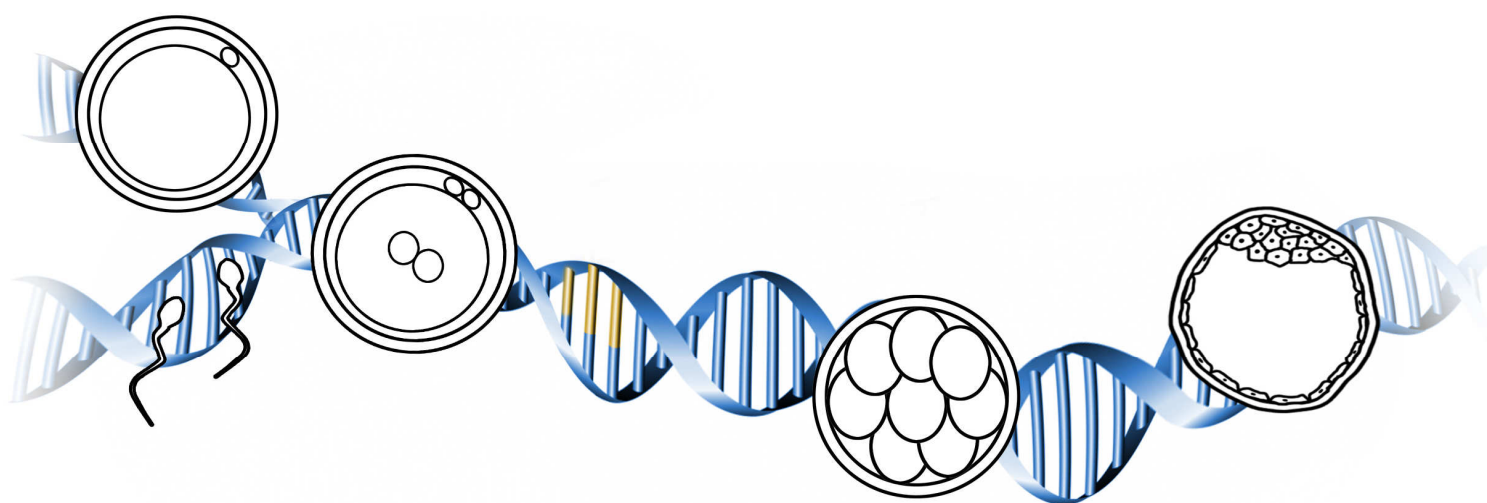




ACADÉMIE NATIONALE DE MÉDECINE



Genetic editing of human germline cells and embryos

April 2016

In 2015, the “Académie Nationale de Médecine” appointed a Working Committee* to discuss research and the medical implications of altering the human germline and early human development. The specific objective was to identify potential medical indications for these new molecular genetics methods, to assess the risks and uncertainties of their use based on current knowledge, and to consider the ethical issues they raise. The resulting report has been submitted to the plenary session of the Academy on April 12, 2016 and has been adopted: 50 votes in favor, 20 against, 14 abstentions.

Abstract: Interventions causing genome modifications that can be passed on to descendants have been prohibited in France since 1994. New methods, such as CRISPR-Cas9, have been developed, raising questions about their potential use on human germline cells and embryos. The only acceptable medical indication would be to prevent the transmission of a disease gene to the child. However, the necessary conditions have not yet been met for this technology to be considered for clinical use, particularly as concerns the efficacy and safety of these methods. There are also other ways for couples to achieve the goal of having children. The ethical questions raised by these technologies will require multidisciplinary discussions within the wider debate on all assisted reproductive technology procedures, which may affect the genome of the unborn child, and, possibly, of subsequent generations. However, this research, including that on germline cells and human embryos, should be carried out provided that it is scientifically and medically justified.

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1 Introduction

There has been tremendous progress over the last 50 years in our understanding of the role of gene expression in cellular function and dysregulation. Initially, tools were developed to unravel the genome, generating a wealth of information on genetic variations and mutations underlying diseases. The next step of the research effort was to engineer the DNA for experimental or therapeutic purposes, by preventing, adjusting, or modifying gene expression. Clinical trials were progressively undertaken to treat patients with hereditary diseases and certain forms of leukemia and lymphoma [1, 2].

