**Editing human germline cells sparks ethics debate**

May 6, 2015

Sci-fi novels and films like Gattaca no longer have a monopoly on genetically engineered humans. Real research scripts about editing the human genome are now appearing in scientific and medical journals. But the reviews are mixed. In Gattaca, nearly everyone was genetically altered, their DNA adjusted to prevent disease, enhance intelligence and make them look good. Today, only people treated with gene therapy have genetically engineered DNA. But powerful new gene editing tools could expand the scope of DNA alteration, forever changing humans' genetic destiny.

Not everyone thinks scientists should wield that power. Kindling the debate is a report by scientists from Sun Yat-sen University in Guangzhou, China, who have edited a gene in fertilized human eggs, called zygotes. The team used new gene editing technology known as the CRISPR/Cas9 system. That technology can precisely snip out a disease-causing mutation and replace it with healthy DNA. CRISPR/Cas9 has edited DNA in stem cells and cancer cells in humans. Researchers have also deployed the molecules to engineer other animals, including mice and monkeys. But it had never before been used to alter human embryos.

The team’s results sparked a flurry of headlines because their experiment modified human germline tissue. While most people think it is all right to fix faulty genes in mature body, or somatic, cells, tinkering with the germ line — eggs, sperm or tissues that produce those reproductive cells — crosses an ethical line for many. Germline changes can be passed on to future generations, and critics worry that allowing genetic engineering to correct diseases in germline tissues could pave the way for creating designer babies or other abuses that will persist forever.

“How do you draw a clear, meaningful line between therapy and enhancement?” ponders Marcy Darnovsky, executive director of the Center for Genetics and Society in Berkeley, Calif. About 40 countries ban or restrict such inherited DNA modifications.

Rumors about human germline editing experiments prompted scientists to gather in January in Napa, Calif. Discussions there led two groups to publish recommendations. One group called for scientists to “agree not to modify the DNA of human reproductive cells,” including the nonviable zygotes used in the Chinese study. A second group called for a moratorium on the clinical use of human germline engineering, but stopped short of saying the technology shouldn’t be used in research. Those researchers say that while CRISPR technology is still too primitive for safe use in patients, further research is needed to improve it. But other groups disagreed.

“Are there ever any therapeutic uses that would demand … modification of the human germ line? We don’t think there are any,” says Edward Lanphier, president of Sangamo BioSciences in Richmond, Calif. “Modifying the germ line is crossing the line that most countries on our planet have said is never appropriate to cross.”

If germline editing is never going to be allowed, there is no reason to conduct research using human embryos or reproductive cells, he says. Sangamo BioSciences is developing gene editing tools for use in somatic cells, an approach that germline editing might render unneeded. Lanphier denies that financial interests play a role in his objection to germline editing.

Other researchers, including Harvard University geneticist George Church, think germline editing may well be the only solution for some people with rare, inherited diseases. “What people want is safety and efficacy,” says Church. “If you ban experiments aimed at improving safety and efficacy, we’ll never get there.”

The zygote experiments certainly demonstrate that CRISPR technology is not ready for daily use yet. The researchers attempted to edit the beta globin, or *HBB*, gene. Mutations in that gene cause the inherited blood disorder beta-thalassemia. CRISPR/Cas9 molecules were engineered to seek out *HBB* and cut it where a piece of single-stranded DNA could heal the breach, creating a copy of the gene without mutations. That strategy succeeded in only four of the 86 embryos that the researchers attempted to edit. Those edited embryos contained a mix of cells, some with the gene edited and some without.

In an additional seven embryos, the *HBB* gene cut was repaired using the nearby *HBD* gene instead of the single-stranded DNA. The researchers also found that the molecular scissors snipped other genes that the researchers never intended to touch.

“Taken together, our work highlights the pressing need to further improve the fidelity and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications,” the researchers wrote.

Viable or not, germline cells should be off limits, says Darnovsky. She opposes all types of human germline modification. The U.K. prohibits all other germline editing. Such unproven technologies shouldn’t be attempted when alternatives already exist, Darnovsky says, such as screening embryos created through in vitro fertilization and discarding those likely to develop the disease.

