Gene editing: a new issue for bioethics?*

Editing genético (edición genética): ¿nueva cuestión bioética?*

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Abstract

Controlled genome mutations are made possible through several techniques since the '70s. Zinc finger nucleases, TALE nucleases and above all CRISPR-Cas9 system are "gene editing" techniques which have made mutations easier. Particularly, CRISPR-Cas9 system seems to be extremely profitable in terms of accessibility, efficiency and versatility.

The aims of the present article are: 1. to reconstruct the main "facts" about the birth of the topic on "gene editing"; to seek to answer a first question about the novelty of issues raised by this topic.

Our conclusion is that, from an ethical point of view, using these techniques does not raise new ethical questions. Perhaps, the only exception refers to the specific mutations produced through these techniques which cannot be distinguished from natural mutations and makes GMO classification more difficult.

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* Original Title: *Editing genetico*: nuova questione bioetica? Published in the *Medicina e Morale* Magazine 2017/3 pp. 291-304. The translation has not been reviewed by the author.

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Received by the *Medicina e Morale* Magazine on April 10, 2017; accepted on June 7, 2017.

Received on July 27, 2018. Accepted on August 1, 2018.

Key words: CRISPR-Cas9, gene editing, genetic engineering

1. Introduction

"It seems that the gene editing is all over the place. In a relatively short time, particularly since the arise of CRISPR-Cas9 in 2012, the techniques to manipulate specific sequences of DNA, have not only attracted the attention of magazines specialized in life sciences, but also occupied a place in the foreground in the news of the communication media" [1, p. 1].[1] With these words the Nuffield Council on Bioethics has begun its big Report [1] of September 2016, about *gene editing* (that in this place we shall call it more generically "genetic", in order to avoid having to go into the most subtle distinctions of terms such as genetics, genomics, epigenetics or epigenomes) and of ethical questions, to point out, on one hand the widespread resonance acquired by the topic at media level, and on the other hand, the worries of the specialists. In fact, also a crude research in the *WEB Internet* performed through key words such as "CRISPR-Cas9" or else "genetic editing", it produces an impressive number of results. On the other hand, the issue has become in such degree "fiery" as to be the object, in December 2015 of an International SUMMIT of specialists, organized by the U.S. National Academy of Sciences, the English Royal Society and the Chinese Academy of Sciences, and for the purpose of updating the October 2015 meeting, of their own reflections about the genomics issue, and about the human rights by the UNESCO's International Bioethics Committee [2].

In Italy, the issue of the genetic editing has been widely retaken in the last years by numerous newspaper headings, particularly by its sections dedicated to the scientific information diffusion. Except for isolated cases [3-4], it doesn't seem however, having received great attention by the bioethics type specialized literature, nor having generated a big passion in the general public opinion.[2]

The objectives of this contribution, are very restricted and fundamentally consist of: 1. Reconstruct the highlight facts which have determined the rise of this *TOPIC*. 2. Try to give an answer to a first and fundamental question about the originality of the ethical dilemmas elicited by this sector.

Our wish is that this paper can be useful in the debate that can be foreseen to develop in the next few years, also in Italy, and in later deepening of these issues much more specific and specialized.

2. From genetics to the *genetic editing*

As it is known, genetics, that is, the science which studies the heritage features and their transmission, has given its first steps with the observations of Gregor Mendel (1822-1884), an Augustine monk, and his world famous experiments with green peas, which allowed him to identify the constants (Mendel's laws), with which certain factors (so called dominant and recessive) could or could not be transferred from one generation to the next one.

Since then, genetics has suffered enormous transformations: «from the macro scope has gone to the microscope, has reached the nuclear and cellular analysis, achieving this way, by means of technologies each time more refined, to identify the molecular basis of the genetic patrimony. From an imposition prevailingly observational, it has been articulated in phases each time more experimental and clarifying, manipulative and predictive; until the last, so to speak, frontier of the so called genetic engineering […]» (7, p. 101). This last one refers to, in particular, to the wide range of techniques that allow to manipulate DNA molecules, for the purpose of provoking predetermined changes in the genotype of an organism.

It is perfectly clear the impossibility to state an account even summarily of all these techniques with their respective deve-

lopment. According to the purposes of this paper, it is worthwhile to evoke at least two fundamental discoveries in this area.

