children housed at the Willowbrook State School in Staten Island, New York, were intentionally given hepatitis in an attempt to track the development of the viral infection. The United States Public Health Service uncovered that over 400 participants of the Tuskegee Syphilis Study (predominantly African American males) were denied anti-syphilis treatments. The investigation lead to the creation of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research whose charge was to identify the basic ethical principles that should underlie the conduct of biomedical and behavioral research involving human subjects. Furthermore, guidelines were proposed to assure that such human research is conducted in accordance with ethical principles. In 1978, Casper Weinberger, President Gerald Ford's Secretary of Health, Education, and Welfare, drafted the Belmont Report. *This crucial* document outlines the guidelines for protecting human subjects in both clinical and research environments.

Considerable debate has recently emerged, however, regarding whether the principles and guidelines proposed in the Belmont Report adequately addressed the broader ethical issues related to biomedical research in grappling with situations where technology confronts ethics. A number of prominent bioethicists such as Dr. Daniel Callahan, cofounder of The Hastings Center and Gilbert Meilaender, a member of the President's Council on Bioethics, all questioned whether these medical ethical principles that often clashed, could be applied to "real life" bioresearch ethical issues. Even Dr. Thomas Beauchamp,

[1] http://www.ushmm.org/research/doctors/Nuremberg_Code.htm for more information

one of the pioneers in bioethical education, questioned the role of classical ethical theories in resolving modern issues of research bioethics (Beauchamp, 2007).

The Need to Redefine Biomedical Research Ethics

Since the 1970s, new biotechnologies in the areas of molecular biology, genomics, and reproductive biology, have been developed, affecting life-science research. These new technologies have challenged the basic definitions of human life, such as when personhood begins and how we define ourselves as an individual species. Embryonic stem cell research and human cloning are important contemporary examples of evolving biotechnologies that require informed discussions about the scientific implications of this research and the bioethical issues that inevitably arise. Recent biotechnological discoveries, such as genetic manipulations, the development of bio-chips, and creating embryos from three genetic parents necessitate the development of a discipline with rules, strategies, and definitions that address the real and never-before-seen bioethical dilemmas that scientists as well as society must confront. The genetic engineering of plants, for example, may not be a relevant problem for the patient-doctor guidelines of medical ethics, but it raises the issue of changing the "natural environmental order."

The first step in differentiating research bioethics from medical ethics is developing an operational definition of bioethics. Today, a broader definition that may better fit contemporary biotechnological innovation is necessary. Bioethics is defined in this book as a broad field of study that examines the ethical issues emerging from biotechnologies that affect human beings, the animal world, the plant kingdom, and the environment. We coin the term *research bioethics* as **the study of ethical dilemmas arising from the acquisition of scientific knowledge and its impact on life forms and the environment.** This definition helps to establish an ethical approach to the acquisition of scientific knowledge as it positively and negatively influences and interacts with human society and the environment at large.

The new definition of research bioethics distinguishes it fundamentally from medical ethics in one critical area. Research bioethics focuses on scientific discoveries that affect human society, animals, plants, and the environment as a whole. In contrast, medical ethics is more circumscribed, and generally focuses on any condition that involves an individual or volunteers participating in medical experimentation or any condition that creates a provider-patient relationship.

If one accepts this definition of research bioethics, then a reformulation of the basic guidelines for research bioethics is required to deal with the specific and unique ethical concerns relating to science, society, and the environment. Historically, scientists who have attempted to apply bioethical-medical ethical principles (as defined by the Belmont Report) (Beauchamp, 2007) to research

settings have discovered that the principles may not provide a useful framework for addressing many relevant ethical **research** concerns. For example, the first Belmont principle, respect for persons (or autonomy), can have a utilitarian, rather than a moral goal. The Belmont Report incorporates John Stuart Mill's utilitarian views of personal autonomy that, "only fully conscious, rational adults capable of acting autonomously are considered moral agents with moral responsibilities" (Callahan, 1994). However, those incapable of acting autonomously (such as infants, comatose patients, or patients with Alzheimer's disease), were defined under the Belmont bioethical principles as non-moral agents and are thus "non-persons" who lack any rights of self-determination. In addition, there are many situations in medical ethics that focus on how the individual infringes on the general welfare of society. Confidentiality and the individual right to privacy in the diagnosis of HIV infection, for example, may compromise public health needs. These needs include surveying the infected in order to protect the uninfected along with notifying individuals of the possible risk of infection.

The second principle, beneficence, incorporates a Hippocratic understanding of beneficence as doing good for the patient. However, the Belmont Report also included a second definition of beneficence that is utilitarian and involves, "one doing good for society at large" (Callahan, 1994). The Belmont Report further declares "citizens have a strong moral obligation to take part in experimental research for the greater good of society." This contradicts the Hippocratic interpretation of beneficence and violates time-honored international medical ethical guidelines such as the Nuremberg Code and the Helsinki Declaration, which oppose physicians experimenting on volunteer subjects unless the subjects **directly benefit** from the procedure.

The third Belmont principle, justice, is also defined in terms of a "fairness" that allocates the benefits and burdens of scientific research equitably across the different social and economic populations. This principle varies a great deal from the classic Aristotelian definition of justice used in medical ethics that emphasizes the fair and just treatment of every human being. Applying fairness in biomedical research is often difficult to ensure. How are decisions made to allocate research funds for Huntington's disease, which affects fewer than a million people around the world, when millions of people are dying of AIDS or malaria? Heart transplants provide another challenge associated with the principle of justice. In the USA about 3,000 heart transplants are performed at a cost of close to 1 million dollars per year per patient. Would this money (three billion dollars) be better allocated to develop new drugs that could benefit the 500,000 people who develop heart problems each year?

Differentiating research bioethics and medical ethics can also manifest into practical ramifications. First, these two disciplines can target two distinctly different participants. The classical audience for medical ethics has been health care providers, clinical researchers, insurance companies, and health care institutions that require guidelines to make complicated decisions regarding the value of human life and patient care. In contrast, biomedical and life science researchers

both in academia and corporate America need ethical guidelines appropriate and relevant for the testing and application of new developing biotechnologies.

A second ramification of the differentiation between research bioethics and medical ethics relates to education via case studies. Medical ethics case studies are generally obtained from real-life situations. Seeing the patient in the health care facility is essential to resolving and/or managing medical ethical issues. In contrast, real-life situations in bioethics are less opportune when the technology is still under development. Society and government are hesitant to allow research with novel biotechnologies to progress without discussing end results. Consequently, bioethical dilemmas are often hypothetical with regards to patient applications. For example, no accessible research facilities are currently engaged in human reproductive cloning to provide a real situation where bioethicists can assess the health and behavior of a cloned human. Furthermore, new biotechnologies are often introduced in a corporate setting where governmental access is also limited.

