Editing mammalian genomes: ethical perspectives

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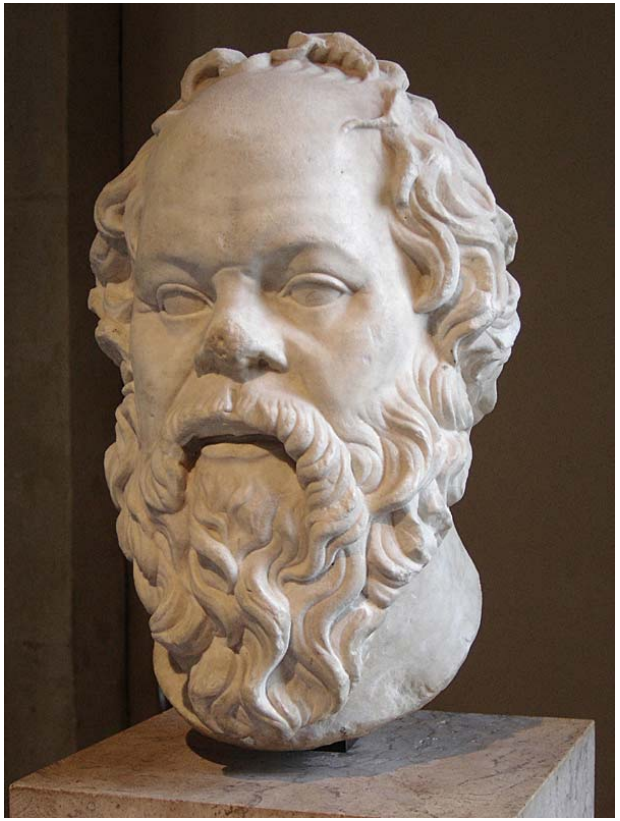

