Manipulating the Genome of Human Embryos: 
Some Unforeseen Effects

Craig Holdrege

In recent years scientists have developed powerful tools to create specific breaks in DNA sequences. They can then either repair those breaks or introduce new DNA into the sequence at the site of the break. These are called genome editing techniques. Two main techniques at present are the CRISPR-Cas9 system and zinc-finger nucleases. In principle, researchers can modify any part of the genome. They have achieved intended modifications in experiments with human and animal cells and also with mouse embryos. But the specific alterations do not always occur and there are also unintended effects.

The techniques are not as precise as they are sometimes made out to be, so there is every reason for caution in their application, especially in connection with the manipulation of human cells or human embryos. But over and beyond technical issues is the pressing ethical concern: should researchers cross the line into genetically manipulating human embryos?

Knowing that scientists in China were performing these experiments, two groups of researchers and others published comments in Science and Nature in March 2015 warning about genome editing in human embryos (Baltimore et al. 2015; Lanphier et al. 2015). Since then, one Chinese research group has published the results of its experiments (Liang 2015).

Using the CRISPR-Cas9 system, the group’s aim was to create a break in the beta-globin gene (β-globin or HBB; a key hemoglobin gene) and then repair that break. The context for the experiment is the heritable disease beta-thalassemia (β-thalassemia, also written “β-thalassaemia”), a blood disease that is related to a mutation in the β-globin gene. If researchers could create a targeted break in the mutated part of the gene and then repair it, they could theoretically prevent the disease from occurring. The current experiment was a first step in this direction, a test of the efficacy and precision of the technique in human embryos.

In this experiment the researchers used 86 abnormal human embryos that they had obtained from fertility clinics that perform in vitro fertilization (IVF); the embryos were donated by the parents. Each of the single-celled embryos (zygotes) contained two sperm nuclei, instead of one sperm nucleus that normally fuses with the egg nucleus. Such abnormal embryos do not develop further when transferred into a woman’s womb and would have been discarded by the fertility clinics. For this reason the researchers state that their experiment avoids any ethical issues, since they were working with embryos that would have been thrown away anyway. (It is a symptom of the distancing effect of technology that it is possible to casually
speak of discarding or throwing away human embryos. In stating that their experiment is ethically unproblematic, they do not question the ethics of IVF techniques that engender abnormal human embryos in the first place.)

The 86 embryos were injected with the genetic construct intended to create the break and then repair the β-globin gene. After 48 hours (at about the 8-cell stage in early embryonic development) 71 of the embryos were viable. Fifty-four of these were tested to see whether the editing had occurred as intended. The creation of the break succeeded in 28 embryos, but only in four had the gene been repaired in the intended fashion. In seven other embryos the “repair” was based on a similar gene (\textit{HBD}) in the embryo and not on the introduced construct. This indicated that the embryo itself was active in trying to put right what had been disturbed by the intervention. From a technical point of view, the embryo got in the way of the manipulation, since an experiment is most successful when only that occurs which is intended.

There were other unforeseen effects. Each of the embryos was in respect to the edited part of the genome a “mosaic.” This means that the sequence of nitrogenous bases in the DNA strands of the “repaired” part of the gene varied in different cells of the same embryo. In other words, the intended uniform and controlled editing process did not occur. In addition, the researchers discovered mutations in two other genes that were directly caused by the genetic manipulation. Since they only looked at selected sites in the genome to screen for mutations, they surmise that they “likely underestimated the off-target effects” in the embryos.

The results show that the technique used is far from trustworthy in producing clearly circumscribed and uniform effects. From a purely technical perspective there can be no doubt that it would be wholly irresponsible at this time to use the technique to genetically alter a human embryo. There is also little doubt that scientists will continue research in this area and improve the techniques. And some scientists will argue that to truly improve the techniques, experiments on human embryos will need to be performed. How could you prove the methods are safe for humans without having tested them on humans? The urge to do what is potentially doable, regardless of any larger context, is not to be underestimated as a powerful driver of technology-oriented scientific research.

At the same time as this drive motivates the development of ever finer manipulative techniques, the broader field of genetics is increasingly discovering how all genetic processes are highly context dependent. For this reason, David Baltimore and 17 co-authors, including some who are leading efforts to develop gene-editing technologies, wrote recently that the “potential for unintended consequences of heritable germline modifications” will remain a serious concern since “there are limits to our knowledge of human genetics, gene-environment interactions, and the pathways of disease (including the interplay between one disease and other conditions or diseases in the same patient)” \cite{Baltimore2015}.

We can take as an example the fairly common blood disorder, β-thalassemia. As mentioned above, it is normally connected with mutations in the β-globin gene that is involved in the formation of hemoglobin, the vital iron-binding molecule in red blood cells. When little (or no) β-globin is synthesized due to a mutation, then hemoglobin formation and function are compromised, which can lead to a variety of symptoms in the affected person. Over 200 different types of mutations in the β-globin gene or in DNA that is related to its production have been discovered. The β-globin gene is located in chromosome 11, of which there are
two. If there is a mutation in only one of the β-globin genes, a person usually has no symptoms. (In genetic terms, they are called carriers.) When there are deleterious mutations in both β-globin genes, then a person normally has symptoms, but they can range from light anemia to the need for ongoing blood transfusions. As a review article states,

> For more than 25 years researchers have questioned why patients who are homozygous for identical molecular defects in the β-globin genes can have such remarkably different phenotypes. Some patients need regular blood transfusion (β-thalassaemia major), whereas others are transfusion independent (β-thalassaemia intermedia). (Higgs et al. 2012)

So at first one imagines a straightforward Mendelian recessive disease in which a person has deleterious mutations in both of its β-globin genes (is “homozygous”) and therefore has the disease. But it turns out that there is no one-to-one correlation between β-globin mutations and how the disease actually develops. There are many additional factors that influence the disease, which has also been found to be the case in other “simple” Mendelian diseases.