The third ramification relates to the different compositional structures of regulatory agencies dealing with medical ethics versus bioethics. Medical ethics committees are typically composed of individuals involved in health care including practicing physicians, nurses, other health care professionals, hospital administrators, medical ethicists, insurance experts, theologians, and lawyers. Medical ethics committees often focus on the influence of managed care with respect to the patient's best interest or deal with issues that may interfere with the daily operations of health care or medical institutions. In contrast, research bioethics committees should also include basic research scientists, physician-scientists, bioethicists, political analysts, environmentalists, and sociologists. An example of this type of committee is the President's Council on Bioethics that focused on human cloning and stem cell research. While the recommendations of the council have been controversial and may never be implemented as originally designed, the Commission was thorough in dissecting the ethical issues and arguments relevant to these technologies.[2] Many Institutional Review Boards (IRBs) established in universities, are expanding their focus to include bioethical issues emerging from new biotechnologies.

The final distinction between medical ethics and research bioethics includes the time frame that is necessary to propose practical ways to resolve the ethical dilemmas. In cases involving patient - health care medical ethics, there is a more immediate need for resolution. The classical case-study whether a neurologically brain dead patient should be removed from a respirator or whether a terminal cancer patient should be denied the option of euthanasia requires an immediate response. In contrast, many bioethical dilemmas related to embryonic stem cell

[2] http://bioethics.georgetown.edu/pcbe/reports/cloningreport/ and http://bioethics.georgetown.edu/pcbe/reports/stemcell/.

research, reproductive cloning, or genetically modified organisms have been debated over the last decade, often without the need for immediate resolution.

Translational Science and Bioethics

Translational science is a relatively new concept (Hostiuc, 2016) that can be divided in two categories, translational medicine and translational research. Translational medicine is to a practical, outcome-oriented research and can be viewed as research on human specimens, whose findings may inform basic science research and lead to a transfer of the results towards clinical therapeutics. It starts with fundamental research (genes, molecular processes, biochemical pathways) and ends at a macro level (social healthcare, access to healthcare, and access to education. Translational research is the application of basic scientific research to non-medical applications. An example of translational science is synthetic biology where scientists have created two new DNA base pairs. In this new system the DNA is now composed of six base pairs. While its application to medicine and health care remains to be defined, it is being applied to enhance the power of DNA-based chip technology in biocomputers.

As discussed below, the bioethical assessment of translational medicine and research can be different especially in establishing the hierarchy of bioethical guidelines. In medicine, one could argue that autonomy may be the most important guideline. In research, on could argue that non-maleficence may be the most important ethical guideline. The new research in synthetic biology that may allow scientists to rewrite the genetic code can elicit fear that tampering with the Holy Grail, DNA, may lead to disastrous consequences. The application of gene drive technology to eradicate Zika born mosquitos, discussed in Chapter 9, may have severe ecological consequences that we will not detect for decades.

Four Additional Principles for Research Bioethics

The following four additional research bioethical guidelines are proposed to regulate the pursuit of scientific inquiry: 1) respecting the value of human life and balancing the needs of the society versus the needs of the individual; 2) Respect for the bio-environment; 3) using scientific research to alleviate specific bioethical concerns; and 4) the “yuck factor” where a technology is deemed unethical for intuitive, rather than logical, reasons.

Human Dignity: At times, respecting human dignity or the value of human life and balancing the needs of society versus the needs of the individual has been invoked in contemporary bioethics regarding issues of human genetic enhancement as well as the generation of human-nonhuman chimeras (de Melo-Martin, 2008; Loike and Tendler, 2008). Regarding this guideline there are two controversial parameters that must be delineated. The first is to define human dignity and the second is to

identify cases when it is appropriate to apply this principle.

Human dignity can be viewed either within a secular or religious perspective. Immanuel Kant proposed a secular definition that human dignity is associated with the capacity to think for oneself and direct one's actions. Using a Kantian moral framework of human dignity, human beings possess an unconditional and incomparable worth that is independent of metaphysical or religious precepts (Macklin, 2003; Karpowicz et al., 2005). According to Kant, human beings have dignity because of their reasoning faculties, which give them the freedom and ability to distinguish moral from immoral actions. Using this Kantian definition; however, some scholars have argued that not all human beings have dignity. The Kantian principle suggests that patients in a permanent vegetative state, for example, who have irreversibly lost their autonomy may no longer have dignity (Loike and Tendler, 2011).

In contrast to this secular definition of human dignity, a theologically-based definition formulates or characterizes human dignity as an inviolable right invested by God in all human beings including fetuses, comatose patients, and patients in a permanent vegetative state (Kass, 2006; Loike and Tendler, 2011). In its simplest religious formulation, human dignity can be equated with the sanctity or infinite worth of human life and assumes that there is something uniquely valuable about human life. From a religious Judeo-Christian view, human dignity emanates from the first chapter of Genesis that records how human beings were uniquely fashioned and divinely created (Soloveitchik, 1983). Several Biblical scholars comment that the Bible describes that God created human beings using two different processes (Soloveitchik, 1983). The first process was biological/genetic as indicated by the fact that human beings were created on the same day as other animals. The second process was metaphysical as God infused into human beings a spiritual entity that differentiates human beings from all other creatures. This metaphysical, and almost divine quality of human beings confers a sanctity that exists within each human being from the beginning of life as a zygote until natural death.

Irrespective of the origins of respecting human dignity, there are moral virtues, such as courage, compassion, and altruism that people often consider as being good. Without implement such moral virtues within a cooperative platform, a society cannot survive.

If one accepts the principle and outcomes of human dignity, then it is appropriate to examine the role human dignity may play in bioethics. On the one hand, bioethicists, such as Ruth Macklin, point out that respecting human dignity is a vague restatement of other bioethical guidelines, beneficence or autonomy, and brings no significant value or greater understanding to bioethical dilemmas (Macklin, 2003). Ruth Macklin states,

> "[Human] dignity is a useless concept…A close inspection of leading examples shows that appeals to dignity are either vague restatements of

> other, more precise, notions or mere slogans that add nothing to an understanding of the topic."

