Second International Summit on Human Genome Editing
NAS/NAM/RS/ASHK

“ Introduction to Genome Editing "

Let’s Talk About Genomics and Genome Editing

The University of Hong Kong

29 November 2018

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Manipulating and understanding DNA sequences

- **Recombinant DNA techniques in bacteria** (1972 -)
- **DNA sequencing** (1977 -)

- **Adding genes to mammalian cells in culture** (1977 -)
- **Better understanding of radiation- and chemical-induced mutations in mice and in cancer treatments in humans.**
- **Development of viruses as vectors** for introducing genes into mammalian cells (1981 -)

These technologies stimulated ideas of how to treat patients with genetic disorders - **“somatic gene therapy”**:

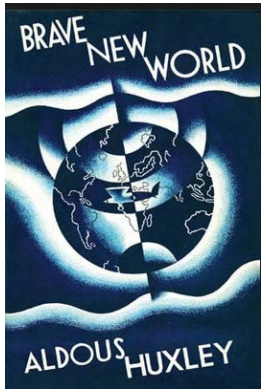
- Can a version of the missing gene be introduced in a way that it should be active in the relevant tissue over prolonged periods ?

But at the same time, and almost every new technology since then, has prompted debate about the possibility of genetically modifying humans …….

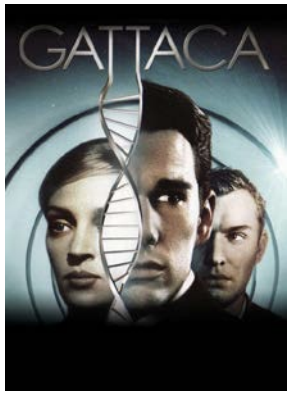
“Germline or potentially heritable genetic alterations”

1978: Birth of Louise Brown, the first IVF baby.

“Frankenstein”
Mary Shelley
1816/1818



1931/1932



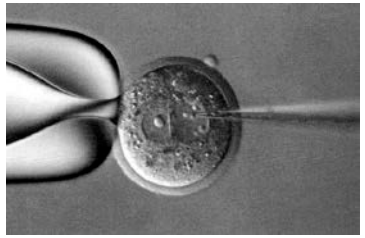
Andrew Niccol
1997


Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

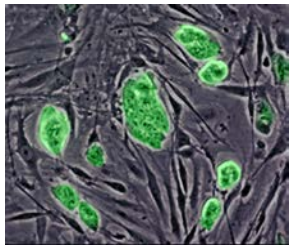
Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

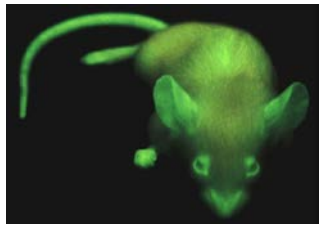


1981: **Transgenic mice** made by injecting DNA into fertilized eggs (zygotes). Inefficient, haphazard.

rat growth hormone transgene

1984: Mouse **embryonic stem (ES) cells** grown in culture are shown to contribute to fertile “chimeras” after being introduced into early embryos (chimeras are made from cells of two or more genotypes).

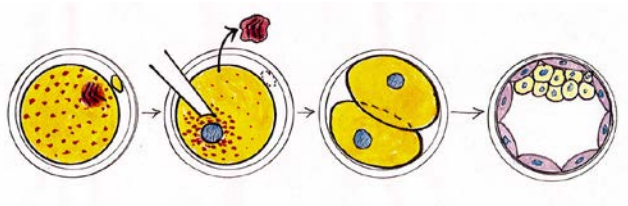




1989: **Gene targeting** via homologous recombination in mouse ES cells.

A precise method to alter genes, but very inefficient, unsafe, and not feasible in humans.

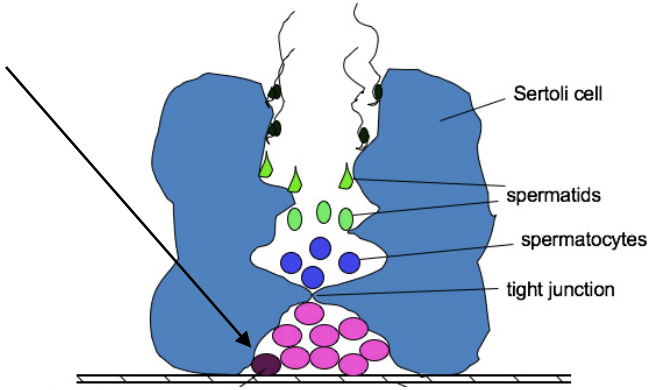
1997: Somatic cell nuclear transfer (**SCNT**) or “**cloning**”.

