Comparative ethical evaluation of epigenome editing and genome editing in medicine: first steps and future directions

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ABSTRACT

Targeted modifications of the human epigenome, epigenome editing (EE), are around the corner. For EE, techniques similar to genome editing (GE) techniques are used. While in GE the genetic information is changed by directly modifying DNA, intervening in the epigenome requires modifying the configuration of DNA, for example, how it is folded. This does not come with alterations in the base sequence ('genetic code'). To date, there is almost no ethical debate about EE, whereas the discussions about GE are voluminous. Our article introduces EE into bioethics by translating knowledge from science to ethics and by comparing the risks of EE with those of GE. We, first (I), make the case that a broader ethical debate on EE is due, provide scientific background on EE, compile potential use-cases and recap previous debates. We then (II) compare EE and GE and suggest that the severity of risks of novel gene technologies depends on three factors: (i) the choice of an ex vivo versus an in vivo editing approach, (ii) the time of intervention and intervention windows and (iii) the targeted diseases. Moreover, we show why germline EE is not effective and reject the position of strong epigenetic determinism. We conclude that EE is not always ethically preferable to GE in terms of risks, and end with suggestions for next steps in the current ethical debate on EE by briefly introducing ethical challenges of new areas of preventive applications of EE (III).

I. INTRODUCTION

(1) Structure of this article

As a first step towards a broader bioethical discourse on epigenome editing (EE), this article introduces the reader to the scientific background about EE and summarises previous debates (I.2–4).

It then evaluates whether EE is ethically preferable to genome editing (GE) in terms of risks it poses to edited subjects (II.1) and how EE compares with GE with respect to potential risks to future generations (II.2). For a comprehensive ethical evaluation of the risks of these two gene technologies (EE and GE), it is required to differentiate between several possible approaches to how EE and GE can be undertaken. Thus, instead of broadly comparing EE with GE, we ethically evaluate and compare different EE and GE approaches. These are in vivo somatic EE, ex vivo somatic EE, in vivo somatic GE, ex vivo somatic GE and germline GE.[i] We discuss the risks of potential unintended edits to edited subjects and the severity of such risks focussing on the comparison between in vivo and ex vivo somatic editing in general (II.1.i.a–c). We, furthermore, assess whether in vivo somatic EE is ethically preferable to in vivo somatic GE (II.1.i.d) and suggest that contrasting in vivo and ex vivo editing be one of three important *criteria for risk assessments of new gene technologies* (II.1.i.e). In section II.2, we compare EE and GE in terms of potential risks for the edited subjects' offspring and explain why EE is risk free in this regard as it cannot lead to germline changes (II.2.i). In this context, the assumption of epigenetic inheritability as well as the associated claim of strong epigenetic determinism are evaluated (II.2.ii), and the risks of generating inheritable effects when performing in vivo somatic GE are described (II.2.iii). Section II closes with a summary of the risk assessment to then answer the questions of whether somatic EE is always preferable to somatic GE in terms of risks to edited individuals (II.3.i), and whether somatic EE is preferable to germline GE (and to somatic GE) in terms of risks to future generations (II.3.ii).

In a concluding section (III), we recommend future directions for an ethical debate about EE. We suggest interdisciplinary cooperation to further refine our suggested three criteria for risk assessment of gene technologies, and to learn from challenging areas of application potentially unique to EE with a special focus on preventive approaches.

(2) Why we need a broader ethical debate about EE

In the past decade, several novel gene technologies have been developed, among them are GE and EE.[1] There is extensive ethical debate about GE.[2–5] In 2021, in a report on GE,[6] the *Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing* by WHO briefly mentioned EE acknowledging its moving closer towards clinical applications. As soon as clinical trials to test EE are prepared,[6 7] bioethicists will be among those who need to assess whether, and with which precautions, these trials can be undertaken. Several scholars have suggested that a broader ethical discussion of EE should take place.[6–9] In our view, first steps for a broader ethical debate on EE should, on the

[i]In somatic editing (EE/GE), cells of a person's body that are not part of the so-called germline are modified, while germline editing changes the heritable genetic information by modifying DNA of early embryos, gametes (egg cells/sperm cells), or precursor cells of gametes. In vivo somatic editing means editing inside the body; ex vivo somatic editing means collecting cells and editing them outside of the body.[53] These terms are further discussed and explained throughout this article.

