ETHICAL CHALLENGES IN GENETICS

http://www.lebanesemedicaljournal.org/articles/67-3/ethics5.pdf

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Bartnik E. Ethical challenges in genetics. J Med Liban 2019 ; 67 (3) : 138-140.

The last years have brought many new advances in the study of human genetics and development. Some things which a few years ago would have seemed impossible are now either already being done or may be done in the near future. The ability to sequence DNA cheaply and rapidly is also something which is worth thinking about when reflecting on the ethical challenges caused by the progress of genetics.

HUMAN CLONING FOR RESEARCH PURPOSES

The first example is human cloning for research purposes (also called therapeutic cloning), which was successful for the first time in 2013 [1]. The ethical issues related to human cloning have been discussed for almost 20 years, since the birth of the first cloned mammal, the sheep Dolly. However, these discussions for a long time were purely theoretical, as no one succeeded in obtaining viable embryos by the process used for Dolly – somatic nuclear transfer. In general there is a consensus in respect to reproductive cloning – where the outcome would have been a baby – which is considered to be unethical. The opinions are not as clear in respect to cloning for research purposes where the aim is to obtain an embryo which is not to be implanted but just allowed to develop long enough to obtain stem cells. This is banned in many countries, though some (for instance the United Kingdom) do allow it if approval is obtained from a special body (The Human Fertilization and Embryology Authority in the UK) and the development is not allowed to proceed beyond 14 days.

It is essentially impossible that a consensus can be reached pertaining to therapeutic cloning. On the one hand there is the potential to obtain embryonic stem cells, which may in the future be used for therapeutic purposes. On the other, embryos are created so they can be used and destroyed, and for some people this is unacceptable.

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GAMETES FROM SOMATIC CELLS

The second example is obtaining gametes from somatic cells. This has been successful to various degrees in laboratory settings [2-3], both using somatic cells directly and using inducible pluripotent stem cells (which in turn can be derived from any somatic cells). So far the success rates are low, and it is included here just to mention that this may become a greater problem than it is now. From a scientific point of view, application of these methods to obtain human embryos would be irresponsible, as normal mammalian development requires a process called imprinting, which occurs during normal gamete formation; if this process is aberrant various human diseases may occur. However, this method of obtaining gametes has been mentioned as a way of helping infertile couples, same sex couples or persons unable to produce sperm or eggs for various medical reasons. The debate on this subject has not yet been widespread, possibly because so far there have not been that many successful applications and also because a lot of the debates have focused on human reproductive cloning.

GENOME EDITING

The third example is the incredible progress in DNA editing through the CRISPR-Cas9 system[4-5]. The progress in itself is not the problem, as this relatively new technique has incredible potential for obtaining better crops, for gene therapy and as a research tool. The ability to direct changes in genes which are specific and relatively easy to obtain has been a boon for research. It has enabled the correction of a disease mutation (hereditary tyrosinemia) in adult mice, suggesting that is could lead to progress in human gene therapy [6]. On the other hand, it has also given rise to fears that as modifying genomes has become relatively easy, it could be used for purposes which are ethically problematic. In the spring of 2015 a group of scientists including two Nobel Prize laureates (Paul Berg and David Baltimore) who had signed the famous letter calling for a moratorium on genetic engineering in 1973 called for broad discussions on the use of this technique for manipulation of the human germline [7]. Another letter on the same subject was published at about the same time by a group including people affiliated with a company working on CRISPR-

Cas9 commercial applications [8]. These letters were probably to some extent caused by rumours that Chinese scientists were applying the CRISPR-Cas9 technique to modify human embryos.

In April 2015 the results of using CRISPR-Cas9 on human in vitro embryos incapable of further development were published by a group of scientists from China [9]. These embryos were tripronuclear, and had been obtained by in vitro fertilization, but the oocytes had been fertilized by two sperms, and therefore were not able to develop normally. The obtained results indicate that the technique is neither highly efficient nor very successful. The method is highly selective, but in addition to the planned changes other – off-target ones – may occur. This is a technical problem which may in the future be solved, and which is not really a problem when experiments are done in the laboratory and appropriate cells or animals may be selected. However, ethical and technical problems are not the same thing, as there rarely is an easy solution to ethical problems. The fact was that the experiments were performed on human embryos, but on the other hand these embryos would not have developed normally as they were triploid.

