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Responsible Innovation in Human Germline Gene Editing.

Background paper to the Recommendations of the European Society of Human Genetics (ESHG) and the European Society of Human Reproduction and Embryology (ESHRE)

Guido de Wert¹, Björn Heindryckx², Guido Pennings³, Angus Clarke⁴, Ursula Eichenlaub-Ritter⁵, Carla G van El⁶, Francesca Forzano⁷, Mariette Goddijn⁸, Heidi C Howard⁹, Dragica Radojkovic¹⁰, Emmanuelle Rial-Sebbag¹¹, Wybo Dondorp¹, Basil Tarlatzis¹², Martina C Cornel⁶. On behalf of the European Society of Human Genetics and the European Society of Human Reproduction and Embryology

1. Department of Health, Ethics and Society, Research Institutes GROW and CAPHRI, Fac. of Health, Medicine and the Life Sciences, Maastricht University, The Netherlands; 2. Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium; 3. Bioethics Institute Ghent, Department of Philosophy and Moral Science, Ghent University, Belgium; 4. School of Medicine, Cardiff University Cardiff, UK; 5. Institute of Gene Technology/Microbiology, Faculty of Biology, University of Bielefeld, Germany; 6. Department of Clinical Genetics, Section Community Genetics and Amsterdam Public Health research institute, VU Medical Center, Amsterdam, The Netherlands 7. Clinical Genetics Department, Great Ormond Street Hospital NHS Trust, London, UK; 8. AMC, University of Amsterdam. The Netherlands; 9. Centre for Research Ethics and Bioethics, Uppsala University, Sweden; 10. Laboratory for Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia; 11. University Paul Sabatier Toulouse, France; 12. University Thessaloniki, Greece.

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Address for correspondence:

Prof. Guido de Wert

Department of Health, Ethics and Society,

Research Institutes GROW and CAPHRI,

Fac. of Health, Medicine and the Life Sciences, Maastricht University,

PO Box 616

6200 MD Maastricht

The Netherlands

Email: g.dewert@maastrichtuniversity.nl

Abstract

Technological developments in gene editing raise high expectations for clinical applications, including editing of the germline. The European Society of Human Reproduction and Embryology (ESHRE) and the European Society of Human Genetics (ESHG) together developed a Background Document and Recommendations to inform and stimulate ongoing societal debates. This document provides the background to the Recommendations.

Germline gene editing is currently not allowed in many countries. This makes clinical applications in these countries impossible now, even if germline gene editing would become safe and effective. What were the arguments behind this legislation, and are they still convincing? If a technique could help to avoid serious genetic disorders, in a safe and effective way, would this be a reason to reconsider earlier standpoints?

This Background document summarizes the scientific developments and expectations regarding germline gene editing, legal regulations at the European level, and ethics for three different settings (basic research, preclinical research and clinical applications). In ethical terms, we argue that the deontological objections (e.g. gene editing goes against naturalness) do not seem convincing while consequentialist objections (e.g. safety for the child thus conceived and following generations) require research, not all of which is allowed in the current legal situation in European countries.

Developing this Background document and Recommendations reflects the responsibility to help society understand and debate the full range of possible implications of the new technologies, and to contribute to regulations that are adapted to the dynamics of the field while taking account of ethical considerations and societal concerns.

1. Introduction

Gene editing has attracted major attention of scientists and media in recent years. While scientists in laboratories witness a revolution, much of the conversation about CRISPR–Cas9 has revolved around its potential for treating disease or editing the genes of human embryos (Ledford 2016). After experiments with zinc-finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), the CRISPR-Cas9 system turned out to have unprecedented ease and finesse. Treatment of cancer patients using gene editing has started (Qasim, 2017; Cyranoski D, 2016). Expectations are that many more potential applications will follow. Ongoing trials are reported for thalassemia and sickle cell disease, mucopolysaccharidosis I and II, and hemophilia B amongst others (www.clinicaltrials.gov). These trials are preparing treatment possibilities, for instance by studying treatment at the human cellular level. Somatic gene editing may prove to be a game changer not only in the treatment of a whole range of serious genetic, esp. Mendelian, disorders, but also in the treatment of cancer and infectious diseases. Gene editing is a medicinal product for human use, where regulations apply. The European Medicines Authority (EMA) that oversees access of medicinal products to the market, including advanced therapy medicinal products (ATMP s), has identified scientific and regulatory guidance on gene editing as an issue for its future agenda (EMA 2017). Ethical, legal and societal issues for somatic gene editing include safety and accessibility, and meaningful stakeholder engagement, education, and dialogue must be organized (Howard submitted). However, if somatic gene editing would be safe and cheap, there will be few other ethical or legal issues left.

For editing the genes of human embryos the situation is different. The prospect of technologies coming available that would allow making changes in the (human) germline has been heavily debated in recent decades, and in many countries germline interventions have been prohibited, sometimes even accompanied by criminal sanctions (Isasi 2016). In the previous decades legislation has been developed not allowing changes in the human germline, including the Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine, often referred to as Oviedo Convention (Council of Europe 1997) and the recent Clinical Trials Regulation No. 536/2014. What were the arguments behind this legislation, and do these still apply? If a technique can help to avoid serious genetic disorders, in a safe and effective way, would this be a reason to reconsider earlier standpoints? Discussion with relevant stakeholders is needed, including professional health care workers, patients and citizens, legal and ethical experts. Globally and also within Europe there is diversity, as several countries have not ratified the Oviedo Convention, which prohibits any (human) germline modification. Meanwhile in the USA a summit was organized in 2015 that brought together scientists, ethicists, legal experts and patient groups from around the world. The report published by the National Academies argues that “Scientists should be permitted to modify human embryos destined for implantation in the womb to eliminate devastating genetic diseases such as sickle-cell anaemia or cystic fibrosis — once gene-editing techniques advance sufficiently for use in people and proper restrictions are in place”. Furthermore, altering human embryos in the lab for the sake of basic research should be acceptable (Reardon 2017). The document outlines strict limits under which scientists could proceed in the future. It recommends restricting the technique to severe medical conditions for which no other treatment exists.

We thus witness that initiatives have been taken worldwide to exchange views about responsible innovation using human gene editing, including GLGE. Changes in the germline genome have been considered much more sensitive than somatic gene editing. The recent scientific advances rekindle ethical and policy questions surrounding the acceptability of germline modification (Isasi 2016). While in the past some may have considered the question of acceptability of germline gene editing not urgent because it was technically impossible, the current scientific developments make the issue more urgent than ever. Also the international differences and potential for cross border movements of patients make societal debate urgent.

The European Society of Human Genetics (ESHG) and the European Society of Human Reproduction and Embryology (ESHRE) consider it to be their professional responsibility to contribute to further discussion on GLGE. As in earlier collaboration between ESHG and other professional societies, a background document and recommendations have been developed. The current text is the background document. Its aim is to inform and stimulate ongoing societal debate and serve as background for ESHG/ESHRE recommendations. Drafts were prepared by a joint writing group. These were discussed in both committees and in a joint meeting of the two societies on September 20, 2016. A draft of the Recommendations has been online on the ESHG and ESHRE website from October 17 until December 2, 2016 forming the backdrop for discussion at the ASHG meeting in Vancouver. This Background document together with the Recommendations were subsequently posted online on the ESHG and ESHRE websites to solicit comments from experts and the membership of both ESHG and ESHRE from April 3 until May 5 2017. The authors integrated the suggestions to the Background document where appropriate.

2. Scientific developments and expectations regarding GLGE

It is first of all important to distinguish the target population of cells on which gene editing can be performed, being somatic cells, pluripotent stem cells or the germ cells. Gene modifications in somatic cells or mostly in pluripotent stem cells are intended for the patients themselves and would not usually be transmitted to the progeny. In contrast, gene editing in the germline, and in some cases in pluripotent stem cells that become differentiated to germ cells in vitro, would change the individual's hereditary genetic profile and would thus be transmitted to future generations (see Figure, Vassena et al., 2016). Besides the gene editing in nuclear DNA, also experiments aiming at gene editing in the mitochondrial DNA (mtDNA) can be considered as gene editing in the germ line. Like editing nuclear DNA, transmission of genetically edited mtDNA can be used to eliminate mutant mitochondria or alter the mtDNA and be transmitted to offspring. The use of donor cytoplasm to replace one set of mitochondria with a different set is a distinct method of germline modification that does not involve gene editing technologies. In theory, gene-edited somatic cells could be used for somatic cell nuclear transfer (SCNT) aimed to produce genetically modified descendants (Zhou et al., 2015). There is a worldwide moratorium on human reproductive cloning, so the latter use of SCNT technology is not considered ethically acceptable in humans at present.

As for the stage at which germ line modifications could be introduced, scientists envisage that this would be carried out at the level of the germ cell or its progenitor cells during gametogenesis in vitro

(see Figure Vassena et al., 2016), or later at the stage of the fertilized oocyte (zygote) or in the early embryo. State of the art literature will be provided for these possible germline modifications.

