A briefing on

Gene editing and frontiers in genetic technologies: Innovations, impacts, and implications

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Information Briefs

Non-Invasive Prenatal Testing (NIPT)

In the 1990s, it was discovered that small amounts of DNA fragments from a fetus (technically, from the placenta, which arises from the same fertilized egg as the fetus) circulate within the blood plasma of pregnant women. This "cell-free fetal DNA" makes up around 10% of the cell-free DNA in the pregnant woman's bloodstream. It appears as early as 4 weeks of pregnancy and rapidly disappears following childbirth. Recent advances in DNA sequencing technology and analytic computer algorithms have made it possible to analyze this cell-free DNA to reveal information about the developing fetus. A common way by which the technique is performed is to use massively parallel DNA sequencing to count and compare DNA fragments from each chromosome. Because this test is done on a blood sample from the mother's vein (usually obtained after 10 weeks of pregnancy), it is a non-invasive prenatal genetic test (NIPT) that does not increase the risk of miscarriage.

NIPT has been available for clinical use in the US since October 2011. NIPT is most commonly used as a screen for extra or missing copies of any chromosome, a condition known as "aneuploidy." The most common liveborn aneuploidies are Down syndrome (caused by three copies, or "trisomy", of chromosome 21), Patau syndrome (trisomy 18) and Edwards syndrome (trisomy 13). NIPT also detects extra or missing copies of the sex chromosomes X and Y. Some NIPTs also analyze for duplication and/or deletion of smaller regions of specific chromosomes.

Studies have shown that, for trisomy 21, NIPT has both a *sensitivity* (a measure of how correctly the test identifies those with the condition) and *specificity* (how correctly the test excludes those without the condition) of over 99% (Dondorp *et al.*, 2015). Follow-up of positive NIPT results with the standard invasive prenatal screening methods reveals that the *positive predictive value* (PPV, the proportion of positive test results that are actually positive) is, however, less than 99%. One reason for this is because the PPV depends on the prevalence of the condition in the population. The PPV is higher in women of advanced maternal age, who are more likely to have a fetus with trisomy 21, but in a general-risk population, the PPV ranges between 45-80% (for comparison, the PPVs for screening tests that measure proteins or ultrasound markers are around 4% (Bianchi *et al.*, 2014)). In most studies, the PPVs for NIPT for the other aneuploidies appear to be lower. As a result, it is important to emphasize that NIPT is only a *screening* and not *diagnostic* test.

Most professional and academic societies, such as the American Society of Human Genetics, recommend NIPT as an alternative screen for trisomies 13, 18 and 21 in pregnant women who are at high risk for fetal aneuploidy, including those who are over 35 years of age or where ultrasound examination has detected possible fetal anomalies. They also recommend that positive results be confirmed with traditional, invasive tests such as amniocentesis. The benefits and risks of using NIPT as a primary screen for the common fetal aneuploidies in the general (low-risk) population are still being actively evaluated.

NIPT results can be complicated, particularly when unusual findings occur. For example, in very rare cases, NIPT has detected cancer in the mother. This can present challenges in education, counseling and informed consent for doctors, genetic counselors and patients.

Gene Editing and CRISPR

Genome engineering refers to a range of techniques for making targeted changes to an organism's genetic information. These are valuable tools for biomedical research, and may one day be used in the clinic to treat the genetic causes of disease (an approach referred to as "gene therapy"). For a long time, genome engineering techniques were time-consuming and inefficient, and worked only in a limited number of organisms. In the past decade, dramatic developments in a class of engineering technologies known as "gene editing" have advanced the field significantly. One of these technologies, called CRISPR, has generated particular excitement due to its relative efficiency and ease of use.

The basic principle of gene editing is to employ an enzyme called a "nuclease" to make a cut at a specific site in a living cell's DNA. The cell's natural DNA repair mechanism will then close this gap. Depending on how this occurs, the repair may either render a gene nonfunctional or replace the original DNA sequence with an alternate version. In the case of CRISPR, a nuclease (e.g., Cas9) derived from bacteria is directed to its target site by a "guide RNA," which forms complementary base pairing with the target DNA sequence. Because scientists can easily "program" guide RNAs with different sequences, the CRISPR system has made it much simpler to modify a large variety of DNA sites within the genome for different research and, potentially, therapeutic purposes. Because of the relative ease with which CRISPR gene editing can be performed pretty much by anyone with basic molecular biology training, the technology has the potential for widespread applications in fields including biomedical research, medicine and agriculture.

