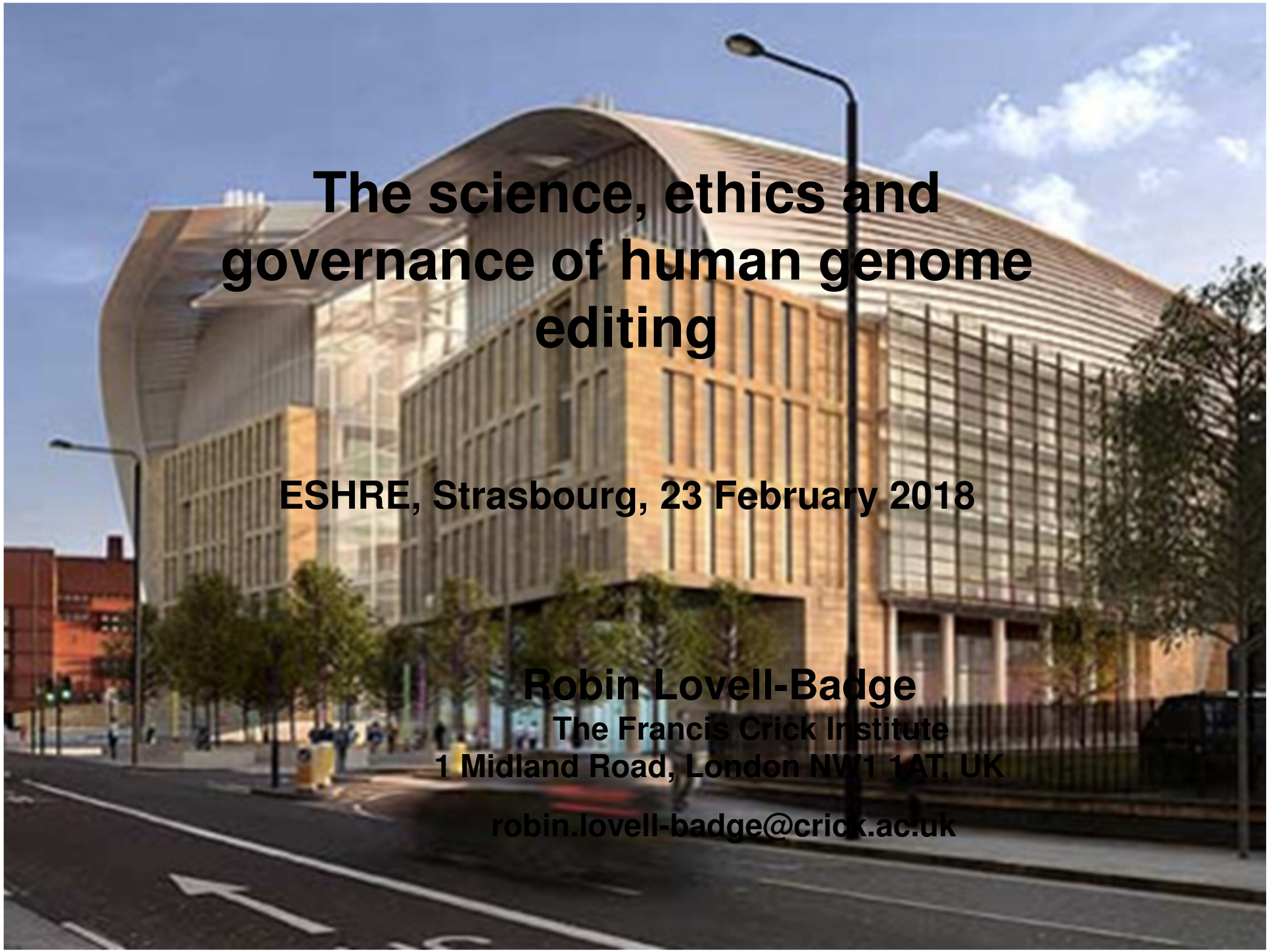

The science, ethics and governance of human genome editing

ESHRE, Strasbourg, 23 February 2018

Robin Lovell-Badge
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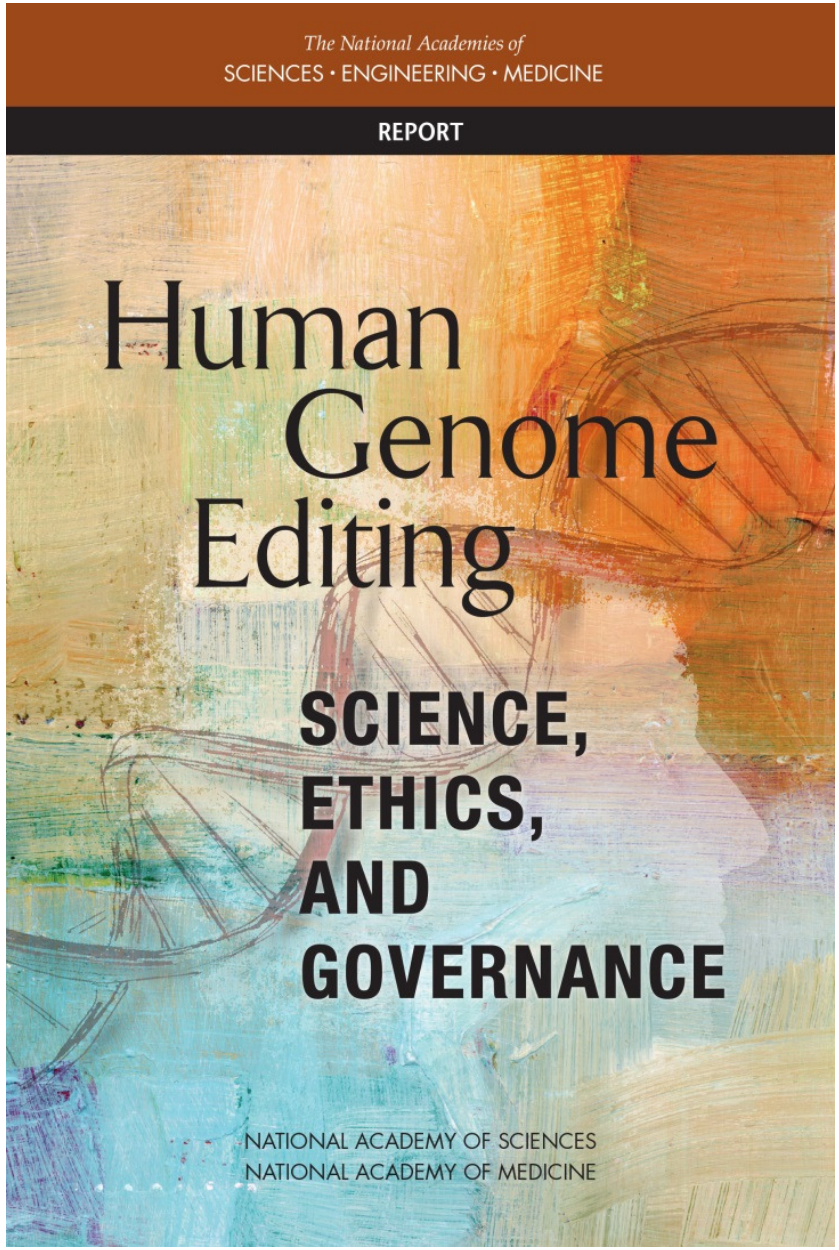