Some people produce no β-globin and some produce reduced amounts, but this “does not necessarily predict disease severity, however; people with both types have been diagnosed with thalassemia major and thalassemia intermedia,” as the description of the disease at the National Institutes of Health (NIH) website states (http://ghr.nlm.nih.gov/condition/beta-thalassemia). Carriers who have β-globin mutation in only one of the two β-globin genes are normally healthy and, interestingly, have been found to be “relatively protected” against malaria (Cao and Galanello 2010), a fact that calls into question the idea of totally eradicating β-globin mutations. However, carriers sometimes also have a mild form of the disease β-thalassemia.

That a seemingly simple genetically conditioned disease is turning out to be highly complex is brought home by a study of β-thalassemia in China that examined 117 individuals with a mild form of the disease (β-thalassemia intermedia). They found, as have other studies, “a high degree of heterogeneity in both phenotypic and genotypic aspects” (Chen et al. 2010). Most surprising was the discovery that among the patients were three individuals who produced no β-globin at all and nonetheless had only mild anemia requiring occasional transfusions. The bodies of some individuals evidently have possibilities of compensating for the lack of β-globin, while in other individuals still other factors play a role leading to a worse condition than a genetic analysis would indicate. Researchers are finding an increasing variety of such “genetic modifiers.”

So what is in any case clear is the gulf that yawns between genetic diagnosis and the actual disease. Increasing knowledge about the genetic factors will not necessarily help patients who are suffering from the disease. What might a couple learn, for example, from a genetic diagnosis they choose to have performed because both individuals have family histories in which β-thalassemia occurred? For example, if both are diagnosed as carriers of one β-globin mutation and want to have a child, then there is a 25% likelihood that the child will have a β-globin mutation in both of its β-globin genes. They could have the embryo (or later the fetus) genetically screened to see if it does in fact carry both mutant genes. If it does, then the parents know that the child will likely have some form of the disease.

But it does not tell the parents about how this genetic condition will actually affect their particular child—will it be mild, will there be serious complications, will the child need frequent transfusions? The parents now have some abstract information and hopefully a clear
sense of how little they actually can know on the basis of the genetic diagnosis. They can choose to have the child or they may decide to have an abortion. That is where we stand today. As genetic diagnostic techniques are further refined, couples will have more and more information. But will it really tell them much, or mainly increase their feeling of facing uncertainty?

And what if one could “repair” the mutated part of the β-globin gene right at the beginning of conception? That is the goal that stands behind the genome-editing experiment performed by the Chinese scientists. The parents would have to agree to in vitro fertilization so that the gene-editing process would take effect before the zygote begins to divide into a multicellular organism. But at this stage you cannot know whether that particular zygote actually has no, one or two mutant β-globin genes. There is a 75% chance that the manipulation would be performed on an embryo that will not develop the disease (that has no mutant β-globin genes or only one as a “carrier”). In other words, the scientists/physicians would most likely be manipulating a completely healthy zygote and have no way of knowing what side-effects (which could be heritable) the manipulation would have. As geneticist Rudolph Jaenisch states, “it is unacceptable to mutate normal embryos. For me, that means there is no application [of this technique in human embryos]” (quoted in Kolata 2015).

What stands behind Jaenisch’s statement is the foundational conviction that the medical profession exists to help patients who are suffering. A physician does not make an intervention in a healthy person that may cause illness. What could be clearer?

That scientists are nonetheless pursuing research that would lead to doing just this is as remarkable as it is disturbing. It shows how disconnected the seemingly logical or “elegant” idea “correct a gene, prevent a disease” is from biological reality. They may truly have the best intentions and they may also be seduced by the Sirens of technology that invite us to pursue the pathway of the technologically doable for its own sake. When we accept this invitation, we are guided by a de-contextualized idea of a disease and the seductive promise of a technique viewed in isolation from concrete life.

The history of technology shows that society has often allowed technology to take on a life of its own, only to be confronted later with all the unintended consequences that had often been foreseen. Will we be any wiser when it comes to manipulating human embryos?

Sources:


Craig Holdrege, Ph.D., is the director of The Nature Institute in Ghent, NY. The Institute works through education, research, and publications to inspire a new paradigm for science and technology — a paradigm that encourages us to strive for a healthy future by embracing nature’s wisdom. Craig is also author of *Thinking Like a Plant: A Living Science for Life* (Lindisfarne Books, 2013) and co-author of *Beyond Biotechnology: The Barren Promise of Genetic Engineering* (University Press of Kentucky, 2008). He can be reached at craig@natureinstitute.org.