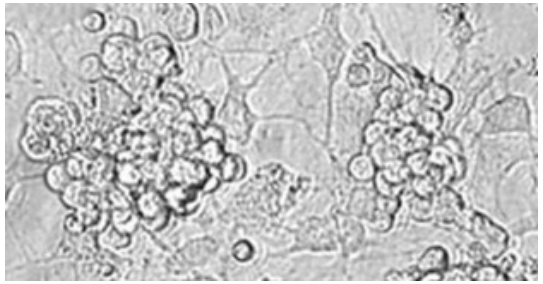


Genes can be altered in the cells, which can then be used to derive cloned animals. But cloning methods are inefficient and very unsafe.

Spermatogonial stem cells as a route to altering the genome

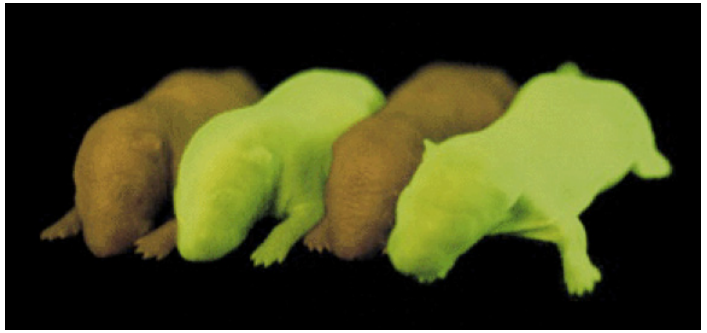
The testis contains special stem cells that divide to make more of themselves and to cells that will give rise to sperm.





1994: Ralph Brinster developed methods to culture spermatogonial stem cells, genetically alter them, and then transplant them back into testes.

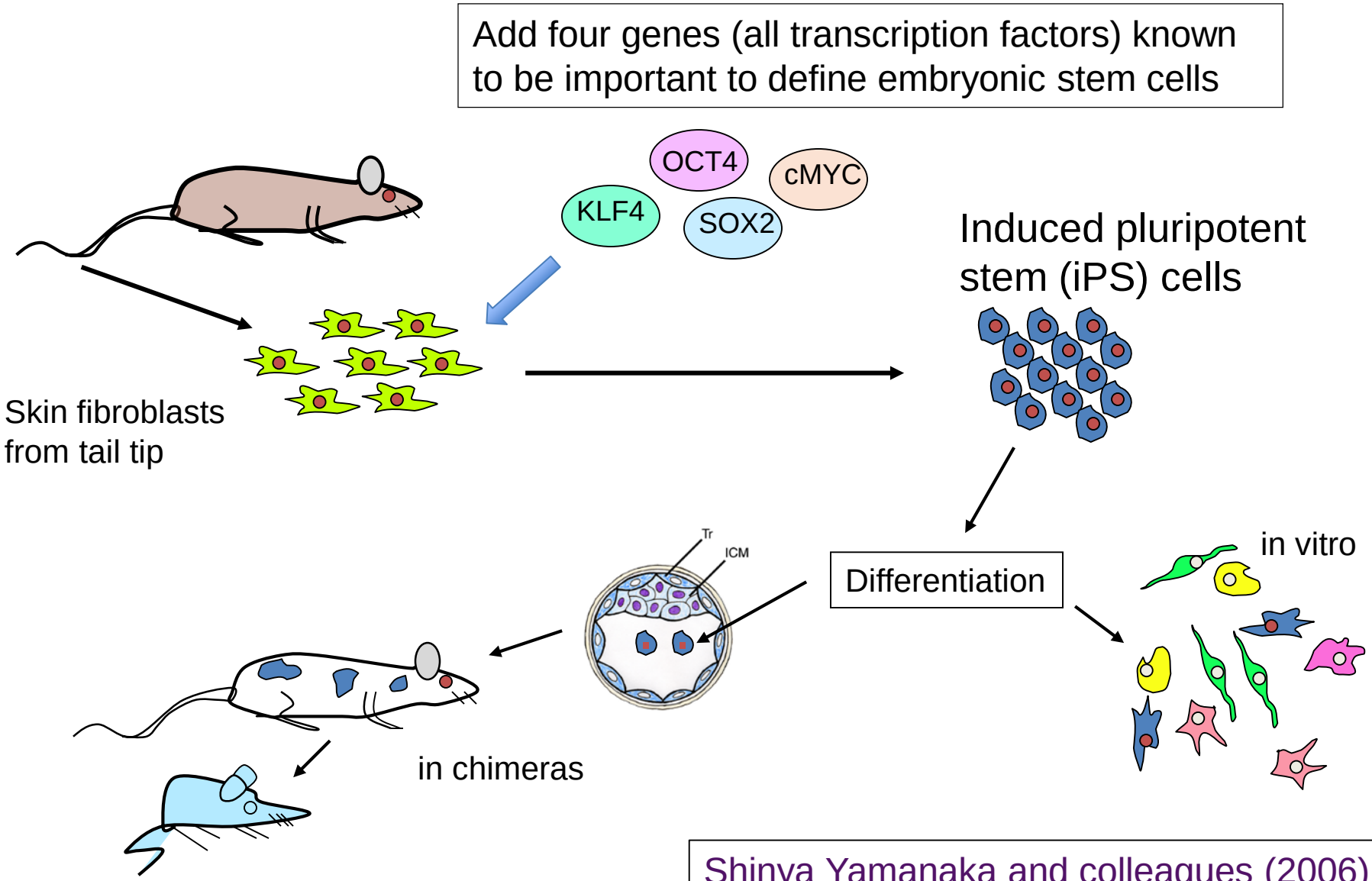
2007: Generation of genetically altered rats by this route:



It has also been shown to work in macaques

This might work in humans, but it has not yet been tried.

Direct reprogramming of adult somatic cells in vitro to give induced pluripotent stem (iPS) cells



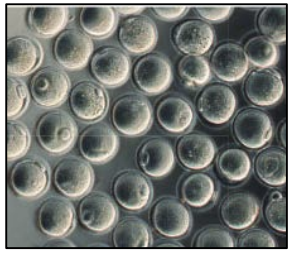
Shinya Yamanaka and colleagues (2006)

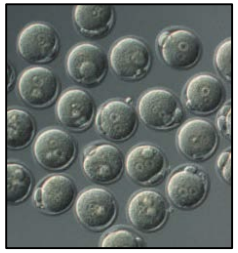
iPS cells and lab-derived gametes as a route to altering the genome

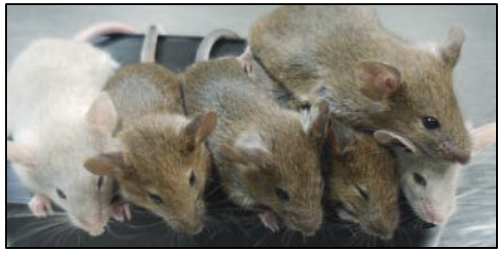
iPS cells can also give rise to “germ cells”, which, in female animals go on to give rise to eggs and in males to sperm.

2016: Mouse eggs and sperm, each capable of fertilisation, were derived from iPS cells in culture (Saitou and colleagues):

From XX iPS cells







From XY iPS cells

iPS cells have been derived from many mammals, including humans.

It may be possible to derive gametes (eggs and sperm) entirely in vitro, beginning with patient-specific iPS cells.