The first is the one of "restriction endonucleases", nuclear enzymes capable of recognizing and cutting the DNA in specific places, characterized by a well determined sequence of nucleotides. Since 1969, when the first of these enzymes from the *Haemophilus influentiae*, bacteria was isolated for the first time, several hundred were identified, and today they are even produced by the industry. This discovery has put in the hands of the molecular biologists, species of "chemical scalpels" with which they cut and analyze chunks of DNA [8].

The second discovery is the "recombining DNA" by the United States biochemist Paul Berg. In the years 1967-1968, spent in the labs of another famous scientist, Renato Dulbecco, he had been convinced that the model of tumor viruses such as the Simian 40 virus (SV40), could have been able to reveal significant aspects of the genetic chemistry of the mammals. Having returned to Stanford, he began a research plan being able shortly to identify, by means of the use of endonucleases of restriction, the identification of the 5 genes contained in the micro chromosome of the SV40 and to define the temporary sequence of these genes, after the virus would have reached the nucleus of the infected cells, and during the vital cycle. He was able to achieve after demonstrating that this viral DNA was capable of "integrate" totally or partially with the DNA of the host cells of rodents, transforming them in neoplastic cells. Thus the idea later performed, of linking the DNA of the SV40 with fragments of the DNA of an *Escherichia coli* plasmid, with the setting of the first "hybrid DNA" (recombining DNA).

If from an operative point of view the recombining DNA technology is complex, conceptually, is based on fairly simple criteria: to identify a gen; to cut it and isolate it from the DNA molecule; to link the gen to a vector; transfer it to the inner part of a receptive cell.

After this first success, Berg projected to include a hybrid DNA of the same type of that recently described in the *Escherichia coli* from which came the plasmids, and to verify if in a bacterial environment, the virus would be capable of replicate itself, and its genes to express themselves [8]. Such experiment was initially suspended by a decision of the researchers themselves, for the fear that bacteria containing oncogene viruses, would disseminate in an uncontrolled manner. In 1973, The National Academy of Science asked Berg and to other authorized researchers, to meet in order to discuss the ethical problems raised by the new discoveries. This symposium, known as "Asilomar I", led to the publishing of an open letter in three authorized scientific magazines ("The Proceedings of the National Academy of Sciences", "Nature" and "Science"), through which they made an invitation to researchers to aratorium to interrupt research that would imply genetic manipulations, up until having an International Conference [9, p. 235]. This last one was developed in February 1975, also in Asilomar, and declared the interruption of the moratorium, and the issuing of a series of rules related to the use of recombining DNA.

The explosion of research following after the Berg experiments, has led to the development of techniques each time more precise to prepare, synthesize, analyze and hybridize specific DNA segments and of methods each time more workable in order to transfer them to specific sites, to clone them –that is to say make them to replicate in an unlimited number of copies– increasing their quantity, sort and select them obtaining the corresponding fragments to desired genes, and connect them until obtaining artificial chromosomes [8]. There were uncountable falls in applicative terms of such achievements, which in turn have opened a series of new fields in the industry, allowing the production of "advantage" substances under diverse profiles. Thereon, it will suffice to think in the vast range of applications in the pharmacologic, cosmetic, agro-alimentary, energetic, military, environment sectors –only to ate some examples–, that the massive and unlimited production of

"re-oriented" cells industry by means of the insertion of specific genes, has allowed in the last few years.

Along this large path of enormous and fast transformations, a new stage would seem to have initiated in the middle of the 90's, with the introduction of a special type of artificial restriction enzymes, called *zinc finger nucleases (ZFNs)*. One of the main problems with which the researchers have had to deal with along the years, has to do with "precision" at the time of performing a mutation, due to the possibility that the vectors would integrate to "functiona" portions of the genome, generating toxicity.

In order to overcome some limits, it has been tried to take advantage of the natural DNA mechanisms of repair, that is the non-homologous end-joining, and the homology-directed repair [10].

The nucleases with a zinc finger, are included in this type of approach: it is about a system that, based on discovery by Aaron Klug in 1996 [11], joins the capability of cutting the DNA of a nuclease enzyme of a bacteria, and the ability of the zinc fingers to link themselves to the double helix.

Always in the context of this approach, more recently, instead of the zinc fingers, similar structures have been used to those produced by the bacteria to defend itself from the viruses, that is to say the TALEN (*Transcriptor Activator-Like Effecto Nucleases*). Introduced in the mid of the year 2010 [12], and extracted from the bacterial proteins of the *Xanthomonas,* these have provided a more efficient instrument to direct tears of the double filament towards DNA specific positions.

