TESTIMONY

OF

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to the

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INTRODUCTION

On behalf of Northwestern University, I would like to thank Chairwoman Comstock and Ranking Member Lipinski for inviting me here today to speak at this hearing on Genetically Engineered Human DNA. I would like to also thank the Subcommittee for convening this hearing.

I am the Elizabeth J. Ward Professor of Genetic Medicine, and I direct the Center for Genetic Medicine at Northwestern. I am a cardiologist who specializes in providing care for patients and families with inherited forms of heart disease. I established one of the first Cardiovascular Genetics Clinics in the United States. Over the last decade, we have seen a dramatic increase in available genetic testing, and we now routinely provide genetic diagnosis, risk assessment, and risk reduction of genetic diseases that affect the heart. Many of the inherited diseases that we diagnose and manage are also those that affect muscle. The same gene mutations that cause heart muscle to weaken may elicit the same effect on skeletal muscle, causing those who carry the mutations to develop heart failure, life threatening irregular heart rhythms and muscle weakness. Genetic diagnosis is not restricted to heart and muscle disorders as nearly every area of medicine is influenced by genetic diagnosis.

Genetic Diseases

Diseases like Cystic Fibrosis, Duchenne Muscular Dystrophy, and Sickle Cell are those that are caused by mutations in single genes. It has been possible for some time to genetically diagnose these disorders. There is considerable effort directed at devising targeted therapies to correct the underlying genetic defects responsible for causing disorders like these, and herein I will discuss the potential application of gene editing techniques for the treatment of genetic diseases.

The first draft human genome sequence was completed just 15 years ago. Now, with advances in DNA sequencing technology it is now possible to sequence an individual human genome in a matter of days. Moreover, human genome sequencing can be completed a comparatively low cost, less than the cost of an MRI, and can be analyzed with high accuracy. It is becoming routine to pinpoint single gene mutations responsible for devastating disorders, including those diseases that affect children. With this explosion in genetic analysis, the number of genetic disorders is increasing. The National Institutes of Health (NIH) Office of Rare Diseases identifies nearly 7000 rare diseases[1], and many of these are genetic in origin, often arising from single mutations. The ORD estimates that nearly 30 million Americans are affected by rare diseases. More than half of rare diseases affect children.[2]

Gene Editing

Concomitant with advances in genetic diagnoses, there are parallel leaps in genome editing. The concept of genome editing is not new. Genome editing has been technically possible since the first reports of inserting genetic material in fertilized eggs of mice, reported by three independent groups in 1981.[3-5] It was around this very same time that the first successes in human in vitro fertilization were reported.[6] In the more than three decades since genetic editing became possible, there has been scientific and technological progress that has improved the proficiency and fidelity of genome editing. Early success in genomic editing relied on random insertion of new DNA sequences into fertilized eggs, stem cells, and cell lines. Random insertion allows new genes to be expressed but does not correct genetic defects. Homologous recombination refers to the process by which sequences can be exchanged between a vector that carries new sequences of insert and the genome to be edited. For most organisms, especially humans, homologous recombination is a remarkably inefficient process. However in other organisms, homologous recombination occurs at much higher rate. Understanding the precise means by which organisms can alter genetic structures has allowed researchers to isolate the machinery that edits genomes. In the last decade, there have been several key discoveries made to improve the ability to precisely change specific sequences. The precision of gene editing remains at the center of these discussions. Precise gene editing refers to producing the desired genetic change, and importantly doing so with high efficiency and with few off target effects.

The most recent advance capitalizes on the tools used by bacteria to ward off viral infection. This newest technology, referred to as CRISPR/Cas9, isolates the sequences and enzymes used by bacteria, and then applies these methods into complex cell types like those in mice, rats and humans.[7] First described in 2012, CRISPR/Cas9 is changing the path and pace of scientific discovery. Research depends on model systems, and model systems include

cultured cells, as well as organisms like mice, yeast, flies, worms and other species. Genetically tractable systems are preferred, and mice remain a standard for the field of human biology. Cell models of disease are also highly useful since experiments can be completed with comparative ease and speed. The timeline of discovery is tied tightly to the model system of choice. The importance of CRISPR/Cas9 cannot be overstated. CRISPR/Cas9 offers a precision heretofore unseen. Cells and animals can be manipulated to more precisely to facilitate the ability to ask and answer critically important scientific questions.

Gene editing in Stem Cells

Alongside these advances in genome editing, it is worthwhile to consider gains in stem cell biology. The application of genome editing goes hand in hand with stem cell biology, and because of this co-evolution of gene editing and stem cell biology, there is significant potential clinical application. The ethics, merits, and implications of human embryonic stem cell biology have been debated and will not be reiterated here. For the purposes of this testimony, it should be acknowledged that some human stem cell lines retain the ability to contribute to human germ cells. In contributing to human germ cells, there is the possibility to transmit stem cell-derived genetic material into new generations. Therefore, genome editing in certain stem cells, in principle, may have the ability to alter human germ lines. However, many stem cells only theoretically have the capacity to contribute to human germ lines. In practice, human stem cells are used in many experiment with no intent or possibility of contributing to human germ lines. Induced pluripotent human stem cells can be made from blood, skin and other mature somatic human cells. Induced pluripotent stem cells, in theory, could contribute to human germ lines but are not used for this purpose. In many laboratories, the true stem cell capacity of such stem lines is never evaluated, even in mice, as the germ line potential is irrelevant to the research.