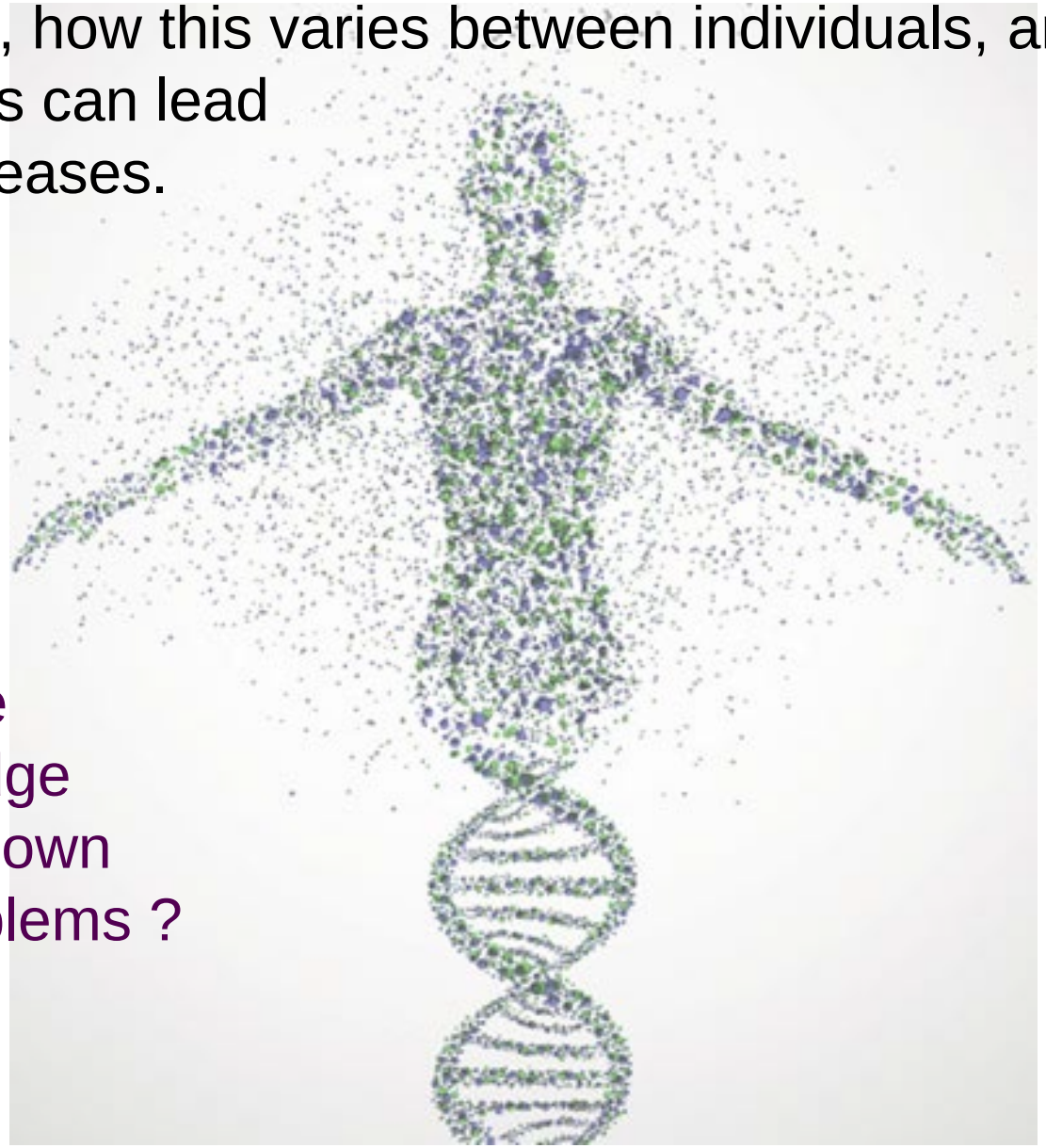
With each new method of manipulating genes in mammals, the same questions have arisen about the possibility of using them to:

- Treat or avoid heritable genetic disorders.
- Make “designer babies”.
- Practice “eugenics”.

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

But at least two areas of science have opened up these debates again:

1. 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.



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

2. The precise and efficient means of altering DNA sequences provided by **genome editing** methods

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Genome editing generally makes use of endogenous DNA repair mechanisms

And it most often requires:

(i). "Molecular scissors": a nuclease enzyme to make a double-stranded (or in some methods a single-stranded) cut in DNA

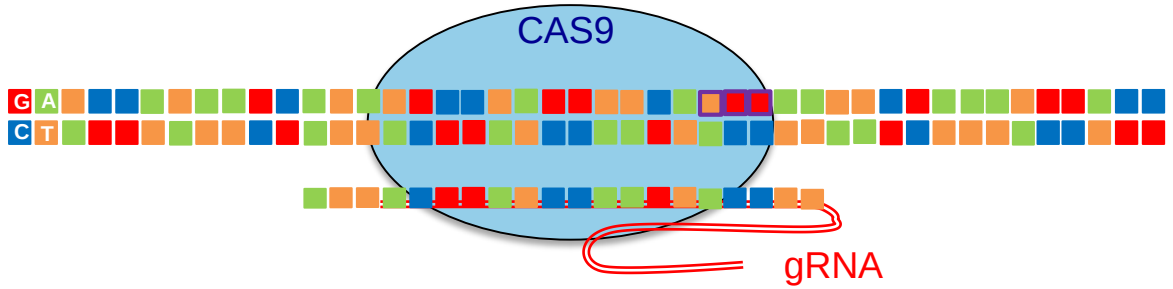
(ii). "Homing device" : a mechanism to recognise specific DNA sequences – derived from DNA binding proteins such as transcription factors (ZFNs, TALENs) or complementary RNA (CRISPR)

(iii). "Template": if more than a simple mutation is required, a DNA template with homologous arms is needed to allow *homology directed repair.*

Using CRISPR/Cas9 to make an inactivating mutation via NHEJ

CRISPR-Cas9

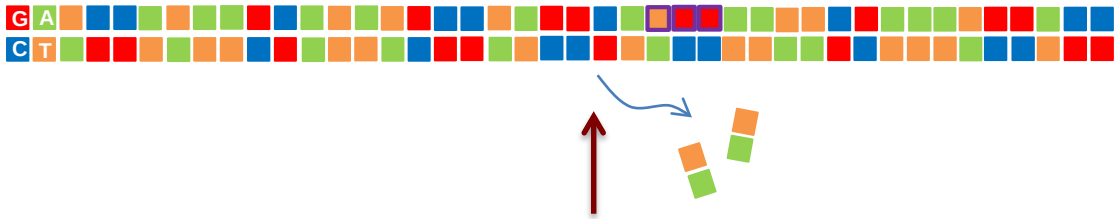
guide-RNA + CAS9 + protospacer-adjacent motif = PAM