National Academies of Sciences and National Academy of Medicine

Report, Released on 14 February 2017

Study Committee co-chairs:

R. Alta Charo, J.D.

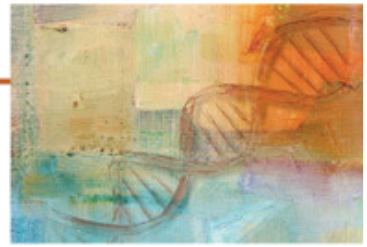
and Richard O. Hynes, PhD

nationalacademies.org/gene-editing/consensus-study

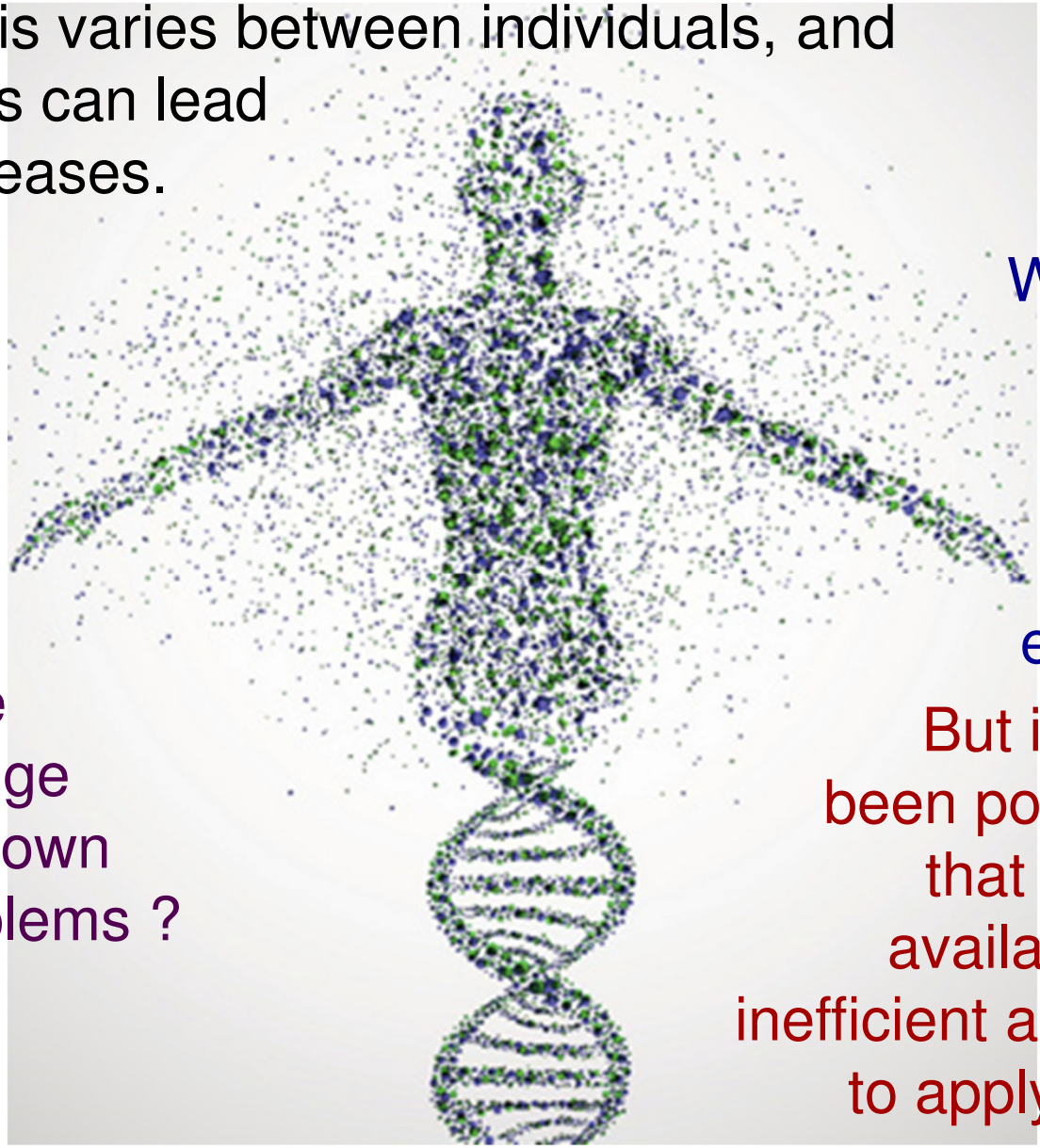
Overarching Principles for Governance of Human Genome Editing

- Promoting well-being
- Due Care
- Transparency
- Responsible Science
- Respect for Persons
- Fairness
- Transnational Cooperation

Any nation considering governance of human genome editing can incorporate these principles—and the responsibilities that flow from them—into its regulatory structures and processes.



Over the last few years we have accumulated a lot of information and understanding of the human genome (our entire genetic code), how this varies between individuals, and how mutations can lead to genetic diseases.



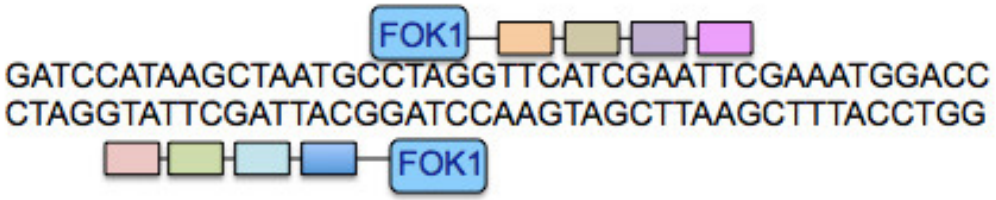
We have had methods to alter DNA in mammals since the early 1980's.

But it has always been possible to say that the methods available were too inefficient and/or unsafe to apply to humans.