Embryonic and induced pluripotent stem cells are an obvious venue in which to test and evaluate genome editing techniques. The value of stem cells lines is that we can study how mutations act in many different cell types. Cells can be induced to form beating heart-like cells in a dish. How a disease-causing mutation affects beating and function can now be readily understood in cell culture. Introducing new mutations into stem cells generates highly valuable models for human disease. These models are then used to identify and test new therapies. The human population is not placed at risk by these experiments in cells. It seems fair to state that the human population would actually be harmed by not doing these experiments since this research offers a potent opportunity to improve human health. This is not an opportunity that should be missed. Having genome-edited cell lines allows more rapid scientific advance and reduces the need for certain types of animal experimentation. At the same time, correcting defective genes in stem cells allows investigators to determine whether genomic correction is possible. In principle, a corrected stem cell could prove useful in cell transplant experiments to treat some diseases.

Gene editing is not restricted to pluripotent stem cells. Stem cells of the bone marrow, muscle, skin and other organs and tissues can be isolated and edited. In this case, editing and correction could be accompanied by transplant into a host human in order to treat

disease. With this method, it would be possible to cure Sickle Cell Anemia or Duchenne Muscular Dystrophy. At present, the methods CRISPR/Cas9 require optimization in order for this to be reality. But the advances of CRISPR/Cas9 bring this approach into discussion. In mice, CRISPR/Cas9-mediated correction in fertilized oocytes corrected the defect of Duchenne Muscular Dystrophy.[8] The method, while imperfect, was associated with remarkably high correction.

Germ line gene editing

Recently a group of distinguished scientists called for a moratorium on gene editing in human fertilized oocytes fearing the potential of germ line gene editing and, ultimately, human eugenics.[9] These discussions were enhanced and prompted by the recent report of Liang et al. described efforts using CRISPR/Cas9 gene editing in fertilized human zygotes.[10] To limit concerns regarding human eugenics, the authors used tripronuclear zygotes that are genetically limited from progressing through development into humans. Notably, the authors concluded that CRISPR/Cas9, while an improvement over previous gene editing technologies, still has limited efficiency and importantly has serious off-target effects. The major off-target effect is the introduction of unintended mutations at sites throughout the genome at an unacceptably high rate for clinical purposes. Whether CRISPR/Cas9's efficiency and off-target effects differ across cell types is not well known at present. However, these same issues are present in all cell types subjected to gene editing to date. With knowledge of the enzymes, sequences and structures of the CRISPR/Cas9 system, optimization is an active area of research in academic, government and private industry laboratories in the United States and throughout the world.

Regulating Gene Editing

A regulatory framework for gene editing should encompass several key points:

1) Permit research to optimize and improve CRISPR/Cas9 and related technology.

2) Permit in vitro, cell-based gene editing technologies, including those in embryonic and induced pluripotent stem cells, respecting regulations currently protecting human embryonic stem cell lines.

3) Permit in vitro, cell-based gene editing with the intent to re-introduce into humans as a therapeutic measure for somatic cells. For example, this would apply to gene edited bone marrow derived stem calls. The treatment of a human with a gene edited cells would fall under the existing regulatory framework.

4) Permit the generation of gene-edited animals for the purposes of scientific research.

5) Limit or prohibit gene editing under circumstances where human transmission of gene-edited germ lines would occur.

Why consider germ line gene editing?

With current technology, it is difficult to envision any justifiable use of gene editing in fertilized human zygotes where the resultant edited genome would be transmitted to future generations. Yet, we should consider the scenario of pre-implantation genetic diagnosis (PGD). PGD is pursued by families to avoid transmitting genetic diseases. Most commonly PGD is only pursued related to genetic diseases associated with significant early onset morbidity and mortality. With more widespread use of genetic diagnosis, as a clinician, I am asked about options to avoid passing deleterious genetic mutations to the next generation.

PGD involves in vitro fertilization coupled with genetic testing. In PGD, in vitro fertilization is used to create a fertilized oocyte that undergoes several rounds of cell division to become an embryo.[11] A single cell is removed from the embryo and tested genetically to identify those embryos that do not carry a specific genetic mutation. PGD allow parents to implant only those embryos free of the mutation in question. PGD is limited by the number of available embryos. PGD is typically not covered by insurance, and yet some families make this choice. These may be families who are already struggling with caring for one disabled child who cannot care for a second disabled child. These may be families where the parent is significantly afflicted with a genetic disease, and the parent wishes not to have his or her child burdened with the same diagnosis. PGD is a personal option and one that is made by solely by parents and families. PGD is not new and has been an available option for the last decade. A relatively small number of families choose this option and the choice to do so is often limited by technology, cost, religious and personal preference. PGD relies on nature to provide embryos free of a specific genetic mutation. Genetic altering of human embryos has occurred in the form of adding mitochondria from an external source, which introduces new mitochondrial DNA. In principle, it is possible that the efficiency of genome editing will improve so that preimplantation genetic correction could accompany PGD. With this process, gene editing to correct and eliminate a genetic disease could become reality. While the temptation may be to ban or limit this possibility, we should do so only with caution.

In my many years of working with patients and families with genetic disease, I can report that many parents of children with genetic disease express significant concern and responsibility for having passed on mutations to their children. A parent's desire to protect children is undeniable. As a society and as a nation, we embrace and endorse the importance of protecting children. It may be tempting, and perhaps easiest, to ban all gene editing where germ line transmission could occur. Yet, the justified use of this approach is certainly conceivable and may one day be appropriate.

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