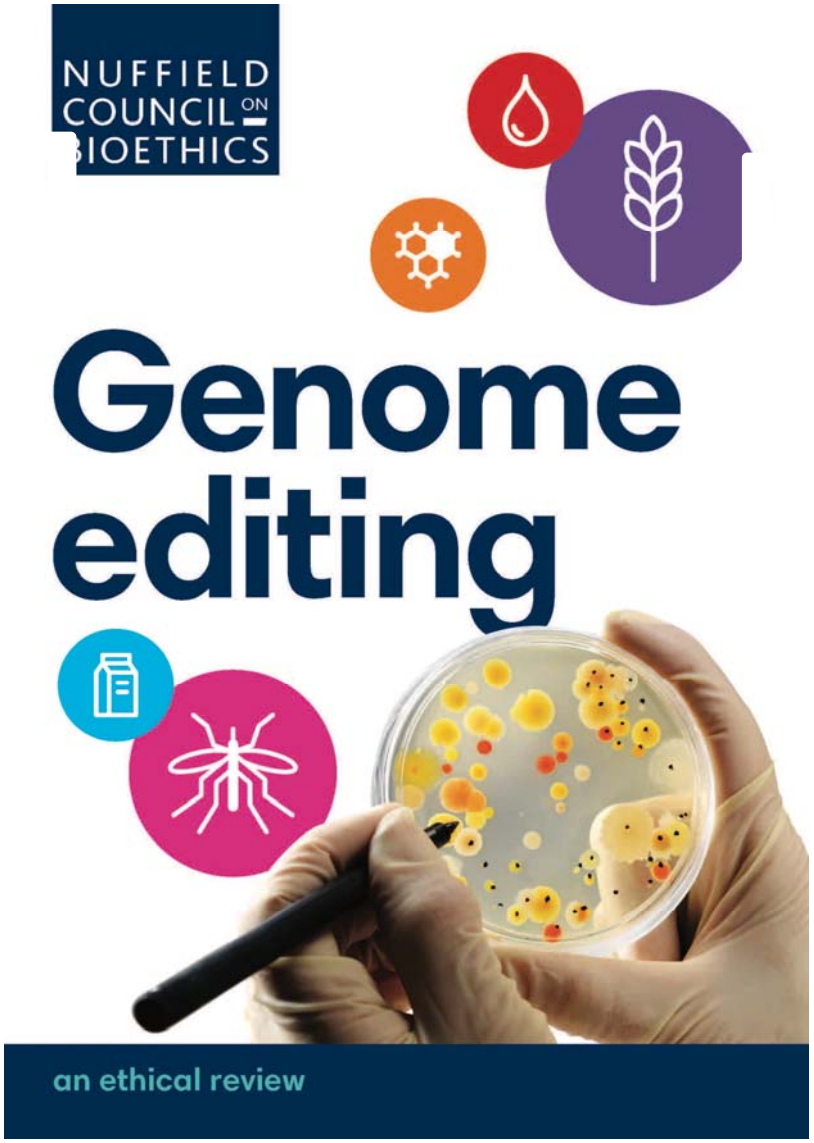

NCOB, 2016

An International Centre for Mouse Genetics

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Ethics 101

1. We are using mouse genome editing to improve human health

2. We ought to use mouse genome editing to improve human health

Importance of engaging stakeholders & ‘publics’

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- Involves more engagement from scientists on this issue
- involves a familiarisation with literature on the ethics of animal experimentation (speciesism, utilitarianism, animal rights etc)

Genome editing

- Is genome editing a disruptive technology?
- Will it change how we implement the ethical imperatives of the 3Rs?

3Rs

- **Reduction**: Distinguishing between per-experiment reduction and overall reduction

3Rs

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- **Replacement**: increased accessibility of other models, including human and other species – places a greater onus on scientists to justify use of a particular animal model

Particular considerations

- The bespoke mouse model of a rare disease: patient perspective? Counselling?
- The increased sophistication of the animal house
- Comparisons with GM plant debate
- The mouse as a pre-clinical research tool for exploring the safety and efficacy of human assisted reproductive technologies

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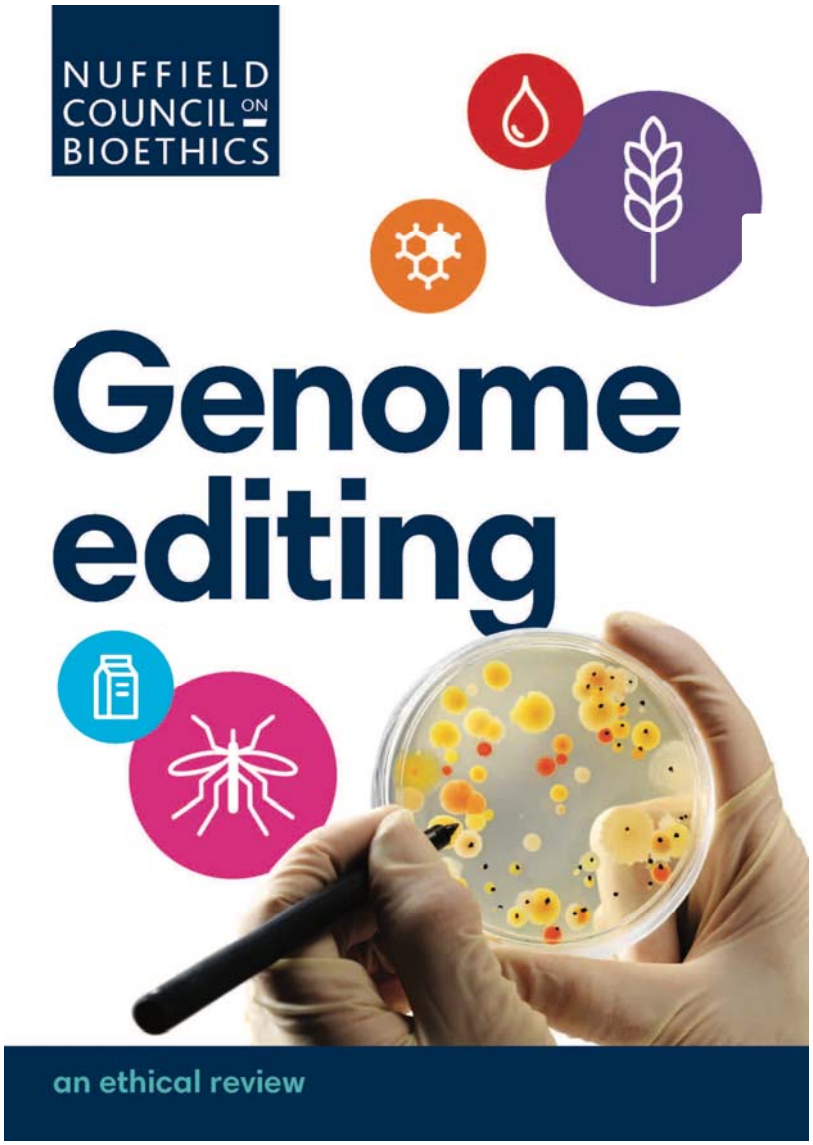