Table 1 Potential applications of EE in comparison with GE in medicine

Disease groups	Potential future applications of SEE in medicine—based on reviews of preclinical research	Groups of diseases in which EE and GE are likewise conceivable—based on reviews of preclinical research on SEE and on preclinical and clinical research of SGE
Imprinting disorders (IDs)	Angelman syndrome; Beckwith-Wiedemann syndrome; Prader-Willi syndrome; Silver-Russell syndrome; transient neonatal diabetes mellitus type 1[60 73]	Because IDs have genetic and epigenetic causes,[60] they might be targeted by either SGE or SEE in future therapeutic approaches.
Genetic disorders	Duchenne muscular dystrophy; congenital muscular dystrophy type 1a; myotonic dystrophy types 1 and 2; haploinsufficiency induced obesity; Dravet syndrome; high-cholesterol disease; retinitis pigmentosa; diabetes mellitus (especially type 1); fragile X syndrome[73–77]	Genetic disorders present a potential target for germline GE as well as for SGE, with several ongoing SGE clinical trials for blood disorders (haemophilia B; sickle cell anaemia; β-thalassaemia).[44 78 79]
Neuropsychiatric diseases and application in brain tissue	See above (some of the IDs and genetic disorders); Parkinson's disease; Alzheimer's disease; intellectual disability; autism spectrum disorder[60 73–77 80–82]	SGE to treat Parkinson's disease is currently studied preclinically.[78]
Metabolic diseases	See above (some of the genetic disorders); hereditary fructose intolerance; glycogen storage disease type 1a[73 77 82]	SGE clinical trials for mucopolysaccharidosis types 1 and 2 are currently ongoing.[44 78 79] SGE to treat further metabolic diseases is currently studied preclinically.[83]
Oncological diseases	Leukaemia (AML, ALL); B cell lymphoma; prostate, breast, liver, colon, cervical, endometrial cancer[73 74 77 84]	SGE clinical trials for cancer have already been completed and are also currently conducted.[44 78 79 84]
Inflammatory diseases	Degenerative disc disease; idiopathic pulmonary fibrosis; acute kidney injury[73 74 76]	SGE to treat inflammatory diseases is currently studied preclinically.[85]
Infectious diseases	HIV; HBV[74]	There are several SGE clinical trials for infectious diseases, especially HIV, as well as HBV and EBV.[44 78 79]

ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; EBV, Eppstein Barr virus; EE, epigenome editing; GE, genome editing; HBV, hepatitis B virus; HIV, human immunodeficiency virus; SEE, somatic epigenome editing; SGE, somatic genome editing.

one hand, consist in introducing bioethicists to what EE is—by translating EE knowledge from science to ethics—and, on the other hand, in providing suggestions for bioethics—like criteria for risk assessment—how to evaluate concrete applications or research proposals using EE.

There is currently not only a broad interest in the ethics of GE, but also in the ethics of epigenetics.[10 11] Therefore, it is surprising that, to date, there are only a handful of contributions to the ethical debate about EE. This might indicate knowledge gaps about what EE is and substantiates the need for 'propagating epigenetic editing knowledge'[9] (p. 206) not only to the public,[9] but also to bioethicists. The recent assertion of Blumenthal-Barby *et al*[12] that bioethics 'needs people who can translate empirical and scientific findings and their implications into philosophical and theoretical discourse'[12] (p. 14) might be applicable to EE.[13] By explaining what EE is—at least how we, as ethicists, understand it—this article serves such a translational purpose.

(3) What is EE?

In the following, we very briefly explain the mechanisms of EE and compare them with the mechanisms of GE as background for the more detailed assessment of risks of EE in comparison with GE in section II.[ii]

In philosophical and ethical debates, the term 'epigenetics' is commonly associated with the interaction between genes and their environment, broadly understood, for example, what persons eat, what pollutants are in the air they breathe, etc. Instead, EE is conceptually based on a more specific and recent understanding of epigenetics related to the molecular configuration of DNA. Changing the epigenome with EE means changing the molecular configuration by employing an editing system very similar to GE. In GE, the CRISPR/Cas system[iii] or other gene editing tools (ZFN, TALEN)[iv] are employed to bind to targeted DNA sequences, cut and intentionally modify them.[14–24]

Like GE, EE is a novel gene technology, but EE does not alter the genetic sequence, instead, it alters the way DNA is structured three-dimensionally, specifically how densely it is folded.[9 25 26] If EE is done with CRISPR, a Cas enzyme with a 'deactivated' nuclease is employed. This Cas enzyme is called 'dCas'. The fact that dCas has a deactivated nuclease means that it cannot cause DNA breaks. A combination of the CRIRSPR/dCas complex and so-called 'epi-editor enzymes' can be used for modifying gene expression.[9 26–32] Upregulating or downregulating how often a gene is expressed alters the number of resulting proteins encoded by the gene, thereby affecting health. EE alters gene expression by modifying DNA methylation,[29 31 32] histone acetylation[28 32] or other epigenetic marks.[27 30 33] The methylation and acetylation patterns determine how DNA is folded and how dense it is. In simple terms: genes from very dense DNA cannot be transcribed. EE therefore operates at a different level than GE but can achieve similar results.

Our analysis of current preclinical approaches employing EE reveals many potential applications in medicine that are also GE use cases (table 1).