But banning genome-altering technology could leave people with genetic diseases, and society in general, in the lurch, says molecular biologist Matthew Porteus of Stanford University.

“There is no benefit in my mind of having a child born with a devastating genetic disease,” he says. Alternatives to germline editing come with their own ethical quandaries, he says. Gene testing of embryos may require creating a dozen or more embryos before finding one that doesn’t carry the disease. The rest of the embryos would be destroyed. Many people find that prospect ethically questionable.

**Enzyme responsible for obesity-related high blood pressure identified**

May 5, 2015

Obesity is a serious health problem affecting approximately one-third of the adult population in the United States. Obese individuals have an increased risk of diabetes and cardiovascular disease, including hypertension. A recent study led by a University of Missouri researcher has identified the enzyme responsible for obesity-related hypertension - a finding that could lead to new treatment options.

"Hypertension is a condition in which arterial blood vessels are exposed to persistently elevated blood pressure, making the heart work harder to pump blood to the body," said William Durante, a professor of medical pharmacology and physiology at the MU School of Medicine and lead author of the study. "Hypertension can lead to severe health issues such as heart attacks, kidney failure, organ damage, and weakened or ruptured blood vessels. By comparing genetically obese rats to lean rats, we discovered that obese animals were deficient in the amino acid arginine due to elevated activity of the enzyme arginase, which breaks down this molecule."

Although arginase is present throughout the body, it is primarily found in the liver. Its role is to assist in the breakdown of ammonia, which is eventually flushed out during urination. However, Durante's team found significantly increased arginase activity within blood vessels and in the blood of obese rats compared to lean animals.

"The problem with this development is that arginase depletes arteries and blood of arginine, which is needed to generate nitric oxide," Durante said. "Nitric oxide is a gas formed from arginine that relaxes blood vessels and lowers arterial blood pressure. The destruction of arginine by arginase reduces nitric oxide levels, leading to the constriction of blood vessels and high blood pressure."

Using two methods to correct the arginine deficiency, Durante's team first supplemented the diet of obese animals with the amino acid L-arginine. The second method involved using drugs that block the activity of arginase. Although both approaches restored nitric oxide production and reversed hypertension in obese rats, the use of arginase-inhibiting drugs may be a better solution.

"Blocking arginase activity offers a more specific approach in treating hypertension, because you are directly targeting the underlying biochemical defect in obesity," Durante said. "L-arginine is a natural amino acid commonly found in red meat, poultry, fish and dairy products. It is also manufactured and used as a nutritional supplement or medication. However, a dietary approach using L-arginine may not be the best treatment option. Yes, arginine increases nitric oxide, but it also exerts other biological effects, and it can be converted by arginase to alternative compounds that counteract its benefits to the circulation."

In future studies, researchers plan to investigate what causes the increase in arginase activity in blood vessels and in the blood of obese animals. However, Durante feels that identifying the role of arginase in the development of obesity-related hypertension will ultimately benefit obese patients.

"Obesity is a significant health issue not only in this country, but worldwide," Durante said. "The key to reversing the effects of obesity-related hypertension will be to effectively block arginase activity. This new knowledge may help in the discovery of treatment options for obese patients with high blood pressure."

**Gene found that is essential to maintaining breast and cancer stem cells**

May 11, 2015

The gene and hormone soup that enables women to breastfeed their newborns also can be a recipe for breast cancer, particularly when the first pregnancy is after age 30. The findings point toward new therapeutic targets for breast cancer and potentially using blood indicators as a way to diagnose early breast cancer, the researchers report. Researchers have now found that the gene DNMT1 is essential to maintaining breast, or mammary, stem cells, that enable normal rapid growth of the breasts during pregnancy, as well as the cancer stem cells that may enable breast cancer. They've learned that the DNMT1 gene also is highly expressed in the most common types of breast cancer.

Conversely, ISL1 gene, a tumor suppressor and natural control mechanism for stem cells, is nearly silent in the breasts during pregnancy as well as cancer, said Dr. Muthusamy Thangaraju, biochemist at the Medical College of Georgia at Georgia Regents University and corresponding author of the study in the journal *Nature Communications*.