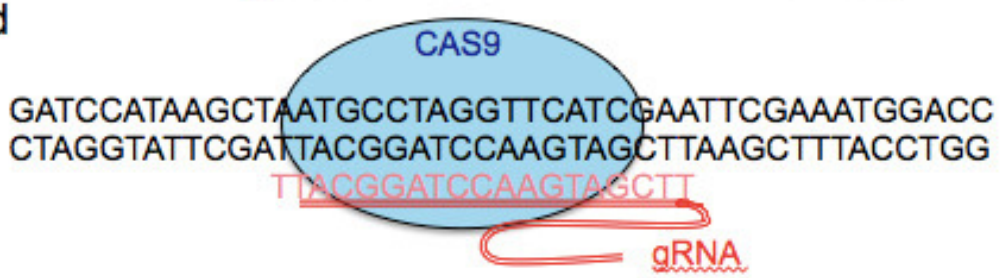
Why not use this knowledge to solve our own genetic problems ?

Genome editing methods can provide precise and efficient means of altering DNA sequences or to control gene activity.

SYSTEM	DNA-sequence recognition	Nuclease
ZFNs (Zinc Finger Nucleases)	modular Zinc Finger domains.....	FOK1
TALENs (Transcription Activator-Like Effector Nucleases)	modular TALE domains.....	FOK1
CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats)	guide-RNA +	CAS9



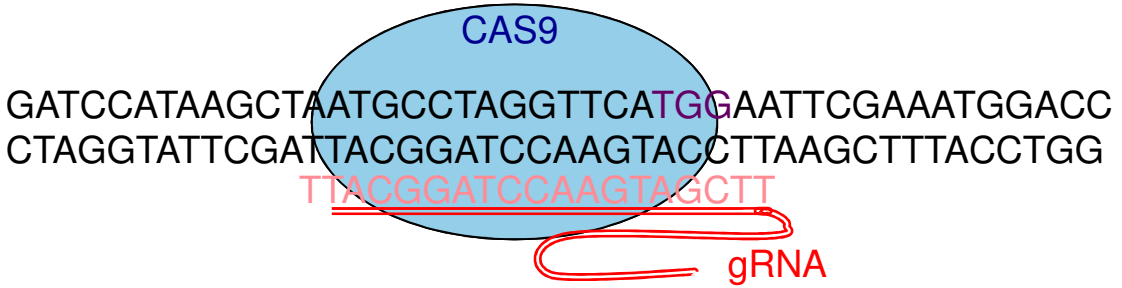
(Or: RNA-guided genome editing)



Using CRISPR/Cas9 to make an inactivating mutation

CRISPR-Cas9

guide-RNA + CAS9 + protospacer-adjacent motif = PAM



Double-strand break in DNA

GATCCATAAGCTAATGCCTAGGT TCATGGAATTCGAAATGGACC
CTAGGTATTCGATTACGGATCCA AGTACCTTAAGCTTTACCTGG

Non-homology end-joining (NHEJ) repair

This leads to small insertions or deletions (INDELs)

Ku70/80-dependent

GATCCATAAGCTAATGCCTAGGCATGGAATTCGAAATGGACC
CTAGGTATTCGATTACGGATCCGTACCTTAAGCTTTACCTGG

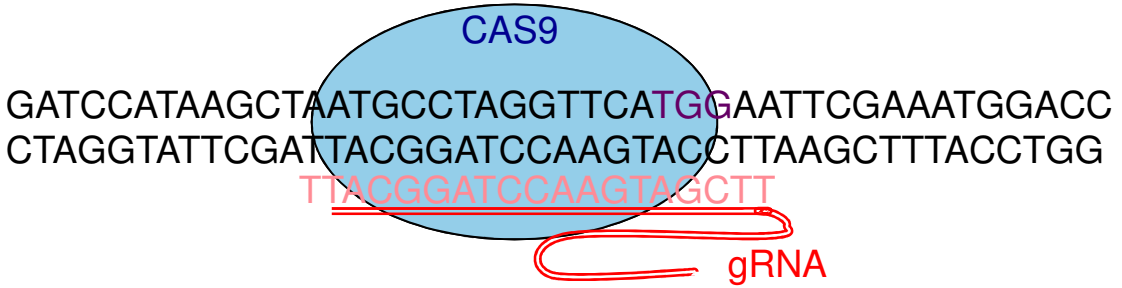
2 base pair deletion
If this is in the coding region, it will prevent the protein product of the gene being made

But it can also be used in some cases, e.g. with DMD, to promote skipping of an exon with a nonsense mutation to allow a functional protein to be made.

Using CRISPR/Cas9 to exchange sequences

CRISPR-Cas9

guide-RNA + CAS9



Double strand break in DNA

+

DNA template : (single- or double-stranded)

GATCCATAAGCTAATGCCTAGGT
CTAGGTATTCGATTACGGATCCA

CTAGGTATTCGATTACGGATATG

TCATGGAATTCGAAATGGACC
AGTACCTTAAGCTTTACCTGG

CTGATCTTAAGCTTTACCTGG

Mutant PAM

Homology directed repair (HDR) leads to precise exchange of sequences

GATCCATAAGCTAATGCCTATACGACTAGAATTCGAAATGGACC
CTAGGTATTCGATTACGGATATGCTGTACTTAAGCTTTACCTGG

Rad51-dependent

8 base pair substitution
But it can be anything from 1 bp to many 1000's, or to insertions or deletions.

Using CRISPR/dCas9 “base editing” to alter C:G to T:A

Cas9 with inactivated nuclease activity (dead.Cas9 or dCas9) linked to a relevant enzyme

↓

A specific C is chemically modified to become a U (without a break in the DNA)

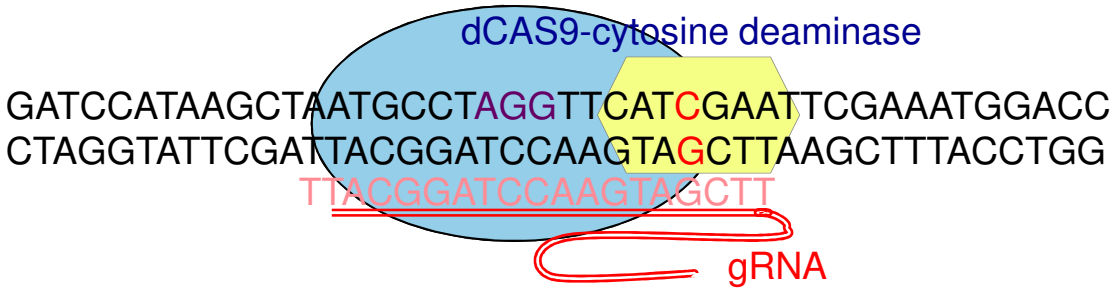
↓

Endogenous processes substitute the U with a T

↓

DNA mismatch repair mechanisms detect a problem and substitute the G with an A to restore base pairing.