(4) Current ethical debates

The ethical discourse on the permissibility of gene therapy has increased significantly in the past decades, particularly around the Asilomar conference in 1975,[2 34] with debates exploding following the development of CRISPR/Cas for more effective and more efficient gene editing in 2012.[35–37] EE, on the contrary, has given rise to only a few ethical commentaries,[8 38] opinion papers,[7] a review incorporating ethical aspects[9] and regulatory considerations.[6 39 40] These contributions focus on EE for purposes of athletic enhancement,[6 7] on the merely hypothetical application of EE on germline cells[38] and on evaluating ethical-epistemic challenges associated with EE.[9v]

[iv]'ZFN' is short for 'zinc-finger nucleases'; 'TALEN' is short for 'transcription activator-like effector nucleases'.

[v]In addition, EE in military contexts is ethically assessed, as discussed in a report[86] of an interdisciplinary workshop at the Centre of Genomics and Policy at McGill University—from this workshop furthermore emerged a joint unpublished manuscript of the participants.[87]

While acknowledging that risks of EE should be further discussed, earlier ethical evaluations tend to emphasise the potential benefits of EE compared with GE over the potential risks. Two of these presumed benefits are that

1. in EE, other than in GE, '[t]he DNA sequence is unchanged. This means that there is little chance of damage from DNA repair (such as deletions, insertions, or chromosomal rearrangements)'[6] (p. 25). This implies a risk reduction for the edited individual.

The other presumed benefit is that

2. EE will not generate effects that can be passed on to descendants of edited individuals, which is a problem associated with germline GE, and a problem that can result as an unintended effect when somatic GE is used.[7][vi] This would mean a reduction of unintended effects for the offspring of edited individuals.

Refining previous rather short ethical assessments of EE and endorsing the call for caution by Huerne *et al*[9] (p. 205) not to 'create a false sense of security, leading scientists and regulatory stakeholders to be less proactive in considering the potential ethical or safety pitfalls unique to epigenetic editing during the adaptation of this technology for human use', we henceforth assess whether EE is preferable to GE in terms of risks it poses to edited individuals. We also assess whether EE comes with any risks to descendants of these individuals.

II. COMPARATIVE ASSESSMENT OF RISKS ASSOCIATED WITH EE AND GE

(1) Risks to edited individuals (comparison of primarily somatic editing approaches)

(i) In vivo versus ex vivo somatic EE and GE (with excursus to in vitro germline GE)

a) Risks of unintended edits

Unintended off-target modifications occur when the genome—in GE—or the epigenome—in EE—is altered at a locus other than the intended one. This happens frequently even with new 'tools' like CRISPR/Cas or CRISPR/dCas, respectively. In both, GE and EE, the CRISPR editing tool uses a guide RNA that 'guides' the epi-editors, or the nucleases that will change the genome, to a specific locus on the DNA strand, but guide RNAs are not very specific. Therefore, they can 'guide' the epi-editors or nucleases to parts of the DNA other than the intended ones and hence introduce changes where they were not planned. It follows that these unintended on-target or unintended off-target edits provide a risk not only in GE, but also in EE.[41–43] More specifically, in EE this is the case if the methylation or acetylation of DNA, but not the DNA base sequence, is changed at unintended loci. This means that genes can be silenced or activated that were not intended to be silenced or activated (unintended off-target effects of EE). Therefore, even though DNA breaks do not occur in EE, as the *Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing* by WHO[6] (p. 25) noted (see I.4), EE can nevertheless result in other unintended off-target effects. Furthermore, even if the editing takes place at the intended locus, EE could silence other genes placed close by, besides the targeted gene (unintended on-target effects of EE).

Thus, not only GE, but also EE can come with unintended on-target and off-target effects.[41–43] The changes can result in severe health conditions but might be less stable after EE than after GE, as we discuss ahead (see section II.1.i.d).

b) Reducing unintended edits by editing outside the body (ex vivo/in vitro)

Whether treatment with EE or GE is accompanied by unintentional edits depends less on the editing approach and more on whether there is a possibility to check for and correct unintended edits. If editing is performed in vivo, that is inside the body of the patient, unintended edits cannot be identified and corrected. In contrast, when cells are edited ex vivo,[vii] cells with unintended edits can be sorted before transferring them into the body so that it is possible to only return cells with intended changes. Furthermore, problems associated with the cytotoxicity and the immunogenicity of the editing tools or with the viral vectors that are used for the transfer of the editing tools[viii] are avoided if an ex vivo editing approach is chosen, both in EE and GE.[7 44–46]