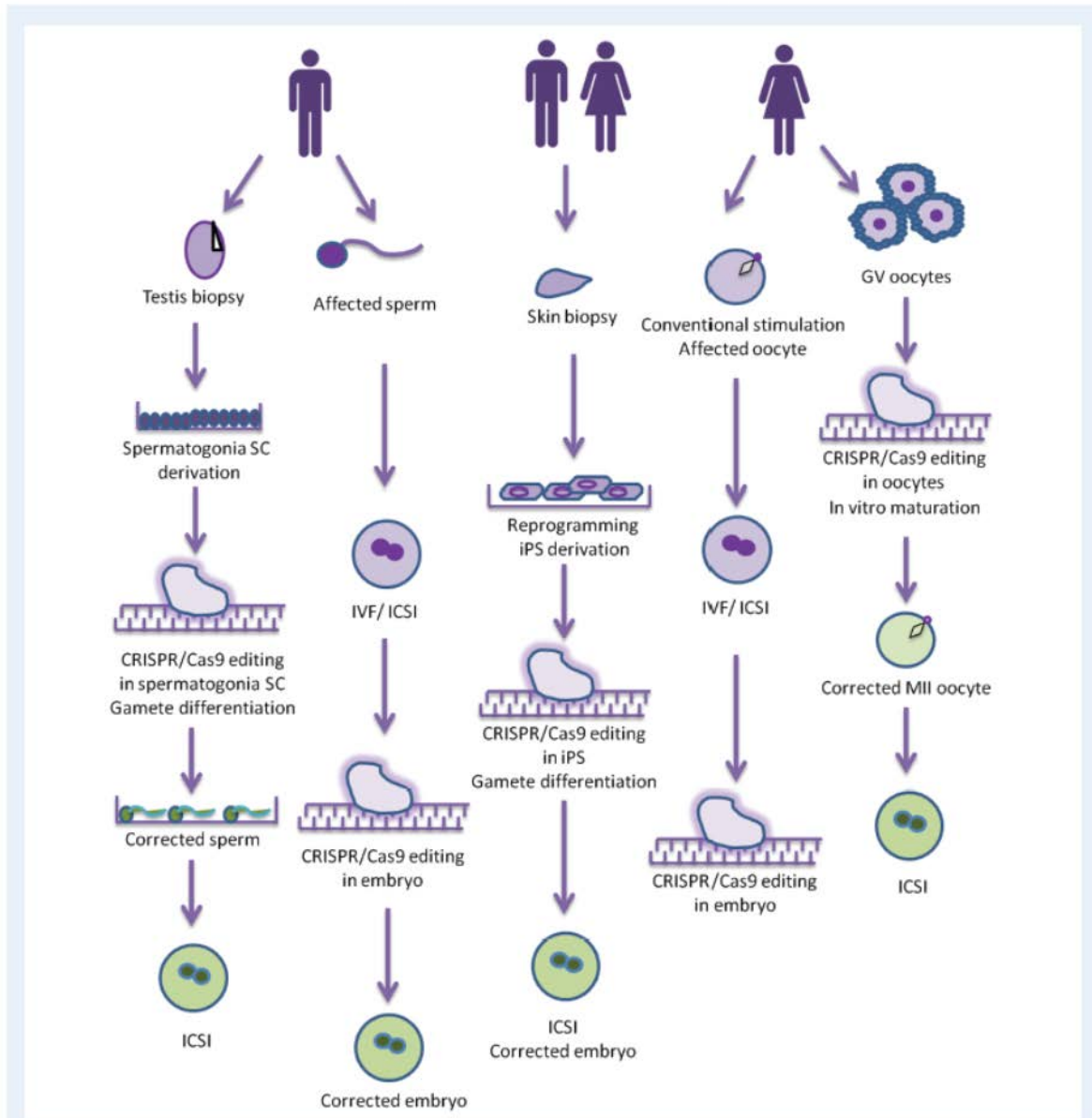


Figure 1 Summary of possible routes to editing of the germline genome in men and women. SC, stem cell; MII, metaphase II; GV, germinal vesicle; iPS, induced pluripotent stem cell; CRISPR, clustered regularly interspaced short palindromic repeats.



2.1. GENE EDITING IN ZYGOTES/PRE-IMPLANTATION EMBRYOS

For germ-line modifications, the genomic editing system is mostly injected into the cytoplasm or pronuclei of zygotes or into pre-implantation embryos, after which genetic screening is used to select the embryos with a corrected genomic pattern in the absence of detectable off-target genetic modifications. Subsequently, prenatal testing using either cell-free fetal DNA from the pregnant woman’s blood or one of the more invasive methods (chorionic villus sampling or amniocentesis), could verify whether or not a fetus shows molecular or genomic mosaicism. Mosaic embryos arise

due to inefficient cutting of the nucleases or the inaccurate DNA repair before the embryo has reached the stage of cleavage. The pre-implantation embryo stage is generally not favoured for genome editing as it would most likely lead to a mosaic individual and possibly to more unforeseen and unforeseeable detrimental effects. Various studies in different animal models have demonstrated the feasibility of gene-editing in animals at the zygote stage (Yoshimi et al., 2014; Heo et al., 2015; Han et al., 2014, Kang et al., 2014). The potential of the technology to prevent the onset of a genetic disorder in mice was demonstrated by the studies of Wu et al. (2013) and Long et al. (2014), respectively for cataract and Duchenne muscular dystrophy. Also in non-human primates, microinjection of Cas9 or TALENs into zygotes led to the birth of targeted gene-modified offspring (Niu et al., 2014; Liu et al., 2014). In mammalian zygotes, the efficiency of the correction of an indel in a single gene by TALENs or Cas9 ranges from 0.5% to 40.9% per injected zygotes (Araki and Ishii, 2014). Ran et al. (2013) reported that the double nicking by RNA guided CRISPR Cas9 nickase treatment can lead to 80% to 100% efficiency in mouse blastocysts. Regarding gene modification in subsequent neonates, the efficiencies of indel and gene addition were 0 to 41.7% by TALENs or Cas9, and 1.7 and 3.0% by Cas9, respectively (Araki and Ishii, 2014). In the targeted gene modifications, the efficiency is 2.0 to 6.0% in mouse offspring, coinciding with off target mutations (Araki and Ishii, 2014).

In the human, researchers are restricted by the limited availability of embryos due to ethical and/or legal constraints. Most supernumerary human embryos available for research will have progressed beyond the cleavage stage, giving rise to more mosaicism when genomic editing is attempted. Only in countries where the creation of embryos for the exclusive purpose of research is allowed, could this technique be applied at earlier stages and with fresh oocytes/embryos. One alternative source would be failed fertilized oocytes, which could be fertilized or artificially activated and subsequently used for genomic editing for research purposes. Alternatively, abnormally fertilized zygotes (mostly 1PN or 3PN) could be used as it was the case in two recent Chinese reports (Liang et al., 2015; Kang et al., 2016). Of the 86 injected 3PN human zygotes, four (4.7%) contained the correct genetic material repaired through non-homologous end joining (NHEJ; Liang et al., 2015). The genomic edited embryos were mosaic, similar to findings in other model systems (Shen et al., 2013; Yang et al., 2013; Yen et al., 2014). In addition, a substantial number of 'off-target' mutations were observed, either due to the inefficient CRISPR/Cas9 complex acting in other parts of the genome and/or caused by the abnormal triploid chromosomal content originating from the abnormally fertilized 3PN zygotes. Also in another more recent study (Kang et al., 2016), the efficiency of homology directed repair (HDR) of the CCR5 Δ 32 allele was low, and the edited embryos originating from 3PN zygotes were mosaic. It has to be remarked that neither of the studies in the human (Liang et al., 2015; Kang et al., 2016) used the most up-to-date CRISPR/Cas9 methods. Recently, some alternative methods used in mice significantly increased the efficiency of genomic editing technology while, at the same time eliminating mosaicism in reconstructed embryos.

Even if ethical/legal barriers for embryo research could be removed and the supply of zygotes or embryos were fully resolved, significant technical hurdles would remain. These need to be tackled to gain control of the genotype of both alleles in a viable embryo. For example, microinjection of the genomic tool machinery into zygotes only (not embryos) is a technically demanding and labor-intensive procedure which can hamper subsequent embryo development. As an alternative, a simple electroporation-based strategy to deliver Cas9/sgRNA ribonucleoproteins into mouse zygotes was proposed with 100% efficiency for in vivo genome editing and no decrease in embryo viability (Chen

et al., 2016).. A similar technology was recently used by Hashimoto et al. (2016), claiming that electroporation of the Cas9 protein/sgRNA into early pronuclear zygotes generates non-mosaic embryos in the mouse. The key to success was performing the electroporation into the fertilized zygotes very early, enabling the genomic editing to occur before the first replication (S-phase) of the mouse genomic DNA.

Next, identifying genome-wide off-target sites is important to avoid and prevent or at least reduce false-positive and false negative sites; concomitantly, accurate methods to measure off-target mutation frequencies have to be developed. Current genome wide sequencing platforms often cannot detect off-target sites at frequencies below 0.1%. Nowadays Next-Generation Sequencing (NGS) technologies have been successfully applied to reveal off-target genetic modifications after application of for example CRISPR/Cas technology. Through Whole Genome Sequencing, thousands to millions of sequencing reactions can be performed in parallel allowing a genome wide screening more rapidly in a high throughput manner. The data can be analysed by the CRISPR Genome Analyzer platform (CRISPR-GA), currently a popular bio-informatics tool that provides information on size and location of indels and on the efficiency of NHEJ and HDR events. A recently developed, easy-to-use bioinformatics tool for batch analysis of NGS-generated genome editing data allows detection of indel mutations and other precise genome editing events and the rapid calculation of the corresponding mutagenesis efficiencies (Boel et al., 2016). This progress in off-target detection will help ensure that the CRISPR Cas or similar gene editing technologies work in a safe and accurate manner.