New molecular tools have recently been developed, based on the bacterial defense system against exogenous DNA (bacteriophages or plasmids), the CRISPR (clustered regularly interspaced short palindromic repeats) system [3]. A guide RNA, coupled to an endonuclease (Cas9), precisely targets any specific sequence in the genome. Then the two strands of DNA are cut and, depending on the application, the targeted fragment of the DNA molecule is removed or replaced by inserting a new DNA sequence. The modified DNA molecule is then repaired by one of the two DNA repair systems present in all cell types: homologous directed recombination (HDR) or non-homologous end-joining (NHEJ). CRISPR-Cas9 has proved to be more effective than previous methods (TALEN, zinc finger nuclease, meganucleases). It is relatively simple to perform and affordable, explaining its rapid spread to many laboratories since its discovery [4-7]. There is considerable potential for further improvement, as for any new technology, including the recent use of bacterial nucleases with different modes of action [8, 9].

CRISPR-Cas9 research has been conducted exclusively in animal models or cell cultures. Clinical applications for this technology have been suggested for repairing single-gene disorders, treating cancer, or inducing potential therapeutic or protective effects against infectious diseases [10, 11]. These applications of CRISPR-Cas9 technology would be appropriate only for somatic cell-based gene therapy [12]. In 2015, a Chinese peer-reviewed publication made waves because it raised the possibility of editing the genome of human embryos. The purpose of this research, using triploid and therefore non-transferable embryos, was to determine to what extent the CRISPR/Cas9 system could be used to replace the mutated β -globin gene responsible for thalassemia [13]. The results were unconvincing, demonstrating low efficiency for modification of the target gene, with numerous undesirable modifications. Reactions were heated, criticizing the approach, and calling for a moratorium or even a ban on any research aiming to modify the genome of human embryos [14, 15]. In addition to the scientific discussion about the effectiveness and safety of the method, ethical and social concerns have been raised about the use of the technique for trivial or eugenic practices (the “slippery slope” argument) [16]. The presence of the induced DNA modifications in all cells, including those of the germ line, leading to their being passed on to subsequent generations, was also of concern while many uncertainties about unintended consequences remain.

2 Legislative, institutional and organizational environment

In accordance with French law, interventions seeking to introduce any modification into the genome of any descendant have been clearly prohibited since 1994. Article 16-4 of the Civil Code states: “... *Without prejudice to research seeking to prevent or to treat genetic diseases, no alteration can be made to genetic characteristics with the aim of modifying a person's offspring*”. In addition, the Oviedo convention, ratified by France in 2011 and by most European countries, clarifies, in article 13, that “*in every case, any intervention which aims to modify the human genome must be carried out for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants*”. Finally, although the Universal Declaration on the Human Genome and Human Rights published by UNESCO in 1997 does not explicitly apply to alteration of the human genome, the International Bioethics Committee of this institution published a report on October 2, 2015 specifying: “*The international community of scientific researchers should be entrusted with the responsibility of assessing and ensuring the safety of procedures that modify the human genome. A thorough and constantly updated investigation on all the consequences of these technologies is required.*” and that “*It is important for States and governments to renounce the possibility of acting alone in relation to engineering the human genome and accept to cooperate on establishing a shared, global standard for this purpose.*”

The international position on this topic is heterogeneous, but many countries have taken steps to prohibit any modification of the genome that can be transmitted to subsequent generations through human germline cells. In addition, the provisions concerning genomic changes to the human germ line may become unclear when combined with other provisions concerning embryo research or genetically modified organisms (GMOs) in general [17].

Since the publication of the article by Liang *et al.* in 2015 [13], individual statements and concerns have been increasingly voiced. These statements come from scientists, scientific societies, but also from governments.

In the U.S., the NIH stated that no clinical research protocols “using germline gene transfer” will be considered. On May 26, 2015, the White House declared that it supports any serious evaluation of the ethical issues in the field, while emphasizing that the modification of the human germ line for clinical purposes is a limit that should not yet be crossed. The U.S. National Academies of Science, Engineering and Medicine have launched a joint study in December 2015, with an international summit at which the scientific, ethical and political issues raised by “genome editing” were examined.