For a comprehensive ethical evaluation of risks associated with in vivo GE and EE versus ex vivo GE and EE, it is important to note that, technically, in vitro editing also takes place outside of the body, that is, ex vivo. This means that, just as in ex vivo somatic GE or ex vivo somatic EE, the risk of unintended on-target or off-target effects is considerably smaller here than in in vivo somatic EE or in vivo somatic GE. To avoid implanting genetically edited embryos with unintended edits following germline GE in vitro, a 'control PGT (preimplantation genetic testing)'[47] would have to be carried out as recommended by NAM, NAS, and The Royal Society.[48] (p. 142) 'Control PGT' is a preimplantation genetic test prior to embryo transfer to control if germline GE was successful. This implies selecting embryos with intended edits but discarding those with unintended edits. Contrary to what some scholars[49–51] have argued, germline GE would, thus, *not* replace selective embryo transfer, as others[36 47 52] have pointed out. Embryo selection raises important ethical concerns. One of these concerns relates to the question of whether the human preimplantation embryo has a moral status that does not allow it to be discarded after embryo selection. This is an important concern that is relevant for an ethical debate that primarily focuses on germline GE, which lies outside the scope of this article.[ix] Selection of embryos therefore can be associated with a potentially successful attempt to reduce

[vi] Cf. 'One of the primary concerns with changes to germ cells is that deleterious changes could be potentially passed on to future generations, for whom germline changes would be irreversible. Such germline modifications are therefore typically unwanted for somatic editing in general, and e-GE [ie, epigenome editing, comment by KA and ECW] in particular. While a comprehensive scientific review of human e-GE is beyond the scope of this forum, it is important to note that several lines of evidence suggest that it is unlikely to result in changes that lead to enduring germline effects'[7] (p. 1652).

[vii] In ex vivo editing, cells that are to be genetically/epigenetically modified are first collected, second, edited, third, edited cells are screened for unintended edits, fourth, edited cells are only retransferred if unintended on-target/off-target edits (at least those detected by screening) have been excluded.[53]

[viii] This means that, for example, the Cas enzyme destroys cells or alerts the patient's immune system.

[ix] Another issue currently emerging from ethical discussions that we do not discuss here is related to the question whether germline GE is a technology of genetic selection in itself because of the control PGT. If germline GE is in essence genetic selection, the same child would not exist without germline GE being used that exists after germline GE was used. In other words, a whole different individual comes into existence if parents decide to use germline GE than if they reproduce without the assistance of germline GE. This, as some argue,[36 47 52] is because germline GE is in essence genetic selection since it uses a control PGT followed by selective embryo transfer, and this might support the position that germline GE can neither harm nor benefit edited individuals.[36 47 52]

the risk of creating individuals who suffer from unintended edits by discarding embryos with off-target edits.

c) Challenges associated with ex vivo editing

In view of these challenges associated with in vivo somatic GE, in vivo somatic EE and in vitro germline GE, ex vivo somatic editing of the epigenome or genome seems least problematic. However, an ex vivo somatic editing approach is restricted to specific diseases, such as blood disorders, where it is actually feasible to obtain affected cells from the patient, edit cells outside of the body and retransfer the edited cells following an assessment of whether unintended off-target or on-target edits have occurred.[53]

d) Reversibility of EE: is in vivo somatic EE preferable to in vivo somatic GE?

If in vivo somatic EE and in vivo somatic GE are compared, it must be considered that off-target and on-target effects of somatic EE are indeed different from those of somatic GE. EE does not come with risks of DNA cuts, false repair of them[6 54]; and the risk for integration of viral vectors is presumably lower in EE than in GE.[27] Nevertheless, research on EE is ongoing. As far as we can assess the current status, more evidence is needed to prove that in vivo somatic EE is overall less risky and preferable to in vivo somatic GE.

The presumption that changes to the epigenome are easier to actively reverse than changes to the genome[6 7 55] might be an optimistic interpretation of the current lack of stability of epigenome edits. Because of this instability, EE is not yet fit for clinical use.[26 56 57] To be considered fit, it will be required that EE can generate stable effects. Research in this regard indeed advances quickly.[6 58] However, should instability remain a problem, since instability necessitates repetitive interventions, EE might not be applicable to diseases that have a limited time window for interventions. Wherever such a time window does not exist and flexible treatment planning is possible,[59] EE could be carried out—even if it required repeated interventions—and is then preferable to GE in terms of risks. The price one must pay for this benefit of reversibility of unintended edits in potential cases of in vivo somatic EE would be multiple treatments instead of only one edit in in vivo somatic GE. This is a potential burden to the patient physically, emotionally and financially.[7]

e) Choice of in vivo or ex vivo editing as a criterion for risk assessment

Focusing on risks for edited individuals, EE is therefore not always ethically preferable to GE, or, in other words, choice of EE or GE is no general criterion for assessing how severe the risks to edited individuals are. A far better criterion would be (i) the choice of in vivo or ex vivo editing. Furthermore, severity of risks depends on the time of intervention (ii) and the targeted diseases (iii), which shall be assessed subsequently.

