Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing: call for contributions

About you

1. What is your name?

Desideria Mini

2. What is your affiliation?
On my own

3. What is your country of residence?
Italia

Your contribution

4. Have you read the DRAFT Governance Framework (https://www.who.int/docs/default-source/ethics/governance-framework-for-human-genome-editing-2ndonlineconsult.pdf)?

- [x] Yes
- [ ] No

5. Please comment on the approach taken by the Committee in developing its DRAFT Governance Framework?

Although the approach You have taken is interesting, I have some notes for You. In Your draft You have done a long overview about genome editing but You have not taken any positions. You have asked yourself many questions but You have not answered practically any of them. Furthermore, I believe that in Your discussion You have left out some important topics. What will follow are my considerations.

6. Please provide your opinions on the specific proposals relating to governance of human genome editing specific considerations for good governance in the DRAFT Governance Framework (Part 3)?

Point 26
Heritable genome editing would be particularly useful for nuclear DNA (how I am going to explain later) but less for mitochondrial DNA. You have not considered that about mitochondrial DNA, indeed, already exist mitochondrial replacement techniques (MRTs), better known to public like “mitochondrial donation”. MRTs are the only concrete possibility available to date able to prevent the transmission of mitochondrial DNA-linked diseases from mother (who has a dangerous mutation in the mitochondrial DNA of her oocytes) to her children and from her daughters to future generations, at the same time allowing the mother to have children genetically related to her nuclear DNA. MRTs prevent mitochondria DNA-linked diseases for all the descendants. MRTs have already successfully passed both basic and preclinical trials. Not only. Some of them have already been tested in the clinic leading to the birth of children. Although there are very few cases, these children are all healthy. The MRTs have been criticized because the mitochondrial DNA brought about by this technique is external to the couple, to the point of talking about "three-parents babies". This objection is countered by the fact that the genetic information brought by the mitochondria is very small, much less than the genetic information external to the couple brought by heterologous fertilization, and that in any case the parents are those who educate and grow children, not those who provide the DNA. All this to say that in the case of mitochondrial DNA the usefulness of a inheritable mitochondrial genome editing, among other technically more complicated, is for the moment more difficult to see and probably is not necessary to prevent mitochondrial DNA-linked diseases.

Point 28
Like pre-implantation genetics analysis, heritable genome editing is often accused to be against sick people by erasing their existence. This claim is countered by the fact that these techniques do not discriminate or eliminate sick people. The use of assisted reproduction techniques to prevent hereditary diseases is or should be based on the fact that being healthy is better than being sick and that all possible measures should be able to be taken to ensure health for future generations avoiding heritable disease transmission. It is evident they prevent diseases can not be honestly compared to intent to harm sick persons. They are two things very different.
Of course ethical prudence is needed and strong regulation will be also required both to avoid abuse on human person, who must always be protected, and discrimination between riches and poor and people from different countries. In particular three things will be very important. First, there will have to be no coercion in these techniques but future parents should be able to freely choose them. Second, no ethnic purpose will have to be permitted. Third, the results of all these researches will have to be shared among the people and to benefit the entire world population. However, given the differences of each country, each of them should be able to freely decide to legalize or not the hereditary modification of the human genome, preferably after an internal ethical debate involving the whole of its society.

Point 29
The inheritable nuclear DNA linked diseases can be prevented through pre-implantation genetics analysis (PGA) which divides in pre-implantation genetics testing for monogenic diseases (PGT-M), pre-implantation genetic testing for aneuploidies (PGT-A) and pre-implantation genetic testing for structural diseases (PGT-SR). Because of that it is often declared that germ-line genetic modification would not be necessary but in reality the matter is more complex.
First, PGT-M of embryos (on its own) has limits that do not always make it useful to deliver a "healthy" child.
About nuclear genetic diseases PGT-M is useless when both partners are homozygous for the

