Letter

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CRISPR -Cas9: a groundbreaking new technique which ushers in new prospects and just as many doubts

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Abstract

Human germline engineering arguably constitutes one of the most promising and at the same time controversial prospects in the realm of gene editing overall, and particularly in the context of the current state of research. The issues raised by such techniques have sparked heated debate worldwide: the scientific and industrial establishments have been strongly supporting CRISPR-Cas9 research, but a well-balanced approach needs to be adopted in order to reconcile the needs of scientific research with the life and dignity of human embryos. ***Clin Ter 2021; 172 (1):e52-54. doi: 10.7417/CT.2021.2281***

Key words: *Cellular and gene therapy, bioengineering, Research ethics*

Dear Editor,

We have read with great interest the paper authored by Susanna Marinelli and Alessandro Del Rio, titled "Beginning of life ethics at the dawn of a new era of genome editing: are bioethical precepts and fast-evolving biotechnologies irreconcilable?", published in issue 5 of 2020 of Your distinguished journal, on which we would like to offer a few brief remarks herein (1). Human embryos have drawn growing attention over the years, particularly in terms of the potential to harvest embryonic stem cells, which are pluripotent, thus able to grow into all derivatives of the three primary germ layers ability, as it has been shown at the experimental level (2). Still, such a use of embryonic stem cells undoubtedly poses ethical issues stemming from the eventual destruction of embryos following experimental procedures.

Nonetheless, stem cell research has broadened the horizon of regenerative medicine, by offering new potential opportunities to treat and cure various as yet incurable diseases. Since 2012, a new technique named CRISPR Cas9 (clustered regularly interspaced short palindromic repeats) has been gaining traction (3). Such a procedure has opened up extremely interesting prospects, by virtue of its being relatively inexpensive and pliable, with a wide range of possible applications both in basic research and biotechnologies overall. Furthermore, CRISPR-Cas9 may pave the way for the eradication of genetic diseases (4, 5). As a matter of fact, CRISPR-Cas9 may enable scientists to intervene and edit human genome by "cutting out and replacing" DNA strands, thus deactivating defective genes by eliminating individual snippets and supplanting them with new ones in an extremely precise and targeted fashion (6). Genome-editing will therefore allow science to "cure" embryonic stem cells with genetic anomalies, thus removing such abnormalities at their earliest stage of development; once the "flaw" has been corrected, the embryo will develop normally (7).

The vast majority of the scientific community has expressed concerns over the news that such procedures had been used, given its inherently risky nature at this point in time. CRISPR-Cas9 interventions may in fact get "off-target", thus modifying parts of the genome other than those meant to be fixed, with unforeseeable unwanted consequences. The newly-acquired ability to edit human genome inevitably gives rise to major philosophical and ethical quandaries centered around the blurred lines between therapy and eugenics, the core value of human dignity and the role of science in earliest stages of human life (8, 9).

The fundamental issue is what exactly the technique's range of application will be, once it is perfected and fully reliable. Where should the line be drawn between the therapeutic use of gene editing practices and other kinds of applications, such as genome modifications aimed at achieving human enhancement according to undefined standards? Human germline engineering could in fact not only be used to repair damaged DNA, but also to modify the characteristics of embryos produced *in vitro*, leading to human beings with predetermined cognitive and physical traits. In time, such research could lead to the creation of a "super race" of genetically enhanced humans, whose characteristics would inheritable. The consequences of such dynamics are currently impossible to foresee. CRISPR-Cas9 techniques still has unknown aspects and uncertainty; it is nonetheless capable of modifying the human genome within embryos, i.e. at the initial stages of development (10, 11). The embryo's genetic profile thus modified is then passed on to future generations.

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Serious concerns have been voiced in the scientific community as to the scope of such techniques even before the first experiment was announced. Scientific journals such as "Nature" and "Science" released editorials in which many researchers asked for an international moratorium on the clinical use of genome editing carried out on human embryos, oocytes and spermatozoa, which are the very applications that could pave the way for inheritable genetic modifications (12, 13), in order to prevent unregulated and lax trials on human embryos, particularly for non-therapeutic purposes. The fundamental principle of precaution would call for science to refrain from altering human genetic structures until all doubts and concerns are put to rest. That same approach is also codified in a European directive which bans the use and patenting of human embryos for scientific research purposes, and in article 2 of the Oviedo Convention, which states that «*the interests and welfare of the human being shall prevail over the sole interest of society or science*».

