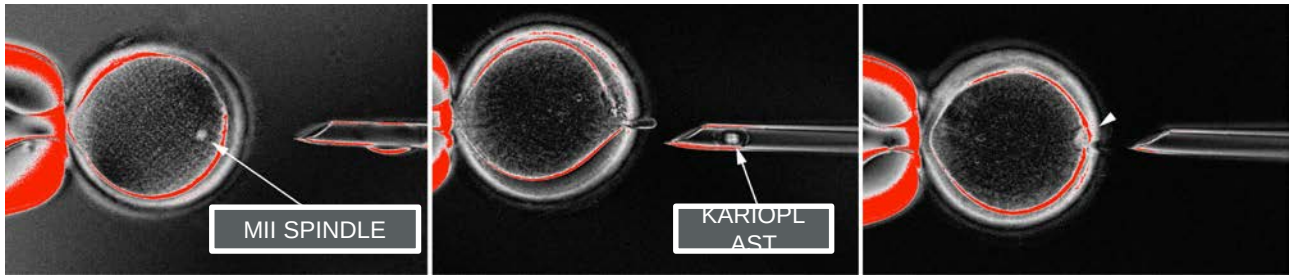
Human Germline Gene Therapy

Paula Amato, MD
Mitalipov Lab
Oregon Health & Science University

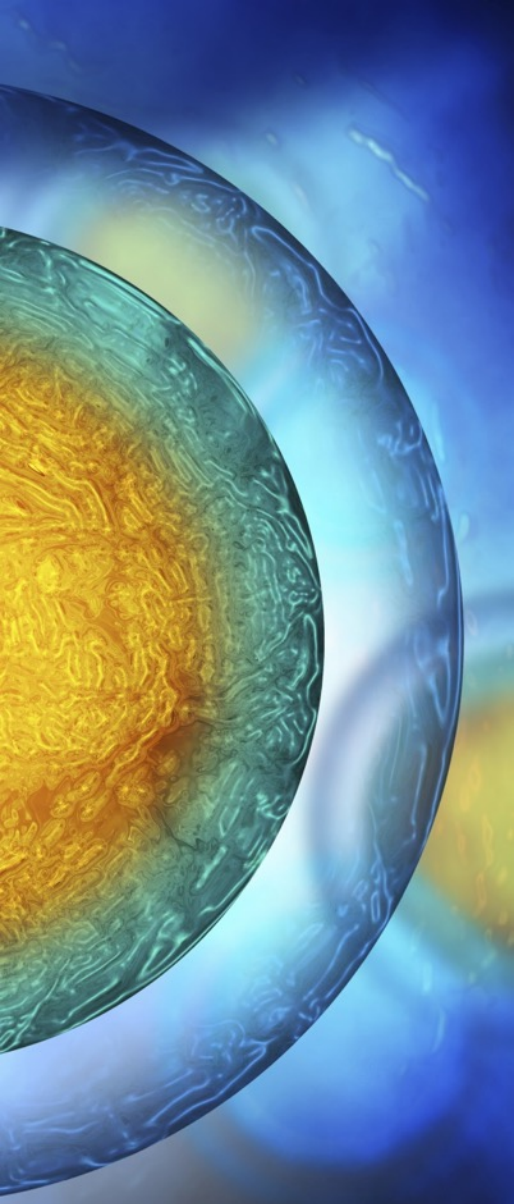
Second International Summit on Human Genome Editing, 27-29 November, 2018, Hong Kong