recessive gene disease and when a person is homozygous for a not-recessive gene disease (dominant, incomplete dominant or codominant).
Second, also when PGT-M can be useful to deliver a "healthy" child not always it is useful or sufficient to deliver a child not carrier of a dangerous recessive allele transmissible to her\his future children, so grandchildren could again risk being sick if the future son\daughter's partner will be also carrier.
In the case in which a parent is homozygous and the other is heterozygous for a recessive gene disease, PGT-M can deliver a healthy child but him\her will be carrier of the dangerous recessive allele.
In the case both parents are heterozygous for a genetic disease, despite PGT-M is able to deliver a not-carrier child, on average only a quarter of embryos will be homozygous for the “healthy” allele. In this situation, if it is a recessive disease, two-thirds of the embryos suitable to deliver a healthy child (from here on “not-affected” embryos) are carriers, so, from these embryos, children who could be born will be carriers. Given the difficulty to obtain non-carrier embryos their number can be often low or zero and so also recessive disease carrier but “not-affected” embryos are transferred in uterus.
Third, unfortunately sometime also the “not-affected” embryos produced by in vitro fertilization can be low or zero. Generally to face the problem of the insufficiency of the "not-affected" embryos number the cycles of in vitro fertilization (IVF) and pre-implantation genetic analysis can be repeated, nevertheless there is no certainty but only the probability of reaching “not-affected” embryos. Furthermore repeating the IVF cycle more times need new oocytes, to obtain them repeating hormonal stimulation of the ovary is needed but that poses several risks to the woman's health.
About nuclear genetic and chromosomal diseases an other possibilities is gametes selection through analysis of first and second polar bodies to try to know the genotype of the oocyte or perhaps in future through analysis of secondary spermatocytes or spermatids grown in vitro for the male gamete. Nevertheless polar bodies biopsy are more limiting than embryos analysis because it allows the selection of genetic or chromosomal makeup coming only from female gametes, does not exclude possible cases of gene conversion, and are completely useless in case of aspiring mother's homozygosity.
Selecting the secondary spermatocytes or the spermatids, something still never attempted, could be hard due to their cytological features, besides presenting limits analogous to those of polar bodies biopsy.

Point 31
It is surprising how You have considered selective abortion an alternative to heritable genome editing (point 29) and not to in utero somatic human genome editing. You have not affirmed anything about in utero somatic human genome editing and abortion. You should ask yourself why.

Points 42 and 43
You have to consider that enhancement through heritable genome editing ought not to aggravate social inequality if was made available to all people who want children. However, as it would be absolutely fundamental that there is no coercion but that it is a free choice, considering that some people may choose using enhancement in reproduction and others not, disadvantages could arise in the children of those who have not used it compared to others. This would be a possible problem to be addressed.
Solved somehow the problem of inequalities, I think performance in sport and in academic endeavours would be welcome because they benefit the individuals but the science will not be capable to induce similar improvements for a long time and society is not ready for a similar change. It would be premature. It has also been theorized about endowing the human species with visual perception in infrared or ultraviolet rays but endowing the human species with advantageous characteristics that it naturally does not have is socially even more premature. About space mission, the long space travel era is very distant and therefore it would be really too premature to create better astronauts.

Such decisions may be made in far future times, although differently distant.
For now focusing on prevention of heritable diseases would be better .
Then about military mission and stronger soldiers I am very against because war is a very ugly things, a threat for Mankind. We must go towards global disarmament not towards super soldiers. "Do not make the war, make the love."
I hope You will agree with me.

7. Please comment on the tools, institutions, and processes for human genome editing governance in the DRAFT Governance Framework (Part 4)?

Point 48
You have forgot to say some important things about creating embryos for research.
First, the Oviedo Convention bans also creating embryos for research purpose as it stipulates in the same Chapter IV - Human genome Article 18 – Research on embryos in vitro: "1. Where the law allows research on embryos in vitro, it shall ensure adequate protection of the embryo. 2. The creation of human embryos for research purposes is prohibited."
Second, You have not said why creating embryos for research is fundamental to develop or test the technology for germline genome editing.
The cryopreserved human embryos left over from previous medically assisted procreation cycles are at the at a stage of many cells.
Modifying and correctly modifying a lot of cells all together is practically impossible and attempting to do so on embryos of many cells would certainly lead to mosaicism. To reduce the probability of mosaicism, it is necessary to act at the moment of fertilization, at the very early stage of zygote or embryo (of 2-4 blastomeres) but the cryopreserved zygotes are in a very minority and in any case today they are no longer frozen. Therefore for this kind of experiments, having to start from a single cell stage, embryos must be produced specifically.
In some country which did not ratified Oviedo Convention scientists are experimenting genome editing on human oocytes at the moment of fertilization, zygotes or very early embryos. These are some most permissive country which permit the creation of embryos for scientific purpose.
An other future possibility will be experiment genome editing on precursor stem cells of the gametes, as soon as it will be possible to produce them in vitro but when in vitro gametogenesis will become reality it will not be possible to bring it immediately to the clinic by transferring in uterus the embryos obtained by fertilization through the gametes produced in vitro but it will be necessary to study these embryos in the laboratory, and this both if the progenitor cells have been genetically edited or not. So also this path requires the in vitro production of embryos for research.
In those countries that have ratified the Oviedo Convention, or in any case where producing embryos for research purposes is prohibited, scientists are forced to limit themselves to conducting genome editing experiments on triploid zygotes or derived embryos. (Triploid zygotes are formed by erroneous fertilization with two spermatozoa or by failure to expel the second polar body.) Also in these countries although producing in vitro gametes, edited or not, could be legal, to fertilizing them (to study the embryo that would form) will not be possible.
In order not to slow down the research and to allow the parties to the Oviedo Convention to participate in it, it would be hoped that Council of Europe amends Article 18 of the Oviedo Convention to allow creation of human embryos for research purpose in cases where it cannot be done without.