Professional societies, such as the Society for Developmental Biology, have called for a moratorium on any manipulation of the preimplantation human embryo by “genome editing” [18]. The International Society for Stem Cell Research has also called for a moratorium restricted to clinical applications, with rigorous scientific studies evaluating the risks of the

method undertaken as part of a broad public discussion on the societal and ethical implications of the technique [19]. A view similar to that of the International Society for Stem Cell Research was also expressed in a review of EMBO Reports on this topic [20]. The Hinxton group formulated the same recommendation, while recognizing that once the questions of efficiency, safety, and governance have been settled, morally acceptable applications of the technology could be found in the field of human reproduction [21].

In the United Kingdom, the five principal agencies responsible for biomedical research, all of which believe that this technology has potential clinical applications, declared that they would support preclinical research using "genome editing", including the human embryo and germline cells. [22]. The Nuffield Council on Bioethics has also established a working group on this topic. In Germany, the government allocated funds for a debate on this issue. The Federation of European Academies of Medicine will organize a workshop in 2016.

3 Challenges of human genome editing techniques that can affect the germline

3.1 Potential clinical applications

This technique could be used to prevent the transmission of a particularly serious hereditary disease to a child, if the causal genetic abnormality has been clearly identified and there is no treatment. The transmission of monogenic changes to the child can be avoided by prenatal diagnosis, potentially followed by medically assisted termination of the pregnancy or by preimplantation genetic diagnosis (PGD), generally performed on the third day of embryo development (8-cell stage). This makes it possible to transfer only embryos not carrying the genetic defect responsible for disease into the womb [23]. There are, however, rare cases in which PGD is not the answer to the problem. For example, if one of the two parents-to-be is homozygous for a dominant autosomal disease (Huntington's chorea), or if both parents-to-be are homozygous carriers of a recessive autosomal disease (cystic fibrosis), PGD cannot be used. In addition, some homoplasmic mutations of mitochondrial DNA (as in Leber's hereditary optic neuropathy) also prevent the use of PGD.

Other cases, in addition to these indisputable but rare occurrences, are those of couples who tried PGD but for whom no embryos could be transferred. Among the 119 PGDs performed at the Necker-Antoine Bécclère Center between 1-1-2015 and 11-15-2015, embryo transfer was not possible for 22 couples (18%). In most cases, all the analyzed embryos were affected by a genetic disease. For these couples, PGD was suggested because of an autosomal dominant disease (11), autosomal recessive disease (8), an X-linked disease (2), or an abnormality of the mitochondrial DNA (1). The concerned patients often asked whether embryos could be "treated" rather than destroyed. This request from patients could become increasingly common [24].

Even if modifying the genome of embryos to avoid transmitting a genetic disease to a future child were acceptable, this approach is not currently clinically feasible (see below). It is also

important to note that there are other ways for couples to achieve their parental objectives: adoption, gamete donation, embryo donation, with all these options being legal and often used in France. Finally, one of the possible outcomes of research using methods such as CRISPR-Cas9 is the development of somatic gene therapies that could benefit the affected child after birth.

Other indications could become integrated into an approach aiming to reduce the risk of common diseases developing or to "protect" the individual, sometimes referred to as "medical indications". This approach has also been described as "transhumanism" by some, because it could be used to "enhance" the human being. There are, indeed, natural variants of the genome that can play a strong "protective" role against diseases such as diabetes (SLC30A8 gene), hypercholesterolemia (PCSK9 gene), and some viral infections (CCR5 gene). The introduction of these variants into individuals, by targeted modification of the germ line, could be viewed as "protection" [25]. The elimination of the e4 allele of the APOE gene may also decrease the risk of developing Alzheimer's disease [26]. Targeted modification of these variants would, thus, be a way to improve the performance of humans, by making them less vulnerable to certain diseases. However, this approach may have some flaws, because we do not yet completely understand the various roles played by these variants. For example, it has been suggested, but not yet confirmed, that the APOE e4 allele is associated with better memory in young adults [27].

Countless interventions on the human genome would be required to introduce new qualities because of the number of genes involved and the number of disease to which humans are susceptible, such as cardiovascular diseases, cancer, neurodegenerative diseases and infectious diseases. In addition, several identified genetic variants are poorly correlated with phenotype, are generally not the main variants involved (these variants often remain unidentified) and often interact with other genes. Their modification in the human genome would make such physiological changes to prevent the occurrence of disease completely illusory.