(ii)Time of intervention and intervention windows (somatic EE and GE)

EE has been suggested as an option to prevent early symptoms of imprinting disorders (IDs; table 1).[60] These are complex diseases with symptoms ranging from metabolic dysfunctions to neurological impairment and growth-related malformations.[61] IDs can either have genetic, or epigenetic causes.[61] EE could (in theory) be used in both scenarios. GE could be employed if the cause is genetic. Should somatic EE or somatic GE be used to treat IDs, a prenatal intervention, or an intervention soon after birth, that is, perinatal, would be required. This is unfortunate since if the intervention window to prevent IDs closes soon after birth,[60] the time window to reverse off-target editing is presumably then closed, too.

As shown above (II.1.i), somatic EE and GE can cause unintended off-target and unintended on-target edits. Whereas the criterion of choosing an in vivo versus ex vivo approach determines the likelihood of unintended edits, the criterion of 'time of intervention and intervention windows' determines the likelihood that such unintended effects are reversible. Therefore, the timing of the intervention and the existence of limited time windows for intervention is another important criterion for assessing the risks of new gene technologies such as EE and GE.

(iii) Targeted diseases (somatic EE and GE)

Unintended off-target edits can of course have completely unknown effects. It can, however, be expected that unintended on-target effects associated with EE or GE differ depending on which disease is targeted. For instance, in the case of targeting neurological impairments, they might affect neurological development. This would be challenging since, just as neurological impairment associated with the targeted disease itself, unintended neurological effects are presumably not treatable. Reversibility and treatability, and with this severity, of unintended edits of both EE and GE therefore probably depend not only on the time of the intervention, but also on the targeted diseases, or rather, on the targeted locus on the epigenome or genome and the treatability of unintended effects.

Which locus of the epigenome or the genome is targeted depends on the targeted diseases. For example, in the case of the severe ID Angelman syndrome, no effective symptomatic treatment exists yet.[60] The main purpose of using gene technology to treat this ID would be to prevent neurological impairment caused by this ID. Unintended on-target effects would likely also affect neurological development. In the case of other IDs, such as neonatal diabetes (table 1), potential unintended on-target effects, such as metabolic dysfunctions, are likely better manageable than effects at a neurological level.

With this, we have identified three criteria for assessing the severity of risks of new gene technologies like EE and GE to edited individuals: (i) in vivo/ex vivo approach; (ii) time of intervention and intervention windows; (iii) targeted diseases. These criteria are summarised in table 2. This analysis illustrates that not only GE, but also EE poses severe risks to edited individuals, and that EE is, therefore, not always ethically preferable to GE. Such a result challenges the presumed, and above outlined, ethical preferability of EE over GE in terms of effects on edited individuals based on the fact that EE does not come with DNA breaks. As shown, even without DNA breaks, unintended effects can occur and can be severe risks of EE.

(2) Risks to edited individuals' descendants

(i) Why EE does not generate inheritable (germline) modifications

To our understanding, it is more than just unlikely, but, based on current scientific views, impossible to envision that EE will impact the germline.[x] Epigenetic modifications introduced through EE are not transferable to future generations because epigenetic information is widely erased during germ cell maturation and again during early stages

[x]Lewens[88] seems to deny this (similarly Tompkins[89]). However, in the study by Lewens,[88] EE is not discussed in detail.

Table 2 Risks, challenges of implementation, further ethical concerns of different editing approaches

Editing approaches	In vitro germline genome editing	In vivo and in utero somatic genome editing **(in utero referring to editing of a fetus, ie, in vivo approaches where the fetus is the patient/research subject)**	In vivo and in utero somatic epigenome editing	Ex vivo somatic genome editing and ex vivo somatic epigenome editing
Severity of risks depends on	(i) In vivo/ex vivo: whether the intervention is performed inside or outside the body			
	(ii) Time of intervention and intervention windows: how early in development the intervention takes place			
	(iii) Targeted diseases: risks specific to the targeted locus on the genome or the epigenome			
	inheritability: risk factors (i)–(iii) are multiplied each time an artificially introduced modification would be inherited			
Risk of unintended off-target and unintended on-target effects	Yes, but at least some unintended edits can be detected in edited embryos, embryo selection before transfer of genome edited embryos will likely become state of the art	Yes	Yes	Yes, but at least some unintended edits can be detected before edited cells are retransferred to the patient (which significantly minimises risks)
Inheritability of unintended off-target and unintended on-target effects (see also table 3)	Yes	Potentially (as off-target effect)	No	No
Risk of cytotoxicity/immunogenicity of editing tools and viral vectors	No	Yes	Yes	No
Further challenges of implementation and further ethical concerns (selection)	Yes, but not discussed in detail in the article (eg, embryo selection following 'control PGT': non-identity considerations, embryo protection)	Yes, but not discussed in the article (eg, implementing prenatal screening for early diagnosis if in utero application is envisioned, new-born screening if perinatal interventions are envisioned because therapeutic windows might close)		Limited applicability (feasible, eg, for treatment of β-haemoglobinopathies and other blood disorders)
			Tension between reversibility and lack of long-term stability (effectiveness)	