In addition, Dr. Macklin presents other philosophical arguments that weaken the validity of the principle of respecting human dignity (Macklin, 2003).

Other scholars and bioethicists (Kass, 2006; Loike and Tendler, 2011) argue from a secular and religious perspective the paramount importance of applying the principle of respecting human dignity in bioethical matters. Francis Fukuyama (Fukuyama, 2002) blends a secular approach of human dignity with the distinct nature of the human species.

> "[Fukuyama] He defines human nature as "species-typical traits" of human beings (such as language and cognition, which provide the grounds for feelings such as pride, anger, shame, and sympathy), arising from genetic factors. …. these species-specific traits of humans differentiate us from all other nonhuman species, and this differentiation constitutes the basis of human dignity. The reduction of shared traits among humans will result, in the degradation of human dignity (Bhuiyan, 2009)."

Under what situations should respect for human dignity be applied? Research programs, for example, designed to examine whether cows can be genetically altered to develop human uteri and serve as surrogate incubators for human embryos should not receive priority over programs engaged in examining artificial incubators for premature babies. Ethicists will argue that gestating human embryos in cows raise the issue of respecting human dignity and should not receive government funding or support. In another situation publicized in April 2008, British researchers claim to have created human embryos using human cells and the egg cells of cows. The researches stated that they had hollowed out egg cells obtained from cattle and inserted human DNA into the hollowed cells to create a growing embryo for the purposes of later isolation of human embryonic stem cells.[3] A final example involves transplanting precursor human astrocytes into mouse embryos to reconstitute human astrocytes into the brains of mice. Such human-chimeras have been reported to be more intelligent than normal mice raising the issue whether it is ethical to create mice that express genes that are associated with human intelligence?

There is also an intimate connection between respecting human dignity and infringing individual rights. For example, obtaining the genetic fingerprint of every individual in a population for the purpose of crime control or prevention of terrorist attacks infringes on the individual's right to privacy and confidentiality; however, it may be a practical method to reduce or solve crimes. Genetic profiles and fingerprints of potential criminals or terrorists have been shown to help manage crime control and potential terrorist attacks and may serve to improve the safety of society in general (Barber and Foran, 2006; Berger, 2006). Another example involves the genetic testing of newborns or adults. Currently, New York State

[3] http://www.sciam.com/article.cfm?id=scientists-make-human-cow

screens every newborn for cystic fibrosis and several other genetic disorders. This appears to reduce the number of children born with these diseases. However, additional genetic screening for certain types of cancers or neurological diseases is more controversial, especially when these tests may not medically benefit the individual research subject. Sometimes, such results could harm research subjects who are not properly educated or prepared to handle the psychological implications of the results of the screening. Should the results of genetic testing done within the context of a research study be shared with the volunteer subjects participating in the study? A great deal of time and effort would be required to properly educate the volunteers about the nature of these genetic exploration studies. Identifying the gene for Familial dysautonomia (Anderson et al., 2001) was clearly accelerated by using a DNA database established exclusively to screen for Tay Sachs disease. Those individuals who originally provided samples for the Tay Sachs database were never informed that their DNA samples would be used for other research purposes. Were the scientists justified in using this database? What protective measures of confidentiality or informed consent were implemented for this study? Is it justified to screen for new disease markers utilizing genetic data banks that were obtained from other studies without obtaining permission (informed consent) from the donors? The underlying justification for such screening is the belief that the more genetic information obtained regarding a disease process, the greater that possibility is that scientists will be able to design more effective future therapies. The countervailing opinion is that individuals may choose not to engage in certain genetic testing for a variety of personal motivations including prescribing to the idea that their life unfolds in a predestined manner. Moving forward, it is clear that it is important to obtain permission from donors to extend the use of their genetic material in other genetic research studies that examine any disease markers, not only the ones that they signed an informed consent for.

A final example relates to the ongoing debate over how to handle the publication of scientific research findings that could threaten national security (see Chapter 14). 'Publish or perish' has always been a guiding characteristic of the academic life of investigators in the sciences. However, since the Anthrax mail attacks of 2001, there have been debates regarding which results of biological research should be published. Similarly, there is concern that research in synthetic biology in which scientists are attempting to build all-new life forms from artificial DNA may pave the way to create new powerful bioterrorist weapons. There is a fear that publishing the underlying methods behind these types of scientific projects could fall into the hands of terrorists, possibly jeopardizing national security.

Policies should be established enabling the scientists to publish research without revealing details that could endanger the safety of the nation. Who should oversee exactly what information is published: governmental agencies, authors, research institutions, journals, or some combination? Policy guidelines should establish strategies for preventing the misuse of biotechnology while preserving scientific inquiry and the dissemination of appropriate scientific data.

In summary, this first additional guideline assumes that all human beings have infinite or immeasurable value and that saving lives is a significant long-term objective of current scientific research activity. Thus, a primary objective of research must be to utilize and develop new life-science technologies to improve health care, disease treatment, and disease prevention. In fact, the recent roadmap proposed by the National Institutes of Health[4] reflects these objectives. Biological research with unclear societal applications should not receive equal priority as research with clear societal applications.

Respect for the bio-environment and biological order: Respecting the environment is a critical concern for bioethics, but is not typically relevant in discussions of medical ethics. The use of biotechnology to improve the color, taste, nutrition, and production of food began in ancient times, when farmers first cross-bred different plant strains and realized that they could produce varieties with the optimal characteristics of both of the original plants. Today about 2-4% of farmlands are planted with genetically modified (GM) crops and most of these GM crops are planted on US soil.[5] In addition, GM plants can serve as a source for manufacturing recombinant proteins to be used for therapeutic purposes. Plant-based production of therapeutic proteins is predicted to cost 4-5 times less than production by classical cell culture techniques. However, the general concern over any genetically modified plant or organism is that transgenes will spread through the environment and ultimately affect non-targeted organisms. In addition, there is a fear that introducing genetically modified organisms could disturb the ecological balance of other plants and animals including humans. Scientists have only begun broadly examining the effects of genetically modified plants on the environment as recently as the 1990s. Finally, as of 2016, there is still considerable debate whether GM plants actually improve yield and reduce the use of pesticides.[6]

This guideline (respect for the bio-environment) would ensure that research into GMOs incorporates safety measures in addition to studying the possibilities of how a genetically modified organism could affect factors of the bio-environment such as the consumer, other plant life or insect habitats. In 2003 and 2008, The Food and Drug Administration (FDA) concluded that meat from **cloned** animals is as safe as conventionally bred animals. Clones are genetic copies of donor animals; unlike genetically modified animals, their DNA is not changed, but used to introduce desirable traits into herds. In contrast, Australia's current policy is that cloning is restricted to breeding stock cattle and sheep that are not entering the