Both, the use of nucleases with zinc fingers, as well as the TALEN, require a thorough work of "engineering" of the proteins, for each DNA sequence to be hit. Furthermore, with both methodologies a consistent probability of cuttings performed in nonspecific genomic regions remains, the so called *off-Targets*, including deleterious effects over the target cell, such as the inactivation of genes essential for the cell life, or chromosomal translocations.

In even more recent years, a path has been opened to an ulterior technology, which is the CRISPR-Cas9 *(Clustered, Regularly Interspaced, Short Palindromic Repeats-Associated Endunucleasa 9),* which instead of proteins that link the DNA, uses an RNA guide. Thanks to this methodology, the DNA molecules manipulation, has become notoriously more precise and simple to the point that it would be metaphorically comparable to the function "search and replace" of the well-known text processor "Word".

Thereon the expression "genetic EDITING", which represents no other thing than, but a suggestive metaphor –appeared in the journalistic area in January 2013 [13]–, in order to highlight the easiness with which the actual genetic engineering techniques (nucleases with zinc fingers, TALEN nucleases, but above all CRISPR-Cas9) make possible the identification and substitution of the four "letters" (A, C, G and T) with which the biochemical information is written.

3. The CRISPR-Cas9 System

The *Clustered, Regularly Interspaced, Short Palindromic Repeats (CRISPRs),* are particular segments of DNA, containing brief repeated sequences, which it has been discovered that, they are present in the inner part of prokaryotic cells. These have been found in nearly 40% of the bacterial genomes, and in 90% of the Archaea genomes submitted to sequencing [14].

Identified for the first time in 1987 for the *Escherichia coli* bacteria by the Japanese Yoshizumi Ishino, without understanding their function, it has been called to the attention of researchers, when it has been found that, in its surroundings, are located small genic CLUSTERS, which in association precisely with the CRISPERs (from here the expression *CRISPER-associated system "cas"),* constitute a kind of "immune system" through which the bacteria recognizes and "destroy" the viruses of infectious background.

In recent times, the properties of this association, have been taken advantage of by two researchers –the French Emmanuelle Charpentier and the American Jennifer Doudna– in order to tune up a method (the so called CRISPR-Cas9 system) capable of editing the genome by means of RNA molecules programming.

Without getting into its complex functioning mechanism, this system is fundamentally constituted by two components, a "sentinel" protein, Cas9, which is an enzyme capable of cutting the DNA, and a "compass" RNA, which conducts the enzyme to the place to be cut [6, p. 17].

Regarding the background methods of genetic engineering, the CRISPR-Cas9 system is revealing itself extremely favorable under various points of view [6]. The main advantage consists of the fact that, regarding to the past, the researchers don't have the need any more to produce a personalized protein for each DNA sequence to be hit, but that they must much more, simply program an RNA molecule. This is the basis of considerable savings both in terms of time (it has been gone from several months to a few weeks, to perform the experiments) but also about resources (the cost of the nucleases with the zinc finger rounds about $5,000 USCy, versus the $30 for the CRISPR/Cas9 system; [3] besides, in order to program an RNA molecule, it is not necessary to have labs particularly equipped, nor high competences of molecular biology) [15].

To the foregone it is added the fact that the CRISPR-Cas9 system, it has revealed itself as capable of modifying several genes at a time; it is much more precise to cut the DNA in specific sites, allowing a drastic reduction of the *off-Targets* cuts (even if recent studies are reducing a little bit, the initial triumphalist estimations, remaining well firm, nevertheless, that there are in course trials of perfecting this technique [16]); and it is extremely versatile, having shown to be able to function in almost all the organisms in which it has been tested [6, p. 12].

CRISPR-Cas9 represents therefore, an example of technology, so to speak, of "second generation", efficient, simple, ductile, low

cost, and therefore, easily accessible and with enormous possibilities for application.

It is not by any chance that the two researchers to whom it generally attributed the discovery,[4] that is Charpentier and Doudna, they have been granted prizes such as in 2015 the prestigious Breakthrough Prize; they have won the Gruber Genetics Prize also in 2015; the Oreal-Unesco Award for Women in Science, in 2016; and the Japan Prize in 2017. In 2015, they were included by the "Time" magazine in the listing of the one hundred more influential persons in the planet: CRISPR-Cas9, besides, resulted in the first place in the classification of scientific achievements of the year 2015, performed by the "Science" magazine.