Finally, this strictly genetic view of disease ignores other factors involved in disease susceptibility and development, and other existing ways to prevent or treat disease. Thus, the limitations of a project involving the targeted modification of the human genome to generate a "superman" are apparent, and such a project belongs to the realm of science fiction.

Would it be possible to modify the genome of germline cells or the embryo to favor specific characteristics or traits in the unborn child? All new technologies developed for intervention in the process of human procreation (from *in-vitro* fertilization (IVF) to PGD) over the years have been debated and contested because they might be used to satisfy the aspirations of parents and physicians wishing to create "designer babies" or to promote a new form of eugenics. This threat has been raised again by many since the description of techniques for the effective modification of DNA structure, particularly since the publication of the article by Liang *et al.* on human embryos. [13]

The risk of misusing these technologies to modify the genome of the embryo to choose the

physical or other early characteristics of the unborn child cannot be ignored, but success would be far from guaranteed in such a project. Indeed, most features would be unpredictable on the basis of a simple DNA modification during embryonic development for several reasons. First, the genomic structure of an individual is not stable throughout development [28]. Second, the expression of a gene is usually controlled by the expression of other genes and by epigenetic and/or environmental factors. There is, thus, no simple and direct relationship between the nucleotide sequence of an embryo and the phenotype of the resulting child, although some variants could clearly lead to striking phenotypic changes.

The other risk is that changes might be made to the germline genome to "improve" humans or "enhance" their performance. This issue, raised by the theoretical possibilities offered by CRISPR-Cas9 and similar techniques, is covered by the broader debate on the evolution of medicine. Medicine is increasingly being asked to act outside its traditional mission of care. It now intervenes to replace faulty organs, tissues, cells, or genes. It can also intervene, with various degrees of invasiveness, to stimulate function or to improve performance in many areas, such as sports or military activity. These various forms of intervention have been facilitated by considerable technological progress. How do the new techniques altering DNA structure fit into this context? A classification of all technologies used to "enhance" the human body into four categories, according to their scope and duration, has been proposed [29]:

- Localized and temporary or reversible actions, such as the use of removable prosthesis;
- General and temporary actions, such as the use of doping agents;
- Localized and definitive action, such as some somatic gene therapies;
- General and definitive action, such as germline gene therapy.

This last category is the most interventionist, and would affect not only the "treated" individual, but also their progeny.

3.2 Mode of action

For decades, the modification of animal genomes at the embryonic stage in the laboratory has constituted an important area in basic science. This type of research has helped to identify genes correlated with a given phenotype, to create models of human diseases, and has been used for agronomic purposes [30]. The yield and efficiency of these techniques have been greatly increased by the use of nucleases, and, more recently, the CRISPR-Cas9 system.

3.2.1 Targeted modification of the embryo genome

In the last three years, the birth of animals obtained after targeted modification of the embryonic genome by the CRISPR-Cas9 method has been reported for many species (Table 1). In most cases, the molecular material was microinjected *in vitro* at the zygote stage (first embryonic cell), either into the cytoplasm or directly into the pronuclei. Embryos were then transferred into the womb of surrogate recipients, either immediately or at the blastocyst stage. The number of live births was often very small. The aim was to abolish the expression of the targeted gene, to overexpress that gene or to modify it. In most cases, the desired genomic

modification was found only in a minority of newborn animals. Furthermore, mosaics were often observed. This incomplete modification of cells in the offspring may be explained by either the late action of CRISPR-Cas9 during embryonic development or by the genetic modification of the paternal and maternal alleles occurring at different times during the transition from gamete to embryo [31]. Finally, unexpected phenotypes often occurred in animals with modified genomes, particularly if the DNA molecule was repaired by non-homologous end-joining (NHEJ) as opposed to homologous directed recombination (HDR).

