Royal Society of Biology

A response from the Royal Society of Biology to Nuffield Council for Bioethics inquiry on Genome editing

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The Royal Society of Biology is a single unified voice, representing a diverse membership of individuals, learned societies and other organisations. We are committed to ensuring that we provide Government and other policy makers, including funders of biological education and research, with a distinct point of access to authoritative, independent, and evidence-based opinion, representative of the widest range of bioscience disciplines.

The Society welcomes the Nuffield Council on Bioethics interest in Gene Editing; we are pleased to offer these comments which have been informed by discussions with our members and Member Organisations from across the biological disciplines. The contribution is not made as a detailed or extensive review, as we expect that others will provide examples of the current state of the field, rather we provide high-level comment on implications for the field, with selected examples where appropriate.

Key points

- The advent of widely applicable genome editing techniques requires genuine decision-making among biologists, social scientists, the legal profession, ethicists, policy-makers and publics. Encouraging and empowering life scientist to join in these developments will be crucial.
- Scientists utilising gene editing techniques should do so with well-informed and developed understanding of the ethical and legal implications of their work. The provision of skills and training for scientists will be essential to creating an informed workforce.
- Regulation of genome editing must be tailored and adaptable as our understanding of the operation, potential and implications of these techniques improves. As the possibility of release or use of genome edited organisms approaches, the characteristics of the regulatory system must be considered.

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Perspectives on genome modification

Is there anything special about the genome that makes intervening in it different from other ways of manipulating nature (e.g. selective breeding of plants or animals)? To what extent can the development of genome editing techniques be regarded as distinct from or continuous with existing techniques? In what way are the differences significant?

Many practices that are widely accepted, including selective breeding of both plants and animals, result in changes to the genome that will be inherited and passed on to future generations. The genome represents the heritable material of a cell or organism. Therefore, changes to the genome are inherited when a cell divides or when an organism reproduces. In the case of somatic alterations, these changes will be effective for the lifespan of the organism or its clones; germline alterations can be passed on to subsequent generations through reproduction.

Research use

The use of genome modification techniques is widespread and long-standing in research communities, both academic and private/business research and development (R&D). Genome editing techniques, including site directed nucleases utilising zinc finger modules or TALENS have been in existence for several years. These techniques have been used to generate cell lines, plant strains and animal models to study gene function, generate potential crop variants and disease models and to identify putative drug targets, among other things. More recently, further development of these technologies, in particular CRISPR Cas9, have been rapidly and widely adopted and used in research settings. This technique, adapted from a bacterial "immune system" function allows the targeting of the Cas9 nuclease to specific points in the genome where it can cleave DNA and induce the cell's own DNA damage repair response. The main difference between CRISPR Cas9 and previous similar techniques is the ease, speed and reduced cost of use, leading to successful outcomes, rather than any categorical difference. Nevertheless, these factors have led to an increased access to these techniques and an effective mainstreaming of genome editing.

Additional benefits of the CRISPR Cas9 system include its lack of inhibition by epigenetic modifications which hampered previous techniques[1]. Furthermore, since its initial application as a genome editing tool in 2013[2], CRISPR Cas9 has been modified in various ways to reduce inaccuracies and off-target effects [3,4], making it more reliable and more specific.

Potential clinical use

Genome editing techniques have potential applications in both somatic cell and germline alterations. Studies in animals have demonstrated the proof of principle that CRISPR Cas 9 can be used to modify the genome of somatic cells in adult organisms[5,6]. Whilst both instances reported issues relating to the delivery

[1] DNA targeting specificity of RNA-guided Cas9 nucleases. Hsu et al., Nat. Biotechnol, 2013
[2] RNA-programmed genome editing in human cells. Jinek et al., eLife, 2013
[3] Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Ran et al, Cell, 2013
[4] High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Kleinstiver et al., Nature, 2016
[5] Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype, Yin et al., Nat. Biotechnol, 2014
[6] In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Nelson et al. Science, 2015

of genome editing components to target tissues and a low efficiency of modifications, they did demonstrate the potential to edit the genome of somatic cells in an organism.