The field of application of this technique is spreading out very rapidly. It will suffice to consider that in 2013, CRISPR-Cas9 has been the object of 282 scientific publications; in 2014, more than 600 have been added, and more than 1,200 in 2015, surpassing altogether the amount of 5,000 in 2017 [6].

It is impossible to account for, in this site, of all the applications of which this system has been an object. Just to site a few examples, CRISPR-Cas9 has been used for the reproduction of mini-pigs, super strong beagles, long hair goats, or for the production of anti-allergic hazelnuts, of grape seeds resistant to the peronospore, or of rapeseed resistant to the sulfonylureas. CRISPR-Cas9 is also at the center of numerous experiments to give life to mosquitoes capable of eliminating malaria, bovines more resistant to tuberculosis; of studies about the xenotransplantation and of a series of researchers in animal models for the autism cure and neurodegenerating illnesses [6].

The news regarding the first application of the CRISPR-Cas9 system in men, goes back to April 18, 2015, when a group of scientists, coordinated by the Chinese Junjiu Huang of the Sun Yat-sen University of Guangzhou, announced having used it in 86 frozen human embryos, for the purpose of correcting the muta-

tion that causes beta thalassemia. The results nevertheless have been deceiving –as it has been admitted by the research team itself–; as far as the 86 injected embryos, only 28 have been immunized, but with the presence of numerous *off-Targets* cuts, of which there could have originated other illnesses.

If the researchers would have declared that their intention to make developments of embryos beyond the fourteenth day (which is the actual limit after fertilization, within which, researches in human embryos produced *in vitro*, can be performed, which is foreseen by the international regulations as a consequence of the "Warnock Report" of 1984) and used embryos with anomalies, and thus not aimed at the implantation, the experiment has raised numerous controversies. On the other hand, before being published in the "Protein & Cell" "magazine" [18], the study would have been rejected, partly due to ethical character motivations, either by "Nature" as well as by "Science", but anyway they would have received the approval of the local ethics committee.

At the bottom of the decision of the two prestigious magazines has been, truly, the intention to preserve themselves from eventual controversies, regarding the spreading of research results about the application in men of a technique around which many doubts and perplexities were growing.

A few days before (March 12, 2015) of the announcement by Huang, an editorial [19] was published in the "Nature" magazine, signed by five eminent scientists (Edward Lanphier, Fyodor Urnov, Sarah Ehlen Haecker, Michael Werner y Joanna Smolenski), in which an international moratorium was asked to the application of genomic *EDITING* to cells of the germinal human line. The moratorium was relaunched a few days later (on April 3, 2015) in "Science" [20] by another group of scientists (among which there were Paul Berg and the Doudna herself).

The international [21] SUMMT, was held on December 2015, organized –as it has already been reminded– by the U.S. National

Academy of Sciences, the English Royal Society and the Chinese Academy of Sciences, to discuss about the ethical questions linked to the use of *Human Gene Editing,* an event which has represented a kind of reediting in modern times, of the Asilomar Conference.

Notwithstanding the making known of a whole series of fears and perplexities, other intents of application of the system CRISPR-Cas9 in men have been performed. A year after the Huang announcement, in the "Journal of Assisted Reproduction and Genetics" [22] the results of the study were referred by a group of Chinese researchers of the Guangzhou Medical University, which has seen the intent of immunize from HIV 26 human embryos which were not aimed for implantation. Also in this case the results have been disappointing, as long as the wanted mutation has been found only in 4 of the 26 embryos injected with CRISPR-Cas9.

Numerous controversies have been generated [3; 23] due to the authorization granted, in February of 2016, by the English Authority for embryology and human fertilization (Human Fertilization and Embryology Authority, HFEA) to the research group lead by Kathy Niakan of the Francis Crick Institute of London, to perform a study where the CRISPR-Cas9 system would be applied in healthy human embryos, in order to understand the role of the genes implicated in the first stages of the embryonic development: the experiment foresaw in particular, to deactivate by means of the CRISPR-Cas9, one at a time, the zygote genes for the purpose of understanding which one is determinant and which is not, and eventually explain the reason.

Lastly, it is worthwhile to mention the first clinical experimentation based on CRISPR-Cas9: pointed out in November 2016 by "Nature" [24], which has been performed by a group of oncologists of the Sichuan University at Chengdu in China and has seen the provision of cells modified with CRISPR-Cas9 to a patient affected by an aggressive form of lung cancer.