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NUFFIELD COUNCIL ON BIOETHICS

Genome

SAFETY…….SOCIETY

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Human Fertilisation & Embryology Authority

Scientific review of the safety and efficacy of methods to avoid mitochondrial disease through assisted conception: 2016 update

Report to the Human Fertilisation and Embryology Authority (HFEA)
November 2016

Review panel Chair: Dr Andy Greenfield, Medical Research Council (MRC) Harwell Institute and HFEA member

www.hfea.gov.uk

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ARTICLE

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Correction of a pathogenic gene mutation in human embryos

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Genome editing has potential for the targeted correction of germline mutations. Here we describe the correction of the heterozygous *MYBPC3* mutation in human preimplantation embryos with precise CRISPR–Cas9-based targeting accuracy and high homology-directed repair efficiency by activating an endogenous, germline-specific DNA repair response. Induced double-strand breaks (DSBs) at the mutant paternal allele were predominantly repaired using the homologous wild-type maternal gene instead of a synthetic DNA template. By modulating the cell cycle stage at which the DSB was induced, we were able to avoid mosaicism in cleaving embryos and achieve a high yield of homozygous embryos carrying the wild-type *MYBPC3* gene without evidence of off-target mutations. The efficiency, accuracy and safety of the approach presented suggest that it has potential to be used for the correction of heritable mutations in human embryos by complementing preimplantation genetic diagnosis. However, much remains to be considered before clinical applications, including the reproducibility of the technique with other heterozygous mutations.

More than 10,000 monogenic inherited disorders have been identified, affecting millions of people worldwide. Among these are autosomal dominant mutations, where inheritance of a single copy of a defective gene can result in clinical symptoms. Genes in which dominant mutations manifest as late-onset adult disorders include *BRCA1* and *BRCA2*, which are associated with a high risk of breast and ovarian cancers[1], and *MYBPC3*, mutation of which causes hypertrophic cardiomyopathy (HCM)[2]. Because of their delayed manifestation, these mutations escape natural selection and are often transmitted to the next generation. Consequently, the frequency of some of these founder mutations in particular human populations is very high. For example, the *MYBPC3* mutation is found at frequencies ranging from 2% to 8%[3] in major Indian populations, and the estimated frequency of both *BRCA1* and *BRCA2* mutations among Ashkenazi Jews exceeds 2%[4].

HCM is a myocardial disease characterized by left ventricular hypertrophy, myofibrillar disarray and myocardial stiffness; it has an estimated prevalence of 1:500 in adults[2] and manifests clinically with heart failure. HCM is the commonest cause of sudden death in otherwise healthy young athletes. HCM, while not a uniformly fatal condition, has a tremendous impact on the lives of individuals, including physiological (heart failure and arrhythmias), psychological (limited activity and fear of sudden death), and genealogical concerns. *MYBPC3* mutations account for approximately 40% of all genetic defects causing HCM and are also responsible for a large fraction of other inherited cardiomyopathies, including dilated cardiomyopathy and left ventricular non-compaction[6]. *MYBPC3* encodes the thick filament-associated cardiac myosin-binding protein C (cMyBP-C), a signalling node in cardiac myocytes that contributes to the maintenance of sarcomeric structure and regulation of both contraction and relaxation[2].

Current treatment options for HCM provide mostly symptomatic relief without addressing the genetic cause of the disease. Thus, the development of novel strategies to prevent germline transmission of founder mutations is desirable. One approach for preventing second-generation transmission is preimplantation genetic diagnosis (PGD) followed by selection of non-mutant embryos for transfer in the context of an *in vitro* fertilization (IVF) cycle. When only one parent carries a heterozygous mutation, 50% of the embryos should be mutation-free and available for transfer, while the remaining carrier embryos are discarded. Gene correction would rescue mutant embryos, increase the number of embryos available for transfer and ultimately improve pregnancy rates.