Double-strand break in DNA

Non-homology end-joining (NHEJ) repair

This leads to small insertions or deletions (INDELs)



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

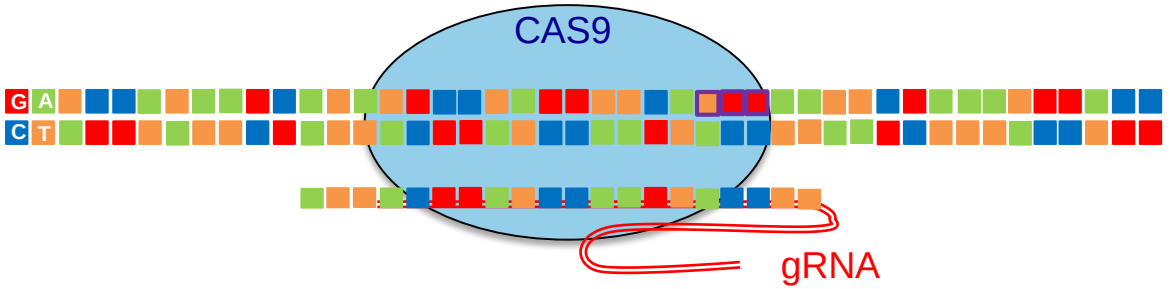
Ku70/80-dependent

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 via HDR

CRISPR-Cas9

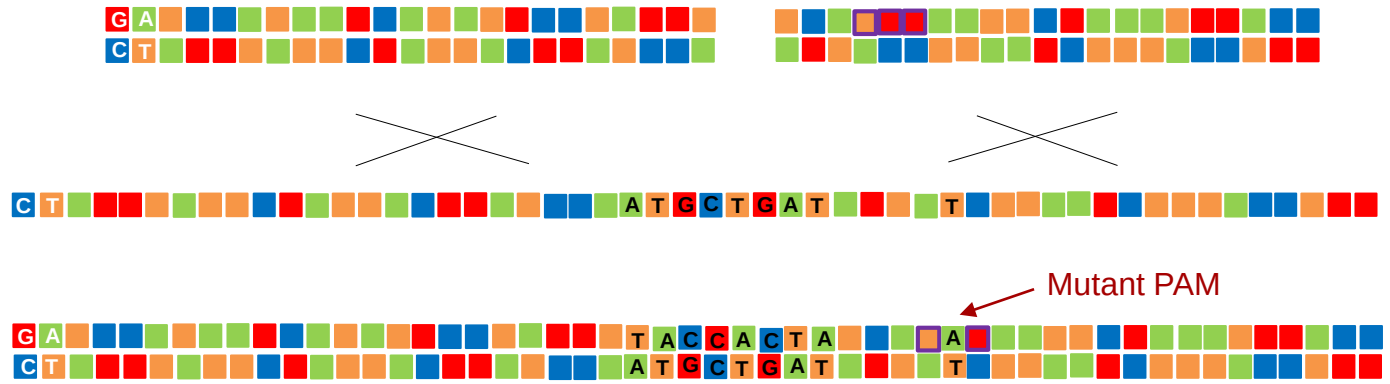
guide-RNA + CAS9



Double strand break in DNA

+

DNA template : (single- or double-stranded)



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

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 (dead.Cas9 or dCas9) linked to relevant enzyme