Application of the technique to germline cells, bringing about heritable changes, has the possibility to prevent or promote transmission or expression of particular characteristics to in progeny. This possibility attracted immediate interest because it could provide a means to block the inheritance of disease.

What obligations do scientists involved in developing and using genome editing technologies owe to society and what freedoms should society allow to these scientists? Do genome scientists have any special obligations to society that are distinct from those of other scientists?

Research should be conducted responsibly and applied ethically, and with due consideration to public attitudes and norms. Genome science is no different in this respect and is neither exempt from these considerations, nor should it be held to different standards. However because the genome both largely determines phenotype and is the heritable material passed between generations, manipulation of the genome raises particular considerations because of the potential power and significance of the outcomes for microorganisms, animals, plants or even humans. The recommendation is not new but, scientists who employ techniques that alter the genome should do so with full awareness of the ethical and legal and societal implications of their work. To embed this in research culture requires early training for researchers to ensure they are an aware and informed community.

In parallel to these concerns, scientists have the obligation to be open about their work. Scientists must be willing not only to explain the technical details of what they are doing, but also why they are doing it and engage constructively with societal responses to the technology and its potential uses, including emotional responses. This is not unique to genome science. Examples of successful engagement and interaction can be seen taken from synthetic biology where social scientists also helped ensure that discussion about new developments was not solely about science and technology[7]. Similar engagement will be invaluable in relation to genome editing. In addition honesty about potential applications will be imperative in public engagements, so that potential benefits are neither overstated nor underexplored. As well as engaging on convenient or attractive innovations, publics need to consider the full landscape of research challenges and the development of enabling technologies to address them. This development usually requires public investment of trust and resources (often in the form of time and money) in science, but this does not pre-suppose suitability or acceptance of the output.

To what extent is the development of genome editing valuable as a pure research tool, and to what extent is its value dependent on envisaged practical applications?

Genome editing techniques have enormous value and potential as pure research tools. The speed and ease of use of these techniques make previously impractical projects possible. The uptake of CRISPR Cas9 by the academic and biotechnology community has been rapid and influential and also is an indicator of its utility.

In order to take full advantage of this technology the research community must be aware of and have access to relevant skills and training to support appropriate and responsible use of the editing capacity as well as the ethical and legal implications. To exploit the full value of these techniques requires an informed and engaged research community.

[7] The role of social scientists in synthetic biology. Science & Society Series on Convergence Research. Calvert et al., EMBO reports, 2009

What obligations do governments have towards society to ensure 'safe' science or otherwise to shape the scientific research and development?

The regulatory system in the UK has dealt capably with new, transformative technologies in the past and continues to perform this role. The bioscience sector is accustomed to embracing new technologies, it is important that discussions about the capacity for genome manipulation delivered by the current technological revolution occur in a timely, open and informed fashion. It is important that the research and possible therapeutic applications of these techniques are not unduly stifled, whilst at the same time recognising the need for potential dangers to be considered and managed.

As the application of these techniques matures to potentially generate products, this may be a point at which to regulate, for example around risk/benefit assessment of sufficiently novel characteristics. This is of particular relevance to breeding techniques in plants as discussed below.

Genome Editing in Plants

Current research

What is the current state of the art in the field? What are the current technical limitations and constraints/ bottlenecks? What are the main directions of travel? What are the envisaged endpoints/ applications? What is the rate of travel? What are the expected timescales for realising the envisaged endpoints? Are gene drives an area of particular interest or concern and, if so, why?