2.2. GENE EDITING OF MALE AND FEMALE GERM CELLS

An alternative to the zygote/embryo approach is to perform gene modifications during early gametogenesis. In this manner, growing immature oocytes or sperm or even precursor cells (primordial germ cells) can be gene targeted by using the Crisp/CAS system, producing genetically corrected mature sperm or oocytes that subsequently can be used for assisted reproductive technology. In the male germ cell line, spermatogonial stem cells (SSC) can be harvested more and more efficiently, and in vitro culture systems are being developed also in the human, and optimized for efficient production of sperm in vitro. So far, animal models have indicated that SSCs can be propagated as clones in culture and then transplanted back into the testis to generate mature and functional sperm (Goosens et al., 2013). So a potential strategy would be to select SSC clones which have undergone correct genomic editing and are free from off-target mutations. These can then be transplanted to undergo final maturation in vivo. Alternatively, the gene edited SSCs can be directly differentiated in vitro to mature gene corrected sperm to be used for in vitro fertilization. Still, optimisation of in vitro culture systems is warranted, especially in the human so that gene editing technologies can be employed safely and with high efficiency.

In the female germ line, the oocyte is more easily accessible for genetic manipulation, but at this stage technical hurdles remain, such as the small number of oocytes that are available (Vassena et al., 2016). It has been suggested that oogonia-like stem cells could be harvested, cultured and expanded (Woods and Tilly, 2013) followed by in vitro culture to the mature MII stage. However, there is still controversy over the existence of such oocyte precursor cells in the female and the efficiency at which mature, developmentally competent oocytes can be derived from them.

A last possibility is to produce gametes in vitro from pluripotent stem cells (PSCs). These PSCs could either be human embryonic stem cells, made genetically identical to the prospective parent by somatic cell nuclear transfer, or PSC obtained through the direct reprogramming of differentiated cells (induced pluripotent stem cells, iPSCs.). One advantage of this approach is that pluripotent stem cells in culture are easy to manipulate to correct genetic abnormalities. Pluripotent stem cells can be grown easily in bulk amounts, especially in the naive state of pluripotency (Hanna et al., Duggal et al., 2015; Van der Jeught et al., 2015), and sustain single cell passaging which makes them an ideal source for gene editing experiments with the CRISPR/Cas system. Ultimately, these PSCs have to be differentiated towards oocytes or sperm that would contain the genetically corrected information and can thus be used in assisted reproductive technology. PSC-derived gametes were successfully established in mice (Hayashi et al., 2011; 2012), although this approach still required an in vivo maturation step to obtain functional oocytes or sperm. Nowadays, full differentiation of mouse embryonic stem cells into mature functional sperm (Zhou et al., 2016) and oocytes (Hikabe et al., 2016) has been achieved in vitro. If/when this strategy proves successful in the human, despite the system's greater complexity, genomic editing might well be successfully applied to produce gene edited gametes and offspring in the future.

2.3. GENOME EDITING IN THE mtDNA

Another form of DNA modification, albeit not in the nuclear DNA, involves the transfer of donor mitochondria containing mitochondrial DNA (mtDNA) from one cell to another (Hyslop et al., 2016), or the use of genomic editing in the mtDNA to eliminate mutant mitochondria or at least change the heteroplasmy ratio (the proportion of abnormal copies of mtDNA) in progeny, e.g. in order to rescue deficiencies in oxidative phosphorylation (Bacman et al., 2013; Jo et al., 2015; Diot et al., 2016). This mitochondrial DNA modification (whether achieved by mitochondrial replacement technique (MRT) or by editing of the original mtDNA) should be considered as a genome-altering procedure which can be passed on to future generations. Note that in MRT the genome is not edited, and different safety and efficacy considerations apply when compared to gene editing. In theory, mitochondrial DNA modification can also cause heteroplasmy containing two or more sets of genetically different mtDNA in the resulting oocytes, as a result of an incomplete elimination of mitochondria with mutant mtDNA or of undetected off-target effects (genetic or epigenetic). More recently, MRT has been proposed to overcome maternal transmission of serious mitochondrial DNA disorders. For nuclear transfer, the nucleus of an oocyte from a patient with a known mtDNA mutation is transferred into a healthy enucleated donor oocyte (spindle transfer) or zygote (pronucleus transfer) to overcome mtDNA disorders. The safety and efficacy of this nuclear transfer technology has been mostly studied in animal models but has also been shown to cause little if any heteroplasmy in human tripronuclear embryos (Hyslop et al., 2016). These NT technologies appear to be efficient in terms of embryonic development and safe in terms of minimal mtDNA carryover (Yamada et al., 2016; Hyslop et al., 2016). In April 2016, the first child resulting from maternal spindle transfer was born (Zhang et al., 2017). The mother was an asymptomatic carrier of a mitochondrial mutation that caused Leigh syndrome, a fatal neurological disorder. The child, who has 1% of its mother's mtDNA, was healthy at three months, although it is not known if any abnormality might appear later on. The use of gene editing technology appears also to correct mtDNA disorders. In a heteroplasmic mouse model containing two genetic backgrounds of mtDNA, selective prevention of germline transmission of one kind of mtDNA has been accomplished using either mitochondria-targeted restriction endonucleases or TALENs (Reddy et al., 2015). CRISPR/Cas9 has also been successfully employed in

mtDNA editing, and mitoCas9a (a new version of the enzyme Cas9) with specific localization to the mitochondria has been developed (Jo et al, 2015).

2.4. RATIONALE FOR GERM CELL GENOMIC EDITING

The following lines of basic research involving genome editing technology in the germline can be considered: 1) addressing fundamental questions of early human and animal developmental biology 2) gaining information to understand and improve the technique and safety of genome editing itself, and 3) engineering specific disease-related mutations in embryos used as experimental animal models, to subsequently analyse the genome edited offspring or derivative PSCs for the development of drugs or other treatments for disease. These research applications are important for gaining more insight into basic developmental biology regardless of any potential future human reproductive GLGE. So far, most of our knowledge of early embryonic development is based on animal models, especially the mouse. However, recent studies have shown that the molecular pathways involved during early embryonic development differ between animal models and humans. For example, the SOX17 gene was recently demonstrated, by the use of human embryonic stem cells, to be crucial for the formation of primordial germ cells in the human, while this is not the case in the mouse (Irie et al., 2015). Accordingly, in the UK, a license was approved this year for Dr Niakan (the Francis Crick Institute) to study early lineage segregations in humans. This will hopefully allow us to understand the molecular pathways involved in early embryonic development and differentiation, and - amongst others – might for example also be beneficial to disentangle complex or poorly understood causes of infertility (for example implantation failure) and develop novel routes for treatments.

Germline editing technology could potentially also be applied to other infertility related treatments. For example, it has been shown that mutations in the PLCzeta gene, which is responsible for successful oocyte activation, can lead to failed fertilization after ICSI (Kashir et al., 2010). Two point mutations in the exons of the PLCz gene were identified, one paternally, one maternally inherited (Kashir et al., 2012) which might be corrected by genomic editing during spermatogenesis of the male patient or following the strategy of first deriving patient specific stem cells, followed by gene correction in stem cells and finally differentiation towards functional sperm containing the corrected PLCz gene and function. Similarly, variants in the polo-like kinase 4 gene (PLK4) which predispose to embryonic aneuploidy (Mc Coy et al., 2015) might be corrected in the female germ line and thereby increase the chance of obtaining more euploid embryos for implantation and conception of a genetically normal child.

3. Legal regulations reg. GLGE (European level)

The focus of this paragraph is on transnational, or in particular European regulations of embryo research (3.1.) and germline genetic modification (3.2.). The international regulatory landscape for

developing GLGE has been summarized by Araki and Ishii (Rep Biol Endocrinol 2014) and Isasi, Kleiderman and Knoppers (Science, 2016).

3.1 Embryo research

An important element in this landscape is the variety of national legislation governing embryo research among European countries. On the one side of the spectrum, there are some countries (e.g. Austria, Germany, Italy) forbidding all research using human embryos (only excepting 'non-instrumentalizing' research aimed to benefit the embryo in question). On the other side, a limited number of countries (e.g. the UK, Belgium and Sweden) allow the creation of human embryos for research purposes. In between are countries (e.g. France, Portugal, the Netherlands) allowing human embryo research using left over IVF-embryos, while explicitly forbidding the creation of human embryos for purposes other than pregnancy.

At the European level, an important document is the Convention on Human Rights and Biomedicine (Oviedo Convention) of the Council of Europe that has been ratified by 29 states. Article 18 part 2 of this convention forbids the creation of human embryos for research purposes. As ratification requires further national legislation by the ratifying partners to be in line with the Convention, countries having ratified this document are bound to maintain this ban.