guide-RNA + dCAS9-cytosine deaminase



GATCCATAAGCTAATGCCTAGGTTCAT U GAATTCGAAATGGACC
CTAGGTATTCGATTACGGATCCA AGTA G CTTAAGCTTTACCTGG

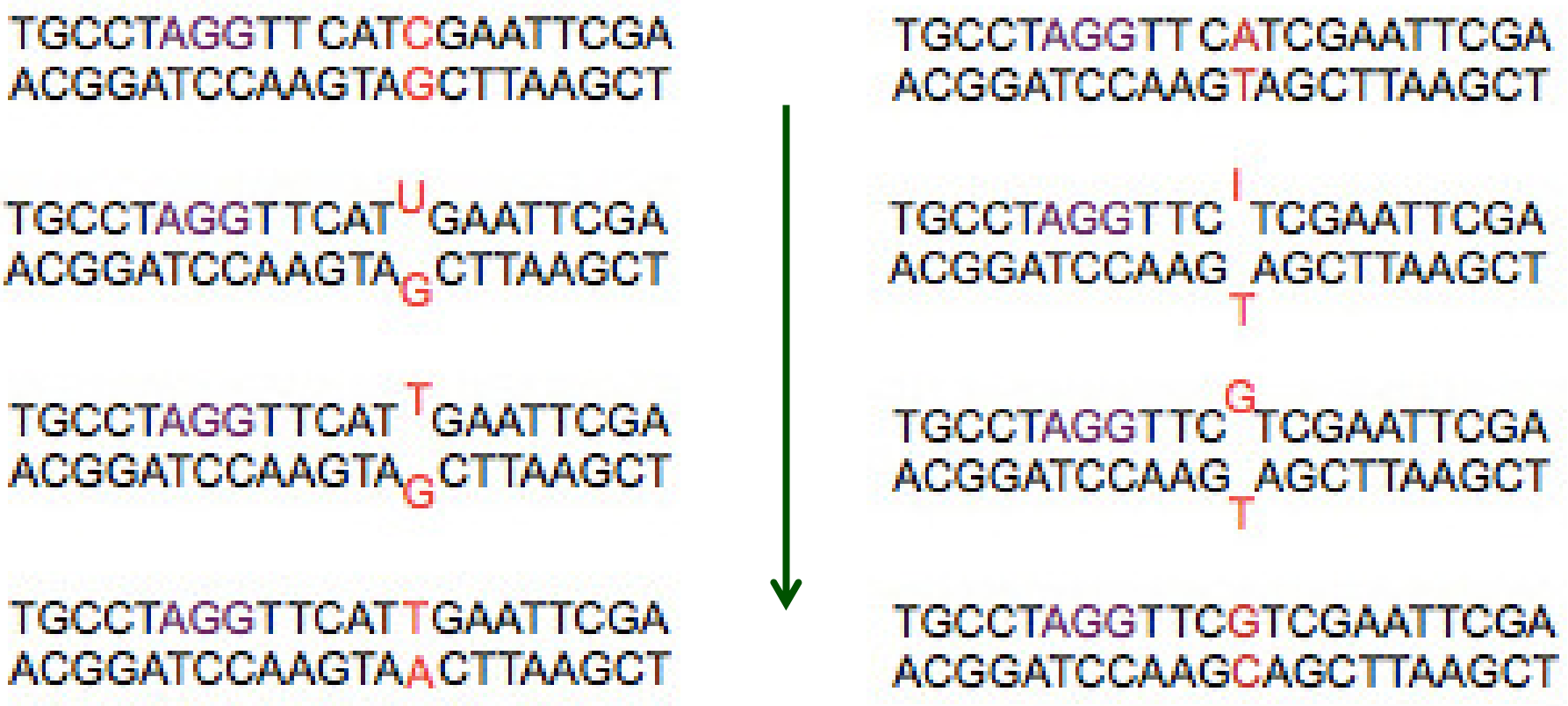
GATCCATAAGCTAATGCCTAGGTTCAT T GAATTCGAAATGGACC
CTAGGTATTCGATTACGGATCCA AGTA G CTTAAGCTTTACCTGG

GATCCATAAGCTAATGCCTAGGTTCAT T GAATTCGAAATGGACC
CTAGGTATTCGATTACGGATCCA AGTA A CTTAAGCTTTACCTGG

↑

Single base pair substitution
This can be used to correct or create a mutation in the coding region or a regulatory region.

Using “base editing” to alter C:G to T:A or A:T to G:C



Kim et al, .. Liu. Nat Biotechnol. 35, 371-6, 2017. Different PAMs and more precise base editing.

Gaudelli et al, ..Liu. *Nature* 551, 464-71, Nov. 2017. Engineering a novel base editing enzyme.

Single base pair substitutions can be used to correct or create a mutation in a coding region or a regulatory region.

~ 50% of inherited diseases are due to single base pair substitutions

The CRISPR/Cas9 methods are now sufficiently precise and efficient that the old arguments, about the methods of altering DNA being too unreliable and unsafe to use with humans, may well no longer apply.

Three major applications of genome editing with human cells

RESEARCH

Basic research (purely laboratory) work on cells and tissues

CLINICAL

Somatic (non-heritable) interventions in patients to treat or prevent disease

Germline (heritable) interventions to treat or prevent disease

Experiments in vitro to understand human biology

- The role of specific genes can be studied in different contexts.
- The methods can be used to make a mutation or correct a mutant gene in patient tissue-specific stem cells or iPS cells.
- Such cells can also be used for screening drugs.

Already common with a variety of human cells systems in vitro:

- Organ-specific stem cells, e.g. neural stem cells, gut stem cells, which can be used to make “organoids”.
- Embryonic Stem (ES) cells and induced pluripotent stem (iPS) cells, which can be differentiated in vitro to:
  - Specific cell types: neurons, primordial germ cells, etc.
  - Complex tissues: cortical brain structures, optic cups, kidney-like structures, etc.

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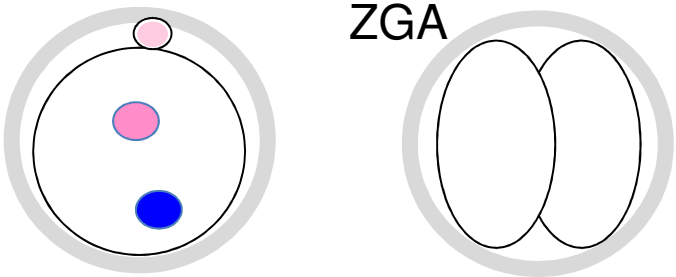
  - Complex tissues: cortical brain structures, optic cups, kidney-like structures, etc.