of embryo development.[62] This process is called 'epigenetic reprogramming' and even includes genes in which 'either the maternal, or paternal allele is normally silenced due to epigenetic mechanisms'.[6] (p. 25) The latter is called 'imprinting of genes'. Imprinted genes are therefore genes that are silenced on one allele. This silencing is associated with specific DNA-methylation patterns regulating the expression of these genes. Even those methylation patterns that cause the gene silencing through imprinting are erased during epigenetic reprogramming and are later re-established, so that they are sustained over human generations. It is yet not fully understood how imprinting works, but genetic factors seem to play a role in the re-establishment[xi] of epigenetic marks on imprinted genes.[62] Therefore, without an additional germline edit at the level of the genome, that is, without germline GE with the purpose of changing genetic factors regulating genomic imprinting, EE, even if it ever were performed on germ cells or embryos, will not result in epigenetically modified offspring in humans.[xii] EE of early embryos would therefore not have effects lasting longer than just through the very early embryonic stage and is, thus, to our understanding, not effective.[62xiii]

If EE were to be performed in the setting of assisted reproductive medicine, it could only be envisioned as modification of the epigenome of gametes, specifically, of 'infertile' oocytes (egg cells), provided that one found a way to make the oocyte 'fertile' by EE so that an embryo can develop.[xiv] This would *not* classify as a germline intervention. Germline effects are effects that can be inherited. The modification of unfertilised oocytes' epigenomes would be reversed soon after fertilisation and would therefore *not* be inheritable, nor present in the resulting embryo itself. The goal of creating an embryo would still be achieved.

EE on oocytes is, although hypothetical, and although no germline intervention, of interest for ethical debates. If EE becomes an alternative to the ethically contested technology of egg cell (oocyte) donation, oocyte donation might not be ethically justified anymore.[xv] Addressing questions of applicability of EE as an assisted reproductive technology in advance is furthermore advisable for establishing whether EE should be mentioned in future legal documents regulating germline GE.

(ii) Why epigenetic determinism is problematic

The hypothesis that epigenetic inheritance is possible in humans has been discussed for centuries (cf. Dupras, Saulnier and Joly[11] (p. 786) and Takahashi *et al*[63] (p. 727) as well as Jablonka and Lamb[64]), but remains controversial even for other mammals.[65xvi] Although there is much speaking against

[xi]This might then be described as 'reconstructive inheritance'.[65] However, we prefer not to use the term 'inheritance', but rather describe imprinting as 're-establishment' of methylation patterns in every (human) generation.

[xii]We base this view on theories about how new imprinted genes might evolve, requiring the presence of ZFP57-motifs, proteins for which a gene (*ZFP57*) must be available.[62]

[xiii]Differently Huerne *et al*.[9]

[xiv]Cf. also case 4 in the study by Savulescu *et al*.[90]

[xv]Similarly, Carter-Walshaw[91] argues oocyte donation is not justified in most cases if in vitro gametogenesis becomes available.

[xvi]Recently, Takahashi *et al*[63] have shown that newly acquired DNA methylation is transgenerationally stable, that is, inherited to more than two/three generations, in mice. However, the researchers did not use EE to introduce the DNA methylations in the first generation of mice but an approach that, as we understand, comprises methods of GE (the insertion and excision of DNA). They furthermore note that they were not able to achieve the same results when they used an EE editing approach (editing of DNA methylation), as Takahashi *et al*[63] write: "when we only induced de novo DNA methylation […] using the dCas9-DNA methyltransferase

Table 3 Different editing approaches' potential for generating inheritable modifications, and suggested dimensions for ethical evaluation

Editing approaches	Germline genome editing	In vivo (especially in utero=in vivo on fetuses) somatic genome editing	1. Epigenome editing of gametes or early embryos (clinical application on gametes unlikely, on embryos not feasible)
			2. In vivo and ex vivo somatic epigenome editing
			3. Ex vivo somatic genome editing
Intervention results regarding inheritability	Intervention results are inheritable.	Intervention results are potentially inheritable.	Intervention results are not inheritable.
Suggested dimensions for ethical evaluation of inheritability	1. Outcome: is the intervention result inheritable?		
	2. Intentionality: is editing performed with the direct (primary) intention of producing germline effects? (to be discussed in more detail in future ethical evaluations of genome editing)		
	Germline genome editing might be the only option to generate a genetically related child without a specific disease, effects to offspring of that child (and further generations) would, then, not be the primary intention. Potentially inheritable in vivo somatic genome editing never comes with the primary intention of generating germline effects as these are off-target events.		