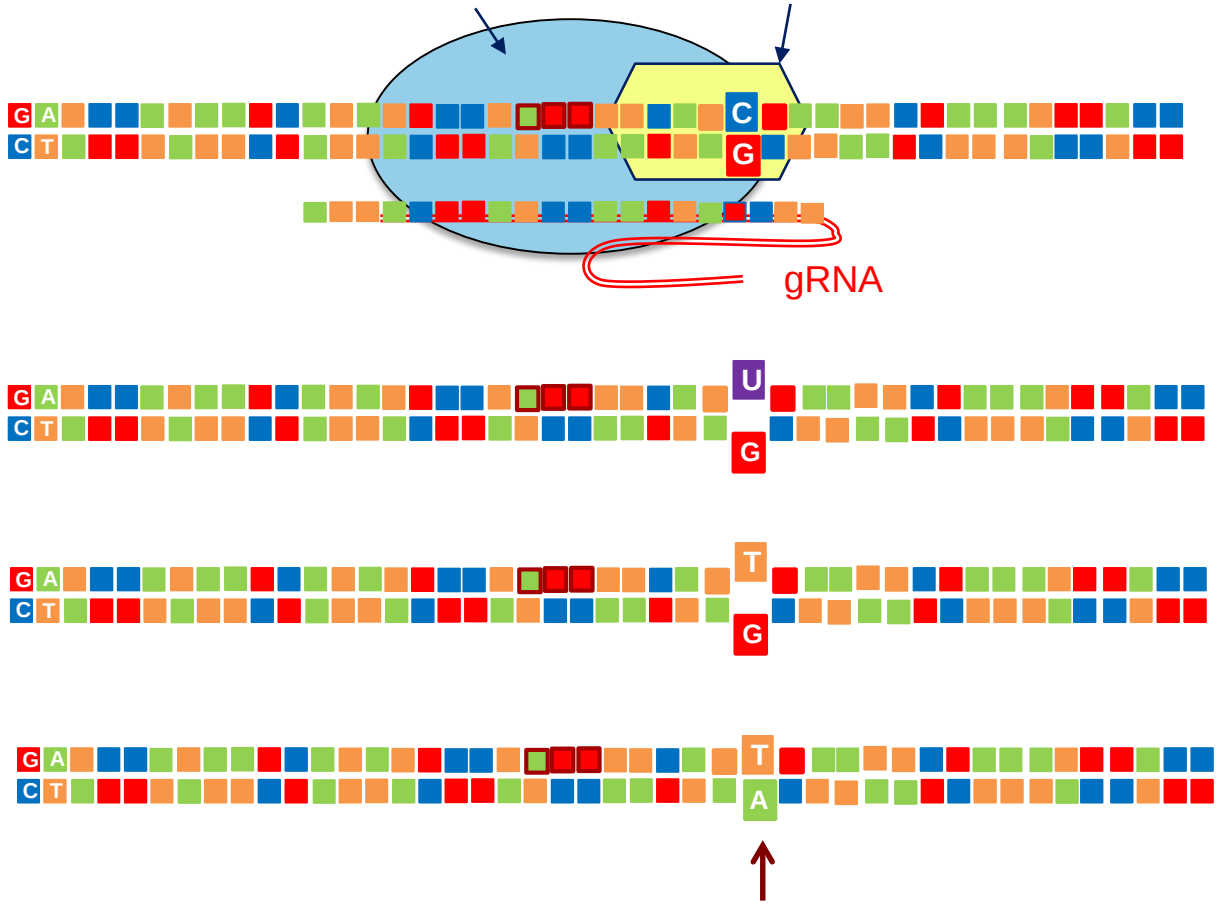
↓

A specific C is chemically modified to become U and then T (no double strand break in DNA)

↓

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

guide-RNA + dCAS9-cytosine deaminase



Single base pair substitution
This can be used to correct or create a mutation

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

Some advantages of CRISPR/Cas9 methods

- CRISPR/Cas9: Simple to make components: guide RNAs, Cas9, and DNA templates
- Relatively simple to introduce these into cells and early embryos.
- Highly specific — Few if any off-target events
- Highly efficient — But mosaicism ?
- Ability to “multiplex”
- Versatile:
  - Alterations to DNA: including “indels” and deletions, insertions or substitutions from single base-pairs up to many kilobases.
  - Cas9 DNAse activity can be mutated and other proteins linked to it to permit “base-editing”, but also by using transcriptional activators or repressors or chromatin modifiers, it is possible to manipulate specific gene activity without altering DNA.

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 and “pre-clinical” research (purely laboratory) work on cells and tissues

CLINICAL

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

Germline (potentially 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.

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

Experiments in vitro to understand human biology

The genome editing techniques are now widely used around the world with a variety of human cell systems in the lab to understand the role of specific genes, how they lead to disease, and to screen for drugs, etc.

These include:

- Simple cell cultures
- Organ-specific stem cells, e.g. neural stem cells, gut stem cells.
- ES and iPS cells, which can be cultured in vitro to give many specific cell types and even complex tissues, including brain structures, optic cups, kidney-like structures, etc.

Human embryos need OCT4 to correctly form a blastocyst

The genome editing techniques are now widely used around the world with a variety of human cell systems in the lab to understand the role of specific genes, how they lead to disease, and to screen for drugs, etc.

These include:

- Simple cell cultures
- Organ-specific stem cells, e.g. neural stem cells, gut stem cells.
- ES and iPS cells, which can be cultured in vitro to give many specific cell types and even complex tissues, including brain structures, optic cups, kidney-like structures, etc.