Germline Gene Therapy

- mtDNA → Mitochondrial Replacement Therapy



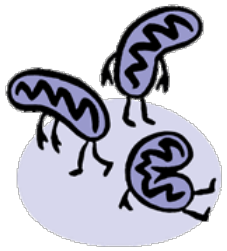
- nDNA → Nuclear Gene Correction

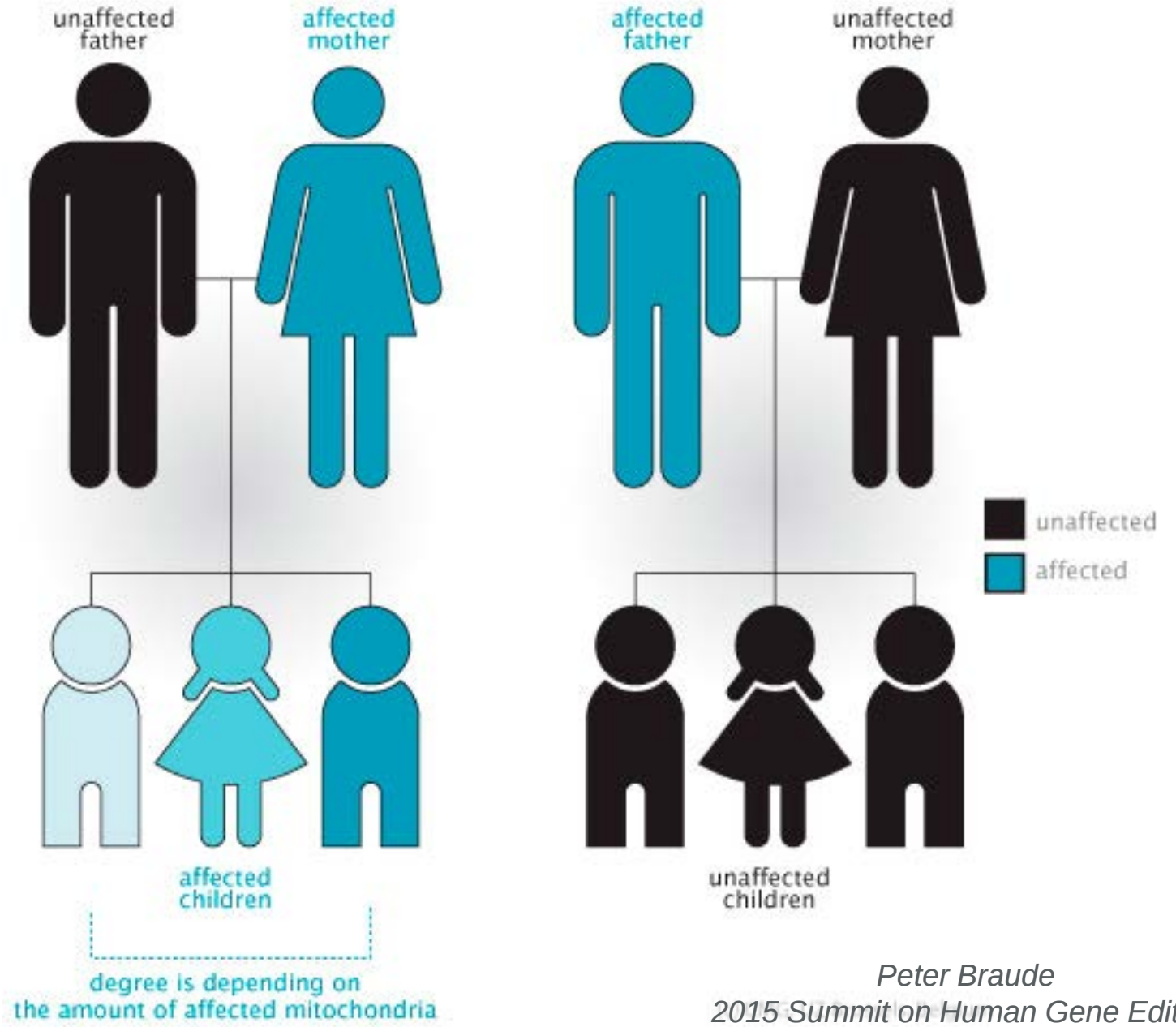


Prevention of Genetic Disease Strategies

- Adoption
- Oocyte, sperm, or embryo donation
- Preimplantation genetic diagnosis (PGD)
- Germline gene therapy
  - Prevention of genetic disease transmission by correcting disease-causing gene mutations in reproductive cells (sperm, oocyte, or embryos)

