Gene editing and germ line modification. Medical and ethical debate

Genetic modification of the human germline that will pass to children, and to future generations, has sparked a lively debate in the international scientific community.

Genome editing consists of the modification or removal of specific DNA sequences in order, for example, to correct a disease-causing mutation. Early approaches were based on the recognition of specific sequences using oligonucleotides, small molecules or self-splicing introns. New techniques were later developed based on DNA sequence recognition by proteins, such as site-directed zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). These methods are based on the binding of a protein DNA cleavage domain to a zinc finger or TALE DNA binding domain, respectively, which has been modified to target the desired DNA sequence. However, difficulties in the design, synthesis and validation of these proteins are an obstacle to the widespread adoption of these artificial nucleases.

The use of CRISPR/Cas9 to modify the human germline could discredit the great tool of gene editing

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CRISPR technique

Genome-editing technique is CRISPR (clusteredregularlyinterspaced short palindromicrepeat)/Cas9,which is based on a system discovered in bacteria that gives them adaptive immunity against viruses. In natural bacterial systems, some genome sequences of viruses that infect the bacterium are incorporated into the bacterial genome between the CRISPR sequences, so that if the same virus attacks it again, the bacterium produces an immune response that includes a copy of the "remembered" sequences, called crRNA, to bind to the virus DNA, and a second RNA, called tacrRNA, which recruits a Cas endonuclease to cut the virus DNA. The technique, patented by Jennifer Doudna and Emmanuelle Charpentier in 2012, consists of modifying the tacrRNA:crRNA pair as a single guide RNA (sgRNA) with a sequence at the 5' end that determines the DNA target sequence and a duplex RNA structure at the 3' end, which binds to Cas9. Thus, the leader sequence can be modified to carry the Cas9 endonuclease to any DNA sequence. This method is simple, cheap and effective, so that the scientific community hopes to use it in a wide range of applications.

Human Germline Modification

Germline modification in mice. However, as the potential applications of genome editing grow, risks appear which must be rigorously analyzed,primarily the real possibility that these techniques, especially CRISPR/Cas9, could be used for genetic modification of the human germ

line, i.e. the introduction of a foreign DNA in gametes or the early embryo, which will be passed on to children, and to future generations. This possibility has sparked a lively debate in the international scientific community. In order to discuss the scientific, medical, legal and ethical implications of these advances in the field of genome editing, Doudna convened a meeting with scientists, ethicists and lawyers in Napa (California) in January 2015, whose conclusions were published in Science in March (read NATURE *Don't edit the human germline)*

Four immediate steps were identified:

1. Strongly discourage any attempts to genetically modify the human germline for use in clinical practice until all social, environmental and ethical issues have been discussed among scientific and governmental organizations;
2. Create forums in which scientists and bioethicists can provide information in this field;
3. Encourage and support transparent research to evaluate the efficacy and specificity of these technologies;
4. Convene a globally representative group of experts in genetics, lawyers, ethicists, members of the scientific community, the public, and relevant government agencies and interest groups to further consider the issues surrounding the use of these new techniques, and to propose recommendations for their regulation.

However, in April, a group of Chinese researchers published an article, in which they reported having edited embryo genomes using the CRISPR/Cas9 technique. Although the researchers used non-viable zygotes with three pronuclei, the publication has generated considerable controversy. Also in April, another article was published describing the use of TALENS for the removal of mutations in mitochondrial DNA in the germline.

Genome editing. Implications of germline modification

The implications of germline genetic modification have already been widely discussed in the literature on gene therapy. This has led to widespread acceptance of the technique when its action is directed to somatic cells, being considered comparable to surgery since it does not alter the global genome and is not transmitted to offspring, but to generalised disapproval when it is directed to gametes or embryos, as the risks are very difficult to predict. However, it is expressly prohibited in only 25 countries, 15 of which are European.

Genetics and bioethics opinion

It should be noted here that the implications of acting on nuclear DNA (nDNA) are not the same as acting on mitochondrial DNA (mtDNA). As far as we are aware at this point, mtDNA is only responsible for the production of cellular energy, and has no effect on the phenotype, so its modification does not raise the same ethical issues as modifying nDNA, which could alter essential characteristics of the individual. One of the main risks arising from the modification of nDNA in the germline is that phenotypic consequences for the individual could be harmful, since we still know very little about how the genetic background affects the various allelic variants. This is compounded by the fact that the modification carried out will be transmitted to offspring. Another serious risk posed by lifting the ban on modifying the germline is that this could be exploited for non-therapeutic use, i.e. for human "enhancement".

The use of CRISPR/Cas9 to modify the germ line has also been criticised, because it could discredit technology which is highly promising in other fields of application. In addition, it presents its own challenges in this field. First, the modification efficiency depends not only on the target sequence and cell type, but also on the type of change to be made, a base deletion

or correction, being much less efficient in the second case. Another major challenge is the appearance of off-target changes, i.e. outside the target sequence, which can give rise to additional problems. In fact, in the article in which the technique is described, the authors reported that, of the 54 modified embryos that were analyzed, only 4 contained the desired genetic material, and that the number of unwanted mutations introduced by CRISPR/Cas9 elsewhere in the genome was extremely high. Additionally, genetic mosaicism that could result from the division of the germ cells before Cas9 completes its action, or from residual endonuclease activity, would prevent the desired changes from being present in the appropriate adult tissues or at the correct levels to result in a healthy phenotype. Finally, this use of CRISPR/Cas9 is said to be actually worse than in-vitro fertilization with preimplantation genetic diagnosis for obtaining healthy embryos in cases where the parents are carriers of a mutation.

Considering that in-vitro fertilization would be required to apply these techniques in the germline, there does not really appear to be any justification for their application for this purpose.

Update of human *germline* genetic *modification debate (5/11/2018)*, there is a range of policies, from permissive ones in China to intermediate ones in the US and ... see HERE.

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