4. Ethical issues

As it has been seen, control alterations in the genome are possible by means of the various techniques, since the 70's CRISPR-Cas9 and the other technologies of *Genetic EDITING* do not represent other thing but a new method to perform operations, that since long ago have been carried out, or since long ago have been projected, about whose moral legality, bioethics has widely asked itself already, and which are regulated by a whole series of international standards. The NBC points out that «the novelty does not consist much in the idea, but rather in the molecular assembly» [5, p. 5].

Moreover, it cannot be avoided to consider the enormous perspectives of intervention that these new techniques, thanks to their increased accessibility, precision and versatility characteristics, seem to be capable to open. It has to do with developments on which it will undoubtedly necessary to think in a specific manner during the next years, as soon as they are verified, under a strict control, because already in the actual situation do not seem to be covered by a proper theoretical thinking.

In this moment, maybe the only new issue from the ethics point of view –that it is already having its regressions in the judicial aspect– which is related to the particular natural mechanisms (link of non-homologous limbs, and of homologous recombination) over which the functioning of these techniques is based on. The "naturalness" of these processes makes possible that one of the characteristics of the *EDITING* methods, is that there should not remain a mark of the alterations performed by them to the organisms. This puts into a crisis the actual assessment of the one that could be considered a genetically modified organism (GMO), that, as it is well known, leans on the distinction between natural DNA alteration processes, and processes induced by men.

As said by the specialists, the lab analysis of the modified plants with the CRISPR-Cas9 would not be, for example, capable of revea-

ling the presence of genes not belonging to its species. Furthermore, a technique based in the CRISPR-Cas9 has been prepared, thanks to which genetically modified plants do not become transgenic in any phase of their production, and they are not distinguishable of plants which naturally present the same mutations [25].

The argument is complex and, for its assessment, would be necessary a thorough technical-scientific understanding of the differences occurred among the induced mutations, by means of the foregone techniques of genetic engineering and the ones of *EDITING*. It is not by chance that the European Union, has not yet expressed a statement about the consideration of the organisms treated with *EDITING* methods as the GMO while the US Department of Agriculture would have spent more than a year to establish that a fungus treated with CRISPR-Cas9 for not obscuring itself, cannot be considered a GMO [26].

In any event, this issue is aimed to open then a debate about which have to be considered as genetic mutations produced by nature and genetic mutations induced by men, between "natural" and "artificial", or at least to its greater problematization.

Making an exception to this issue, in the actual situation, the use of *genetic EDITING* techniques does not seem to produce new ethical issues. A proof of it is the fact that the main document of bioethical issues which, have confronted the argument (immediately retaken briefly) mainly consists –if that has a value– in reaffirming recommendations/positions shared already for long time by the international scientific community. In an extreme synthesis, it has to do, in particular, with the prohibition of doing experiments in gametes and at the conception and of human embryos aimed at the implantation, in the promotion of research in somatic human cells and of a counter-position about the legality of doing experiments in lab in gametes not aimed at reproduction, and in embryos in vitro not aimed to the implantation.

One of these documents is represented by the final statements of the already mentioned international SUMMIT about the *human*

gene editing of December of 2015, that for its importance it is worthwhile to mention it in full:

1. Preclinical and basic research. An intensive preclinical and basic research is clearly necessary, and should be referred to, in the area of proper standards and of a judicial and ethical supervision to: (i) the technologies to modify the genetic sequences in human cells; (ii) the potential benefits and the risks of the proposed clinical uses and (iii) the understanding of the human embryo's biology and of the cells from the germinal line. If in the research process the human embryos in the first stages of development, and the germ cells suffer a genetic modification, the modified cells should not be used to produce a pregnancy.

2. Clinical use: somatic. Many promising and valid clinical applications of the genetic EDITING, are aimed to alter the genetic sequences only in the somatic cells that is in the cells whose genomes are not transmitted to the next generation. Examples already offered include the genetic EDITING for the sickle-cell anemia in blood cells, or to improve the capability of the immune cells to attack cancer. There exist the need to understand the risks, such as an inexact EDITING, and the potential benefits of all genetic modification that is proposed. In as much the proposed clinical uses are aimed to modify only the individual which receives them, it can be rigorously and opportunely assessed within the standard frames for the existing gene therapy and in a continuous evolution; and in the approval of clinical studies and therapies, the regulatory authorities could calculate the risks and the potential benefits.