To the extent that human embryo research aimed at developing GLGE requires normal one-cell stage embryos, these embryos will have to be created specifically for the purpose. As a consequence, this research can be carried out in a limited number of European countries, depending on further regulatory conditions also with regard to accepted purposes. Should the research move to investigating GLGE in blastomeres, left-over IVF embryos could be used in countries allowing research with those embryos, depending again on further conditions. Given the ethical sensitivity both of creating research embryos and of asking women to donate the necessary oocytes, the principle of subsidiarity (as for instance laid down in the Belgian Embryos' Act, Article 4 part 1) requires that no human embryos should be created for research that can as well be performed with spare embryos.

3.2. Germline genetic modification

The scope for future clinical reproductive application of GLGE will depend on legislation conditionally allowing rather than categorically forbidding procedures aimed at modifying the human germline. Whereas most countries (based on recent surveys) currently prohibit germline modification, many of the concepts used in relevant legal documents are ill-defined and ambiguous, including the distinction between research and clinical applications and basic definitions (Araki and Ishii, 2014; Isasi et al., 2016). For instance, whereas the Belgian Embryos' Act does not contain a categorical ban on germline modification, it contains a provision (Article 5 part 4) forbidding "research or treatment with a eugenic purpose, that is: aiming at the selection or enhancement of non-pathologic genetic traits of the human species" (Article 5 part 2).

Here again, the Convention on Human Rights and Biomedicine (Oviedo Convention) of the Council of Europe is a key document. Article 13 states that "An intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants."

There has been much debate about whether mitochondrial replacement therapy (MRT) aimed at helping women at risk of transmitting a mitochondrial disorder to have healthy children should be regarded as a form of germline modification (Health Council of the Netherlands; Nuffield Council on Bioethics, 2012; Castro 2016). MRT refers to procedures in which the pronuclei, meiotic spindle or polar bodies of the prospective mother's oocytes are transferred to an enucleated donor oocyte (Wolf et al., 2015). While UK legislation prohibits clinical applications of germline modification, this ban does not apply to MRT, given recent regulations specifically allowing the reproductive use of this technology subject to some conditions (2015). As these regulations specify the method used in MRT (germline transplantation rather than editing), this has no implications for a potential use of GLGE of mtDNA.

The legal context is different in the Netherlands, where legislation was also adapted to allow for MRT (Dondorp and Bolhuis, 2002). As this was done by limiting the ban on reproductive germline modification in the Embryo Act (2002) to the intentional modification of the nuclear DNA (Article 24g), it would seem that not just MRT, but also clinical GLGE of mtDNA is beyond the scope of the legal prohibition of germline modification in the Netherlands. Whether the legislator would have accepted this implication is an open question as GLGE was not yet available when the Act was drafted.

These are only a few out of many examples of how legislation in this field tends to lag behind the dynamics of the very technologies that it means to regulate (Isasi et al., 2016). Considering the dynamics of the scientific discoveries and medical developments it is relevant to reflect on what were the arguments behind such legislation. Do these still apply? If a technique can help to avoid serious genetic disorders, in a safe and effective way, would this be a reason to reconsider earlier standpoints? It should be considered to structure a legal framework which could be more flexible and promptly reactive to the evolution of the technologies and possibilities, to ensure an appropriate societal oversight.

From an ethical point of view, scientists and clinicians must respect the legal and regulatory framework in their country. They also have an important responsibility to help society understand and debate the full range of possible implications of the new technologies, and to contribute to regulations that are adapted to the dynamics of the field while taking account of ethical considerations and societal concerns. As the European professional and scientific organizations most comprehensively involved in the development of GLGE technology, ESHRE and ESHG are well placed and willing to take up this responsibility.

4. Ethics

While the scenario of future genetic engineering/manipulation/modification in the human germline has been a topic for debate for decades, recent developments in genome editing give a new impetus to ethical and societal discussions. For an adequate debate and reflection, it is important to make a distinction between (non-reproductive) basic GLGE-research (4.1.), (non-reproductive) preclinical GLGE-research (4.2.), and possible future human reproductive, clinical, GLGE (4.3.), taking account of both moral concerns and objections, and scientific and possible clinical advantages of GLGE. Although the distinction between basic and preclinical research is important, there may well be an overlap

between these types of research. For instance, basic research may aim at e.g. improving the precision of the technology, thereby lowering the risk of off-target effects and enabling future clinical GLGE.

4.1. Basic research

4.1.1. Possible advantages

A general advantage of basic research is that it will generate new scientific knowledge which may contribute to improving human health and welfare. GLGE-linked basic research mostly regards the study of fundamental questions regarding human embryology and the methods applied in gene editing. A good example regards the plans of researchers in e.g. the UK to study genetic factors linked with early embryo development, implantation and problems with both development and implantation. This may help to improve the success rate of assisted reproduction.

4.1.2. Objections and concerns

There are different types of ethical objections, both deontological and consequentialist, to basic research reg. human GLGE, especially as and insofar this involves the research use of human embryos.

A. Deontological objections and concerns

Questioning the 'instrumentalizing' use of human embryos in basic GLGE research relates to a wider legal and ethical debate on embryo research that has been evolving over more than three decades worldwide. Critics of 'instrumentalizing' embryo research mostly argue that such research is at odds with the presumed/proclaimed high, even 'person-like', status of the embryo.

This view is, however, widely contested (ref.). Though the moral status of the early embryo is significant, there is wide support, both in secular ethics and in many religions, for the view that at least the early embryo has a lower moral status than fetuses, and much lower than children and adults. For that reason, legal regulations and ethical principles and guidelines regarding medical research with human subjects, aimed at protecting research participants from serious harm, do not apply to early (preimplantation) embryos in vitro (especially if these embryos will not be transferred). (The arguments for this view differ, and relate to e.g. the lack of sentience and cognitive functions, and/or the lack of so-called 'ontological individuality'; after all, the early embryo may still split or different embryos may combine to build one single embryo.) Against this background, many countries have accepted a regulatory approach which leaves room for at least some embryo research on (more or less strict) conditions, including proportionality and subsidiarity (cf par. 3.1.).

Parthenotic embryos ('parthenotes') and 3PN embryos can and have been used as alternatives for 'normal' embryos in research, including basic research on GLGE (cf par. 2). These alternatives may be used and preferred for different reasons, including the wish to mitigate or even completely avoid the controversy regarding embryo research. Parthenotes and 3PN embryos are regularly not considered to be embryos as they lack the potential to develop into a (viable) child (a condition that is frequently part of the definition of an embryo). Or alternatively, they are seen as embryos with a somewhat

lower status in view of this diminished potential. Postponing a stance on the possible merits of these normative views, it is sufficient for the moment to state that the research use of parthenotes and 3PN embryos does not pre-empt the need to make use of normally fertilized oocytes for research, including GLGE-linked research.

Most controversial is as to whether it would be ethically justified to not only make use of left-over or spare embryos, but to create embryos specifically for research purposes, so-called 'research embryos'. According to amongst others ESHRE, there is no fundamental, decisive, ethical difference between the two (ESHRE Task Force, ..). After all, both the moral status and the fate of spare and research embryos are the same. Still, it is widely - and rightly – accepted that one should not engage in making embryos specifically for research if this research can be conducted by using spare embryos. While spare embryos may be useful for research, including some types of GLGE-linked research, research embryos are sometimes necessary. Most importantly, spare embryos conceived by conventional IVF/ICSI are simply not useful for safety-research regarding new (pre- or peri-conception) reproductive technologies. Examples include in vitro maturation (IVM) and stem-cell derived ('artificial') gametes. Likewise, the making of research embryos is necessary for (some types of) preclinical GLGE research, especially if such research aims at studying the effects of GLGE applied in either gametes or in very early embryos, or when such research requires specific genotypes, not available in spare embryos.

Most jurisdictions accept a time-limit regarding embryo research of 14 days. Recent research suggests that it will become possible to grow human embryos in vitro for longer than 2 weeks. Research beyond 14 days may increase the value and relevance of both basic and pre-clinical safety research. This may hold true for GLGE-linked research as well. As a consequence, a (renewed) discussion about the ethics of a possible extension of the time-limit has started (refs e.g. Morris SA, et al. *Nature Communications* 2012; Pera et al. *Nature Methods*, 2015; Hyun et al., *Nature* 2016; Reardon, *Nature* 2016). Questions include: what, precisely, are the arguments in favor of the present 14-day limit? Are these arguments convincing or could a later time-limit be ethically and legally justified, and if so, which limit – and why? What about a slippery slope? Clearly, the time-limit regarding embryo research should be part of the agenda for further ethical and societal debate and reflection.

Another relevant item for the normative debate on research embryos is the position and protection of candidate donors of oocytes for research. Concerns regard both the autonomy and the welfare of oocyte donors. Amongst others, ESHRE recommended to impose conditions, in line with the regular/widely accepted safeguards to protect participants in research, aimed at minimizing the risk of pressure to donate and avoiding disproportionate medical risks and exploitation (cf Health Council, 1998; Pennings, et al., *ESHRE/Task Force Ethics & Law*, 2007). Such conditions should also be taken into account in the context of non-reproductive GLGE research with research embryos. The future availability of surplus oocytes frozen (but no longer needed) for fertility preservation might eliminate or at least lower some of these risks and concerns.