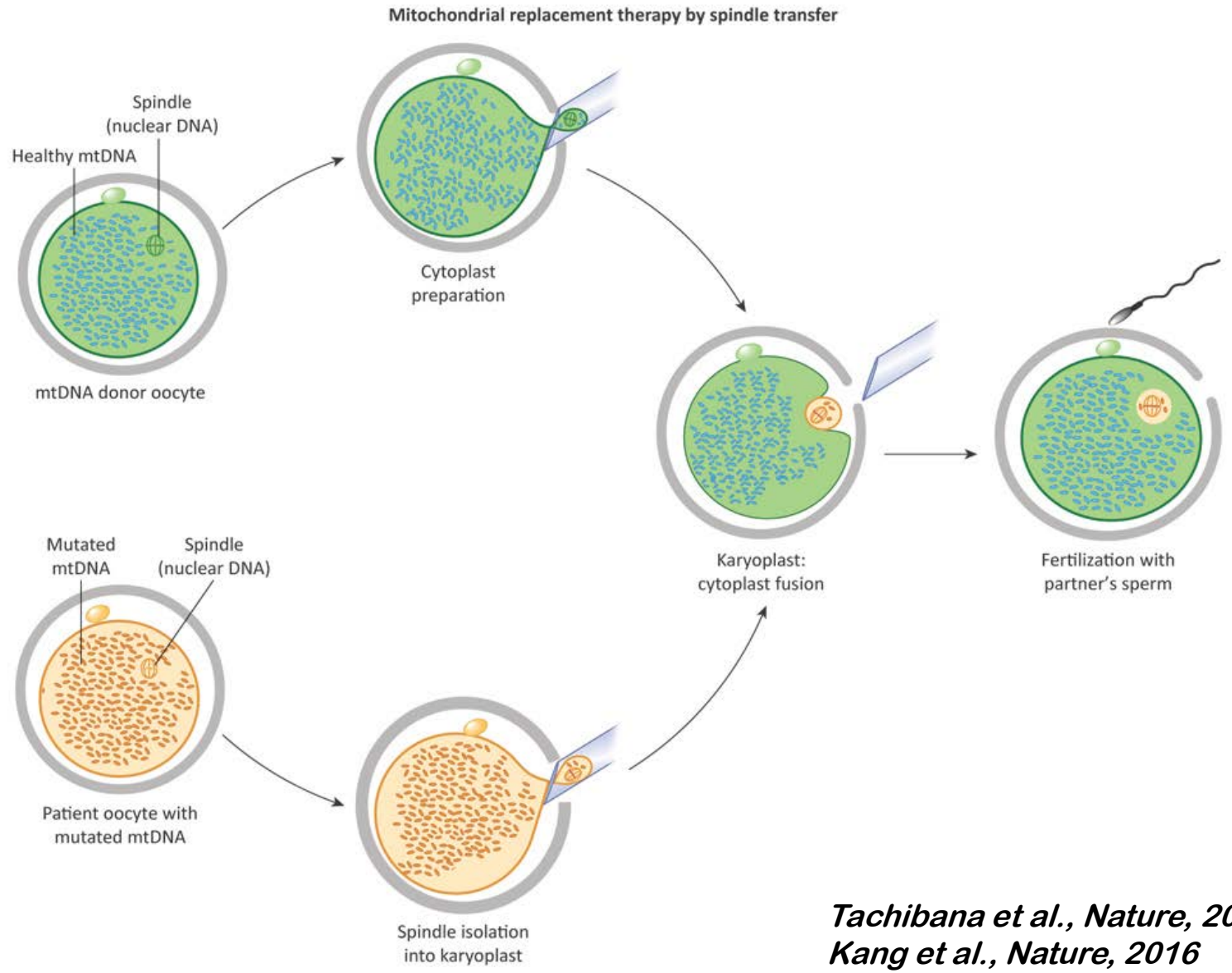
mtDNA disease Inheritance





Peter Braude
2015 Summit on Human Gene Editing

Mitochondrial Replacement Therapy
Spindle Transfer between MII oocytes



Tachibana et al., Nature, 2009
Kang et al., Nature, 2016

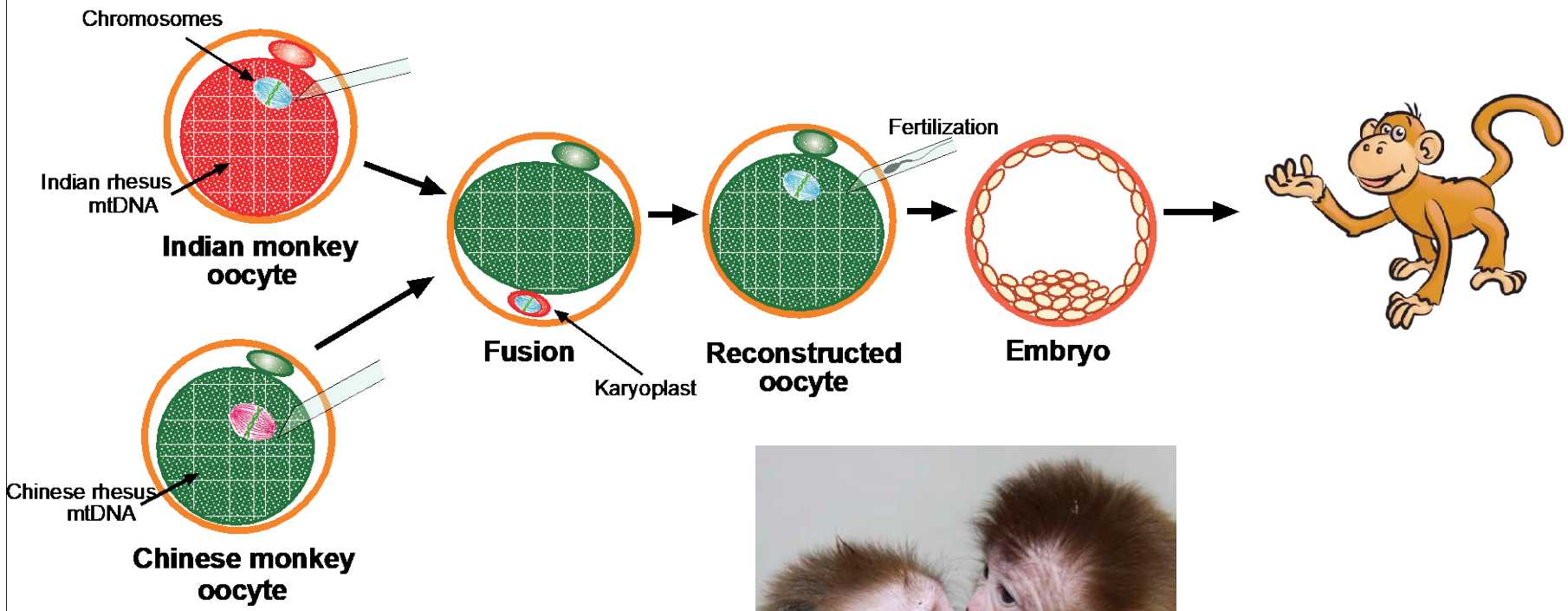
OHSU

Vol 461|17 September 2009|doi:10.1038/nature08368 nature

ARTICLES

Mitochondrial gene replacement in primate offspring and embryonic stem cells

Masahito Tachibana[1], Michelle Sparman[1], Hathaitip Sritanaudomchai[1], Hong Ma[1], Lisa Clepper[1], Joy Woodward[1], Ying Li[1], Cathy Ramsey[1], Olena Kolotushkina[1] & Shoukhrat Mitalipov[1,2,3]