Why not use the techniques to study preimplantation embryos and other germline cells ……

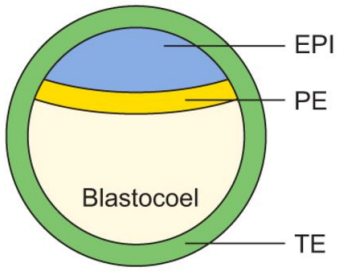
Comparison of blastocyst and early post-implantation development between mouse and human.

MOUSE

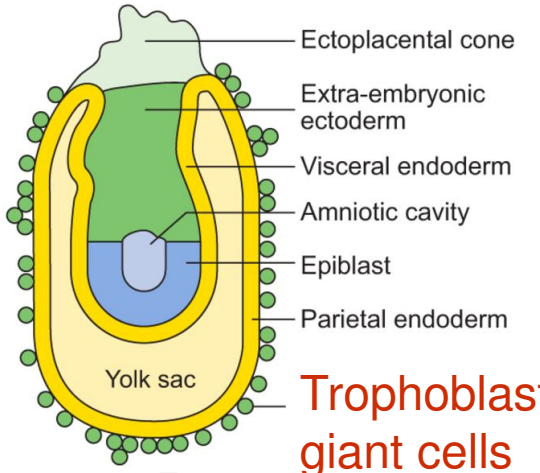


ZGA

0 Embryonic day 1



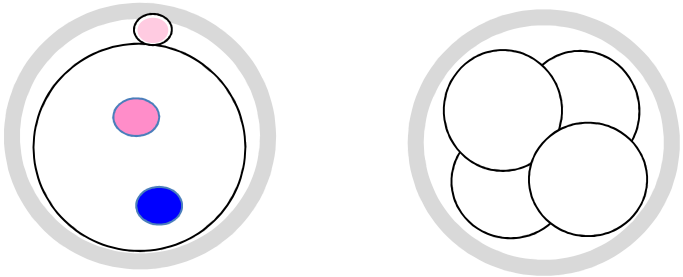
3.5



Trophoblast giant cells

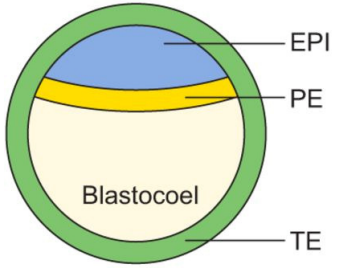
6.5

HUMAN



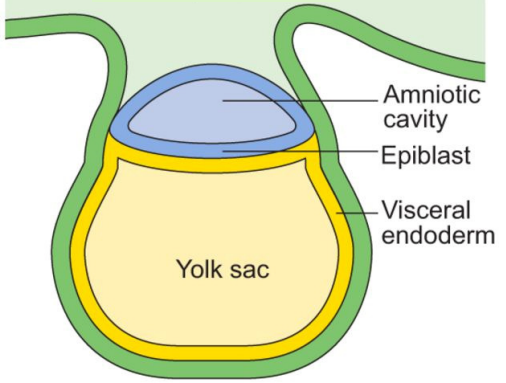
ZGA

0 Embryonic day 2-3



5-6

Syncytiotrophoblast

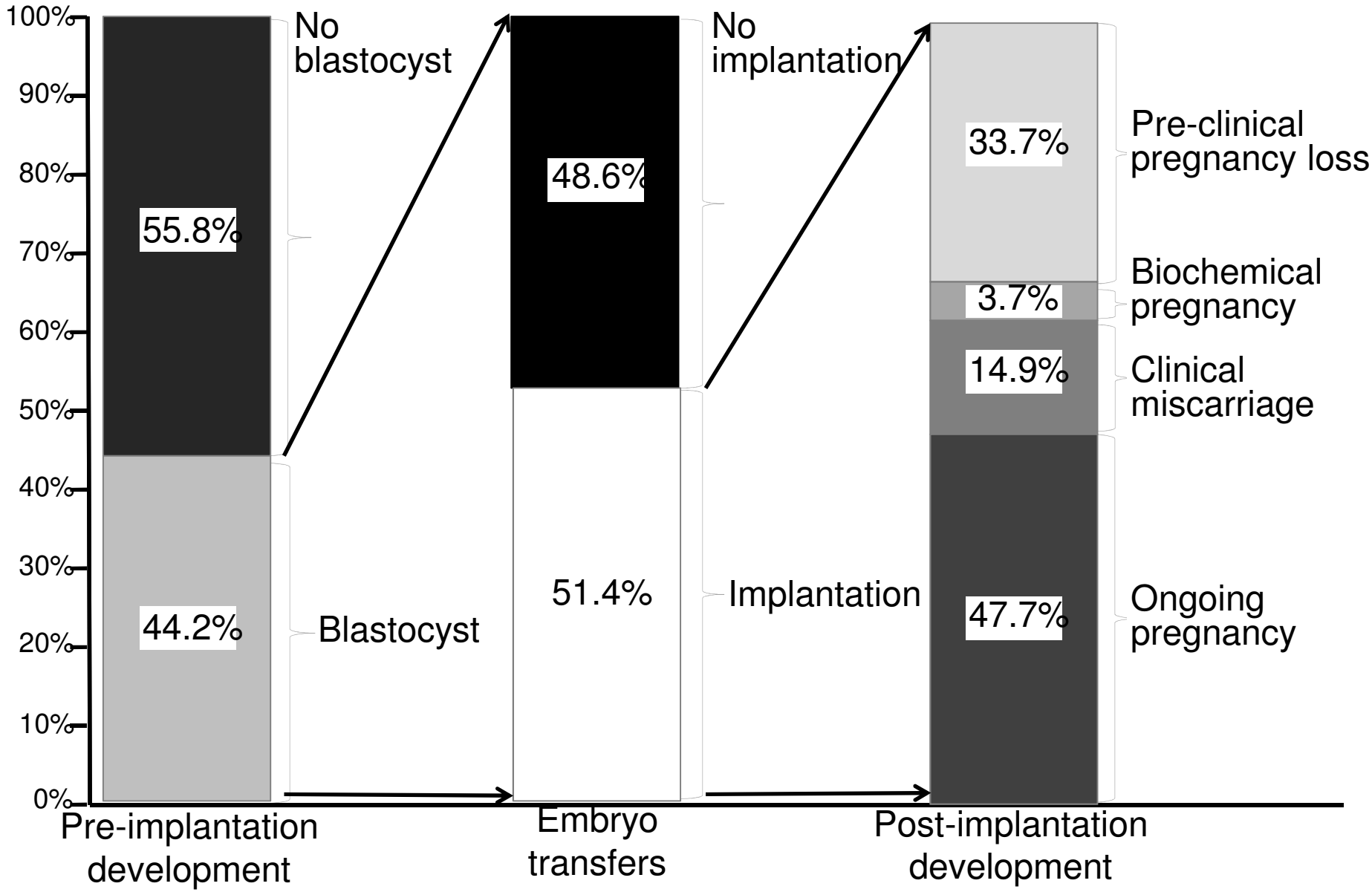


13

RNA expression studies show many differences in gene activity between mouse and human embryos