Genome editing has been used widely in a plant research setting to understand CRISPR Cas9 efficiency and accuracy in plant model systems[8] and to investigate gene function. Experimental approaches have included the development of *multiplexing techniques* to edit multiple regions of the genome at once. The majority of the work to date has been on model organisms, with relatively little gene editing work on economically important crop species. However, the understanding accumulated from model systems is beginning to be applied to commercially relevant crops, including rice[9] and wheat[10].

A number of barriers to the utilisation of CRISPR Cas9 in crop development have been identified in particular a frequently-expressed concern regarding off-target effects. Plant genomes are complex and the quality of genomic information available on crop species is a particular challenge. Whilst reference genomes are available for most major crop species much of this information is recent and differences between cultivars can be significant. In addition, the repetitive nature of some plant genomes may have implications for the capacity to accurately specify alterations and detect unintended changes. This highlights the need for good quality genomic sequence data about any organism being edited.

Gene drives are not a major area of research in crop development, although relevant to crop pests. Many commercially cultivated crops are grown from commercially produced seeds and therefore not propagated for multiple generations[11], although some are.

[8] CRISPR/Cas9-mediated genome editing and gene replacement in plants: Transitioning from lab to field. Schaeffer et al., Plant Sci., 2015

[9] Gene targeting using the Agrobacterium tumefaciens-mediated CRISPR-Cas system in rice, Xu et al., 2014, Rice

[10] Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew, Wang et al., Nat biotechnol. 2014

[11] http://wyss.harvard.edu/staticfiles/newsroom/pressreleases/Gene%20drives%20FAQ%20FINAL.pdf accessed 02/02/2016

Conditions of research and innovation

What are the main 'drivers' and 'obstacles' for plant genome editing in relation to envisaged endpoints? What direct or indirect influence does historical public discussion surrounding genetic modification of plants have? What is (and what should be) the current level and focus of public debate?

Plant varieties developed through genetic modification are widely cultivated and traded in some regions, including the Americas and some parts of Asia. This is not the case in most of Western Europe where they are subject to strict regulation and have seen limited market success. Public concern has been expressed regarding the generation of modified plants by genetic techniques, including those causing the random insertion of foreign genes. Genome editing techniques have the capacity to be adaptable and need not rely on the insertion of any foreign DNA into the genome. Certain applications of CRISPR Cas9 can simply remove undesirable regions from the genome resulting in a product indistinguishable from that generated by traditional breeding techniques. Furthermore, even techniques which do utilise genome editing techniques to insert foreign DNA are targeted to a specific region of the genome and therefore reduce the risk that random insertion may disrupt desirable characteristics.

Genome editing using site directed nucleases is one of several so-called Novel Breeding Techniques (NBTs) that have been developed for use in plants. The European Commission is currently deliberating which, if any of these techniques should fall under the same legislation as conventional genetic modification (GM). This decision is expected before summer 2016. This ruling will have significant implications for the legislation and surrounding debate. The potential classification of genome editing products as genetically modified organisms (GMOs) carries the risk that the increased legislative burden of utilising these techniques commercially makes them prohibitively expensive for all but the largest plant breeders. Furthermore, the decision will both draw on and inform public perception of genome editing techniques. The effective inability to distinguish (by genetic examination) between crops generated by traditional breeding methods and those generated by genome editing raises concerns about definition, implementation and enforcement of any legislative classification. An alternative approach, advocated by European Academies Science Advisory Council (EASAC)[12], is to adopt a legislative process that is based on traits and a regulatory process triggered by the incorporation of novel characteristics. It is acknowledged that transitioning to such an approach would entail its own difficulties, notably the possible cost and delays associated with a major change to the way both GM and, importantly, non-GM plant products are regulated. Concerns have been raised about bringing so many organisms onto scope of regulation that the assessment process would be over-burdened, this also must be considered.