Recent developments in precise genome-editing techniques and their successful applications in animal models have provided an option for correcting human germline mutations. In particular, CRISPR–Cas9 is a versatile tool for recognizing specific genomic sequences and inducing DSBs[7–10]. DSBs are then resolved by endogenous DNA repair mechanisms, preferentially using a non-homologous end-joining (NHEJ) pathway. Obviously, NHEJ is inappropriate for gene correction applications because it introduces additional mutations in the form of insertions or deletions at the DSB site, commonly referred to as indels. In some cases, however, targeted cells activate an alternative DNA repair pathway called homology-directed repair (HDR) that rebuilds the DSB site using the non-mutant homologous chromosome or a supplied exogenous DNA molecule as a template, leading to correction of the

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§These authors jointly supervised this work.

Summary

- Talking about science *matters*
- Talking about ethics *matters*
- Talking about why we want to edit the mouse genome *matters*
- Making ourselves *understood* is important
- Engage with difficult topics; drop unhelpful tropes/metaphors; be honest – others will appreciate it
- Carry on editing (in accordance with the laws/regulations of whatever jurisdiction you are in!)
- Embrace ethics as a way of facilitating your research rather than preventing it

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Editing mammalian genomes: ethical considerations

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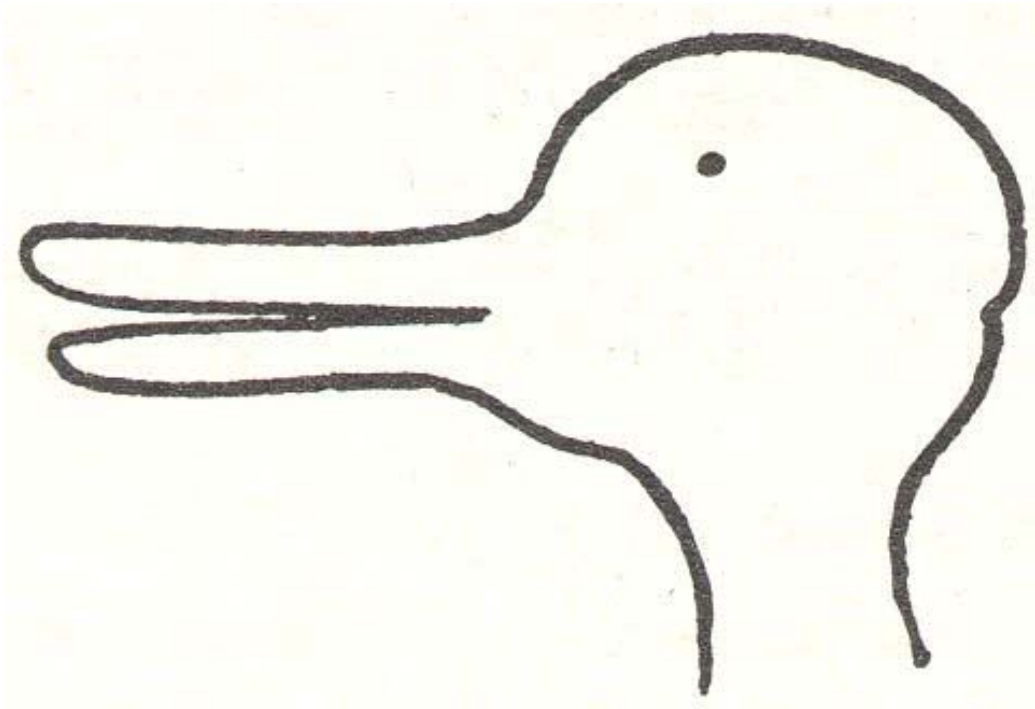
Ye Olde Mammalian Genome Editing…

Aspect change



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Character and culture

"We acknowledge that the UK has the most detailed legislative framework regarding animal research in the world. But…regulation can act as an emotional screen between the researcher and an animal, possibly encouraging researchers to believe that *simply to conform to regulations is to act in a moral way*. It is therefore crucial to promote best practice more actively and to improve the **culture of care** in establishments licensed to conduct experiments using animals."

NCOB, 2005 – "The Ethics of Research Involving Animals"