Point 62 (and 46)
Two of the major concerns of the committee who elaborated the Oviedo Convention were both the possible health hazards of future generations that could result from an intentional modification of human genetic inheritance, without adequate knowledge of the human genome and without using sufficiently precise techniques to not to cause damage, and uncertainty as to when the necessary requirements would be reached. It is clear that until the germ-line modification has research reached safety and efficacy it must be confined in the laboratory. Although improvement with basic and preclinical experimentations is still needed, today research is running fast and in a some years sufficient security and effectiveness could be achieved. However, the role of bioethics experts must not be confused with that of scientists: it is not bioethicists who have to determine if and when the germ-line genome modification is or will be safe enough to be brought to the clinic (heritable genome modification). This task falls to the scientific authorities, of which we must trust. The task of bioethics experts is to determine whether it is morally right to use it and, if so, for what purposes. Reducing the

incidence of hereditary diseases is surely included in the purposes to whom is morally right to use it. For these reasons it would be hoped that Council of Europe amends Article 13 of the Oviedo Convention to allow heritable human genome modification for preventive purpose under the meeting of certain conditions.

8. Please provide your opinions on the scenarios in the DRAFT Governance Framework (Part 5), including whether we have missed any important details?

I would like to try to answer some Your questions in the Table 5.

About “Responsible stewardship of science” and for the question “What assessment will be used to ensure the benefits are greater than unintended effects?” I have something to say.
Unintended effects could result from off-targets editing or wrong on-target editing. There are various different techniques and strategies to analyze the DNA but to know with high confidence whether the editing has gone the right way I think it would be necessary performing whole-genome sequencing of an appropriate sample.
A sample would be more easily representative in the case of gamete precursor stem cell editing because them can be separated even after various cell divisions from the introduction of the editing tools and then analyzed after these have ceased editing, allowing to select the correctly edited cell lines before the gamete generation.
In the case of zygotes and embryos this is not possible and there is the problem of mosaicism. To analyze the embryos subject to editing PGA is needed. Unfortunately mosaicism is a insoluble problem for current PGA techniques which take cells from embryos (not only for those edited) because the cells collected by biopsy may not be representative of the rest of the embryo.
Analysis of the blastocele fluid and/or of the spent culture medium, the second after several washing of the embryo at morula stage in the liquid of culture medium to remove foreign DNA, are new promising PGA techniques*1 which have identified aneuploidies and (few times) monogenic mutations in embryos*2, *3, *4.
These techniques are still experimental, have not yet been used to identify genetic mosaicisms and have only been tested on unedited embryos but perhaps, being able to detect “general state” of embryos, could be capable to identify genetic mosaicism, I speculate. It should be tried.
An appropriate sequence of amino acid residues added to the N-terminus, including ubiquitination*5, to reduce the half-life of the Cas protein and derived versions, preventing it from continuing to act, can reduce mosaicism. This idea has been tested on monkey embryos via N-terminal ubiquitination with good results*6 and could also be tested with the N-terminal amino acid residue rule*7, I suppose.
To interrupt the action of the Cas and derived proteins, it is also possible to resort to “anti-CRISPR agents”. There are several types able to prevent Cas and derived proteins from binding to DNA or to inhibit or block their DNA-cleavage activity*8. The function of the "anti-CRISPR agent" would be to try to prevent the editing proteins from continuing to act and therefore could be useful to be even more sure of stopping its action after a certain very early embryonic stage to prevent mosaicism. I think it's another pathway to test.

About “Responsible stewardship of resources” and for the question “How much time, talent and treasure should be spent on developing heritable human genome editing to change the traits of future people when there are people living among us who might better benefit from somatic human genome editing?” I have something to say.
Both somatic and heritable genome editing should be developed an implemented. Heritable genome editing should be so because hereditary diseases are a threat for Mankind.
About 8000 monogenic diseases are known and each individual, often without knowing it, is a carrier of various mutations that can cause a genetic disease and to date some hundreds of millions of people worldwide are affected by heritable genetic diseases, perhaps more than one person on twenty.
Today, fortunately, the new medical advances sometimes allow people affected by a hereditary disease which reveals itself (or which anyway revealed itself) at young age to live (longer) and have a healthier life and this often allows them also to get to have children. Thank to research, in the future, this will be even more true, and for an increasingly number of hereditary diseases. If on the one hand