[4] http://bioethics.georgetown.edu/pcbe/bookshelf/

[5] http://www.newscientist.com/channel/life/gm-food; http://www.ers.usda.gov/publications/err-economic-research-report/err162.aspx

[6] http://www.nytimes.com/topic/subject/genetically-modified-food and https://www.geneticliteracyproject.org/2016/10/31/danny-hakims-new-york-times-gmo-expose-misleads/

food supply. It is unclear why such a statement was issued without the appropriate scientific studies justifying such a conclusion. Just as new drug investigations require safety controls, research that involves GMOs should include appropriate safety tests. Such safety controls should be instituted regardless whether the GMO is developed by industry or academic institutions. The fact that most European countries are considering, or have, a ban of GMOs highlights the difficulty in scientifically assessing their environmental impact.

The development of genetically modified organisms should include a comprehensive survey of potential environmental impacts. One could envision that routine test phases could be implemented, similar to the test phases implemented with the development of new therapies. Phase I development would examine the effects of GMOs within a test field that examines other plants, whereas phase II development would include the effects of GMOs on larger farms and fields and a study of their impact on insects, animals, humans and other plants.

Another generally adhered component of this guideline is to provide health care and ethical treatment of animals used for scientific research. This issue is becoming more difficult, owing to the fact that as we learn more about animal behavior, science recognizes that many animals exhibit social skills and characteristics that resemble human behavior. As the complexities of animal behavior are revealed, distinctions that differentiate human beings from animals become blurred. In fact, several countries, such as Argentina, confer “personhood” status to certain non-human primates.

Does a "legal person" need to be human, or even alive? American courts routinely extend personhood rights to nonhumans: to corporations, municipalities, and even ships. Therefore, there is a greater need today for scientists to: a) evaluate whether research can only be accomplished using animal models, such as non-human primates, rather than cell models, and b) consider the degree of animal suffering and sacrifice within each experimental design.

Respecting biological order also falls within the second guideline and is rooted in the diverse religious and cultural backgrounds of human beings. A variety of religious groups and cultures believe that while the pursuit of scientific knowledge is valuable, there may be areas where humans should not “play God” by engaging in activities that do not reflect the natural order of life. Examples of inappropriate or low priority scientific investigations may include: research into male pregnancy (e.g., uterine transplants), the creation of two-headed animals, or creating chimeras where human embryonic stem cells are transplanted into mice or chimps to reconstitute part of a human brain in these animals (see Chapter 8), and using germ line gene therapy when research into somatic gene therapy has not been fully developed.

Many cultures believe that some higher power is responsible for creation of the world and that there is a valid reason behind biological order. Other cultures believe that natural evolution has ultimately resulted in a functional biological order that operates efficiently in this world. Therefore, technologies that alter this

biological order are viewed with great skepticism; the fear is that these technologies will destroy humanity or the environment. For example, there is currently a heated debate over whether it is ethical for scientists to create artificial organisms using commercially available DNA. A group led by J. Craig Venter has reportedly created an artificial virus with the identical genetic code of a simple virus already known to infect and kill bacterial cells.[7] The researchers hope that this type of technology will help create genetically-based solutions for treating diseases or dealing with environmental challenges.

There are also significant concerns that scientists do not know enough about the effects of synthetic organisms on biodiversity, the environment, or society. Moreover, there is a fear that this technology could be used to create bioterrorist organisms that are even more destructive than anthrax or smallpox. Developing such technologies should take into consideration that sometimes the unknown may lead to undesired paths.

A major question facing scientists related to this guideline is whether and what types of limitations should there be to scientific research. For example, transplanting human embryonic stem cells containing specific genetic predispositions for disease into mouse embryos creates a mouse model for human diseases. However, examining whether transplanting human brain cells into mice to study human behavior or mental capacity raises issues of animal welfare concerns and whether such a mouse would have human-like consciousness. Clearly, there are many factors that must be considered. Is creating such human-mouse chimeras the only way to examine neuro-biological questions (see Chapter 8)? Does this type of research show disrespect for biological order?

Use of scientific research to alleviate bioethical concerns. The third guideline reflects a current trend in research bioethics. There are times when bioethical concerns appear to be irresolvable. The contentious debate over when a pre-embryo or embryo attains human status or personhood has been ongoing for many decades, restraining the progression of embryonic stem cell research, which is influenced by how one views the beginning of human life (see Chapter 7). The scientific community has responded to this apparently irresolvable issue by trying to utilize creative science to circumvent or defuse the bioethical concerns. For example, research on de-differentiating an adult cell to a pluripotent stem cell or obtaining embryonic stem cells from a morula without destroying the pre-implanted embryo is not as ethically troubling as conventional therapeutic cloning using embryonic stem cells.

The Asilomar Conferences of 1973 and 1974 highlight a unique situation in which life science research was restricted. The first conference was organized in response to the research program of Dr. Paul Berg to determine if the simian virus 40 (SV40) could be used to transfer a foreign gene into a common bacteria found in the human intestine. In 1971, Dr. Robert Pollack contacted Dr. Berg to discuss the safety issues related to Dr. Berg's proposal. One safety issue was the fear that

[7] http://www.economist.com/science/displayStory.cfm?story_id=2224008).

transfecting a common bacteria with SV40 might potentially expose millions of people to this virus, resulting in an increase in the incidence of cancer. Thus, the overall bioethical issue discussed at the first conference was determining the risks of joining DNA from animal viruses with DNA from bacteria. In 1974, a second conference was called when it became possible to safely splice and recombine different DNAs and join DNA from animal viruses with DNA from bacteria.

The Asilomar Conferences proposed a set of scientific guidelines for recombinant DNA research that incorporated safeguards into this technology. The most important guideline proposed was to establish biological and physical safeguards to restrict the viability of these new recombinant organisms within a laboratory environment. The biological barriers mandated the use of bacterial hosts that could not survive outside the laboratory and that physical barriers such as gloves, hoods and filters were required to ensure that recombinant organisms never left the laboratory. The third safety net prohibited the use of highly pathogenic organisms until more knowledge was gained. The Asilomar Conferences challenged the autonomy of biological science and showed that scientists and the public must share the responsibility of preventing the negative effects of scientific research on society in general. Moreover, the proposed guidelines worked as so far, no pathological organism has ever been released from such research. In December of 2015, a summit convened experts from around the world to discuss the scientific, ethical, and governance issues associated with human gene-editing research.[8] Unlike the Asilomar conferences, the there was more cautionary statements than guidelines issued by the organizers of the summit.