Outcomes

What are the main anticipated benefits and costs (including safety and other risks) of genome-edited plants? In what ways, if any, are they significantly different from alternative GM technologies? Are there particular issues raised by genome editing in relation to ecological stability, biological diversity, technology transfer between countries, and equitable sharing of the benefits of research? To what extent, and in what way, does and should the distribution of anticipated benefits and costs of using genome editing in plants influence research and innovation? To what extent are public and commercial interests in genome editing in plants complementary? In what circumstances might they come into conflict? What other important questions should or might we have asked in this section?

[12] http://www.easac.eu/home/reports-and-statements/detail-view/article/easac-statem-2.html accessed 02/02/2016

Genome editing techniques in plant breeding have the potential to significantly reduce the length of time required to generate new strains compared to traditional breeding techniques. Genome editing techniques have the potential to generate crop variants genetically indistinguishable from those bred by conventional techniques, raising potential challenges if regulators require tracing or genetic identification of modified products, but also potentially converging the potential risk profiles of conventionally bred and edited organisms.

Genome Editing in Animals

Current research

What is the current state of the art in the field? What are the current technical limitations and constraints/ bottlenecks? What are the main directions of travel? What are the envisaged endpoints/ applications? What is the rate of travel? What are the expected timescales for realising the envisaged endpoints? Are gene drives an area of particular interest or concern and, if so, why?

Genome editing technologies have been widely used already in research using animals. In particular, CRISPR has been employed in the generation of animal models. The majority of animal models generated in this way have been mice, although genome editing has been applied to other organisms including nematode worms, fruit flies, zebrafish, frogs, mice and pigs.

Recent experiments have addressed the possibility of using genome editing techniques to treat diseases in somatic cells. In one study in the United States CRISPR/Cas 9 was used to treat a mouse model of Duchenne Muscular Dystrophy. Treatment of adult mice succeeded in editing the defective gene in a small percentage of muscle cells and partially restored muscle function[6]. Experts emphasised that whilst this study was important, a number of barriers must be overcome before this technique could be used in the clinic[13].

Developments in multiplexing have recently allowed more than 60 genes to be edited simultaneously in a cell line derived from pigs[14]. This study aimed to reduce the risk of transmission of retroviruses from the pig genome, thereby making pig organs safer for transplantation into humans. The work demonstrates the potential of the technique in animal tissues to not only develop the technology but in the longer term to potentially address unmet clinical needs.

Gene drives, in which a gene is rapidly passed through a population at a rate much faster than described in classical Mendelian genetics, have been proposed as a potential mechanism to control the prevalence of certain characteristics in a population, or indeed to induce population decline by spreading unfit genes. This has particularly been posited for disease vectors such as mosquitos. Genome editing techniques can be used to alter a genome and introduce gene drives. CRISPR Cas9 has recently been used to generate gene drives in *Anopheles* mosquitos[15], the most efficient malarial vector. A strain of the *Anopheles gambiense* mosquito carrying a gene drive leading to female sterility has recently been produced and proposed as a means of possible population control for malaria vectors [16] In addition to disease vectors,

[13] http://www.sciencemediacentre.org/expert-reaction-to-the-use-of-genome-editing-techniques-to-treat-duchenne-muscular-dystrophy-in-a-mouse-model/ accessed 02/02/2016

[14] Genome-wide inactivation of porcine endogenous retroviruses (PERVs), Yang et al, Science, 2015

[15] Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi.,Gantz et al., PNAS, 2015

[16] A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae, Hammond et al., Nat. biotechnol, 2016

gene drives could prove to be a means of controlling agricultural pests. Scientists have highlighted the possibility that accidental or inappropriate release of gene drive containing organisms could have unintended or dangerous consequences.[17] This is an area of very active debate and investigation.

Conditions of research and innovation

What are the main 'drivers' and 'obstacles' for genome editing in relation to envisaged endpoints? What is (and what should be) the current level and focus of public debate?

Use of genome editing techniques in animal research is predominantly focused on building better models of disease although it has also been employed in the development of mosquitos that may be released into the wild. As with all research using animals, public support is an important factor. All animal research in the UK is conducted under the strict guidelines of the Home Office and both project and research require licensed approval.