Several international research societies such as the National Academy of Sciences, the Royal Society, the Chinese Academy of Sciences, the Committee on Bioethics (DH-BIO) at the Council of Europe, the European Group on Ethics in Science and New Technology (EGE), the European Group on Ethics in Science and New Technology (EGE), the Nuffield Council on Bioethics and the Italian Committee for Bioethics have spoken out in favor of *in vitro* and animal research trials of genome editing techniques meant to test effectiveness and safety, whiling declaring their opposition to any such trials on gametes destined for conception and on embryos to be implanted in uterus, thus backing the international moratorium on clinical or in vivo research, as long as acceptably high high safety and effectiveness standards are not met. All of the associations' heads have concluded that further studies are necessary before clinical applications can be allowed: gene editing meant to engineer human germlines of humans at the stage of conception, or to design new DNA-based characteristics for human beings yet to be conceived makes the changes thus attained irreversible and inheritable by the future human offspring. It is undoubtedly an extremely promising technique, but rather inaccurate at the time being (14, 15).

It is in fact also possible that such intervention could engender mutations in the expression of nuclear DNA genes: that outcome may have potentially catastrophic consequences, unforeseeable and not immediately detectable, since they would manifest themselves over the years. Hence, an extremely cautious approach is necessary when dealing with scientific innovations of this magnitude.

A key ethical boundary to scientific research is that it should never expose its human participants to unnecessary and unreasonable risks (16). Research hazards should instead be kept to the minimum level necessary to provide science with answers, while the benefits should always be in proportion with the expected risks. In order to determine whether a risk is acceptable or not, it is essential to assess not only the likelihood of reaping a benefit, bur also the extent of the benefit itself. The higher the scope of expected benefits, the higher the risk worth taking. One might even argue that in order for any risk to be deemed acceptable, it should be specifically defined. Conversely, CRISPR-Cas9 may result in the accidental alteration of genes other than those targeted for editing. Consequently, the risks involved are impossible to define and assess. That in turn entails the inability to map out a risk-benefit analysis, hence the inadmissibility of any such clinical application irrespective of the benefits hoped for and of the adverse repercussions of the disease if the technique is not used. The paper's authors contend that genome editing may follow the same path that medically-assisted procreation (MAP) has, which was initially harshly criticized and ethically contentious. Nowadays, MAP is widely available worldwide, despite its inherently controversial nature (17), and through procedures such as *in vitro* fertilization (18-20), gamete donation (21, 22), egg and sperm freezing (23-25), it has fundamentally changed the very core essence of the family and parenthood (26-30). Medically-assisted procreation has not been regulated evenly in every country: some nations passed permissive laws as opposed to others that opted for a more restrictive position, such as Italy (31, 32). That has led many to travel abroad, a trend dubbed "procreative tourism". Being mindful of the dangers posed by modern biotechnology is the wise thing to do. Nevertheless, if on the one hand it is advisable to tread lightly, by taking into account pros and cons, it is not right to be biased against and adamantly opposed to any procedure that entails a degree of risk. It would in fact be unthinkable to operate under totally risk-free conditions, since any technique or trial can lead to potentially adverse outcomes or unforeseeable consequences for those involved. In order to assess the tenability of each and every choice, it is necessary to weigh the risks against the possible benefits.

It is also worth pointing out that so far, using embryonic stem cells has not produced the effects scientists had hoped for; as of today, no known therapy is based on embryonic stem cells. Professor Wilmut himself, the scientist who created the sheep known as Dolly, has voiced his doubts about achieving substantial results through embryonic stem cell research. Such doubts and concerns may steer scientific research towards adult stem cells. Ultimately, *scientists should not decide to refrain from action, bur to act differently (...).* In that regard, Japanese scientist Shinya Yamanaka, winner of the Nobel Prize in Physiology or Medicine, has focused on "induced pluripotent stem (iPS) cell" research, unveiled in 2007, a technique capable of genetically reprogramming mature somatic cells till they "regress" to a state close to the embryonic stage, without the need to destroy embryos in the process (33).

It is quite complex for lawmakers to legislate on sensitive issues such as embryonic stem cell research, on which no international consensus exists. As for embryo research, among other controversial fields, lawmakers from different countries need to find common ground and cooperate, in order to stave off trends such as "healthcare tourism", which has recently manifested itself for stem cells based alleged treatments as well. Clearly defined public rules should be devised in order to properly direct and support scientific research as a whole, and medical research in particular (34, 35). Any clinical application of a given technique should only be allowed in full compliance with the rule of law. Currently, such high standards cannot be met, due to the absence of safety requirements; in addition, the laws currently in force in many countries do not allow for human germline engineering (36). It would therefore be utterly irresponsible to carry on genome editing research, at least until the safety concerns are not put to rest, the risks are properly evaluated and weighed against the possible benefits and alternatives (37).

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