Adapted from Rossant (2015), Development 142:9-12

Development of human embryos after IVF



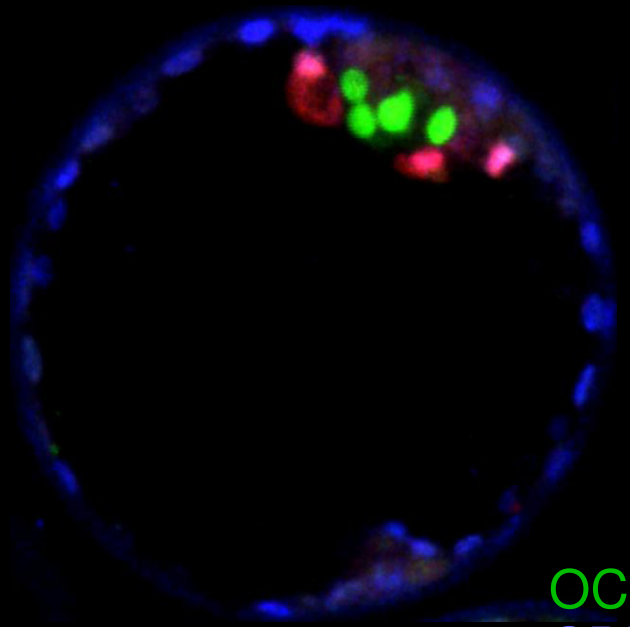
Adapted from Koot *et al., Biochimica et Biophysica Acta* 2012

Kathy Niakan

Francis Crick Institute

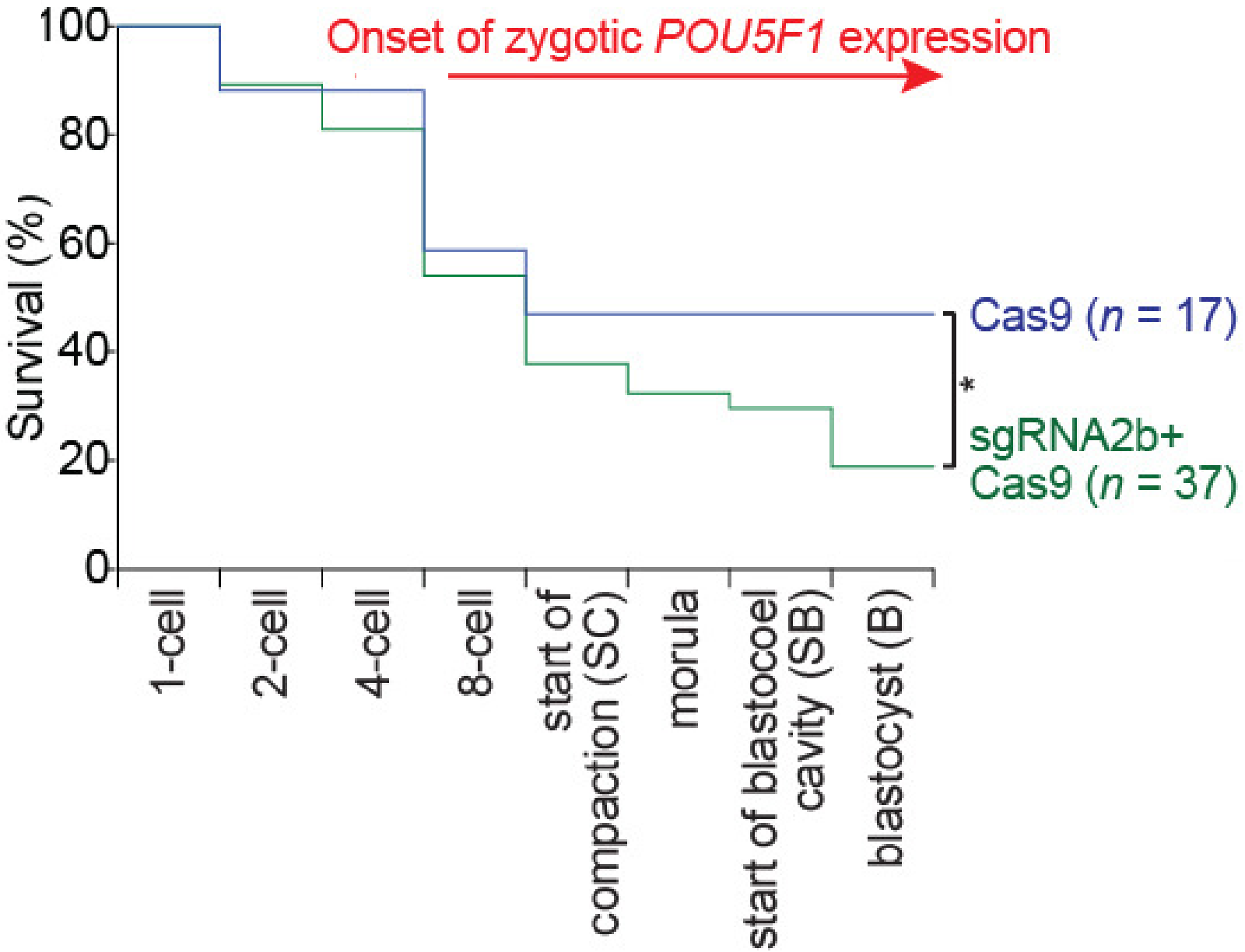
Holds an HFEA license to carry out this research

Mechanisms of lineage specification in human embryos



What is the role of OCT4 in human embryos?

Human embryos need OCT4 to reach the blastocyst stage



Summary and future implications

- First time genome editing has been used to study gene function in human embryos, revealing an important function for OCT4 for differentiation of trophectoderm as well as the ICM and epiblast.
- Studying the role of genes during human development will advance our understanding of human biology.
- In turn, this knowledge could lead to improvements in stem cell biology, IVF treatment and help understand and prevent some causes of miscarriage, …...

  … using methods that come from understanding of pathways, metabolism, etc, not genome editing.

Are there any “cons” to research using genome editing on early human embryos in vitro ?

- I think not, as long as the work is conducted well and with appropriate ethical approval and oversight.
- After all, genome editing in this context is just another method to ask questions about the biology of early human embryos.