Authors	Species	Target gene	Transferred embryos	Newborn animals	Newborn with modified gene
Wu 2013 [32]	Mouse	<i>Crygc</i>	472	78	36 (46%)
Mizuno2014 [33]	Mouse	<i>Tyrosinase</i>	205	60	28 (46%)
Whitworth 2014[34]	Pig	<i>CD163</i>	93	4	4
		<i>CD1D</i>	110	4	2
Niu 2014 [35]	Cynomolgus monkey	<i>Ppary,Rag1, Nrob1</i>	83	2	2
Ménoret 2015[36]	Rat	<i>Anks3</i>	156	22	4 (18%)
Zou 2015 [37]	Dog	<i>Myostatin</i>	35	27	2 (7%)
Kou 2015 [38]	Ferret	<i>Dcx</i>	117	15	11 (73%)
		<i>Aspm</i>	64	12	8 (75%)
		<i>Disc1</i>	18	4	1
Crispo 2015 [39]	Sheep	<i>Myostatin</i>	53	22	10 (45%)
Honda 2015 [40]	Rabbit	<i>Tyrosinase</i>	67	9	2
Wang 2015 [41]	Sheep	<i>Myostatin, FGF5</i>	416	98	26 (26%)

Table 1: Genomic changes observed in newborn animals after the induction of genomic changes *in vitro* by the CRISPR-Cas9 method and microinjection at the zygote stage of embryonic development (one cell).

The introduction of the components of the CRISPR-Cas9 system into zygotes by electroporation has been suggested, because microinjection is technically difficult and potentially dangerous for the embryo. In mice and rats, electroporation and microinjection have yielded similar results [42-44].

No attempts at targeted modification of the embryonic genome appear to have been made after the zygote stage and before embryo implantation. There are, thus, currently no experimental results indicating that it would be possible to act on embryos subject to PGD, at the eight-cell stage or beyond. In any case, from this stage onward, microinjection into the

embryonic cells would be difficult. Could other vectors, such as retroviruses, which have been successfully used in other cellular systems [46] and in somatic gene therapy, be used at this stage? Again, there are no experimental results for embryos to suggest that such techniques would be useful.

3.2.2 Targeted modification of the germline genome

Another approach to modifying the genome in all the cells of a person would be to intervene before fertilization, by injecting the various components of the RNA-Cas9 complex into the mature oocyte (metaphase II) at the same time as the sperm cell or sequentially [32]. This technique of gene modification in oocytes has been experimentally tested in mice, to reduce the rate of mitochondrial mutation (using the TALEN system and not CRISPR-Cas9 as the endonuclease). However, in this experiment, there was no embryo transfer so no offspring [47]. Application of the technique at an earlier stage of oogenesis is not possible because the oocytes are not easily accessible in the ovary. In addition, oocytes in adult ovaries are at the meiotic prophase stage, with oogonial stem cells present only in the fetal ovary.

The requirements are different in males, because the stem spermatogonia can easily be removed from the postpubertal testis. The cells can be treated *in vitro* by CRISPR-Cas9 and cultured to ensure their proliferation and the formation of cell colonies, on which all the necessary controls can be performed before transferring the modified spermatogonia into the testis, where *in vivo* spermatogenesis will occur. This technique was successfully used in mice to correct a mutation of the *Crygc* gene (*Crygc*^{-/-}) causing cataracts. The modified gene was present in all newborns, which had a normal phenotype and in which no off-target effects were observed on whole-genome sequencing [48]. Other experiments in mice and rats have been less successful [49-50].

Indeed, it is necessary to transfer the modified spermatogonia into the testicles of a living animal to obtain gametes, because spermatogenesis cannot be reproduced *in vitro*. However, to ensure that all the produced sperm cells carry the desired genic modification, native germline cells present in the host testis must be eliminated, and this is not easy to achieve. This approach is useful for the production of transgenic animals or for studying male infertility of genetic origin. It is unlikely, however, that it could be used in humans to obtain "correct versions" of gametes in a homozygous carrier of a gene mutation, to prevent the transmission of a genetic defect to descendants. It would also be necessary to ensure that germline cell differentiation and maturation in the adult testis, and the *in vitro* processing and proliferation of these cells in culture did not lead to epigenetic abnormalities or genomic imprinting defects that might be transmitted to future generations.

3.2.3 Efficiency and safety of genome editing techniques in embryonic and germline cells

When the objective is to modify all the cells of an individual, regardless of the timing of genome engineering (before or after fertilization), the efficiency and safety of the method must be much higher than for an experimental approach, or even somatic gene therapy. When

cells are modified *in vitro*, the necessary checks on the efficiency and safety of the intervention can be made on colonies of the resulting cells with the required qualities, which are then transferred *in vivo* to act. In addition, when creating transgenic animals, not all the neonates need to be modified, because it is possible to select those with the required genic attributes after birth, for subsequent use.