"DNMT1 directly regulates ISL1," Thangaraju said. "If the DNMT1 expression is high, this ISL1 gene is low." They first made the connection when they knocked out DNMT1 in a mouse and noted the increase in ISL1. Then they got busy looking at what happened in human breast cancer cells.

They found ISL1 is silent in most human breast cancers and that restoring higher levels to the human breast cancer cells dramatically reduces the stem cell populations and the resulting cell growth and spread that are hallmarks of cancer.

When they eliminated the DNMT1 gene in a breast-cancer mouse model, "The breast won't develop as well," Thangaraju said, but neither would about 80 percent of breast tumors. The deletion even impacted super-aggressive, triple-negative breast cancer.

The findings point toward new therapeutic targets for breast cancer and potentially using blood levels of ISL1 as a way to diagnose early breast cancer, the researchers report. In fact, they've found that the anti-seizure medication valproic acid, already used in combination with chemotherapy to treat breast cancer, appears to increase ISL1 expression, which may help explain why the drug works for these patients, he said. The scientists are screening other small molecules that might work as well or better.

Mammary stem cells help maintain the breasts during puberty as well as pregnancy, both periods of dynamic breast cell growth. During pregnancy, breasts may generate 300 times more cells as they prepare for milk production. This mass production may also include tumor cells, a mutation that seems to increase with age, Thangaraju said. When the fetus is lost before term, immature cells that were destined to become breast cells, can more easily become cancer, said Rajneesh Pathania, a GRU graduate student and the study's first author.

DNMT1 is essential for maintaining a variety of stem cell types, such as hematopoietic stem cells, which produce all types of blood cells. But, its role in regulating the stem cells that make breast tissue and enable breast cancer has not been studied, the scientists write.

While the exact reasons remain unclear, there is an increased risk of breast cancer if the first pregnancy occurs after age 30 as well as in women who lose their baby during pregnancy or have an abortion. Women who never have children also are at increased risk, while multiple term pregnancies further decrease the risk, according to the American Cancer Society.

Theories include that the hormone-induced maturation of breast cells that occurs during pregnancy may increase the potential for breast cancer cells to be made as well. Also, most breast cancers thrive on estrogen and progesterone, which are both highly expressed during pregnancy and also help fuel stem cell growth.

During pregnancy, stem cells also make more of themselves so their population increases about five times. DNMT1 levels experience a similar increase.

In five different types of human breast cancer, researchers found high levels of DNMT1 and ISL1 turned off. Even in a laboratory dish, when they put the ISL1 gene back, human breast cancer cells and stem cell activity were much reduced, Thangaraju said.

**Blood test for early detection of breast cancer metastasis**

May 18, 2015

New hope has been given for a way of detecting metastases significantly earlier than is currently possible. The discovery is based on what is known as cell-free circulating DNA - small fragments of genetic material from different cells which circulate in the blood. It is normal to have low amounts of such DNA material in the blood, but in the case of diseases such as cancer, these amounts can increase. Furthermore, in cancer patients, the circulating DNA contains the genetic mutations which are specific to the tumor.

The chances of being cured of breast cancer have increased in recent decades, however if the tumour has metastasised, the disease remains essentially incurable. One reason for this could be that the metastases are detected late, after they have grown enough to cause symptoms or be seen on a radiological scan. If they could be found sooner, it might be possible to treat the new tumours. Research findings from Lund University in Sweden now provide new hope for a way of detecting metastases significantly earlier than is currently possible.

The discovery was made by a research team led by Lao Saal, M.D. Ph.D, and is based on what is known as cell-free circulating DNA -- small fragments of genetic material from different cells which circulate in the blood. It is normal to have low amounts of such DNA material in the blood, but in the case of diseases such as cancer, these amounts can increase. Furthermore, in cancer patients, the circulating DNA contains the genetic mutations which are specific to the tumor.

Lao Saal and his colleagues used previously gathered material from a breast cancer study which has been underway in Lund since 2002. The material contained samples from surgically removed tumours from patients with non-metastatic disease as well as blood samples taken from the patients at regular intervals during the years in which they were followed up.

The tumour samples contained many genetic changes, which constituted a "fingerprint" specific to each tumour. Researchers then looked in the blood samples for circulating tumour DNA (ctDNA) with the same fingerprint. Although the study is fairly small -- it is based on material from only 20 women -the results are striking.