In some situations, there is a lack of consensus regarding how research should be regulated. Research involving the genetic alterations of pathogens may be important in creating new vaccines but also may offer new approaches to create bioterror weapons (see chapter 14). Other examples include the creation of the first synthetic life form made entirely with pieces of lab-assembled DNA (Moore, 2012), and the creation of a living organism that can grow and reproduce using DNA base pairs that aren't found in nature (Malyshev et al., 2014). Scientists inserted an unnatural base pair, marked X-Y, into the sequence of a plasmid of E. coli. The resulting bacterium is the first organism able to stably maintain DNA comprised of 3 types of base pairs. This scientific accomplishment raises the possibility that scientists might be able to retool nature to create new forms of proteins for therapeutic and other uses. The move from creating new proteins to creating new life seems only a small step away from a long-standing dream, or nightmare, of creating artificial life.

The "yuck factor" in bioethics. Originally termed by Dr. Arthur Caplan, the "yuck factor" was popularized by Dr. Leon Kass in 1997 when he described his position against cloning human beings. Dr. Kass defined the bioethical "yuck factor" as being an unethical technology based on an intuitive negative response rather than

[8] http://www.nationalacademies.org/gene-editing/Gene-Edit-Summit/index.htm

on concrete ethical or moral values. The yuck factor has been applied to other biotechnologies as well, such as generating mice that produce human sperm or eggs, creating cows with a human uterus, or using stem cell technology to produce consumable human hamburgers (See Chapters 8 and 15).

History can serve as a master teacher about research bioethics.

The development of in vitro fertilization (IVF) and embryonic stem cell research raise similar bioethical issues regarding the initiation of human life (see Chapter 8). As IVF became a more accepted treatment for infertile couples, these ethical concerns declined in importance for the American public. One might extrapolate this observation and predict that if embryonic stem cell research or gene editing technologies develop into an accepted therapy, the bioethical issues of whether a pre-implanted embryo is considered a human being or "playing God with our genetic code" will be less of a concern to society in general.

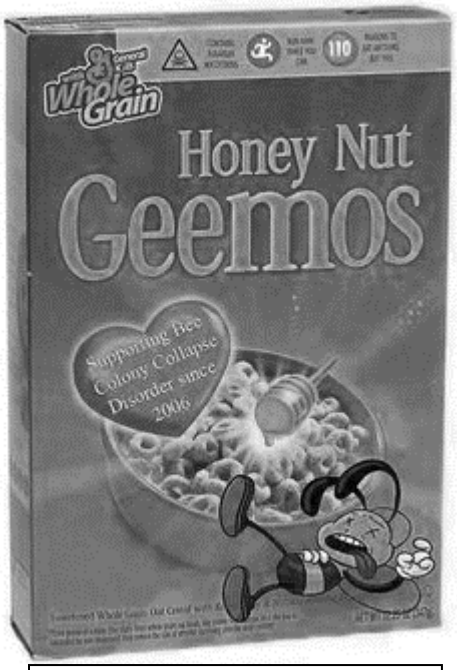
Unfortunately, historical lessons cannot always provide insight into the resolution of bioethical issues. The court of law may not be an effective forum for resolving bioethical issues. Consider the 1973 Supreme Court's *Roe v. Wade* decision regarding a woman's right to abortion. In the majority opinion written by Justice Blackmun, the court granted the right to early term abortions by balancing the interests of the fetus and the mother, during the early term of a pregnancy the woman's right to an abortion outweighed the embryo's/fetus' right to continued existence. Considering this decision, an interpretation of the Court's ruling in *Roe v. Wade* would indicate there should be no law banning or restricting embryonic stem cell research. Similarly, various interpreters of the U.S. Constitution believe that the ability to reproduce is a fundamental human right (See Griswold v. Connecticut, Planned Parenthood v. Casey). Within this context, infertile couples should be allowed to engage in reproductive cloning as long as the medical risks are minimal. Nonetheless, reproductive cloning is not as yet considered acceptable by either the research community or society.

Conclusions

The acquisition of scientific knowledge is a fundamental characteristic of human society and can generate a variety of ethical issues that differ in principle from medical ethics. Thus, the call to conceptually differentiate these two disciplines is the focus of this chapter. The reformulated definition for research bioethics serves as the fulcrum for developing the four principles of bioethics described here. As in any moral and ethical system, there may be clashes between the four principles proposed for research bioethics. Nonetheless, these guidelines are designed to ensure the ethical pursuit of scientific inquiry and to establish a structural framework in research bioethics in order to develop appropriate applications of scientific technologies to society.

The aforementioned guidelines are valid only if they enable ethicists and scientists to respond to bioethical issues related to new biotechnologies in a more effective way than prior medical ethics or bioethical conceptualizations. In this period of economic uncertainty, research bioethical guidelines establish priorities regarding which research activities should be pursued by evaluating how the research will benefit the public or the environment.

In the final analysis, research bioethics is inclusive enough to incorporate genetics ethics, environmental ethics, and neuroethics, among other fields. Bioethics in general would then be the overall subject covering both research bioethics and medical ethics. Despite the differences in philosophical focus between the two, there is a common thread underscoring both life-science research and clinical research that can best be summarized by a famous Hippocratic aphorism: "Life is short, the art long, experience fleeting, experiment perilous, and judgment uncertain."[9]



Obtained from NIH.GOV

[9] Hippocrates. Aphorisms. http://classics.mit.edu/Hippocrates/aphorisms.html

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Chapter Two

Ethical Approaches to Bioethics

Introduction

The term “moral” is derived from the Latin word *mos* or *moralis* meaning custom and the term “value” denotes good, benefit, or truth in cognition. The capacity to reason and think rationally about good, evil, ethical behavior and unethical behavior is one major force motivating humanity to establish a set of beliefs and values that will result in the most good for the greatest number of people.

The word “ethics,” often used interchangeably with morals, is derived from the Greek word *ethike*, meaning habit, action, or character. Ethics is conceptualized as the branch of philosophy that deals with moral aspects of human behavior and is the study of how decisions are made, what is right and wrong. Ethical theory is the process used to define and justify how specific ethical decisions are made because terms like morality, ethics, and values are difficult to define objectively or scientifically.