The most revolutionary aspect of gene editing is the ease with which it can be applied to the cells of many different organisms, including humans. By making targeted DNA changes in somatic cells (cells from the body), such as those in muscles or blood, scientists can establish biological models to study the development of diseases such as cancer. In the future, it may be possible to use gene editing to correct disease-causing mutations within a person's body. Gene editing can also be used to make genetic changes in reproductive cells (known as germ cells) and in embryos. This has been done in many non-human organisms, and in April 2015, a research team at Sun Yat-sen University in China reported that they had performed gene editing in non-viable human embryos. Because genetic changes in an embryo or germ cell could be passed on to the next generations, this application of genome engineering in humans raises particular ethical concerns among scientists, bioethicists, policymakers and broader society. These issues have been the subject of a number of recent meetings worldwide, including an upcoming International Summit on Human Gene Editing at the US National Academies. (See "**Inheritable Genetic Modifications: The International Regulatory Landscape,**" p. 5-6)

CRISPR and Gene Drives

A gene drive is a strategy for efficiently "driving" a DNA sequence to become inherited by all of an organism's offspring. In sexually reproducing organisms (including most animals such as humans), an individual generally inherits one copy of its chromosomes from each parent. Typically, a stretch of DNA present on only one of the two chromosomes will only be passed on 50% of the time to the next generation, assuming its mate does not also carry this stretch of DNA. By contrast, in a gene drive, a genetic element on one chromosomal copy will duplicate itself onto the other chromosomal copy. As a result, that sequence will be passed on 100% of the time to the next generation. Such a genetic change may eventually "sweep" the population.

There is growing interest in gene drives with the advent of CRISPR technologies, which simplify some of the technical aspects of this approach. By placing DNA sequences that encode for the components of a CRISPR system (including the Cas9 nuclease and the guide RNA) directly into its target site in the genome, the CRISPR system can act as a gene drive and catalyze its own incorporation into each new chromosomal copy.

A much discussed application of CRISPR gene drives is for controlling the population of disease-carrying insects, such as the mosquitoes that act as vectors for malaria or Dengue fever. In this theoretical scenario, a mosquito would be engineered with a CRISPR gene drive that is placed into, and so disrupts, a gene controlling the insect's ability to carry the parasite. This mosquito would then be released into the environment and allowed to mate with wild mosquitoes. As the gene drive sweeps through the population, the number of mosquitoes that can carry the parasite would drastically decrease.

While using such a gene drive strategy to control insect-borne diseases has significant potential for public health benefits, it is an environment-altering intervention where the ecological consequences are impossible to know with absolute certainty. Scientists and other stakeholders have called for further deliberation on whether or how such research and application should proceed.

Inheritable Genetic Modifications: The International Regulatory Landscape

With the emergence of technologies that allow us to modify our own genetic information, countries around the world are grappling with how to regulate these technologies to ensure they can benefit society in the safest and fairest way. While different political and cultural contexts shape how different nations approach these issues, understanding the rationales behind each country's regulations can be instructive. Here, we provide a brief survey of the international legal and regulatory landscape for the application of genetic technologies in humans, focusing on the practice of inheritable genetic modifications (IGM, also known as "germline engineering") — changes made to an individual's DNA that can be passed on to his or her offspring. The US National Academies, the Chinese Academy of Sciences, and the Royal Society of the United Kingdom (UK) will convene an International Summit on Human Gene Editing on December 1-3, 2015, for experts to discuss the relevant scientific, ethical and governance issues.

The United States has traditionally approached the issue of regulating human genetic technologies through the research-funding power of state and federal agencies such as the NIH, as well as the FDA's authority to approve clinical studies. As reiterated by NIH director Dr. Francis Collins in April 2015, the agency at present will not fund research that involves genome editing in human embryos.