"For 19 of the 20 women, the ctDNA in the blood samples gave a clear indication of how things would turn out. The women who never got a relapse had no detectable ctDNA, whereas all women who had tumour DNA in their blood eventually had symptomatic relapses that were diagnosed in the clinic," said Lao Saal.

The metastases were also reflected in the blood samples at an early stage. There it was possible to find signs of the new tumours many months before hospital investigations revealed that the patients had suffered a relapse.

"The circulating tumour DNA values in the blood samples identified the metastases on average 11 months before they were diagnosed by standard clinical procedures. In some cases, the blood test detected the metastasis three years earlier. If we could find the cancer recurrences that much earlier, we might be able to treat them more successfully," said Lao Saal.

The study must be followed by investigations with more participants, so that researchers can be sure that the results are sustainable. If they are, ctDNA testing could become a way of detecting breast cancer metastasis much earlier than is currently possible.

In addition to the possibility of treating the women who are about to get metastases, the a potential future use of the new method could be to determine which women do not need to be treated so aggressively. If we know that women with no ctDNA in their blood are not going to get a relapse, less aggressive treatment could be sufficient in their case. Currently, most breast cancer patients are treated not only with surgery, but also radiation, hormone therapy or chemotherapy.

"It is believed that many women with breast cancer are being overtreated, which entails considerable side effects and costs. But as long as we do not know for certain which women will survive without additional treatment, physicians are hesitant to skip the additional therapies. The monitoring of ctDNA could help address that question," says Lao Saal

If supported by further studies, he believes that the monitoring should be carried out at regular intervals after the breast cancer surgery. The quantity of ctDNA, as well as the emergence of specific gene mutations, could be used in the future to steer therapy in a more precise manner. The Lund researchers have already started new studies in which a larger number of women will be monitored from breast cancer diagnosis and onwards, as well as testing ctDNA methods in other cancer types.

**Adenosine receptor can activate 'off signals' for pain**

April 16, 2015

Pain is the most common reason that people seek medical attention, but the available treatments are not always successful at relieving pain in patients with chronic pain. Now researchers found that drugs targeting the A3 adenosine receptor can "turn off" pain signals in the spinal cord to provide relief from chronic pain.

In a study published in the April issue of the *Journal of Neuroscience*, Saint Louis University scientists led by professor of pharmacological and physiological sciences Daniela Salvemini, Ph.D., discovered that drugs targeting the A3 adenosine receptor can "turn off" pain signals in the spinal cord to provide relief from chronic pain.

Pain is the most common reason that people seek medical attention, but the available treatments -- most commonly non-steroidal anti-inflammatory drugs (NSAIDs) and opioids -- are not always successful at relieving pain in patients with chronic pain. For this reason, Salvemini and colleagues teamed up with researchers from the National Institutes of Health, the University of Arizona and two institutes in Quebec, Canada, to investigate a new target for treating chronic pain: the A3 adenosine receptor or A3AR.

In earlier studies, Salvemini's laboratory demonstrated that two drugs which target the A3AR -- IB-MECA and MRS5698 -- were effective in treating several models of chronic pain, including painful chemotherapy-induced neuropathy, metastatic cancer pain, and nerve injury. More recently, the group sought to uncover the mechanism of A3AR pain relief.

"Chronic pain can result from the loss of regulatory mechanisms in the nervous system pathway that transmits pain," Salvemini said. "Adenosine acts as a regulatory signaling molecule in other areas of the nervous system, so we hypothesized that A3AR might also play a role in regulating pain signals during pain processing."

Indeed, Salvemini and colleagues found that A3AR drugs not only relieved pain, but did so by activating an inhibitory transmitter system known as the gamma amino-butyric acid (GABA) system. In areas of the spinal cord and brain dedicated to pain processing, A3AR activation promoted GABA signaling by preventing the breakdown and removal of GABA from neuronal synapses.

"In chronic pain, GABA signaling is often lost or diminished. Our A3AR drugs were able to restore GABA signaling in areas that process pain and 'turn off' the signals that maintain the pain state," Salvemini said.