Medical ethics refers to the application of general and fundamental ethical principles to clinical practice situations including biomedical research. As described in Chapter 3, there are obviously overlapping principles in both research bioethics and medical ethics. We begin this chapter by first summarizing some of the moral/ethical principles that have been applied to bioethics and medical ethics. Those interested in a more comprehensive study of these principles should read from the following books (Beauchamp and Walters, 1999; Bulger et al., 2002; McGee, 2003).

Classical Ethical Theories

Before describing modern theories of ethics, it is important to highlight one continuing controversy underlying many ethical theories. Plato was one of the earliest philosophers to argue that the validity of moral cognition is absolute and objective. Plato believed that ethical laws and principles should be universal and apply to all cultures at all times. Other philosophers question whether emotions or culture should be considered in developing ethical principles. Secular “rationalist” philosophers, such as Socrates and Immanuel Kant, argued that people should primarily rely on intellect when distinguishing right from wrong. In contrast, “sentimentalists”, like David Hume, believed that emotions, such as empathy, should be included to guide moral decisions. Interestingly, brain-scanning technology support the idea that both rational and instinct influence moral choices (Shenhav and Greene, 2010). Green views the moral brain as a camera that comes with manufactured presets, such as “portrait” or “landscape,” along with a manual mode that requires photographers to make adjustments on their own. Emotional responses, which are influenced by humans’ biological makeup and social experiences, are like the presets: fast and efficient, but also mindless and inflexible. Rationality is like

manual mode: adaptable to all kinds of unique scenarios, but time-consuming and cumbersome.

These approaches to ethical theory have permeated bioethics as well. In their classic work, Beauchamp and Childress divided bioethical theory into two major ethical schools: a deontological approach and a utilitarian approach. Deontology is rooted in the Latin word *deon* which means 'duty', and maintains that the concept of duty is independent of the concept of good, and that the correct actions are not necessarily determined by goodness. In this theory, one has to determine what is right or wrong by asking whether an act or sets of action would likely produce the greatest benefit to a society. Deontological theories of ethics state that an act is considered proper and good if it fulfills basic requirements of ethical values, without regard to the expected or anticipated consequences. Many religions are founded on this ethical principle. Immanuel Kant is credited for developing a secular modern approach to deontology. He emphasized that there are ethical values that dictate actions categorically without compromise. Kant asserted that ethical law is not determined by experience but is imperative - objective, absolute, and unrestricted. Kant believes that generally the consequences of actions should not be considered, rather, emphasis should be placed on moral rules of duty, autonomy, justice, and kind acts.

The utilitarian approach, in contrast, emphasizes that actions are morally acceptable when they lead to the greatest possible **balance** of good and harmful consequences. In other words, actions should promote maximum benefits with minimum harm. Utilitarian ethics defines a specific goal and a specific action in order to achieve that goal. The utilitarian approach has its origins in the writings of David Hume, Jeremy Bentham, and John Stuart Mill, who believed that consideration of the consequences of all actions are vital in any decision-making process

The utilitarian approach to ethics has also been challenged. First, in many situations it is difficult to weigh the expected benefit if varying and conflicting actions are occurring simultaneously. Second, utilitarianism can lack ethical consistency in decision-making processes because it changes with different expected outcomes. Third, benefiting the majority may create serious harm to the remaining minority and lead to unjust social distributions of benefits. Finally, utilitarianism is based on the premise that ethical acts themselves have no intrinsic value and outcome and consequence are the prime determinants of action. Hence, some actions could be ethically wrong but still justified because their outcome produced the desired benefit.

Beauchamp and Childress summarize the differences in these two schools quite clearly. "The utilitarian holds that actions are determined to be right or wrong by only one of their features -- their consequences -- while the deontologist contends that even if this feature sometimes determines the rightness and wrongness of acts, it does not always do so" (Beauchamp and Childress, 1979).

In the last fifty years, other ethical theories have been developed in an attempt to create a school of ethics within the context of both bioethics and medical ethics (see Moore, 2012 for a review). None of these theories are universally accepted.

Steps in Resolving Ethical Dilemmas

There is no consensus among modern ethicists which of the above theories is best to resolve issues of bioethics or medical ethics. However, common steps in analyzing bioethical dilemmas include:

1. *Identifying and recognizing the specific ethical issues for any case.*
2. *Identifying the key facts*, *important definitions, and what remains to be discovered in a particular case*.
3. *Identifying the stakeholders.* Are the stakeholders in a case the research scientists, patients, or commercial companies supporting research that will generate profits?
4. *Identifying those ethical principles or guidelines that best apply to the case.* In cases where there are conflicting principles, how would you establish a hierarchy?
5. *Evaluating how a course of action will impact the specific issues and their impact on other related social or biomedical issues.*
6. *Evaluating how would your chosen course of action impact future cases.*

Fundamental Guidelines in Bioethics and Medical Ethics

Ethics and science differ in several aspects. First, specific conclusions and future directions in the pursuit of scientific knowledge are based on objective observations through the process of experimentation. In contrast, bioethical or medical ethical questions cannot be resolved by experimentation. The result is that many ethical theories can be employed to deal constructively with moral disagreements and no single set of ethical considerations will prove consistently reliable as a means of ending disagreements and controversy.

In classical medical ethics, there are four basic guidelines considered in evaluating ethical dilemmas (Bulger et al., 2002):

- *Autonomy, Respect for Persons, or Self-determination* is the right of the individual to determine his/her own destiny. Respect for persons implies that everyone has intrinsic value and incorporates two ethical convictions: 1) a right to personal liberty, i.e., they are autonomous, and 2) a right to be properly informed. The granting of autonomy implies that society recognizes the free choice of each person even if that choice seems inappropriate or even life-endangering. The second is that those individuals who do not have the resources, education, or capacity for self-determination should be protected. The principle of autonomy and respect also assumes that 1) the individual's right to act should be mediated by

reason and not desire and 2) social and political control over individual action requires the prevention of harm to other individuals affected by those actions.

For autonomy to be realized a patient must have the capacity for understanding the situation with its risks, benefits, and alternatives and of reasoning through to a decision that appreciates the consequences. It is a tremendous responsibility for caregivers to educate patients adequately. How much information is material and sufficient? While autonomy is highly valued in the United States, it is often difficult to be confident that the physician has provided all the information necessary for the patient to make complex medical decisions. Even the most educated patient may not have a sufficient understanding of all medical issues and concerns to weigh all risks and benefits correctly. In addition, autonomy has to be modified when dealing with mentally challenged individuals, children, comatose patients, or even those who are highly traumatized who are temporarily or permanently not competent to make decisions for themselves and hence do not have autonomy.