In the case of genomic editing of a human embryo for a clinical application, failure of the intervention would be unacceptable since it could lead to the birth of children not presenting the desired modification. It would, thus, be necessary to check the efficiency of the intervention before transferring the embryos into the womb, at the blastocyst stage, for example.

It would also be necessary to check the safety of the method, including, in particular, the absence of off-target effects of CRISPR-Cas9. Recent results suggest that technological improvements, such as the use of CRISPR-Cas9 variants, could considerably reduce the risk of off-target effects [51], but the only experiment performed on human triploid embryos showed that unwanted effects were not rare. In the case of clinical application in humans, would it be necessary to sequence the whole genome of embryos before their transfer into the womb? Would it be necessary to look for only certain undesirable off-target effects? Would it be necessary to analyze the epigenome of the embryo?

Research may focus on the development of techniques in animals to address the requirements defined in humans, for subsequent clinical application. In addition, recommendations must be formulated concerning the tests to be performed to assess the safety of the technique for both somatic and germline gene therapy.

The use of genome modification techniques on embryos or germline cells, with the goal of creating a child, independently of any ethical considerations, is currently inconceivable. Much research still needs to be done and there is, thus, no reason to consider modifying French legislation forbidding germline gene therapy at the present time.

4 Research

Attempts to create a child with a modified genome are currently prohibited but is it necessary to forbid all research into germline modifications, including that on germline cells and human embryos? As stressed in the conclusions of the international meeting organized in December 2015 by the American Academies of Science and Medicine, the British Royal Society and the Chinese Academy of Science: "there is an urgent need for basic and preclinical research on (i) technologies for editing genetic sequences in human cells, (ii) the potential benefits and risks of proposed clinical uses, and (iii) understanding the biology of human embryos and germline cells" [52].

The law of August 6, 2013, lifted the ban on human embryo research previously in place in the French legal and regulatory framework. For a research protocol to be authorized, the scientific relevance of the research must be established, and it must have a medical purpose. In

addition, as specified in article L 2151-5 of the Public Health Code, the research must be performed "on embryos conceived *in vitro* within the framework of assisted reproductive techniques that are no longer required for planned parenthood" with the consent of the couple whose gametes were used to generate the embryos. According to the last assessment published by the Agence de la Biomedicine (ABM), on December 31, 2013, 19,335 frozen embryos stored in French *in vitro* IVF laboratories had been donated to research by 5,883 couples.

All the conditions have, thus, been met for this type of research to be carried out in France. It would, however, be advisable to clarify, possibly through changes to legislation, the persistent ambiguity in the following texts:

- Article 16-4 of the Civil Code, which excludes any modification of genetic characteristics with the aim of modifying the descendants but "without prejudice to research seeking ... to treat genetic diseases"

- Article L 2151-5 of the Public Health Code, which currently allows embryo research with appropriate authorization and under certain conditions.

- Article L 2151-2, introduced into the Public Health Code by the law of July 7, 2011, which forbids the creation of transgenic embryos.

The Working Committee agrees with the spirit of the law, which clearly distinguishes between any intervention aiming to modify the genetic characteristics of descendants and research which does not lead to the birth of a child with modified genetic characteristics. The first situation, which could be the consequence of a modification of the genome of germline cells or human embryos, followed by the transfer of the resulting embryos into the womb, is inappropriate in the present context as discussed above. On the other hand, research not leading to the birth of a child should be authorized, including that performed on germline cells or human embryos. Finally, the Working Committee considers that research in this field should be supported when scientifically and medically appropriate.

5 Ethics

5.1 Ethical questions relating to research performed on human germline cells and embryos

Research should be allowed if scientifically and medically justified, even if it leads to changes to the embryonic genome, provided that the embryos are not transferred into the uterus. This view is consistent with previous expert opinions emitted by the ANM on research on human embryos and embryonic stem cells [53-56]. Research must be strictly supervised in accordance with the disposition of French legislation and regulations.