With A3AR drugs demonstrating good safety profiles in clinical trials as anti-inflammatory and anti-cancer agents, Salvemini and colleagues are enthusiastic about the potential of these new drugs to treat chronic pain in patients.

"Several lead molecules for prospective clinical use have been identified through our collaboration with Dr. Kenneth Jacobson at the National Institutes of Health and we are very excited about the potential for translational therapeutic impact," Salvemini said.

The lab will continue to investigate the intricate mechanisms underlying A3AR pain relief with the hope of providing better palliative care to individuals suffering from unnecessary chronic pain.

**Birth-weight boost tied to cleaner air during Beijing Olympics**

May 13, 2015

The Olympics are stuffed full of feel-good moments featuring amazing athletic feats, heart-warming backstories and national pride. Now, a new study details another Olympic win: Bigger babies.

Babies whose eighth month of gestation fell during the 2008 Beijing Olympics and Paralympics were born slightly heavier than babies born a year earlier or later. Why? Because those Olympic babies got a break from Beijing’s profoundly polluted air, [researchers suggest](http://ehp.niehs.nih.gov/1408795/) April 28 in *Environmental Health Perspectives.* The results serve as a stark reminder of how pollution can harm fetuses.

Olympic organizers in Beijing went to [great lengths](http://www.nytimes.com/2008/04/15/world/asia/15beijing.html) to clean up their air in advance of the 2008 Summer Games. The government took cars off roads, shuttered factories and even banned outdoor spray-painting. And those efforts worked. Concentrations of certain pollutants dropped.

Researchers led by epidemiologist David Rich of the University of Rochester Medical Center in New York realized that this rare break in pollution was a golden opportunity to study the effects of dirty air on pregnancy.

The team combed through health records of more than 80,000 pregnant women in Beijing to see whether the pollution drop had any effect on the outcome. Women whose eighth month of pregnancy coincided with the Olympics went on to have babies who were an average of 23 grams heavier than the babies of women whose eighth month of pregnancy came the year before or after, the researchers found.

That average birth weight may have responded to such a short bout of clean air makes you wonder what pollution is doing to fetuses who experience it for all 40 weeks. “Even in this 47-day period you see a public health benefit like this,” Rich says. “Imagine what you could do if you could have air pollution levels reduced throughout the whole entire pregnancy.”

The timing of the pregnancy seemed to matter. The researchers found that baby weight went up when clearer skies coincided with the eighth month of pregnancy, a time when a fetus is really packing on pounds. Finding an effect there “makes a lot of sense,” says epidemiologist Beate Ritz of the University of California, Los Angeles, who wasn’t involved in the study.

Twenty-three grams — about 0.8 ounces — isn’t a big difference. In fact, it’s less than 1 percent of these babies’ median weight. But that slight change may serve as a bellwether for more insidious effects.

“It’s really important, not so much as ‘Oh, those few grams, do they really matter in the life of this child?’’ Ritz says. “That’s not really what we’re asking.” Instead, scientists suspect that if pollution is behind the weight difference, then dirty skies could be inflicting damage on other developing organs and systems too. Pollution during pregnancy could be programming these babies to develop in a way that leads to important differences in the immune system, the brain and other systems, she says.

It’s also possible that pollution might do more damage earlier in pregnancy, Ritz says. Dirty air could be contributing to early miscarriages, which would be hard to study.

More research is needed to figure out just how the pollution may be affecting fetuses, and babies, and people for that matter. But what people really need is more than just a temporary reprieve from pollution.

**Major advance in artificial photosynthesis poses win/win for the environment**

April 16, 2015

By combining biocompatible light-capturing nanowire arrays with select bacterial populations, a potentially game-changing new artificial photosynthesis system offers a win/win situation for the environment: solar-powered green chemistry using sequestered carbon dioxide.

A potentially game-changing breakthrough in artificial photosynthesis has been achieved with the development of a system that can capture carbon dioxide emissions before they are vented into the atmosphere and then, powered by solar energy, convert that carbon dioxide into valuable chemical products, including biodegradable plastics, pharmaceutical drugs and even liquid fuels.