- *Beneficence* is the capacity to do good or what is best for the patient. Therapeutic privilege also comes under beneficence: the physician's subjective determination of what seems to be in the best interests of the patient is a critical component of beneficence which may preclude providing fully informed consent to avoid causing anxiety or depression.

- *Non-maleficence*. While incorporated in the concept of Beneficence, this is often considered as a separate guideline. Non-maleficence operationalizes the Hippocratic doctrine to strive to "do no harm," and has three sub-themes: not to inflict evil or harm; to prevent evil or harm; and to remove evil or harmful forces or conditions in society.

- *Justice* demands fairness in distribution of resources (including accessibility and finances) where the benefits and the burdens (risks) are to be shared equally. Justice requires the division of rights and assets in an equitable and appropriate manner. Injustice occurs when some benefit is denied or some burden is imposed without reason or acceptable justification. A historical look at new biotechnologies reveals that often, initial scientific discoveries are highly expensive. The first sequencing of the human genome at the turn of the 21st century cost close to one billion dollars. Fifteen years later, the cost to sequence a human genome is less than $1000 and it is estimated that within the next five years, the costs will go down to less than $100. On the other hand, the costs of in vitro fertilization technologies (IVF) continues to remain quite high averaging between $25,000-$50,000 for one round of IVF.

Hierarchy of Bioethical Guidelines

One major challenge in presenting bioethical guidelines is how to establish a hierarchy of which guideline should take precedence in a situation that involves

multiple conflicts. A classic example relates to end of life issues. Does the autonomy of a dying patient's desire to engage in euthanasia trump over the guideline of non-maleficence? Here beneficence conflicts with autonomy. How to establish hierarchy of these guidelines is often a function of culture. In the United States, autonomy is viewed by many bioethicists as the most important guideline.

A second example relates to gene editing. How should one view the decisions of parents who want to apply gene editing to their embryo for non-medical applications? Do parents have the autonomous right to genetically alter the hair color of their child? Often introducing new biotechnologies into a clinical situation is extremely expensive which limits who can partake in these new procedures. Gestational surrogacy is an example of an expensive technology in the United States costing anywhere between $50,000-$100,000. However, couples can recruit gestational surrogates from developing countries such as India for less than $1500. The ethical problem associated with foreign surrogates is that they are subjected to greater abuse and misuse (conflicting with the guidelines of non-maleficence and justice).

Some bioethicists argue that the principle of utility must be applied to each case that elicits bioethical challenges. The principle of utility states that we should produce the most favorable balance of benefit over harm for all concerned. Various states in the USA allow parents not to vaccinate their children for religious reasons. While this law acknowledges religious freedom, it also can cause severe consequences, such as the many cases of infectious disease outbreaks that could have been prevented via vaccines.

Another example is capital punishment. Currently physicians are not allowed to administer lethal drugs for capital punishment because it violates the guideline of beneficence. So non-medical individuals are now trained to administer the drugs and physicians are allowed to observe treatment. However, there are several reports claiming that administering lethal drugs to prisoners convicted to death is not a simple procedure and unanticipated adverse events occur during executions (Kas, Yim et al. 2015). There are dozens of reports of inhumane executions. Most states employ a three-drug protocol comprising of sodium thiopental, pancuronium bromide, and potassium chloride. In 2016, several companies that produce these drugs are refusing to manufacture them for lethal injections because of their ethical views concerning capital punishment. The ethical unresolved question is whether convicted criminals have the same death rights as everyone else?

What is a disease?

Any discussion of bioethics in the 21st century has to focus on defining what a human disease means in scientific, legal, and social terms. A basic assumption within modern medicine is that health is the absence of disease (Scadding, 1988), and illness is the patient's personal experience of disease. The World Health Organization (WHO) defines health as a state of complete physical, mental, and social well-being, not merely

the absence of disease or infirmity. Yet, these definitions are neither precise nor scientific because it is unclear whether health, illness, and disease are purely biological in nature. In fact, biological approaches to chronic illness often do not produce the anticipated effects. It is now well accepted that psychosocial factors play a major part not only in the experience of illness, but also in the development of disease (Engel, 1977). This has led some scholars to propose a 'reverse view' concept of disease, outlining that the development of disease doesn't start with dysfunction as abnormal function, but with the patient's experience of illness as 'action failure' (Fulford, 1999). Immune/health status is now a form of *habitus* or personal "capital" that increasingly is used in society to establish a general kind of fitness or even moral virtue. Another example relates to the term "disease free survival" in cancer patient that implies that these individuals do not present any outward symptoms of the cancer even though they may harbor cancer cells within their bodies.

Finally, one needs to distinguish between a drug and a cosmetic. The Federal Food, Drug, and Cosmetic Act defines cosmetics by their intended use, as "articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body...for cleansing, beautifying, promoting attractiveness, or altering the appearance". The FD&C Act defines drugs, in part, by their intended use, as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" and "articles (other than food) intended to affect the structure or any function of the body of man. Some products meet the definitions of both cosmetics and drugs. A shampoo, for example, can be defined as a cosmetic because its intended use is to cleanse the hair as well as a drug because of its antidandruff properties.

Culture can have dramatic effects on the categorization of an alleged disease or disorder. In the first half of the 20th century, many physicians viewed homosexuality as an endocrine disturbance requiring hormonal treatments or as a psychiatric disorder that could be treated using conditioning or psychotherapeutic methodologies. At that time it was classified as psychological pathology or abnormality. Yet in 1974, homosexuality was officially de-pathologized by the American Psychiatric Association when they removed it from their list of diseased states. In 2015, the Supreme Court issued a legal and moral decision that the Constitution guarantees a right to same-sex marriage.

Today, our definition of disease still remains imprecise but nonetheless important. Defining a condition as a disease is associated with decisions concerning whether or not to allocate research and medical funds to correct or treat this condition. Defining a disease also has an impact on the system of health insurance. Medical insurance coverage requires that a code specifying a medical condition, symptom, or procedure be entered, and without a code, there is no reimbursement.