The techniques are also being used to study early human embryos in culture

Clinical applications: Somatic Gene Therapy

Genome editing is a relatively new tool for gene therapy

Advantages over old methods:

- Corrects the defective gene, which will be active at the correct levels, rather than introducing an extra copy of the gene, which will insert at random in the genome and may not be correctly regulated.

Approaches for somatic interventions:

- Editing cells outside the body (*ex vivo*) and reinserting them:
  - editing blood cells for treatments of cancer (CAR-T cells) or HIV
  - editing blood cells for sickle cell disease, thalassemias

- Editing cells directly in the body (*in vivo)*, with e.g. viral or particle delivery (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

Somatic gene therapy in humans using genome editing:

- ZFNs to mutate the HIV receptor, CCR5 in AIDS patients

- Layla Richards, with acute lymphoblastic anemia (ALL), was the first in the world to be given TALEN genome-edited immune cells (CAR-T cells) to treat an 'incurable' cancer.

- CRISPR/Cas9 for CAR-T cells for small cell lung cancer:
  Trial in China just starting.

- Sickle cell and β-thalassemia: Preclinical data is very promising.

With all the above, the genome editing is carried out ex vivo.

What about *in vivo* somatic gene therapy via genome editing ?

Challenges include:

- Efficient delivery methods
- Capacity of viral vectors: - Adenovirus Associated Viral (AAV) vectors
  - Adenoviral vectors
- Off target events: - Even if the frequency is low, the number of cells that need to be targeted is very high. (But benefit versus risk ?)
- Immune responses: -To Cas9 and/or viral vectors
- Moving from preclinical (cell lines and animals) to clinical “trials”.

But some preclinical data looks promising:

- **CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington’s disease. Su Yang, et al. JCI. (2017)**
- **Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Amoasii et al. (2018).**

Germline (potentially heritable) genome editing 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 ?

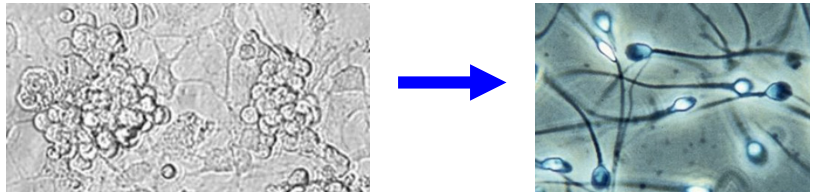
How about deliberately altering our genes and genomes ?

- Can we avoid genetic disease in our children ?
  - In theory, if the techniques are both safe and efficient.
  - But certainly not always – we can do little about spontaneous (de novo) mutations.
- Could we genetically enhance our children ?
- Can we alter our own evolution ?
- Should we do any of these ?

Potentially Heritable Genome Editing

POSSIBLE METHODS: 1

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



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

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

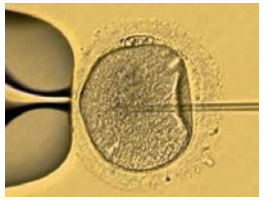
Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells. Wu et al. (2015) Cell. Res. 25, 67-79.

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

Heritable Genome Editing

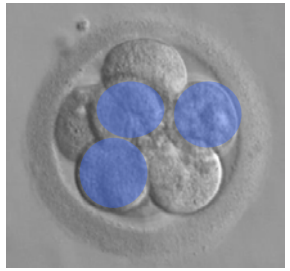
POSSIBLE METHODS: 2

- Editing the fertilised egg (zygote)



- It will be more difficult to verify the edits. Unless these are known to be close to 100% reliable, this would require Preimplantation Genetic Diagnosis (PGD)

- But currently, the methods are not quite efficient enough and there is a risk of mosaicism, where not all cells in the embryo carry the desired genetic alteration.



If this is the case, PGD becomes 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

Correction of a pathogenic gene mutation in human embryos.
Ma, H., … Mitalipov, S. (2017). Nature. 548, 413-419.

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.

Correction of the Marfan Syndrome Pathogenic FBN1 Mutation by Base Editing in Human Cells and Heterozygous Embryos.
Zeng, Y., … Huang, J. (2018). Mol. Therapy.

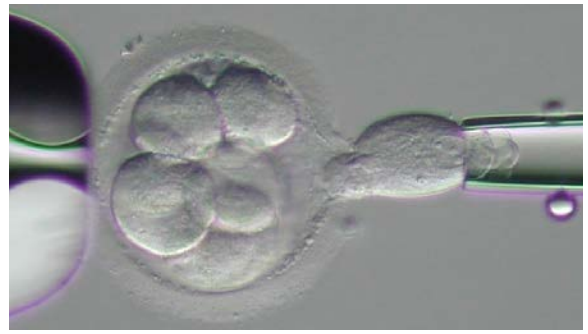
Heritable Genome Editing

The methods are still not safe to use: 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.