Baltimore *et al*. make a number of suggestions on how to go forward with the use of CRISPR-Cas9 and pose the fundamental question – whether even if the technical problems of efficacy and such things as off-target interactions (with other genes than those addressed) are solved, these techniques should ever be used for human engineering [7]. They call for broad discussions and for the convening of a group made up of persons who use CRISPR-Cas9, lawyers, geneticists and specialists in bioethics, as well as other scientists and members of the public, to reflect upon the issues and possibly recommend policies. Lanphier *et al*. stress how important it is to distinguish between research on somatic cells and on the germ line [8]. Some international regulatory issues for CRIPR-Cas9 as well as other techniques of genome editing have been addressed by Araki and Ishii [10]. Lander has discussed this problem in a short paper with the ominous title *Brave New Genome* [11], concluding with the statement "It has only been about a decade since we first read the human genome. We should exercise great caution before we begin to rewrite it".

PREVENTING THE TRANSMISSION OF MITOCHONDRIAL DISEASES

The fourth new issue is the closest to my field of interest, mitochondrial diseases. Mitochondria are organelles in eukaryotic cells. They are maternally transmitted in humans (and all mammals), as the sperm mitochondria, much less numerous than those from the oocyte, are actively destroyed at a very early stage – around the third cell division after fertilization.

Mitochondria have their own circular DNA, 16.5 kb in size. Mutations in this tiny genome, containing only 37 genes, can cause serious diseases affecting mainly the nervous and the muscular systems in humans, which occur approximately in 1:10000 live births. As in the case of most mitochondrial diseases, both healthy and affected mitochondria (that is with and without the mitochondrial DNA mutation) are present in each cell; prenatal and preimplantation diagnoses have been successfully used to select for unaffected embryos [12]. However, this technique is not always effective due to the unpredictable distribution of mitochondria with healthy and mutated mitochondrial DNA in the embryo [13]. Moreover, in one mitochondrial disease, a form of blindness, Leber's Hereditary Optic Neuropathy, in most cases practically 100% of the mitochondrial DNA is mutated, thus there is no way to select for embryos which will certainly not be affected.

After consultations with the general public and many discussions, the Nuffield Council on Bioethics published a thorough evaluation of the problem and ethical implications of a method for preventing the transfer of mitochondrial diseases to successive generations (Nuffield Council on Bioethics) [14]. The British Parliament passed a law that would allow manipulations in vitro, which would lead to a woman with a mitochondrial disease having children, who would essentially not inherit her defective mitochondria. There are two variants of this technique; both start with an oocyte from the woman with a mitochondrial disease and another oocyte from a healthy donor. In the first procedure, called spindle transfer, the nucleus is removed from the donor oocyte, and replaced by the nucleus from the oocyte of the affected woman. The oocyte is fertilized and when it develops into an embryo is transferred to the womb of the affected woman.

The second technique called pronuclear transfer involves fertilization of both oocytes, and then the two nuclei (of the oocyte and the sperm) are removed from the fertilized oocyte of the healthy woman and replaced by the two pronuclei from the fertilized oocyte of the woman with the mitochondrial disease. The number of women who could benefit from this technique is estimated to be 150 per year in the United Kingdom and about 780 per year in the United States [15]. There has been extensive experimentation on primate and human embryos suggesting that this technique would work [16], as well as numerous papers on the clinical and ethical problems of the method, its safety, efficacy, and regulatory, legal and ethical questions [17-18]. Some other concerns have been mentioned, such as the possibility of the com-

mercialization of human eggs in relation to mitochondrial replacement [19] and the discussion of possible risks of disrupting the interactions between the mitochondria and the nucleus [20]. It is very likely that there will be babies born after mitochondrial replacement reasonably soon in the United Kingdom, and currently the possibility to allow this method is being considered in the United States.

Recently, germ line editing was applied in mice to repair the mitochondrial mutations in oocytes, not CRISPR-Cas9 but restriction nucleases and a different method called TALEN were used to eliminate mutated mitochondrial DNA in mouse oocytes, leading to the birth of live mice [21]. Whether this would be more or less of an ethical problem if applied to humans is beyond the scope of this short discussion.

All these new technologies can create ethical problems, but whereas there is fairly general agreement concerning a ban on reproductive cloning the same is not true for the other new methods mentioned above. Some of them are very new, some have been extensively discussed but only in certain countries.

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