Many conditions that heretofore have been considered within normal human variation, such as baldness or short stature, have now become medical conditions. In 2004, Medicare discarded its declaration that obesity is not a disease. According to the Journal of American Medicine (JAMA), one-third of all adults in the United States are obese. Obesity occurs when Body Mass Index (BMI) reaches 30 percent, and morbid obesity is defined by a BMI of greater than 40 percent. An obesity diagnosis alone does not qualify an individual for disability benefits. Yet, there are circumstances under which an obese person may meet Social Security disability medical eligibility requirements. These include cases where a person's BMI is so high that they are unable to move, walk, or complete everyday tasks like preparing food, cleaning their home, or dressing or bathing. This policy change allowed millions of overweight Americans to make medical claims for treatments such as bariatric (stomach) surgery and prescription diet regimens.

Autism is a disease that has been difficult to categorize. Autism was first identified in 1943 by psychologist Leo Kanner who reported aberrant behaviors in children such as "insistence on sameness," and "autistic aloneness." Since then, these criteria have been delineated and reformulated multiple times to yield the current characterization of autism spectrum disorders (ASD) as identified by the DSM-V, the standard manual used in identifying and diagnosing mental disorders. Today, autism spectrum affects about one out of every sixty-eight children in the United States. The incidence of autism seems to be on the rise, yet researchers are still unable to determine the etiology of this disorder or how genetics or environment contributes to disease onset. Is the observed growth of this developmental disorder artificially induced by redefining the disease or by employing better diagnostic tools and earlier screening? Could there be environmental factors that are interfering with normal neurodevelopment? The re-definition of autism via symptoms rather than pathological signs has generated many questions and has required researchers to re-examine genetic and environmental factors that may contribute to the pathology as well as the ways medicine screens for this multifaceted disease.

Human beings in general tend to be prone to black-and-white thinking. It can be very difficult to see something—especially something like autism—in shades of gray. Interestingly, famous individuals, such as Albert Einstein, Darryl Hannah, and Wolfgang Mozart have been described as exhibiting symptoms of Autism spectrum disorder. Would you describe their alleged symptoms as a "disease" or as an "asset" that enabled them to make significant contributions to society?

Today, we are entering an era where DNA analysis, precision medicine (see Chapter 11) and biomarker analysis are used to predict the onset of future diseases, even before any symptoms appear in the targeted individual. The response of the public towards view these types of analyses remain to be determined, especially with regards to early treatment options, sustaining pregnancies where DNA mutations are detected in the fetus and whether early intervention should be covered by medical insurance.

Another issue is how should ethicists deal with pre-natal testing for diseases that have late-in-life onset, such as Alzheimer's disease, breast cancer, or Huntington's disease? Would a Woodie Guthrie, one of the most celebrated and influential folk singer-songwriters of the twentieth century, be born today if his mother terminated the pregnancy

because of genetic testing? Would his parents, who carried the Huntington's disease gene, bear a child with the known risk that can be established by genetic screening? Many have argued that certain individuals born with genetic or congenital conditions that constrain their lives in challenging ways are driven to be more productive in society as a result of their disabilities.

Ethical and definitional quandaries regarding genetic testing are abound. For example, how do we define a person who is either a carrier for a genetic disease or has a genetic predisposition to a disease? As one example, everyone agrees that government funds should be allocated to enhance breast cancer diagnosis and treatment. But is a 16-year old teenage girl with a genetic predisposition to a breast cancer already considered ill or as having a pre-existing condition that should be treated with a mastectomy? The awareness of any serious diagnosis may have traumatic psychological implications on a 16-year-old. At what age should the government to begin fund her preventive care?

Similarly, is a carrier of a genetic disease state such as Tay Sachs disease, considered ill even though carriers appear to have no medical symptoms or adversities? Statistically, if two carriers marry, then 25% of their children will be born with this fatal condition. These medical considerations intersect directly with bioethical concerns with respect to eugenics or designer babies. For example, many ethicists believe it is ethical to undergo pre-implantation genetic diagnosis (PGD) to eliminate those in vitro-fertilized eggs that carry two genes for Tay Sachs disease. How, would they deem it ethical to destroy those in vitro-fertilized eggs that only carry one gene for Tay Sachs and who will not be born with this condition? At the other extreme, can parents who are hearing impaired use PGD to select a child who is also hearing impaired, to better fit into their world? These are just some of the difficult questions that ethicists are currently debating which highlight the need to refine bioethical principles to address these issues.

CASE STUDY-

A married couple is expecting their first child. They undergo fetal DNA testing only to be told that their female fetus is carrying a BRAC1 gene. Statistically, this means that this child will have an 80% chance of developing breast cancer within 70 years. Aside from the issue of autonomy, it is ethical for the parents to terminate the pregnancy?

Financial repercussions of unethical behavior

It is important to consider some of the tremendous financial consequences of unethical practices. The vaccination scandal, is one example where millions of dollars were lost because of a Lancet report in 1998, authored by Dr. Andrew Wakefield, a British surgeon and medical researcher who allegedly found a connection between vaccines and the onset of autism. It took over 10 years until the report was deemed to be fraudulent and retracted by the journal Lancet. Yet the financial damage was huge. The reviewers of Lancet failed to recognize the paper's extreme scientific manipulations, a lack of good statistical analysis, (a small group of 12 children as test subjects), the absence of a control group, and the reliance on people's memories for vaccination records.

From 2003 to 2010, over ten large studies were conducted by the CDC, by other government agencies and medical institutions to re-establish the safety of vaccinations and to try to alleviate the public fears that vaccinations are linked to autism. The costs for these studies ran in the millions of dollars, and highlight the financial repercussions of medical and scientific fraud. In one study, researchers examined 291 articles originating from the United States and published between 1992 and 2012 that were retracted for research misconduct. The total cost for these research studies ran over $58 million including $19 million that were NIH-funded.

In addition, hundreds of thousands of patients have been placed at risk of improper medical care due to enrollment in fraudulent studies or the administration of treatment based on fraudulent studies. The medical costs and health risks these patients encountered are huge. Decreasing vaccination rates are often associated with outbreaks of preventable infections, such as a recent measles outbreak in Wales that resulted in more than 1200 cases and cost an estimated $800,000 US) (Stern, Casadevall et al. 2014). In summary, robust science needs robust processes of review, transparencies, and enforcements to maintain ethical practices in publishing data.

Conclusions

There are many diverse theories regarding medical ethics that have been applied to bioethical dilemmas. In this book, we propose that resolving these dilemmas requires a multidisciplinary approach that ideally should integrate philosophy-based theories with knowledge of the underlying science. In addition, any attempt to resolve bioethical issues should consider an historical review to assess whether there are important lessons that can be learned from previous bioethical dilemmas that our society has already faced.

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