3. Clinical use: germinal line. The genetic EDITING could be used, in line from the beginning, also to provide genetic modifications in gametes or embryos, which would cover all the resulting baby cells, and will be transmitted to the following generations as part of the human genetic inheritance. The examples that have been provided go from the prevention of serious hereditary illnesses to the "improvement" of human capabilities. Such modifications of the hu-

man genomes could include the introduction of natural variants or of genetic changes totally new thought to the useful. The germinal line modification puts several important issues, among which: (i) the risks of an inexact EDITING (such as mutations out of the prefixed target) and the incomplete modification of the embryo cells in the initial phase (mosaicism); (ii) the difficulty to foresee the harmful effects that the genetic changes can imply, in the light of a vast range of situations which are encountered in the human population, including the interactions with other genetic variances and with the environment; (iii) the obligation to take into consideration the implications both for the individual as well as for the future generations which will carry the genetic alteration; (iv) the fact that, once they are introduced in the human population, the genetic changes would be difficult to remove, and they would not remain inside a particular community or country; (v) the possibility that permanent genetic "improvements" for some subgroups of the population, could sharpen the social inequalities, or they could be used cohesively; and (vi), the ethical and moral considerations of the intentional alteration of the human evolution, made with this technology. It would be irresponsible to proceed to any clinical use whatsoever of the modification of the germinal line, unless, and until when, (i) the relevant problems of security and efficiency, would have been solved, based on a proper understanding and a balance of risks, potential benefits and alternatives, and (ii) there exists a wide social consensus about suitability of the proposed resource. Besides, any clinical use should proceed and be executed only under a proper regulatory supervision. In today's situation, these criteria have not been respected by any clinical use proposed: the problems of security and safety have not been yet properly explored; the convincing cases of greater benefit are limited; and many nations have legislative or regulatory prohibitions regarding the issue of modification of the germinal line. Nevertheless, given that the progress of scientific knowledge, and the points of view

of society evolve, the clinical use of the modification of the germinal line, must be reconsidered periodically.

4. The need for a permanent forum. While every nation has in the end, the authority to regulate the activities under their own jurisdiction, the human genome is shared by all nations. The international community should dedicate itself to establish the standards relative to the acceptable uses of EDITING of the human germinal line, and harmonize the standards, for the purpose of demotivate the unacceptable activities, and to make advances in the human health and wellbeing.

Therefore we ask the National Academies which have participated in the vortex –U.S. National Academy of Sciences, U.S. National Academy of Medicine; Royal Society; Chinese Academy of Sciences– of taking the initiative for the creation of an international forum in order to discuss the potential clinical uses of the genetic EDITING; of concurring in order to inspire the decisions of national politicians and of others; to formulate recommendations and guiding lines; and to promote the coordination among nations. The forum should be open to all nations, and inspire a vast range of perspective and competences, including those from biomedical scientists, sociologists, ethical experts, sanitary operators, the patients and their families, disabled people, politicians responsible for the regulatory entities, research financiers, religious leaders, groups carrying instances of public interest, industry representatives and members of the public in general.[5]

The international work group, has been effectively settled and has already produced a big volume presented in Washington in February 2017 (*Human genome editing; Science, Ethics and Governance*), which represents a kind of "global" guiding line in order to carry out experiments that foresee the use of genetic EDITING techniques.

Another document is the *Report of the IBC on updating its reflection on the human genome and human rights* [2] of the UNESCO's International

Bioethics Committee, published on October 2, 2015, that reaffirming the human genome value as "heritage of humanity", underlines the impossibility to arrive to a shared position at the international level about the experimentation in labs with genetic EDITING techniques in gametes not destined to the reproduction and in the *in vitro* embryos not destined to the implementation.

Furthermore, on January 11 of 2016, the European Group on Ethics in Science and New Technology (EGE) has approved a *Statement on Gene Editing* [28] favorable to the moratorium about the *Genome Editing* with reproductive purposes of the embryos and gametes, but with some Distinctions by some of the members of the committee about the legality of the investigation based on gametes not destined to the reproduction and in embryos *in vitro* not destined to the implantation.