  It is not much different from other research that has led to IVF and associated methods, such as PGD.
- I think that the paper from Kathy Niakan’s lab has set the standard at to how this type of research should be done.
- This includes “openess”: making her intentions, all the issues to do with obtaining permission to do the work, and all the data public as soon as it was reasonable to do so.

Are there any “cons” to research using genome editing on early human embryos in vitro ?

But there are several concerns :

1. It may be necessary to create embryos for research, something that is currently permitted in just a few countries:
   - If it is difficult to obtain fertilised eggs (zygotes), and/or:
   - If it is important to know exactly when fertilisation took place, or:
   - It is necessary to introduce the genome editing components along with the sperm (during ICSI), to avoid mosaicism, or perhaps to study mechanisms of DNA repair in very early embryos.
2. It could lead to a significant increase in numbers of embryos used for research – after all there are potentially many genes to study. (This is one reason why it is important to share intentions as well as results.)

Are there any “cons” to research using genome editing on early human embryos in vitro ?

Concerns continued :

3. It will lead to an extension of the “14 day limit” on embryo research.

But there is pressure to do this anyway.

4. Improved efficiency and versatility of genome editing in early embryos (and germline cells) may facilitate attempts to use the methods clinically to make heritable genetic alterations.

But it would be unethical not to use the most efficient methods for research on the biology of human embryos.

And would it be wrong to make heritable genetic alterations …

Somatic Therapy

Genome editing is a relatively new tool for gene therapy

Approaches for somatic interventions:

Outside the body (*ex vivo*) by removing cells, editing them and reinserting them:

- **editing blood cells for treatments of cancer (CAR-T cells) or HIV**
- **editing blood cells for sickle cell disease, thalassemias**

Directly in the body (*in vivo)*, e.g. with viral delivery, which is technically more challenging:

- **editing liver cells for metabolic diseases or haemophilia**
- **editing muscle cells for muscular dystrophy**
- **mutating human papilloma virus in epithelial cells to reduce cancer risk**

Enhancement

Making changes beyond ordinary human capacities; or anything outside of treatment/prevention of disease and disability

- Significant public concern about fairness, if available only to some people, and about creating pressure to seek out enhancements
- But many other kinds of enhancement are tolerated or encouraged: Nutrition, education, cosmetic procedures
- Potential for uses of genome editing beyond therapy

    For example: curing muscular dystrophy versus becoming stronger than normal.

But the range of possible uses of approved therapies for enhancement seems limited

Enhancement

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- Potential for uses of genome editing beyond therapy

  For example: curing muscular dystrophy versus becoming stronger than normal.

But the range of possible uses of approved therapies for enhancement seems limited

Enhancement is unlikely to offer benefits sufficient to offset risks at this time

RECOMMENDATIONS
Enhancement

- Genome editing for purposes other than treatment or prevention of disease should not proceed at this time

- Do not extend genome editing to purposes other than treatment or prevention of disease without extensive public input

Germline (heritable) interventions to treat or prevent disease

- The human genome is not static; changing with ~40 to 80 base pair substitutions and 4 or 5 small insertions or deletions (INDELS) each generation due to de novo germline mutations.
- Given the size of the genome, and that many of these mutations will be silent, this degree of change seems small.
- Nevertheless, it has contributed to human variation and, consequently, to selection for specific traits during our evolution: in response to changing climate, food, and disease.
- It also contributes to the burden of genetic disease, leading to:
    - Miscarriage and prenatal lethality
    - Early postnatal lethality
    - Other congenital diseases
    - Lifetime “disabilities’
    - Cancer, degenerative and late onset diseases

What can we do about inherited genetic disease ?

How about deliberately altering our genes and genomes.

Can we avoid genetic disease in our children ?

Certainly not always – we can do little about spontaneous (de novo) mutations.

Could we genetically enhance ourselves or our children ?

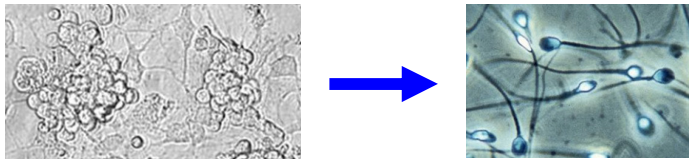
Can we alter our own evolution ?

Should we do any of these ?

Heritable Genome Editing

POSSIBLE METHODS: 1

- Editing cells that give rise to sperm, such as spermatogonial stem cells, or perhaps to eggs or via iPS cells and in vitro-derived gametes ?



- This allows verification of the edits before embryos are made.

Has been done using spermatogonial stem cells in mice, rats and macaques; and via ES and iPS cells in mice.

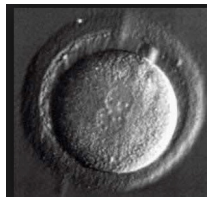
Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells.
Wu et al. (2015) Cell. Res. 25, 67-79.

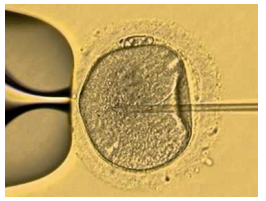
Targeted Germline Modifications in Rats Using CRISPR/Cas9 and Spermatogonial Stem Cells.
Chapman et al. (2015) Cell Rep. 10, 1828-35.

Heritable Genome Editing

POSSIBLE METHODS: 2

- Editing the fertilised egg (zygote) - perhaps coincident with intracytoplasmic sperm injection (ICSI) ?

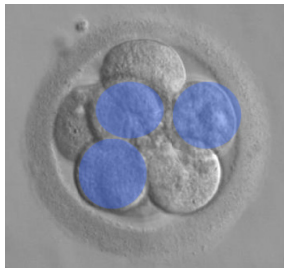




- It will be more difficult to verify the edits. Unless these are known to be ~ 100% reliable, this would require PGD (and perhaps ES cell derivation), but :

- Currently the method carries a risk of mosaicism, where not all cells in the embryo carry the desired genetic alteration.

  If this is the case, PGD is unreliable.



All three main genome editing approaches have now been used in early human embryos

HDR

CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein.

Tang, L., .. Lui, J. (2017). Mol Genet Genomics. 292, 525-533

NHEJ

Genome editing reveals a role for OCT4 in human embryogenesis.

Fogarty, N. et al,.. Niakan, K. (2017). *Nature*. 20 September.

Base editing

Correction of β-thalassemia mutant by base editor in human embryos

Liang, P. et al, .. Huang, J. (2017). *Protein and Cell*. 27 September.

But the methods are not yet safe to use !!

Much more research is needed.

However, it seems inevitable that genome editing via gamete precursors or early embryos will be made to work efficiently and, probably, safely.