A final deontological concern emerges with the possible use of whole genome sequencing ('comprehensive' PGS) of edited embryos, including/in the context of a trio-analysis, as a method to pre-clinically study and reduce possible off-target effects (iatrogenic damage) in edited embryos. This strategy would require specific informed consent of the providers of the spare embryos or gametes

used. The central normative question raised by such screening would be how to respect the gamete or embryo providers' right (not) to know/(not) to be informed about any incidental findings (IFs) regarding their own genetic status. This question is not unique for such GLGE-linked research, as it also arises in the context of e.g. genomic research making use of human material in biobanks. Relevant guidelines for the responsible handling and communication of IFs can be found in recent literature and documents (refs).

B. Consequentialist objection: the 'slippery slope' towards reproductive GLGE

The slippery slope-argument against basic GLGE research presumes both that allowing such research will result in future reproductive applications (the empirical premise), and that such reproductive applications are ethically unjustified (the ethical premise). What about the strength of these premises: are these convincing?

Both premises are contested. With regard to the empirical premise, one may argue that reproductive applications of GLGE will not automatically follow – these could continue to be forbidden as is the case now in most countries worldwide. In scrutinizing the empirical premise of the slippery slope-objection, it may be important to make a distinction between basic and preclinical GLGE-research (even though the demarcation is not sharp) as preclinical research may be more vulnerable to this slippery objection than basic research. For that reason, this objection will be elaborated in that context (see below, par. 4.2.).

4.2. Preclinical research

Pre-clinical research aims at assessing the effectiveness and safety of possible future clinical/reproductive GLGE.

4.2.1. Possible advantages

The general value of preclinical research has been acknowledged in the normative framework for responsible innovation in assisted reproduction and related technologies (ARTs), as proposed by, amongst others, the Health Council of the Netherlands and (the Task Force Ethics & Law of) ESHRE. While new, experimental, reproductive technologies are often introduced in the clinic without proper preclinical research, including safety studies, this framework stipulates that preclinical research, primarily aimed at avoiding or at least reducing health risks for possible future children thus conceived, should be performed as much as is reasonably possible. Such research could involve using cells/tissues, animals, and human embryos in vitro (on strict conditions) (Health Council of the Netherlands, 1997; ESHRE, 2001). The ethical rationale of preclinical safety research is obvious: one should rather experiment on early embryos in vitro than on future children thus conceived and prospective parents/mothers. As Anne McLaren eloquently stated: to refrain from adequate preclinical safety studies is like "making the first test of a new aircraft-guidance system on a crowded Boeing 747" (McLaren, 1989). This framework would be relevant for any proposed future clinical applications of GLGE.

4.2.2. Objections and concerns

A. Deontological objection

If human embryos are used in preclinical research, such research, like basic research involving human embryos, is criticized because of the instrumental use of embryos. As indicated before, this criticism is based on a widely contested view on the moral status of the early human embryo (cf sub 4.1.2.).

B. Consequentialist: slippery slope

As stated before (cf. sub 4.1.2.), preclinical GLGE-research may be more vulnerable to the slippery slope-objection than basic GLGE-research; the step from basic GLGE-research to clinical application of GLGE is much greater than the step from pre-clinical GLGE-research to clinical application. After all, the whole idea of pre-clinical research is to study whether the conditions for sound clinical applications in terms of effectiveness and safety can be met. But crucially, the slippery slope's second, ethical, premise, namely that (any) reproductive GLGE is ethically unjustified, is not self-evident. In fact, there is wide disagreement regarding the ethics of possible clinical GLGE apart from safety-concerns. If one considers e.g. principled, deontological, objections to clinical GLGE to be convincing (cf par. 4.3), then preclinical studies would not only be a complete waste (of money, energy and embryos), but also bring us dangerously close to the edge of the slope. And as long as it is unclear as to whether safe clinical GLGE could be morally justified, preclinical safety research can be easily dismissed as premature (and disproportional). But if only safety issues stand in the way of morally justified clinical GLGE, then, obviously, preclinical safety studies would be justified.

4.3. Future reproductive human GLGE

4.3.1. Possible advantages

GLGE could, if safe and effective, contribute to greater welfare, first and foremost in terms of health gains. Such GLGE could well be more effective than somatic editing when it comes to multi-organ disease, and have a further advantage because of its trans- or multigenerational effects. According to some commentators, GLGE may also have positive - but contested - effects in terms of enhancement (see below) (Savulescu et al. Protein Cell, 2015).

Linked to this is the argument of respect for reproductive autonomy. People at high genetic risk of having a child affected with a serious disorder or handicap may feel more confident to reproduce or may see the editing as a prerequisite of reproduction (Sugerman, 2015). Even though there are other reproductive options to avoid genetic disorders in future children, people may have reasons to prefer GLGE (see below).

Last, but not least, correcting disease-causing genes may be seen as promoting justice: increasing the equality of opportunity of every person. The natural unequal distribution of capabilities (through the genetic lottery) is corrected by modifying the genetic constitution of persons who received less than their fair share of capabilities (Buchanan et al., 2000).

4.3.2. Objections and concerns

Again, it may be helpful to distinguish different types of objections and concerns:

A. Deontological objections

The deontological arguments against germline gene modification have been around for some decades. We will only discuss the more relevant ones and leave aside the general objections such as 'it is unnatural', 'playing God' etc. The main problem with several of the arguments is that they are very broad and would be applicable to many medical interventions that are (very) widely accepted, like contraception, fertility treatments and organ transplantation.

An important argument is that germline modifications threaten human dignity. The Parliamentary Assembly of the Council of Europe stated that human dignity implies 'the right to inherit a genetic pattern which has not been artificially changed' (Parliamentary Assembly of the Council of Europe, 1982) and also the Council of Europe declared germline modifications to go against human dignity (Council of Europe, 1997). However, if GLGE is used to correct defects and to restore health in future children, it is difficult to see how this would show a lack of respect for human dignity. A specific interpretation of the dignity argument is linked to the idea that the human species as such should be respected. Related to this idea is the presentation of the human gene pool as a 'common heritage' that concerns the whole of humanity (UNESCO International Bioethics Committee, 2015). There are at least two problems here. The first regards the concept of the 'human gene pool' (Juengst, 2006). Mistakenly, the human gene pool seems to be seen as a fixed catalogue of all human genes. It is unclear why the present catalogue should receive a special status. Moreover, for the sake of mankind, the gene pool has to continue to evolve. In addition, the present objection would mean that every mutation in every new person is problematic. To avoid this conclusion, one would have to focus on intentional genomic modifications. However, intentional modifications need not entail a change in the human gene pool. Gene editing could for instance be used to repair or reinsert an already existing gene (i.e. a normal allele). Supposedly, critics want to prevent the (hypothetical) introduction of genes that would alter the person to a point where he or she would have features that no other human being has. This may be a valid argument against genetic enhancement (although the line between treatment and enhancement is difficult to draw, see below), but not against the editing of disease-causing mutations. Apart from the fact that human dignity is a very broad concept and notoriously difficult to define, it looks as if this argument cannot serve to condemn all germline gene modifications.

Another objection is that germline intervention would violate the autonomy of future children. Different variants of this objection may be discerned. A first version holds that GLGE would be at odds with the autonomy of children thus edited as they did not consent to having their genome modified (Collins, 2015) nor to being included as research subjects (in clinical trials). Although correct, this is true for all reproduction (including natural reproduction) and it is even theoretically impossible to respect this interpretation of autonomy since there is no person before or at the moment of the decision. A second variant of this objection is that GLGE would undermine (what Joel Feinberg has called) the child's right to an open future (Feinberg, 1980), in that the child would be pre-determined and pressed into some sort of a mold in order to optimally meet his parents'

expectations. This would not only 'instrumentalize' the particular child, but would simultaneously undermine respect for human beings more generally (Habermas, ..). Again, this criticism may be relevant for at least some (theoretical, even largely unrealistic) types of enhancement (see below), but seems not to apply to editing disease-causing mutations.

A somewhat similar argument is brought forward at the species level: future generations have a right to an unmodified gene pool (Frankel and Chapman, 2000). For some authors, the issue of germline modification is linked to human rights (Annas et al., 2002). Altering fundamental human characteristics may lead to inequality and unjust situations in two ways: either the modified 'people' would be superior to the unmodified people and would be unduly privileged, or the modified 'people' would no longer be considered as human and consequently may be deprived of their human rights. But again, this argument seems to be mostly directed at enhancement and is not relevant when it comes to therapeutic or medical editing of disease-causing mutations.

B. Consequentialist objections

These objections and concerns regard the risks of reproductive GLGE. It is important to discern medical (health related) and social risks:

B1. Health/medical risks:

Health risks of GLGE regard not just the particular edited (embryo and) future child, but also next generations (in plural). The types of risk are rather diverse (cf par.2) and include off-target effects, (antagonistic) pleiotropy, genetic and epi-genetic risks. While recent literature seems to be quite reassuring in that new variants of CRISPR are depicted as having increasingly less off-target effects, at the same time some experts worry that 'CRISPR enthusiasts have their head in the sand' about the safety of editing (Begley, July 2016).