Tachibana et al., Nature, 2009

Mito & Tracker

OHSU

Germline transmission of donor mtDNA to F2 offspring

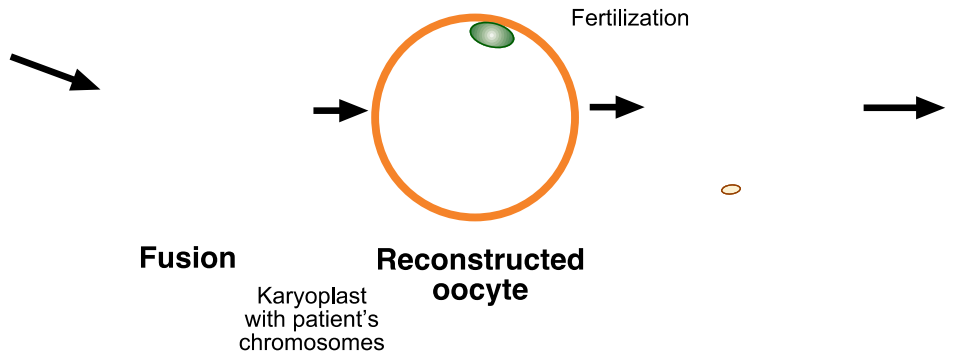
Van Dyken et al., unpublished

ARTICLE

doi:10.1038/nature11647

Towards germline gene therapy of inherited mitochondrial diseases

Masahito Tachibana[1], Paula Amato[2], Michelle Sparman[1], Joy Woodward[1], Dario Melguizo Sanchis[1], Hong Ma[1], Nuria Marti Gutierrez[1], Rebecca Tippner-Hedges[1], Eunju Kang[1], Hyo-Sang Lee[1], Cathy Ramsey[1], Keith Masterson[2], David Battaglia[2], David Lee[2], Diana Wu[2], Jeffrey Jensen[1,3], Phillip Patton[2], Sumita Gokhale[4], Richard Stouffer[1,2] & Shoukhrat Mitalipov[1,2]