Research that may destroy the embryo is authorized provided that the embryos were conceived *in vitro* and are no longer required for procreation by the parents, as stated in article L 2151 -5 of the Public Health Code. Embryos can be used for research if they are inevitably destined to be destroyed and if they are donated for this purpose by the couples concerned. Two types of embryos correspond to this situation:

- Embryos for which the nuclear, cytoplasmic or molecular characteristics are considered, in the days following *in vitro* fertilization, to be incompatible with development. These embryos are therefore neither transferred nor frozen. According to the last ABM report, 288,495 embryos were conceived *in vitro* in assisted reproductive technique (ART) centers in France in 2013, and 145,850 (more than half) of these embryos were not transferable or freezable [52]. Embryos found to carry a genetic or chromosomal abnormality on PGD, which are therefore neither transferred nor frozen (1,416 in 2013, according to the data of the ABM [57]), may also be used.
- Frozen embryos that are no longer desired for the purposes of procreation. This was the case for 2,744 frozen embryos in 2013 that were donated to research by 892 couples [52].

Research on germline cells poses no particular ethical issues provided that the gametes with modified genomes are not used for fertilization. Otherwise, it would be necessary to remove legal provisions prohibiting the creation of embryos for research, which the majority of the working group would consider to be undesirable.

5.2 Ethical issues regarding the clinical use of technologies that can edit the genome of germline cells and human embryos

These issues should be raised now, even if this use of such technology is currently forbidden and unforeseeable. They are of three types:

- Would it be lawful to modify an embryo before its transfer to the uterus?
- Can the intervention be invasive and alter the genome of the embryo?
- Should we exclude, as a matter of principle, any modification of the embryo with consequences that are potentially transgenerational?

The nature of the interventions to which embryos conceived through IVF can be subjected is regularly debated, particularly as the boundaries between care, study, and research are not always well defined. The Académie nationale de médecine has already stressed the obligation to ensure that the embryo receives the best care possible if it is destined to be implanted [53]. This position was recently confirmed by the Constitutional Council ruling on paragraph III of Article 155 of the "law modernizing our health system" of January 26, 2016 relating to biomedical research on gametes and embryos in the framework of ART. The Council considered that the provisions of this amendment could lead to "the prevention or cure of diseases in the embryo" and that clinical trials could therefore be conducted in this situation "for the benefit of the embryo itself" [58]. The "treatment" of an embryo carrying a genetic mutation could be seen as being covered by this disposition, and it would, therefore, not be unethical to treat it rather than destroy it.

The clinical use of CRISPR-Cas9, or a similar technology, would not be the only situation in which medical intervention would have consequences for the genetic constitution of the embryo and the unborn child. Since the advent of ART, diverse possibilities have emerged and have been implemented: choice of donor gametes on the basis of genetic criteria to reduce the risk of disease in the child, IVF by ICSI (intracytoplasmic sperm injection) with sperm from carriers of CFTR gene mutations or Y chromosome microdeletions, preconception screening or

selection of the embryo after testing to detect a disease allele, as practiced in the USA or Israel for the eradication of serious genetic diseases such as Tay Sachs disease or thalassemia, the use of PGD to select the sex of the child (banned in France but a common practice in the US), creation of embryos by transfer of the nuclear genomes of the future parents into the oocyte cytoplasm of a donor to prevent the transmission of mitochondrial diseases (project approved by the British parliament). These interventions are of different types and natures, but all have consequences for the genetic constitution of the embryo and the future child. This genetic dimension of ART should be the subject of comprehensive discussions to establish the conditions for the use of particular approaches and possible limitations. This reflection should involve all of the relevant stakeholders and society as a whole and should not ignore practices in other countries.

The transgenerational transmission of genetic changes with insufficiently or not well known effects is often considered to be a limit that should not be crossed. However, when an embryo carrying a mutation of the CFTR gene is deliberately created by ICSI, this genetic alteration is also likely to be transmitted to subsequent generations. The same applies to the transfer of embryos reconstituted to prevent the transmission of mitochondrial diseases into the womb, as planned in Great Britain. Once the efficacy and safety of CRISPR-Cas9 have been demonstrated, will this technique have additional disadvantages for future generations? Future discussions of this issue should not ignore the potential role of epigenetic and environmental factors during the period around conception, and their possible transgenerational consequences. It is in this wider context that discussions on CRISPR-Cas9, which is only one of a number of techniques, should be conducted and that all the necessary studies should be undertaken to minimize, as far as possible, the uncertainties and risks for future generations.