epigenetic inheritance in humans and the scientific discussion is controversial, the unproven assumption of epigenetic inheritance in humans has a major influence on the ethical debate about epigenetics, and on the debate about GE,[66 67] and even on the debate about EE.[7] (p. 1652)

Epigenetic inheritability is a necessary condition for *strong epigenetic determinism*. The claim of *strong epigenetic determinism* is the following: because of the presumption of *epigenetic inheritability*, that is, that epigenetic effects can presumably be passed on to future generations, environmental conditions (cf. I.3 above) caused by individual persons or states influence present, and future members of society.[66–68] This results in a, what one might call, *epigenetic responsibility* towards future generations, which can be perceived as an additional burden on individual persons or states.[69] Since environmental influences on the epigenome are usually not targeted (I.3), their effects probably cannot be fully influenced by individual choices anyway. This makes it very challenging to support normative claims of epigenetic responsibility even for present generations.

As far as the targeted intervention in the epigenome by means of EE is concerned, *strong* epigenetic determinism should not guide the ethical debate because EE, like some GE approaches, does not generate inheritable risks *for the offspring*.

(iii) The risk of generating inheritable effects following somatic GE

Some GE approaches either always generate inheritable edits (germline GE) or at least come with the risk of introducing inheritable edits (in vivo somatic GE), while other GE approaches do not (as tables 2 and 3 illustrate). One potential off-target effect of in vivo somatic GE and also in vivo somatic EE is that germ cells are unintentionally modified. However, unintended edits of germ cells will only be inheritable if the edits result from GE, not from EE (see explanation in II.2.i). Therefore, there is indeed a clear difference between in vivo somatic EE and in vivo somatic GE. The former does not come with the risk of generating *inheritable* off-target edits, whereas the latter does. Non-inheritable off-target and unintended on-target edits are, however, a risk of both EE and GE (see II.1).

(3) Summary of the comparative assessment of risks associated with EE versus GE

(i) Is somatic EE always preferable to somatic GE regarding risks to edited individuals?

EE is a novel gene technology and, just as GE, comes with several risks to edited subjects. In somatic editing approaches, these risks can be significantly minimised through ex vivo somatic GE or ex vivo somatic EE. However, there are only a few diseases where these approaches can be applied—mainly blood disorders.[xvii]

Besides the choice of ex vivo versus in vivo editing approaches (i), we outlined two further criteria as relevant for an assessment of risks associated with novel gene technologies like EE and GE. These are: (ii) time of intervention and intervention windows and (iii) targeted diseases. A first step for ethical debates about somatic EE and other somatic gene technologies is to conduct ethical risk assessments that align with these three criteria. Our comparative assessment of somatic EE and GE based on these three criteria revealed that EE is not always preferable to GE in terms of the risks it poses on edited subjects. This should be further discussed in future assessments.

As far as we can evaluate the current state of scientific research—as scholars from bioethics in close coordination with epigenetics researchers—there is no sufficient evidence to presume that unintended edits after EE are overall less risky and less problematic than those associated with GE for the edited individual. There seems to be a tension between a current lack of stability of EE edits as such and the supposition that these edits will, in the future, be easier to reverse. Even if off-target and unintended on-target edits will be easier to reverse following EE than GE, this would only make in vivo somatic EE less risky than in vivo somatic GE, whereas ex vivo somatic EE and GE come with similar risks.

(ii) Is EE preferable to germline GE (and to somatic GE) in terms of risks to future generations?

New gene technologies such as EE and GE come with many risks to edited individuals, as we have explained above. These risks comprise unintended edits impacting health in undesired ways. In our view, editing approaches that rule out the possibility of inheriting (the above mentioned) unintended edits to offspring are currently

(DNMT) systems [this would have been epigenome editing; comment by KA and ECW], the acquired DNA methylation was not stably maintained in mouse ESCs".

[xvii] And there may also be pragmatic reasons that make in vivo editing appear preferable to ex vivo editing for blood disorders in underserved countries and communities, which many patients live in.[92]

preferable to editing approaches resulting in germline (=inheritable) effects. Since this article focuses on the comparison of EE and GE (with germline EE not being effective), not on germline GE, the only distinction we want to point out—since it is a less discussed but important difference—as being crucial for the normative assessment of inheritability is the one between the two dimensions of a result oriented versus an intention-oriented evaluation: whether or not inheritable effects can occur versus whether these effects are primarily intended or not. We do not evaluate the latter question further here, but suggest that it be discussed in future GE-ethics debates (see table 3).

Within the small previous ethical debate that compared EE with GE, the view that inheritability of off-target edits is problematic and should be avoided prevails. The respective scholars[6 7 38] maintain that the absence of inheritable effects renders EE ethically preferable to germline GE, at least. That being said, first, the risk of inheritability of unintended edits is clearly ruled out not only for all types of EE, but also for ex vivo somatic GE. Second, not only germline GE, but also in vivo somatic GE can result in unintended inheritable edits.