that is a good news on the other this also means that harmful genetic variants are and will be always passed to children and therefore (also) to future generations.
So, due to medicine, natural selection against inheritable diseases is and will be missing in human species. This will have serious consequences, especially in the long term. Indeed the harmful genetic mutations inevitably accumulate in the absence of the natural selection because possible harmful variations of DNA are much more numerous than the non-harmful ones so harmful mutagenic events occur more frequently than beneficial ones. So, considering all that, about those (many) hereditary diseases whose onset occurs (or may also occur) before or during the usual reproductive period of the individual's life, it is more than predictable that, moreover, their incidence in the human population over the generations will tend to increase so that more and more people will need important therapies. It is also very likely that the birth rate of very unfortunate children who will experience multiple hereditary diseases and the necessary related multiple therapies in their life will tend to increase. Furthermore over the centuries the incidence of heritable diseases could even up to a "point of unsustainability" for human species.
This principle apply to heritable genetic diseases and, in a similar way, to some chromosomal diseases.
For these reasons the fact that with PGA and “gamete test” only rarely is possible to avoid the birth of a not-carrier child is very worrying.
The heritable genome modification would avoid having to cure and heal people for each generation, something of which, moreover, there would be need more and more.
Heritable genome editing would prevent the genetic and chromosomal diseases for all the descendants.

About “Respect for individual dignity” and for the question “How will the interest of future generation be taken into account?” obviously this will happen if the hereditary modification introduced in the genome is simply beneficial and not harmful to future individuals.

About “Fairness” I would report that in two of the related questions You have written “somatic” instead of “heritable”.

*1. Is cell-free DNA in spent embryo culture medium an alternative to embryo biopsy for preimplantation genetic testing? A systematic review - Sophie Brouillet, Guillaume Martinez, Charles Coutton and Samir Hamamah – June 2020 – ScienceDirect

*2. Non-invasive preimplantation genetic testing for monogenic diseases and aneuploidies using cell free embryonic DNA - Svetlana Madjunkova, Ran Antes, Rina Abramov andValeriy Kuznyetsov - October 2018 - ResearchGate

*3. Strategies to achieve combined non-invasive PGT-M and PGT-A on spent culture media using target sequence enrichment – C. Robinson and M. J. Jasper – 13 Semtember 2018 – ScienceDirect

*4. Medium-Based Noninvasive Preimplantation Genetic Diagnosis for Human a-Thalassemias-SEA - Haitao Wu, Chenhui Ding, Xiaoting Shen, PhD, Jing Wang, Rong Li, Bing Cai, Yanwen Xu, Yiping Zhong and Canquan Zhou – March 2015 – Medicine

*5. Short-lived green fluorescent proteins for quantifying ubiquitin/proteasome dependent proteolysis in living cells - Nico P. Dantuma, Kristina Lindsten, Rickard Glas, Marianne Jellne and Maria G. Masucci - June 2000 - Nature Biotechnology

*6. Promoting Cas9 degradation reduces mosaic mutations in non-human primate embryos - Zhuchi Tu, Weili Yang, Sen Yan, An Yin, Jinquan Gao, Xudong Liu, Yinghui Zheng1, Jiezhao Zheng, Zhujun Li, Su Yang, Shihua Li, Xiangyu Guo and Xiao-Jiang Li - 18 August 2016 – NCBI

*7. The N-end rule pathway and regulation by Proteolysis - Alexander Varshavsky - 11 June 2011 – (Review) – ProteinScience

*8. The kill-switch for CRISPR that could make gene-editing safer - Elie Dolgin - 15 January 2020 - (News feature) – Nature

9. Please comment on the questions to be considered when developing governance measures (Annex)?

All is ok. Nothing to declare about.

10. What would you want to see in a decision tree to assist those taking governance decisions? (We are currently consider creating a decision tree based on the questions to be considered when developing governance measures (Annex))?

Nothing to declare about.

11. Are there additional measures we could include to deter or avoid bad practice around applications of human genome editing (such as rogue clinics or other 'bad actors', inappropriate uses of the technology, etc.)?

Nothing to declare about.

12. What else do you want to tell us about good governance of human genome editing?

The criteria for regulating biomedical scientific experimentations and applications must positively consider all those researches and results that can be helpful for treatment of serious diseases in order to guarantee the primary interest of both present sick people and future generations of Mankind.
If in a country a genome editing beneficial research is not permitted, researchers who are citizen of that country should be able to carry out research in another jurisdiction where it is permitted without being prosecuted in their original country, both for somatic, germinal and heritable genome editing, provided that the eventually (somatic or heritable) treatments offered to patients are not deceptive but safe and effective. Otherwise they will still have to be criminally prosecuted in one of the two countries. Also the patients who are citizens of that country should be able to travel in the other more permissive jurisdiction to receive adequate medical treatments both for somatic and heritable genome editing without take legal risk in their country. All countries should however discourage patients from travelling to foreign countries to undergo harmful treatments or of questionable safety and efficacy. Same things for resident people.

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