Impacts

What overall impact might genome editing have on animal lives? Can genome editing be expected to contribute to or inhibit the replacement, reduction or refinement (the '3Rs') of the use of animals in research? Does genome editing give rise to special moral considerations about generating artificially modified animals for research (including disease models in large or highly sentient animals) or for trivial/ commercial reasons (e.g. 'toy' pigs)?

Animal models generated using conventional genetic modification techniques are already widely used in research in the UK. This process is strictly regulated by the Home Office and processes are established to assess the overall harm benefit balance of individual projects, both prospectively in the granting of licenses and now retrospectively within the annual statistical reporting for research institutes. This combination offers a more refined analysis overall of aims and outcomes and can underpin welfare improvement measures. However genome editing will not alter categorically the range or nature of animal research. Thus the application of novel genome editing techniques does not represent a novel category of experimentation or introduce novel moral or regulatory problems. It is possible that sufficiently large populations of model organisms may be produced with fewer generations and fewer overall animals in the breeding programme than previously. Additionally some animal research benefits other animals (or humans) when therapies are developed for use in the community. However, gene editing will not in the near term provide a means of replacing animals in experiments, so some animals will continue to be used. Capacity to address different questions and disease models may arise but may not represent a categorically different set of considerations.

Genome Editing in Microorganisms

Current research

What is the current state of the art in the field? What are the current technical limitations and constraints/ bottlenecks? What are the main directions of travel? What are the envisaged endpoints/ applications? What is the rate of travel? What are the expected timescales for realising the envisaged endpoints?

[17] Akbari O S. et al. (2015). Safeguarding gene drive experiments in the laboratory. Science 349 (6251), 927-929

Genome editing techniques have been applied to a range of microorganisms including the pathogenic yeast *C. albicans* [18] and protozoan parasite, which causes Chagas disease, *Trypanosoma cruzi* [19]. As with other model organisms, use of these techniques has the possibility to greatly facilitate research not possible using conventional genetic manipulation. In addition, these tools may prove useful in the area of synthetic biology, in which numerous genes must be inserted or edited to generate a network of interacting genes.

Conditions of research and innovation

What are the main commercial applications of genome editing in microorganisms and what are the main economic drivers of development?

There is a wide array of potential commercial applications, especially in the longer term. Synthetic biology also has varied and abundant potential for commercial application, from the synthesis of drug compounds to biofuels. Microorganism gene editing and the incorporation of synthetic components into microbial genomes together offer huge potential for product development. A major driver is the potential to produce complex product cheaply, from inexpensive feed stock, or in some cases from waste. Waste management and reclamation have already emerged as important areas of activity. Current applied use of microorganisms may not be very different in principle. The scale of potential use and the required focus on environmental protection and quality assurance will be relevant influences in development.

Biomedical research and human applications

Current research

What is the current state of the art in the field? What are the current technical limitations and constraints/ bottlenecks? What are the main directions of travel? What are the envisaged endpoints/ applications? What is the rate of travel? What are the expected timescales for realising the envisaged endpoints?

Academia and the biopharmaceutical industry have widely adopted these techniques to develop cell lines in which genes have been tagged, deleted, rendered conditionally inactive etc.. Recent adaptations have allowed this technique to be coupled with nuclease-null Cas9 components fused to epigenetic modifiers such that gene expression can be altered without altering the genetic sequence[20]. Methods have also been developed towards the use of these techniques in human pluripotent stem cells (hPSCs) and discussion continues around this potential area of activity.