Another declaration (*Statement on genome-editing technologies)* [29], is the one issued on December 2, 2015, by the Bioethics Committee of the European Council (DH-BIO) in which the international scientific community has been summoned to perform experiments of *EDITING*, according to what it is foreseen by the *Oviedo Convention.*

It has already been mentioned the *report* (*Genome editing: an ethical review*) [1] del Nuffield Council on Bioethics of September, 2016. More specifically, it is a heavy document of identification and precise description of all the ethical issues related to the use of genetic EDITING technologies. In the summary, the issues have been catalogued according to three typologies: issues to be taken care urgently (the ones related to the use of the human germinal line, and those related to an up bringing); issues to be considered in the next future (those related to the liberation in nature of species modified by these techniques, and related to the xenotransplants); issues to be considered (the ones related to research in human somatic cells, to plants and to ulterior uses, as for example the production of weapons). A second document coming from the English organism is expected which deals with the standard profiles for the use of the techniques.

Lastly, it is worthwhile to mention the final recommendations of the opinion (The genetic EDITING and the CRISPR-Cas9 *technique)* [5] coming from the NBC, on February 23, 2017. The Committee has declared itself in favor or the experiments *in vitro*, and the use of *EDITING* techniques in animals, for the purpose of testing safety and efficacy, and to the research in human somatic cells; also against all that concerning experiments in gametes aimed to the conception, and in human embryos aimed to the implantation, concurring about the suitability of the moratorium about the clinical research or the research *in vivo,* until safety and proper efficacy conditions are reached; finally it has expressed conflicting positions related to experiments in labs on gametes not aimed to the reproduction, and in embryos *in vitro* not aimed to the implantation, considered ethically legal by some people, but illegal by others.

5. Conclusions

Nucleases with a zinc finger, TALE nucleases, but above all CRISPR-Cas9 (the so called genetic *EDITING* techniques, have made simpler to control the alterations in the genome. The CRISPR-Cas9 system, in particular, it has shown to be extremely favorable in terms of accessibility, efficiency and versatility.

From an ethical point of view, in today's situation, the use of these techniques do not seem to generate new ethical issues. The only exception would have to be given by the particular type of induced mutations through these techniques, which cannot be distinguished from those produced by nature. This characteristic is generating difficulties in the classification of the OGM obtained from these techniques, and which is worthy of inside deepening especially all that, coming from the technical-scientific point of view.

An analysis of the most important documents in bioethics about the *EDITING* methods, fundamentally reveals the rejection of positions already known in this subject matter: in extreme synthesis, it

has to do with the prohibition of experimentations in gametes aimed to the conception, and in human embryos, aimed for implantation, of the promotion of research in human somatic cells, and of an opposing position about the legality of doing experiments in labs in gametes not aimed to reproduction, and in *in vitro* embryos not aimed to implantation.

The application perspectives that the genetic *EDITING* open are, never the less enormous and, as for any kind of technology, hardly predictable. The legality of each one of these applications will be in the future, as always happens, specifically assessed, "putting it under test", based on the standards in force, which in the case of showing themselves improper, they should be optimized/reconsidered.

Biblilography references

[1] Translation checked by the authors.

[2] During the preparation and writing of the present paper, the National Committee for the Bioethics (NCB) has apparently published L'editing *genetico e la tecnica* CRISPR-*Cas9: considerazioni etiche* [5] that we were able to include in this analysis. A few days after the issuing of the opinion, the first monography in Italian about the topic, has also been issued, written by the science journalist Anna Meldolesi. The book, titled *E l'uomo creò l'uomo.* CRISPR *e la rivoluzione dell'editing genomico* [6], constitutes a punctual reconstruction of all the controversies that have led to the configuration of the CRISPR-Cas9 and the status of the issue about its application, and also looks on some notes of bioethical nature. The book has been particularly useful in the revision stage of this paper in order to confront some information about the CRISPR-Cas9 system.

[3] Only during 2015 the "Addgene" Company has sold more than 60,000 molecular *TOOLS* based on the CRISPR [14] system.

[4] Charpentier y Doudna are two researchers, on ARN studies and coincidentally they have met in a MEETING in Puerto Rico in 2011; immediately after it they began a scientific co-work, that has led for them to be the first ones in making an announcement in an article 2012 [17] of the possibility to take advantage of the CRISPR-Cas9 system in order to edit the genome. Nevertheless, it in progress a legal controversy with the Chinese Feng Zhang, about the rights of intellectual property of the discovery.

[5] Translation taken from the site http://www.lescienze.it/news/2015/12/07/news/editing_genetico_ricerca_cautele-2884732/ (access of 8.4.2017).

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