Regarding the effects on future generations, GE approaches without hereditary risks and all EE approaches might be preferable to GE approaches with hereditary risks. One can hardly argue that only EE be preferable to all types of GE regarding the effects to future generations. Based on the assumption that the risk of generating inheritable edits should be avoided due to the unknown impact of off-target edits on future generations,[xviii] it should coherently be argued that not only the inevitable inheritability of unintended edits after a germline GE, but also the potential inheritability of unintended edits after an in vivo somatic GE is ethically problematic and should be avoided,[5 6] and that therefore—as has not been made explicit by other scholars before—EE and ex vivo somatic GE would be preferable to germline GE and to in vivo somatic GE if inheritability is a problem.

With respect to germline editing, we have also argued that the risks to those individuals that result from germline editing can be minimised if control PGT and embryo selection are used. There are, however, many ethical challenges and moral concerns associated with the selection and discard of preimplantation human embryos which we have not discussed in this article.

We have shown why germline EE is not effective. This is because epigenetic reprogramming will erase any changes introduced to germline cells by EE. We also briefly discussed the hypothesis of epigenetic inheritability in the context of ethical debates about epigenetics, that is, about unspecific environmental influences. The hypothesis of epigenetic inheritability and the resulting claim of a strong epigenetic determinism should neither guide ethical debates about epigenetics nor ethical debates about EE. It is important for an emerging ethical debate on EE to be critical about the validity of the claim that epigenetic inheritance to multiple generations is possible in humans, as a sufficient amount of scientific evidence for this claim does not (yet) exist. Furthermore, we have briefly pointed to a potential application of EE in the setting of reproductive medicine, which would consist in editing egg cells to make them *ready* for creating an embryo. This may be a candidate for alternatives to egg cell donation and would then have to be assessed in ethical debates about assisted reproductive technologies.

III. CONCLUSION AND FUTURE DIRECTIONS

This article has the intention to jumpstart a broader ethical debate on EE. We have provided scientific background on EE and compared risks of EE and GE. Such a comprehensive risk assessment suggested itself as a first step for a comparative ethical assessment of EE and GE since these technologies are similar in many respects: the mechanisms are easily comparable, and the applications in medicine could overlap. As our analysis reveals, the risks are also very similar at least for edited individuals (see the summary of the risk assessment in section II.3). For future directions of the ethical debate on EE, we suggest that the three criteria for assessment of risks of gene technologies that we have proposed in this article ((i) in vivo/ex vivo approach; (ii) time of intervention and intervention windows; (iii) targeted diseases; see table 2 for a summary) be critically discussed and further refined in close cooperation of scientific and ethical researchers. As stated above, we furthermore suggest to be very critical about the supposition that epigenetic inheritability is possible in humans in ethical debates about epigenetics, and specifically in emerging ethical debates about EE.

Finally, we suggest to identify and assess challenging use cases that might be unique to EE. Important but very hypothetical ethical and regulatory questions arise with respect to EE of oocytes. Specifically challenging, potentially unique to EE (not with the same applications as GE), and more realistic than oocyte-editing might be the approach to use EE in a preventive setting. As an outlook for future debates, we conclude this article with briefly describing such a scenario:

In 2022, a news article[70] reported preclinical EE research on rats to epigenetically reverse 'adult anxiety and alcohol use disorders' that resulted from 'adolescent binge drinking'[70] (cf. also Bohnsack *et al*[71] and Pandey *et al*[72]). These are potentially unique 'reversing' applications of EE and a form of tertiary prevention, which is intervening to prevent further deterioration of health when a disease is already symptomatic. There is, however, the risk that these diseases or 'loosely defined disorders'[9] (p. 207) are completely reduced to epigenetic alterations in light of such research. This can foster societally prevalent notions of a *weak* form of epigenetic determinism (see II.2.ii for the discussion of *strong* epigenetic determinism) that 'portray[s] epigenetics as the underlying "cause" of health states, as if epigenetics is the (often sole) reason for the disease'[9] (p. 207). Combined with the general understanding of epigenetics in bioethics and society, namely, that epigenetics is the way genes interact with the environment (see I.3), this can lead to *epigenetic responsibility* being ascribed to the fact that preventive measures, such as inhibiting adolescent alcohol exposure, be recklessly neglected. Public and scientific discourse on these potentially unique 'reversing' applications of EE as a form of preventive medicine should be accompanied by bioethical assessments of EE, and these ethical assessments should in turn be closely informed by up-to-date scientific knowledge.

[xviii] Within the ethical debate on germline GE, there is a special focus on impacts on future generations.[2 14 42 51 84 88 93–110]

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