There is a strong consensus worldwide that, in view of the many unknowns, including the uncertainty about the reversibility of possible adverse health effects, any clinical GLGE would be at least premature. The question is whether it could ever be sound to apply GLGE clinically and if so, on what conditions. In order to reduce health risk for children thus conceived, a combination of measures and safeguards could be considered, including:

- a) performing adequate preclinical research,
- b) embedding possible future reproductive GLGE in a research trajectory,
- c) focusing clinical GLGE on/limiting GLGE to causative genes in order to minimize the risk of pleiotropy (check: already explained in par.2?) and make any such residual risk proportional,
- d) adding back-up WGS/WES-based PGS (and/or similar prenatal screening), and
- e) embedding clinical GLGE in long-term follow-up studies (which may be especially important for risk reduction for next generations).

Preclinical research

There is a strong consensus that clinical GLGE could only be justified after adequate pre-clinical studies and that more pre-clinical GLGE research is needed (REFs). This approach ('patience, not patients') is in line with the general framework for responsible innovation in assisted reproduction as developed by amongst others ESHRE (see par. 4.2). Clearly, questions about risk are not just scientific, but also normative. The vexing question remains when findings of adequate preclinical safety studies would be sufficiently reassuring to justify experimental reproductive technologies in general and clinical GLGE in particular - how safe is safe enough? What is the proper evaluation standard for any such risks? Even after extensive, reassuring pre-clinical safety-research there will always be a residual risk that can only be clarified by engaging in clinical research. A 'zero risk-tolerance' criterion would preclude any clinical innovation (because of its inherent risk) and would be at odds with regular assisted reproduction practice. What is then appropriate evaluation standard? ESHRE has proposed to make use of a so-called 'reasonable welfare standard', aimed at avoiding 'a high risk of serious harm/suffering', combined with the moral responsibility of doctors involved to minimize risks for future children as much as is reasonably possible. But how to make this operational? Nevertheless, it is important to avoid arbitrary and ad hoc decisions, and to develop a coherent and transparent policy.

A clinical research trajectory

Whether we would consider the step towards clinical application, should depend on the outcomes of basic and preclinical research, taking account of further ethical and legal discussion, societal views, risks and implications (see below). If so, this should be embedded in a formal and rigid research trajectory. According to the Clinical Trials Regulation EU No.536/2014, Article 90 "No gene therapy clinical trials may be carried out which result in modifications to the subject's germ line genetic identity." The implication of this regulation, if interpreted strictly, may well be that clinical GLGE research will be impossible in the EU so that such research takes place outside the EU and without proper research protocols and oversight.

Focus on causative mutations/Reduction of pleiotropic risks

While the debate about the health risks of possible future clinical GLGE seems to concentrate on off-target effects, the risk of pleiotropy is as important, including antagonistic pleiotropy, meaning that decreasing the risk of developing a particular disease may simultaneously increase the risk of having another disease. Current knowledge of the human genome is rather fragmentary – which is a reason for concern regarding clinical GLGE:

"Pleiotropy may be a widespread phenomenon if antagonistic pleiotropic effects are pervasive then this suggests that we use caution when we design genetically-based treatments for diseases. ... a full understanding of the risks and benefits of undergoing such gene-targeted treatment is essential to ethical patient care. ... Rather than just focusing on a culprit disease allele and silencing it,

selectively inhibiting the allele's deleterious pathway while allowing the beneficial pathway to persist becomes a more responsible, albeit more difficult, course of action." (Carter and Nguyen, 2011)

This risk seems to be of lesser concern when clinical GLGE would regard well-known highly penetrant (causative), clearly pathogenic, mutations linked with Mendelian diseases - but may be more relevant when clinical GLGE would regard alleles linked with complex disorders and traits, caused by less or not understood gene-environment or gene-gene interactions. In view of this, GLGE of clearly pathogenic mutations can more easily be justified as being proportional than GLGE of less-well-understood mutations and genetic variants.

Back-up testing

One might consider to include WGS/WES-based 'comprehensive' PGS, combined with a trio-analysis, as a safeguard to detect off-target effects (iatrogenic damage) in edited embryos. Clearly, such PGS may simultaneously generate IFs, including both inherited and de novo mutations. The ethics of comprehensive PGS is more complex in a clinical context as compared with using such a safety-test as part of pre-clinical studies (see before). Ethical issues of the former include the feasibility of prospective parents' informed consent, the proper handling of difficult reproductive genetic counseling dilemmas, including possible tensions between transfer criteria and preferences of applicants on the one hand and professionals on the other, and the possible invasion of future children's right to an open future, more in particular their right not to know about their genetic status, i.e. their right to decide themselves, later in life, when competent, about undergoing predictive genetic testing for late(r) onset diseases (De Wert, 2009; Hens et al., 2012). In theory, at least some of these issues could be avoided or mitigated by an additional round of GLGE of any IF or off-target effect found – but this scenario seems to be far-fetched. A more realistic scenario (depending on the results of preclinical research into possible off-target effects of GLGE) may be to target clinical PGS to possible off-target effects and to then select an unaffected embryo for transfer. Maybe, similar WGS/WES-based screening could alternatively be considered during pregnancy.

Follow-up

Part of the framework for responsible innovation in assisted reproduction is the follow-up of children conceived through new, experimental reproductive technologies. A fortiori, any future clinical GLGE should be embedded in such follow-up studies. Experience so far with follow-up studies linked with ARTs shows that there are practical barriers and limits in terms of e.g. lack of funding and tensions with parental autonomy and familial and children's privacy, especially when it comes to long-term follow-up, as would be the ideal in this context.

Many critics of GLGE argue that there are safer alternatives, especially PGD - more precisely: PGD combined with a selective transfer of a 'healthy' embryo. In view of these alternatives, it is sometimes questioned as to whether there is a real need for human GLGE. (See below)

B2. Societal risks and concerns

There are different types of societal risks and concerns. We will focus here on four of these, two of which are often addressed under the name of 'eugenics'. As eugenics is a term with very different meanings, we will avoid this term as much as possible and point to specific societal concerns covered by this term (Paul 2016).

Disability rights

A first concern is that GLGE may have negative consequences for (at least some) people with handicaps and disorders. This concern, which does not regard GLGE specifically, but reproductive and genetic medicine more generally, is often called 'the disability rights critique'. In the background of this critique is the tension between the medical and the social model of disability (Shakespeare, Parens/Asch). While the medical model, aimed at prevention and treatment of disease, is based on an individualistic account of disability that focuses on the biological deficit, the social model stresses societal and cultural co-determinants of disability, like exclusion of people with impairments, environmental barriers to participation, stigmatizing cultural discourse, and discrimination. In recent years, patient organizations (sometimes explicitly under the umbrella of the disability rights movement) have substantially contributed to a public policy agenda aimed at strengthening the societal position, interests and rights of people with disabilities through barrier removal, anti-discrimination legislation and inclusion of people with disabilities. At the same time, patients organizations are very much in favor of research on new therapies.

The disability rights concern comes in different forms (Buchanan et al., 2000). One is the so-called 'expressivist' argument, holding that genetic interventions, including GLGE, express negative judgments about the worth of life of people with disabilities, which would violate their right to be regarded as persons of equal standing. Obviously, society should (continue to) protect the equal rights of people with impairments. However it is difficult to see how this would amount to an argument against developing new (genetic or non-genetic) therapies which may substantially improve their quality of life and avoid serious suffering. A second concern is that both preventive and therapeutic medical interventions, including GLGE, could reinforce the traditional medical model, with its blindness for sociocultural determinants of disability. While a one-dimensional medical approach is, indeed, to be avoided, this is not an argument against developing new treatment options either. A final concern is articulated as the 'loss-of-support' argument, i.e. that to the extent that genetic science, prevention and therapy will lead to reducing the number of disabled people, public and political support for these people will also dwindle. While this concern underlines the continued responsibility of society for also supporting lower numbers of people with particular handicaps, this is again not a good reason to refrain from developing new types of prevention or therapy, like germline or somatic GE. If we would accept the loss-of-support argument, we should also stop current preventative programs, like recommending folic acid to pregnant women in order to reduce the risk of neural tube defects – which would be unacceptable.

Some commentators, taking the social model to its extreme, argue that there is no real need to invest in the development of new treatments, genetic or otherwise. In their view it is society that needs to be treated, rather than people with impairments. While it is certainly true that at least part of the problems that many people with impairments encounter could be diminished or even avoided if society were more inclusive of diversity, the problems of people with impairments cannot simply

be reduced to prejudice and exclusion. The extreme variant of the social model disregards limitations that cannot be erased in even the most 'barrier-free utopia' (Shakespeare, ...; Davis, 2010).

All in all, the disability rights critique forcefully reminds society of its responsibilities towards people with disabilities, more particularly its obligation to remove barriers for inclusion, but it should not be used as an argument against the development of medical therapies, including GE, irrespective of whether it regards somatic or germline GE.

The undermining of reproductive autonomy

Concerns have been raised that reproductive GLGE will increase the pressure to avoid the conception of affected/handicapped babies and as a result undermine prospective parents' reproductive freedom. This may happen in different ways: by more or less subtle moral and social pressure or even by enforcement/coercion. How likely is this scenario, how is to be evaluated and what are the implications for policy-making regarding on clinical GLGE?