- 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, PGD or prenatal diagnosis with selective termination are alternatives.



But PGD is often inefficient and it is not always possible

PGD is often inefficient and it is not always possible :

- 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.
- For some people they may be more acceptable, because embryos are “rescued”, not destroyed.
- 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.
- In all these situations, genome editing may be the only way to retain a genetic connection to the child.

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 and somatic gene therapy, there will be more patients surviving to reproductive ages. They will not want their children to inherit alleles associated with genetic disease.

The methods are still not 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.

Enhancement – somatic or germline

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

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- Potential for uses of genome editing beyond therapy

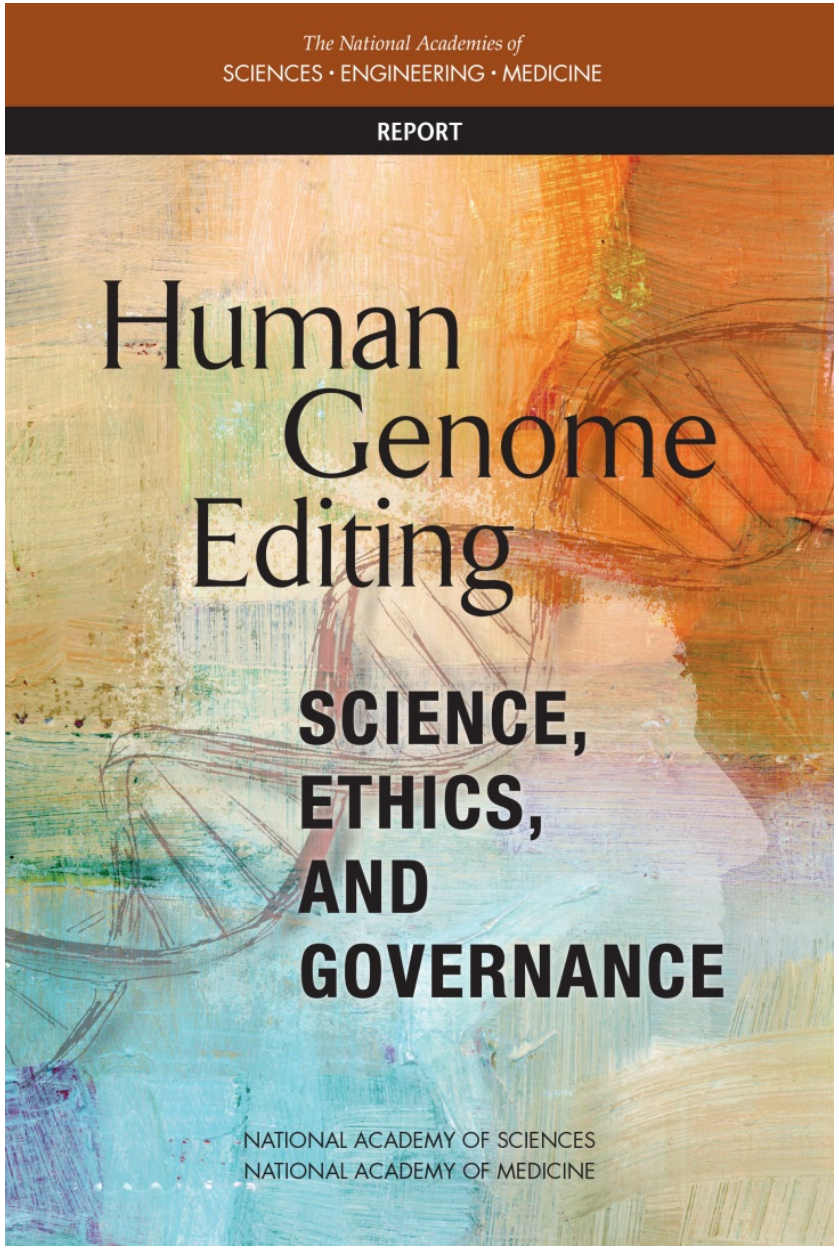
  For example: curing muscular dystrophy versus becoming stronger than normal.

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

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

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 who may need them ?
- How can we obtain good understanding of the views of patients and their families ?
- 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 ?
- 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 ?



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

Heritable Genome Editing Clinical Trials

The NAS Report was framed with a clinical perspective, which allows an approach based on risk versus benefit.

- 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.

Overarching Principles for Governance of Human Genome Editing

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

Embedded within international conventions for protection of human rights & for research with human subjects and clinical care

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.