6 Conclusions

The possibility of using somatic gene therapy has gradually become established, but French lawmakers were ahead of the times when they banned all interventions seeking to modify the human germline genome in 1994. This position was reiterated in the Oviedo Convention, and has been ratified by many countries.

New high-performance molecular tools for the targeted modification of the genome have been described. These tools are now available in many laboratories, and they open up major opportunities for all research in all domains of biology, including medicine. They will probably engender considerable progress in somatic gene therapy.

Methods based on CRISPR-Cas9 or similar procedures have already been used, on the embryos and germline cells of many species, to create animals with edited genome in all cells. Could the same approach be used in humans? The only possible medical indications would be prevention of the transmission to the child of serious monogenic diseases, which are rare.

None of the techniques currently available can be used with the required levels of safety and

efficacy to modify the genome of germline cells or embryos leading to childbirth. There is, thus, no need to modify current French legislation on this point. This position may need to be reconsidered later, in light of advances in our knowledge.

The absence of clinical applications should not, however, stop basic and preclinical research in this area, including work on human germline cells and embryos, to improve our understanding of the mechanisms regulating gametogenesis and early embryonic development and their anomalies. This research should therefore be authorized and supported provided that it is scientifically and medically relevant.

The possible clinical use of these new technologies, amongst other innovations, is part of the current evolution of medicine, in which the stakes are not only technical or medical, but also involve ethical and social choices. However, it is necessary to clearly state that these new technologies should not be used for deliberate eugenics.

7 Recommendations

The National Academy of Medicine recommends:

- Maintaining the current legislation prohibiting all manipulation of DNA resulting in changes to the genome of offspring;
- The development of research using technologies for targeted genome modification, including work on germline cells and human embryos.
- The adaptation of legal and regulatory texts, as necessary, to allow the development of this research in France and Europe, particularly as concerns the ban on creating transgenic embryos, provided that the modified embryos are not transferred to the uterus under the current state of knowledge and legislation;
- The establishment of multidisciplinary discussions on the questions posed by the techniques for the germline and embryonic genome editing. This subject should be considered as part of a wider debate on all the medical technologies and interventions used in assisted reproductive technologies with potential effects on the genome of unborn children and, possibly, that of subsequent generations.

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Annexe 1: Terminology

It is difficult to translate the term "genome editing" into French and to find a precise and unambiguous term that can apply to all situations, and which is explicit enough to be understood by all, regardless of expertise.

The terms have to describe all applications using the techniques that allow the modification of DNA structure by molecular tools. However, "genome editing" concerns only the modifications of genes, or, more specifically, their structure, but not their function or expression, which can be modified by other means. The DNA sequence or the targeted gene can be found in diverse cell compartments (nucleus, mitochondria) and various types of cells, tissues, or organs. Finally, the intervention can have different purposes (treatment, prevention, improvement), with the same word being interpreted differently for different purposes. Furthermore, a recent study analyzing publications in the American press showed that the term "editing" was generally interpreted in a positive way, but was considered to be negative when associated with the term "embryo" [1].

In French, the term "édition" would be inappropriate for the genome. The terms "correction" of the genome or "revision" of the genome would be a more literal translation but do not encompass all the possible interventions. The terms "targeted modification of the somatic nuclear DNA structure" and "targeted modification of the germline nuclear DNA structure" would best describe the mechanisms involved, but their complexity makes their use impractical.

The phrases "engineering of the genome" and "targeted engineering of the genome" are commonly used. The term "engineering", while appropriate, could be misinterpreted and applied with some difficulty to "medical procedures". Other general terms are sometimes used: "correction", "manipulation", "surgery," each of which have their pros and cons. Finally, metaphors are sometimes used: "retouching", "reshaping", "rewriting", "cutting".

The French terms "*modification du genome*" or "*modification ciblée du genome*" should be preferentially used to promote public understanding. Modifications involving the germ line should be specifically indicated, when required. Several members of the working group pointed out that the term "*Ingénierie du genome*" best reflected the scientific terminology used to define the procedures based on molecular tools, such as CRISPR-Cas9. This terminology was adopted by the Ethics Committee of INSERM [2] and a working group set up by the *Société Française de Génétique Humaine* and the *Société Française de Thérapie Cellulaire et Génique* [3], which were also asked to provide an opinion on the question. In the interim, both above terminologies could be used.

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Annexe 2 : Experts Consulted

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