An application by researchers at the CRICK Institute to use genome editing techniques in human embryos was granted on 1st February 2016[21]. This decision recognised the use of CRISPR as a “highly efficient and

[18] A Candida albicans CRISPR system permits genetic engineering of essential genes and gene families. Vyas et al., Sci Adv, 2015
[19] CRISPR-Cas9-Mediated Single-Gene and Gene Family Disruption in Trypanosoma cruzi, Peng et al., mBio, 2015
[20] epigenome editing by a crisPr-cas9-based acetyltransferase activates genes from promoters and enhancers, Hilton et al., Nat. biotechnol, 2015
[21] http://guide.hfea.gov.uk/guide/ShowPDF.aspx?ID=5966 Accessed 02/02/2016

targeted method of gene disruption, potentially superior to other techniques". Furthermore, the review acknowledged that it remains illegal to implant these embryos into a surrogate mother and that experiments will not proceed beyond the current 14 day limit on research using human embryos. Finally the decision noted that "[T]his study may facilitate, in the long term, the development of treatments for serious diseases or other serious medical conditions" by developing human embryonic stem cells (hESC) as a platform for drug discovery.

The applications described above are predominantly related to research applications of these techniques; however a number of researchers and biopharmaceuticals companies are investigating the possible application of genome editing for clinical use either in the form of gene therapy or cell therapy. In fact the precursor technologies TALENS have been used as part of a cell therapy procedure which modifies the genome of immune cells such that have been shown to specifically target drug-resistant cancers[22]. The use of genome editing techniques as a somatic gene therapy is a topic of interest for the biopharmaceutical industry and will entail many challenges of its own. Current delivery techniques for both components of the CRSPR Cas9 system to target cells in an organism are relatively inefficient and will require refinement to be applicable in the clinic, among other considerations.

There is the additional possibility that genome editing techniques could be applied in the germline as a means of treating hereditary conditions, separate consideration of the potentially profound issues raised would be required.

Conditions of research and innovation

What are the main 'drivers' and 'obstacles' in relation to envisaged endpoints? What bearing do international ethical debates and agreements (e.g. high level statements or calls for moratoria) have on the pace or organisation of research? Who should lead and who should be involved in setting policy for research and human applications of genome editing? Is this significantly different from other kinds of experimental or reproductive medicine?

Many of the issues raised by genome editing techniques have been raised during previous debates surrounding gene therapy, reproductive techniques and mitochondrial donation. The UK regulatory environment has dealt capably with these techniques. Whilst genome editing techniques do display some differences to the subject of previous debates, this should not suggest that they be dealt with by an entirely different approach. Research addressing the potential risks and benefits of these techniques should continue and be used to inform discussions surrounding their potential application in clinical settings.

In particular the pace of scientific advance and societal acceptance should be synchronous.

Military and security considerations

Is there a military interest in genome editing research? What is its nature? What can we discover about defence funding for research and development in this area? What are the limits of our knowledge in this area and what implications might this have for decisions about research policy more generally? Are there

[22] http://www.gosh.nhs.uk/news/press-releases/2015-press-release-archive/world-first-use-gene-edited-immune-cells-treat-incurable-leukaemia Accessed 02/02/2016

areas of genome editing research that are or should be classified as 'dual use research of concern' (DURC)? If so, what are they and what applicable measures are there to address these concerns? Are there distinctive concerns about biosafety/biosecurity that are being investigated with respect to genome editing research or applications in particular?

The ease of use and low cost of CRISPR Cas9 genome editing has brought these techniques to a far wider use community than previous techniques. This has raised concerns that it will be possible to generate organisms or pathogens which could be used as bioweapons[23]. It will be essential to consider the potential military and security aspects of genome editing and gene drives by both states and non-state groups. In particular the use of this technology is not particularly resource-dependent and therefore the approach of export and trade restrictions may be of little benefit. High levels of awareness, and appropriate and robust behavioural norms, in the science community are vital to ensure that knowledge and wisdom in its humanitarian use develop together. Training and professional standards will be important and particular attention to the sharing of information and resources.

[23] http://nuffieldbioethics.org/wp-content/uploads/Background-paper-2016-Dual-use.pdf accessed 02/02/2016