For evaluating this objection, it is important to place GLGE in the broader context of repro-genetics and options to avoid the birth of (seriously) affected/handicapped children. Although views about prospective parents' responsibility regarding the handling of possible reproductive genetic risk vary widely, there is a strong consensus in ethics that taking reproductive genetic risks is not ethically indifferent and that prospective parents should in principle try to avoid at least high risks of serious suffering for future children. This is widely considered to be a moral – not a legal – responsibility. Direct coercion (legal enforcement) to make use of 'preventive' options like prenatal testing and selective abortion or PGD, maybe linked with GLGE, would be ethically and legally unjustified – and seems to be rather unlikely, at least in democratic states respecting human rights. Still, socio-moral pressure to avoid high genetic risks of having a seriously affected child may increase as the possibilities for such avoidance grow. In fact, this is a worry already regularly mentioned and encountered in the context of current repro-genetic options, like making use of donor gametes, prenatal testing and PGD, although there may also be pressure, at least in some cultures and families, to not make use of these options. Importantly, however, this risk is not widely considered to be a convincing objection to the offering of such options. On the contrary, these options are considered valuable first and foremost as they may allow prospective parents to take measures to avoid the conception or birth of seriously affected children in their families. In view of this, and taking account of the fact that GLGE would add only a little to any already existing, more or less subtle, social pressure, it would be problematic and inconsistent to prohibit GLGE in order to protect reproductive autonomy. Instead of selectively and arbitrarily prohibiting a particular technology in order to protect future reproductive freedom, society should uphold and materialize its willingness and commitment to provide adequate medical care and societal support for each and every handicapped baby. Any punishment of parents who conceive a handicapped baby, whose conception and/or birth could have been prevented, by withholding (funding for) adequate medical care of such babies would not only undermine the reproductive confidence and autonomy of prospective parents, it would also be highly unjust to these babies (De Wert and De Wachter, 1990; Dondorp et al., ...) and unacceptable at a societal level.

In the context of applying for assisted reproduction, the situation may be somewhat more complex. According to the widely accepted normative framework for genetic counseling and testing in reproduction, the central ethical principle is respect for reproductive autonomy. This is made operational by underscoring the importance of both the prerequisite of voluntariness (as part of informed consent) and the ideal of non-directive counseling, which implies that doctors should support prospective parents at high risk of having an affected child, whatever reproductive option they select, preferably after an exploration of what options are available and what their 'considered feelings and views' are in relation to these. This accepted ethical guidance for reproductive genetic counseling is, however, not entirely applicable in the context of assisted reproduction. After all, doctors involved have the professional responsibility to take account of the welfare of the possible future child and to refrain from medically assisted reproduction in case of 'a high risk of serious suffering/harm' to the child (Pennings et al., ..). In view of this, it may be morally justified to offer PGD to applicants at high genetic risk of having a seriously affected child as a condition for access to assisted reproduction - a so-called 'coercive' offer (De Wert et al., 2014). If so, a case could perhaps be made for offering GLGE (instead of PGD aimed at a selective transfer of a 'healthy' embryo) to prospective parents at high genetic risk as a condition for access to assisted reproduction. There may well be different views about whether this would indeed be acceptable – but, even if clearly problematic, this scenario is not a good reason to refrain from offering GLGE (if proven to be safe and effective) at all.

'Enhancement': designer babies

An important concern is the fear that prospective parents (and clinicians/companies) may engage in making so-called designer babies. Like 'eugenics', the terms 'germline enhancement' and 'designer baby' are often not precisely defined. Mostly, the terms seem to refer to modifying/editing the genome of future children for non-medical reasons, or, maybe more precisely, for the improvement of normal traits. Examples often mentioned include raising cognitive skills/IQ, a social attitude/empathy, and exceptional musical and sporting capacities. But strengthening the resistance to diseases or eliminating carrier status for recessive conditions (in situations where being a carrier has no clinical significance for the health of the carrier himself) can also be regarded as forms of enhancement – which illustrates the conceptual blurring of medical and non-medical, preventative and enhancement-like applications (a.o. Health Council, 1989; Buchanan et al., 2000; Comfort, 2012).

People have different expectations regarding the feasibility of germline enhancement. While 'designer baby'-talk plays a major role both in the imagination of the public and in scenarios discussed by policymakers and philosophers/ethicists (partly as a consequence of the genetic reductionism communicated by many high-profile geneticists in the past), most biomedical experts seem to consider this as science fiction, at least insofar as making a 'designer baby' would require the modification of complex traits. A recent publication clearly states that even with the most accurate and reliable version of CRISPR, programming favorable traits in human embryos may not be possible (Janssens, 2015; 2016). Technology is not the limiting factor in the enhancement of individuals, rather, so it is argued, it is nature. A trait can be edited in the germline only when two conditions are met. First, the trait must be predominantly determined by DNA – its heritability must be close to

100%. According to a recent review, most potentially desirable traits have a moderate heritability; for example, the heritability of intelligence and higher-level cognitive functioning is around 50% (Polderman et al., 2015). Second, for enhancement to be practical, the traits in question must be caused by a single variant or an interaction among a limited number of variants. Although it may become possible to edit DNA accurately at multiple loci, it is unlikely, so the argument continues, that we will learn anytime soon how to successfully edit tens or hundreds of variants simultaneously. GLGE for enhancement purposes should, then, be considered a non-issue: "(...) progress should not be hindered by an ethical debate about a potential misuse of the technology that will not be possible."

Others disagree, however, for different reasons (REFs). First, there may well be some non-a-priori-unrealistic examples of enhancement of less complex traits - X (resistance to particular infections?) and Y (please, feel free to add examples) may be good examples. Second, while successfully editing tens or hundreds of variants simultaneously is not a realistic option at the moment, this may well change. And thirdly: some prospective parents may want to make use of CRISPR – if safe and affordable – for more complex normal traits even if a successful programming of the desired trait cannot be guaranteed – a significant increase of the likelihood of the phenotype may be ‘good enough’ for them to proceed. It is not unrealistic, then, to expect that commercial companies and professional GLGE-‘enthusiasts’ may want to exploit prospective parents’ dreams (or their fears to lag behind) by selling them this technology as a means to at least improve their chances of having a ‘perfect child’ (however that is understood). Even if the designing is still imperfect, this may be a highly lucrative market (think of the analogy with companies which offer DTC-tests). Competition for market share may lead these companies to exaggerate both the heritability of the relevant traits and the probability of the effectiveness of GLGE. They may also see an attractive market in advertizing ‘smart combinations’ of GLGE and life-style modification, thus by-passing any accusations of outdated genetic reductionism.

Ethical evaluations of GLGE enhancement differ, not just because of different normative views, but also because the concepts and examples used differ significantly. In the early days of the debate on GLGE, there was an almost unanimous support for the view that we can and should make a sharp distinction, both conceptually and ethically, between gene therapy and enhancement, of which only the former could be morally sound (Anderson, 1989). But this strong consensus seems to have disappeared, maybe surprisingly quickly (Comfort, 2012). So-called ‘liberal eugenics’, arguing that prospective parents should be largely free to genetically design their future children, has gained considerable support, at least in the academic literature - which is not to say that anything goes. Most commentators who consider the idea of drawing a rigid line between (germline gene) therapy and enhancement to be problematic, both conceptually and ethically, seem to argue in favor of different ethical evaluations of specific types of genetic enhancement. Anticipating a better scientific understanding of the complexity of our genome and further technical developments and breakthroughs in the field of GLGE, it might be helpful to make a distinction between non-medical and medical genetic enhancement, and, with regard to the former, between ‘instrumentalizing’ and ‘non-instrumentalizing’ types of genetic enhancement. A more detailed reflection is beyond the scope of this paper (and, in view of the current state of science, highly hypothetical), so we only add a few remarks:

1) medical genetic enhancement: an often used example is the strengthening of the human immune system. This, so it is argued, may not be a priori unsound, as it is linked with the classical aim(s) of medicine and may in fact be comparable with traditional vaccination. A second example would be the editing of embryos carrying recessive conditions. While some authors simply assume that this would be ethically sound (check Wivel and Walters, 1993), others would probably dismiss this as a problematic form of population eugenics.

2) non-medical genetic enhancement: this is widely considered to be ethically unsound. Major objections are that this would be at odds with human dignity, would instrumentalize the child and undermine its right to an open future (see before; refs. Habermas (citat+comm A), Davis, Sandel, Safranski). But some scholars argue that non-medical germline GLGE would not necessarily instrumentalize the future child and should not be categorically dismissed. Some traits are, so it is argued, 'general purpose' means, i.e. capacities that are useful and valuable in carrying out nearly any plan of life or set of aims that humans typically have (Glover, 2006; Buchanan et al, 2000). A good example is intelligence; a genetically (or otherwise) enhanced IQ would not limit the child's right to an open future.

Even if the reasoning behind the latter view is sound, there are additional issues to be addressed - apart from the fact that precisely the example of genetically enhancing IQ is, because of the extreme complexity of this trait, qualified by genetic experts as totally unrealistic. One further issue is the need to take account of the possible implications of the phenomenon of pleiotropy, especially antagonistic pleiotropy. This is a more relevant issue in the context of possible GLGE of complex traits than when it is about GLGE of causative genes with a clear pathogenic effect (see before). Taking pleiotropic risks can be more easily considered to be proportional when it is linked with avoiding a serious disease than when it is merely about enhancing a normal, healthy future child.