Ma et al, Mitalipov (2017) reported HDR occuring with ~100% efficiency in human embryos, i.e. no mosaicism, when the genome editing components were introduced very early, along with the sperm during ICSI to correct a paternally inherited dominant mutation

The absence of mosaicism may be true, but did they have allele drop-out rather than HDR ?

When might it be appropriate to use genome editing rather than alternative methods, such as preimplantation genetic testing (PGD) ?

Heritable Genome Editing: Concerns

Genetic changes may be inherited by the next generation

Commonly viewed as unacceptable in the past:

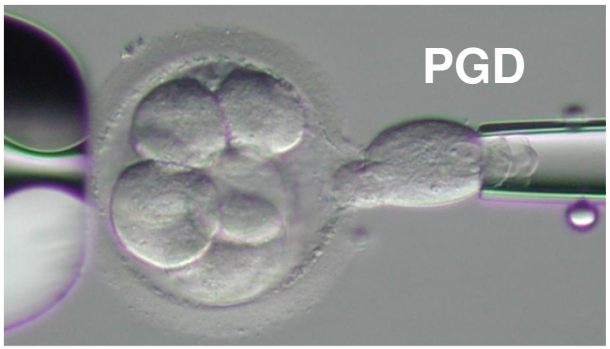
- multigenerational risks (but also possible benefits)
- need for (and possible difficulty of) long term follow-up
- lack of consent by affected persons (future child; generations)
- the degree of intervention in nature
- affecting acceptance of children born with disabilities
- a step toward enhancement for “designer babies”

Heritable Genome Editing

But if it is now a realistic possibility we need a fresh look at these earlier views:

- Interest is driven by the thousands of inherited diseases.
- It would allow individuals to have genetically related children without passing on a known risk of genetic disease.

In many cases, preimplantation genetic diagnosis (PGD) or prenatal diagnosis with selective termination are alternatives.



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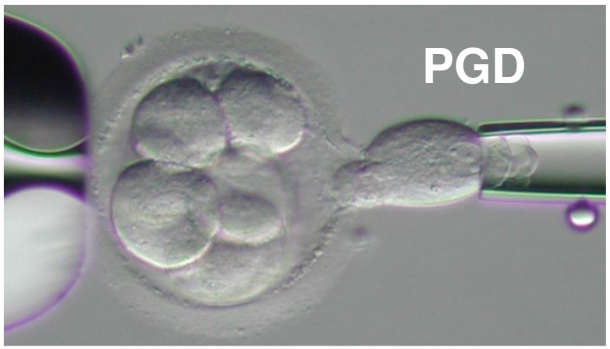


But these methods are unacceptable for some individuals (or even many).

And there are cases where they cannot be used to retain a parental genetic connection, but avoid having a child with a genetic disease.

PGD is not always possible, and it is often inefficient:

- Rare individuals homozygous for any dominant version of a gene that leads to disease, such as Huntington's disease.
- Rare occasions where both parents are homozygous for a recessive mutation leading to a genetic disease.
- Where mutations affect fertility: too few embryos and patients might have to go through many rounds of treatment to find a disease free embryo if ever.
- For "saviour siblings", or where more than one harmful mutation or variant allele makes the probability of finding a "disease-free" embryo very low.

The genome editing methods may turn out to be more efficient and perhaps more reliable than PGD.

And for some people they may be more acceptable, because embryos are "rescued", not destroyed.

Which gene variants (mutant alleles) might be relevant for correction via germline genome editing ?

It is difficult to focus on specific genes:

- Common diseases, such as:

  Cystic Fibrosis, Duchenne Muscular Dystrophy, familial hypercholesterolemia, sickle cell disease, beta-thalassemia, Spinal Muscular Atrophy ?

- Diseases that are generally rare, but occur at high frequencies in specific populations, such as:

  Tay Sachs, Huntington's, etc.

- But there are >10,000 single gene disorders !

Perhaps it will depend on who is standing in front of the clinician asking for help to have a healthy, genetically related child.

As our ability to treat patients improves, including conventional methods or somatic gene therapy, there will be more patients surviving to reproductive ages.

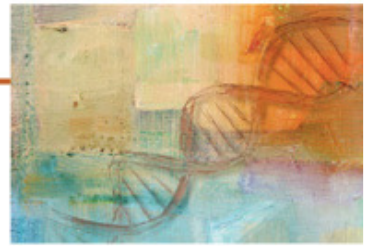
Heritable Genome Editing - Regulations

- Regulations covering laboratory work and human subject protections in clinical trials are applicable.
- If done in the USA with embryos (as opposed to gametes), it would be prohibited in some states; at a federal level there are restrictions on funding.
- At this time, clinical trials are not possible in U.S. due to limitations on FDA authority.
- Other countries vary, from prohibition (including much of Europe) to possible authorization under strict regulation, to no rules at all.
- In the UK it would require a change in the HFE Act via primary legislation.

Heritable Genome Editing Clinical Trials

- Caution is needed, but being cautious does not mean prohibition.

- Heritable genome editing research trials might be permitted, but only:
  - After more research to meet existing risk/benefit standards,
  - under strict oversight, and
  - if they are restricted to specific set of criteria.

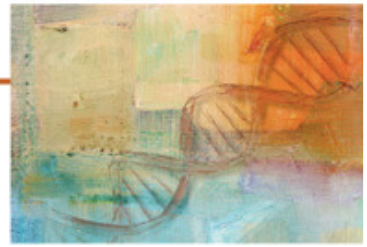


Criteria to Initiate Clinical Trials

- Absence of reasonable alternatives;
- Restriction to prevention of a serious disease or condition;
- Editing only genes that have been convincingly demonstrated to cause or to strongly predispose to the disease or condition;
- Converting such genes to versions that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects;
- Availability of credible pre-clinical and/or clinical data on risks and potential health benefits of the procedures;

Criteria to Initiate Clinical Trials

- Ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants;
- Comprehensive plans for long-term, multigenerational follow-up;
- Maximum transparency consistent with patient privacy;
- Continued reassessment of both health and societal benefits and risks, with broad on-going participation and input by the public; and
- Reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition.
- The establishment of appropriate and robust regulations and oversight will be critical – *indeed it should not proceed in a jurisdiction until these are in place.*



Continuing, but key questions :

- What uses for genome editing in human clinical applications might be permissible ?
- What are the safest methods ?
- Social justice: how can we ensure access of the applications to all ?
- How can we avoid the problems associated with “rogue” clinics offering unsafe, untested, genome editing methods to ‘treat’ or avoid genetic disease or for enhancements – a problem for both somatic and germline genome editing ?
- How can we obtain good understanding of the views of patients and their families (and not to be swayed by dystopian views from science fiction) ?
- How can we have good regulation and good oversight which, if done well should avoid trivial, unjust, or other uses that society as a whole deems unacceptable ?