Some other jurisdictions have established clear bans on IGM. More than 50 countries worldwide, including the vast majority of European nations such as the UK, Germany and France, as well as Canada, Australia, New Zealand, South Korea, Brazil and Mexico, have legislations that prohibit IGM, often as criminal offenses punishable by fine and/or imprisonment. At the same time, the 1997 Convention on Human Rights and Biomedicine of the Council of Europe (CoE) prohibits germline modification, with the provision that the "interests and welfare of the human being shall prevail over the sole interest of society or science." The Convention has been signed by 35 of the 47 CoE member states and ratified by 29, but notably, neither the UK nor Germany has signed the Convention.

The UK is one of the first countries to consider and establish comprehensive legislation on assisted reproduction technologies (ART), including embryonic stem cell research and human genetic engineering. The Committee of Inquiry into Human Fertilisation and Embryology was set up in 1982 to look into these issues, and in 1990, the UK Parliament passed the Human Fertilisation and Embryology Act, which set up a government agency (HFEA) to regulate and license such research and clinical activities. Originally, all forms of genetic modifications in human gametes (sperm and eggs) and embryos were prohibited under the Act, but in 2008 the legislation was amended to permit these procedures in scientific research. More recently, in Feb 2015, Parliament gave approval for therapies that involve the replacement of mitochondria – the cell's energy-producing components – in embryos, in order to treat certain types of genetic diseases. Because mitochondria, which are only inherited from mothers, carry a small amount of DNA distinct from the main genome in a cell's nucleus, mitochondrial replacement would represent a kind of IGM. HFEA has begun licensing and regulating mitochondrial replacement at the end of Oct 2015.

Similarly, Canada established the Royal Commission on New Reproductive Technologies in 1989 to consult Canadians on how to proceed with regulating ART. In 1995, the Canadian government issued a voluntary moratorium on several procedures highlighted by the Royal Commission's report, including human cloning, germline modification, and commercial transactions involving eggs, sperm, embryos and surrogacy. These prohibitions were enacted into law in 2004 with Parliament's passage of the Assisted Human Reproduction Act. The intent of the legislation is laid out in several "Principles," including the idea that "human individuality and diversity, and the integrity of the human genome, must be preserved and protected."

In Japan, genetic modification of gametes and embryos is prohibited under the Guidelines for Gene Therapy Clinical Research issued by the Ministry of Health, Labour and Welfare in 2002. As ministry guidelines rather than legislation, the prohibition might be regarded as less permanent or enforceable. China's former Ministry of Health issued its Technical Standard for Human Assisted Reproduction in 2001, which prohibits IGM "for the purpose of reproduction." However, the enforcement of the guidelines has been left to provincial or local authorities and is seen to be lax. The recent gene editing experiments in human embryos performed at Sun Yat-sen University technically did not contravene government regulation, because the embryos used were not viable and could not have been used for reproduction.

To our knowledge, many other countries in Asia, Africa and Latin America either have no, or ambiguous, regulations regarding IGM.

DNA Nanotechnology: Using DNA as an Engineering Material

DNA molecules have many interesting chemical properties – they are strands of chemical bases, with easily "programmable" sequences, that automatically and specifically bind to complementary sequences and form rigid double helices. These properties have caught the attention of scientists and engineers who see potential for DNA as an engineering material for data storage, computation and robotics. The relative stability, density and energy efficiency of DNA have been exploited for using the molecule as a form of long-term data storage. A variety of data, from text, to audio, to pictures, have already been artificially encoded in DNA – the data is "written" by synthesizing the DNA molecules, and "read" by sequencing the DNA.

Researchers have also used DNA as a material to build "nanobots" that may one day be used, for example, to target drug delivery to specific cells. Because the shape of a DNA molecule can be modulated using small molecules or proteins, such as those on the surface of a drug target cell, the DNA can act as computational "logic gates" to control the opening and delivery of the drug payload (e.g., only opening when both proteins A and B are present, or only when protein A is present and B is not present). With the exponentially decreasing cost of DNA synthesis and sequencing, the appeal of using DNA for engineering purposes will likely continue to increase.

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NIPT

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