No doubt, these conceptual and normative issues require a more detailed analysis and debate. For the moment, it is important to acknowledge that any enhancement-oriented GLGE of complex traits is far beyond what may become possible in the next years or even decades – and may never become a realistic option. That said, at least two policy questions are on the agenda right now. First, how to tackle the risk of commercial exploitation of uninformed, naïve, parental dreams about perfect children? Secondly, is it acceptable or wise to take the slippery slope-risk that allowing GLGE even if only for serious disorders will in the end, irrespective of well-intended mechanisms to avoid this, like strict criteria/indications and procedures for societal oversight of GLGE-practice, result in all sorts of germline enhancements? This question, like the former question about the ethical balancing of residual health risks of possible future clinical GLGE (cf par. ..), should be addressed taking account of another question: what is the added value of GLGE, in view of the currently available therapeutic and reproductive alternatives? (see below)

Inequity

GLGE will in most scenarios be a very expensive procedure. Even if the gene editing in itself may not necessarily be expensive, the accompanying steps (IVF, PGD, safety measures ...) most likely will be.

This raises the question of who will have access to the technology. This question is raised for every emerging technique and should be considered within the context of the distribution of scarce resources. The inclusion or exclusion of an ART procedure in a decent package of reimbursed health care will depend on the wealth of the state and on the status attributed to infertility. At the moment, some countries have generous provisions for medically assisted reproduction while others largely leave this to the private market. However, all countries have limits for the spending of public resources to infertility treatment (female age, number of cycles ...). These limits are justified by different criteria: efficiency, cost-effectiveness and safety. The same criteria could be used for the decision regarding the reimbursement of GLGE. Three general remarks: first, decisions on reimbursement and access could be made in steps. One could for instance at the start only provide reimbursement for certain types of diseases. Second, most affluent societies strive to give equal access to medical care for everyone. That does not mean that when equity is not reached, no one should have access to the treatment. This 'leveling down' justice would in fact block all progress in medicine. Third, affordability may also to a large extent depend on patenting and commercialization of the technique (Newson & Wrigley, 2015).

Beside the matter of access based on financial means, there is a second justice consideration that refers to prioritization of diseases. Here too, several criteria can be used: magnitude and frequency of need, cost-effectiveness, the existence of alternative interventions etc. (Hinxton Group, 2016). The experience with the introduction of other techniques such as PGD may help to design a strategy here.

Likewise, GLGE 'enhancements' to subsets of the population could exacerbate social inequities – (Internat. Summit; this Summit seems to disregard that the same may hold true for therapeutic GLGE), although this threat may be somewhat exaggerated in view of the serious scientific doubts about the feasibility of most, rather hypothetical/theoretical, GLGE-enhancements.

4.2.3. Alternatives for reproductive GLGE: 'no real need' for the latter?

In view of both the medical and societal objections and concerns, alternative options should be taken into account of for parents 'at high risk' of having an affected child and who do not wish to have children affected by the specific condition they are at risk for. The alternatives may be of two different types: first, therapeutic (postnatal)/non-reproductive, and second, reproductive. The most obvious therapeutic alternative may be somatic GLGE. Reproductive alternatives include adoption, prenatal diagnosis (NIPD/CVS/AC), the use of donor gametes and (IVF/ICSI-) PGD aimed at transferring a 'healthy' embryo. The availability of possible alternatives seems to strengthen the reluctance and opposition to future reproductive GLGE (Health Council, 1989; Von den Daele, 1985): 'there is no real need to engage in germline GE' (Mertes and Pennings, 2015). But what about the effectivity and availability of these alternatives? And does the balancing of the pros and cons of these alternatives indeed lead to the conclusion that these are to be ethically preferred?

While expectations regarding the effectiveness and future clinical utility of somatic GE seem to increase, (safe and effective) GLGE could have two comparative advantages: it is more efficient because of its multi-generational preventive/therapeutic potential, and it may be more effective for the prevention/therapy of multi-organ disorders, at least in theory. For at least a subset of such

genetic disorders, somatic GLGE is simply not a realistic option/alternative. Somatic GE may become an effective treatment for less complex (single organ) disorders, and preferred by many, even if GLGE would prove to be safe and effective. But obviously, context matters. If, for example, prospective parents at high genetic risk are sub-fertile and apply for IVF/ICSI anyway, their fertility treatment might be relatively easy combined with GLGE if safe and effective instead of anticipating repeated somatic GE in consecutive generations.

With regard to reproductive alternatives several issues need further discussion, including:

Many prospective parents at high genetic risk do not meet the stringent criteria for adopting a child as accepted in the relevant jurisdictions. Maybe these criteria could be relaxed – but even then, this may not be a real, let alone the ideal, solution for at least some prospective parents:

Most prospective parents prefer to have a genetically related child – adoption or donor gametes are either not an option at all (the use of donor gametes is forbidden in many countries) or only second best. How to morally evaluate this preference? Obviously, this is a fundamental question which regards reproductive medicine generally. Even if one would argue that counseling might help prospective parents to reconsider their preference ('people can be very happy without having children', and/or 'children need not be genetically linked in order to build a happy family'), to disregard people's persistent preferences for a genetically linked child would be difficult to justify in view of the principle of respect for reproductive autonomy. Still, a further debate about the (relativity of the) value of 'genetic parenthood' is important.

No doubt, PGD aimed at a selective transfer of an unaffected embryo can and does help many prospective parents 'at high genetic risk' to have a (genetically related) unaffected child. To argue, however, that therefore GLGE is not really needed, is a non sequitur:

- In some (admittedly: rare) cases, prospective parents can only have affected children, e.g. when both partners are affected by the same autosomal recessive disorder. PGD is, then, a priori pointless/useless.
- Instead of routinely discarding affected PGD-embryos, a differentiated ranking system may be preferable which prioritizes unaffected embryos for transfer while cryopreserving the (good morphology) affected embryo(s) for a possible thawing, editing (if safe and effective) and transfer in a next cycle. This policy may well increase the take-home-baby-rate (THBR) of IVF/ICSI-PGD cycles and be more patient-friendly.
- Furthermore, in a significant number of cases, all embryos in a given IVF/ICSI/PGD-cycle lack the criteria for transfer, either because they all prove to be affected (think especially of so-called 'combination PGD', performed for two indications), or because the embryo(s) proves to be unaffected, but not a good match for a diseased sib needing hematopoietic stem cells (in the context of PGD/HLA-typing, aimed at conceiving a health 'saviour' baby). The couple may, then, be encouraged to engage in a new cycle or to consider to transfer an affected embryo or an HLA-mismatched embryo (assuming the couple's consent). If effective and safe GLGE would become possible in the future, an additional IVF/PGD-cycle would be disproportional in view of its (avoidable) burdens and risks (and costs). And wouldn't the transfer of an affected embryo be unsound if one could avoid the birth of an affected child by GLGE?

- For a comparative moral evaluation of 'standard' PGD and (PGD combined with) GLGE, some deontological aspects may be relevant as well. First, as 'standard' PGD aiming at a selective transfer entails the de-selection (and destruction) of embryos, while GLGE may (avoid or) at least reduce embryo-loss, the latter strategy may be the morally better one (if safe and effective and) if one acknowledges that the embryo has a significant moral status (which is not necessarily to be identified with a strict 'pro-life' view). Second, if prospective parents prefer reasonably safe and effective GLGE instead of PGD aiming at a selective transfer, to not accept this may be at odds with respect for reproductive autonomy.

4.2.4. Comprehensive PGS: a driver for systematic GLGE?

Assuming further technological improvements, comprehensive PGS, making use of high-resolution sequencing technology, could be used not only as an instrument in preclinical safety studies regarding GLGE (cf par. 4.1.2.) and as a safeguard in possible future clinical GLGE (cf par. 4.2.2.), it might also be (routinely) used in the context of future regular IVF. In fact, the latter is sometimes advocated in the medical literature for selecting 'the best embryo' for transfer. Such PGS - which raises a lot of ethical issues (cf par. 4.2.2.; De Wert, 2009; Hens et al., 2012) - might then function as a powerful 'driver' for clinical GLGE (if proven to be safe, effective and applicable to day 3 or even day 5 embryos), as all embryos, like all humans, are 'fellow mutants' and will prove to be 'affected' or 'at risk' in different ways, being amongst others heterozygous for some recessive conditions, and carrying predispositions for more or less common disorders, polymorphisms, and genetic variants. The challenge, then, may be/become to find/edit the embryo with the 'best' risk profile - which would push the application of GLGE far beyond the 'high risk of serious disease'-cases where it is regularly considered to be possibly sound in current ethical literature. Especially private clinics and clinics in countries with a lack of adequate regulation may want to (commercially) offer routine comprehensive PGS combined with gene editing of any 'defect' found. Obviously, this scenario would be problematic in view of the pleiotropic risks of GLGE, as these can be more easily considered to be proportional if linked with avoiding a serious disease than when it is just about editing lower risk factors or, particularly, enhancing a normal, healthy future child. But at the same time, this scenario urges society even stronger (than GLGE of one particular disease characteristic) to engage in a more principled debate about the ethics of and policymaking regarding the conceptually and morally grey area between therapeutic, preventive and enhancing GLGE.

Based on this overview of the ethical and legal considerations, the European Society of Human Genetics and the European Society of Human Reproduction and Embryology consider it to be their professional responsibility to contribute to further discussion on GLGE. Based on this background document Recommendations have been developed and discussed within both societies. We encourage other stakeholders to also engage in this debate.

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