Scientists with the U.S. Department of Energy (DOE)'s Lawrence Berkeley National Laboratory (Berkeley Lab) and the University of California (UC) Berkeley have created a hybrid system of semiconducting nanowires and bacteria that mimics the natural photosynthetic process by which plants use the energy in sunlight to synthesize carbohydrates from carbon dioxide and water. However, this new artificial photosynthetic system synthesizes the combination of carbon dioxide and water into acetate, the most common building block today for biosynthesis.

"We believe our system is a revolutionary leap forward in the field of artificial photosynthesis," says Peidong Yang, a chemist with Berkeley Lab's Materials Sciences Division and one of the leaders of this study. "Our system has the potential to fundamentally change the chemical and oil industry in that we can produce chemicals and fuels in a totally renewable way, rather than extracting them from deep below the ground."

The more carbon dioxide that is released into the atmosphere the warmer the atmosphere becomes. Atmospheric carbon dioxide is now at its highest level in at least three million years, primarily as a result of the burning of fossil fuels. Yet fossil fuels, especially coal, will remain a significant source of energy to meet human needs for the foreseeable future. Technologies for sequestering carbon before it escapes into the atmosphere are being pursued but all require the captured carbon to be stored, a requirement that comes with its own environmental challenges. The artificial photosynthetic technique developed by the Berkeley researchers solves the storage problem by putting the captured carbon dioxide to good use.

"In natural photosynthesis, leaves harvest solar energy and carbon dioxide is reduced and combined with water for the synthesis of molecular products that form biomass," says Chris Chang, an expert in catalysts for carbon-neutral energy conversions. "In our system, nanowires harvest solar energy and deliver electrons to bacteria, where carbon dioxide is reduced and combined with water for the synthesis of a variety of targeted, value-added chemical products."

"Our system represents an emerging alliance between the fields of materials sciences and biology, where opportunities to make new functional devices can mix and match components of each discipline," says Michelle Chang, an expert in biosynthesis. "For example, the morphology of the nanowire array protects the bacteria like Easter eggs buried in tall grass so that these usually-oxygen sensitive organisms can survive in environmental carbon-dioxide sources such as flue gases."

"Our artificial forest is similar to the chloroplasts in green plants," Yang says. "When sunlight is absorbed, photo-excited electron pairs are generated in the silicon and titanium oxide nanowires, which absorb different regions of the solar spectrum. The photo-generated electrons in the silicon will be passed onto bacteria for the CO2 reduction while the photo-generated holes in the titanium oxide split water molecules to make oxygen."

Once the forest of nanowire arrays is established, it is populated with microbial populations that produce enzymes known to selectively catalyze the reduction of carbon dioxide. For this study, the Berkeley team used Sporomusa ovata, an anaerobic bacterium that readily accepts electrons directly from the surrounding environment and uses them to reduce carbon dioxide.

"*S. ovata* is a great carbon dioxide catalyst as it makes acetate, a versatile chemical intermediate that can be used to manufacture a diverse array of useful chemicals," says Michelle Chang. "We were able to uniformly populate our nanowire array with *S. ovata* using buffered brackish water with trace vitamins as the only organic component."

Once the carbon dioxide has been reduced by *S. ovata* to acetate (or some other biosynthetic intermediate), genetically engineered E.coli are used to synthesize targeted chemical products. To improve the yields of targeted chemical products, the *S. ovata* and E.coli were kept separate for this study. In the future, these two activities -- catalyzing and synthesizing -- could be combined into a single step process.

A key to the success of their artificial photosynthesis system is the separation of the demanding requirements for light-capture efficiency and catalytic activity that is made possible by the nanowire/bacteria hybrid technology. With this approach, the Berkeley team achieved a solar energy conversion efficiency of up to 0.38-percent for about 200 hours under simulated sunlight, which is about the same as that of a leaf.

The yields of target chemical molecules produced from the acetate were also encouraging -- as high as 26-percent for butanol, a fuel comparable to gasoline, 25-percent for amorphadiene, a precursor to the antimaleria drug artemisinin, and 52-percent for the renewable and biodegradable plastic PHB. Improved performances are anticipated with further refinements of the technology.

"We are currently working on our second generation system which has a solar-to-chemical conversion efficiency of three-percent," Yang says. "Once we can reach a conversion efficiency of 10-percent in a cost effective manner, the technology should be commercially viable."