Tachibana et al., Nature, 2013

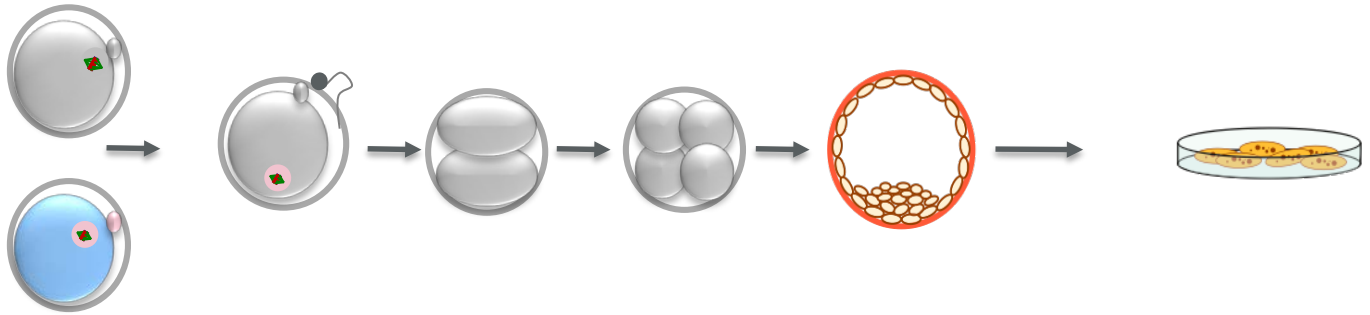
OHSU

LETTER

doi:10.1038/nature20592

Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations

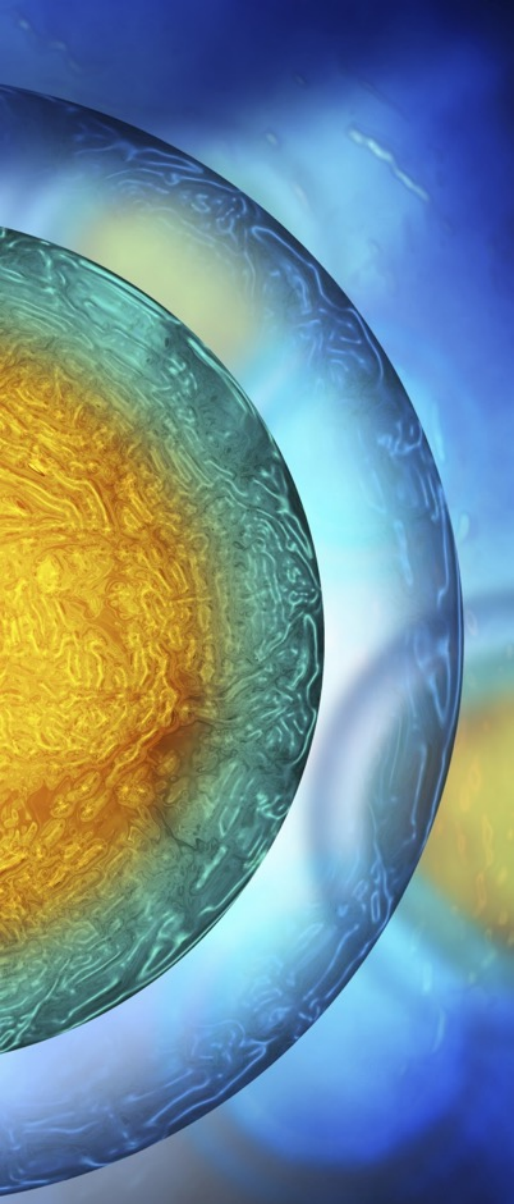
Eunju Kang[1,2]†, Jun Wu[3], Nuria Marti Gutierrez[1,2], Amy Koski[1,2], Rebecca Tippner-Hedges[1,2], Karen Agaronyan[4], Aida Platero-Luengo[3], Paloma Martinez-Redondo[3], Hong Ma[1,2], Yeonmi Lee[1,2]†, Tomonari Hayama[1,2], Crystal Van Dyken[1,2], Xinjian Wang[5], Shiyu Luo[5], Riffat Ahmed[1,2], Ying Li[1,2], Dongmei Ji[1,6], Refik Kayali[7], Cengiz Cinnioglu[7], Susan Olson[8], Jeffrey Jensen[9], David Battaglia[9], David Lee[9], Diana Wu[9], Taosheng Huang[5], Don P. Wolf[1,2], Dmitry Temiakov[4], Juan Carlos Izpisua Belmonte[3], Paula Amato[9] & Shoukhrat Mitalipov[1,2,9,10,11]



OHSU

Reversal Back to Maternal mtDNA

Wolf et al., EMBO J. 2017



Mitochondrial Replacement Therapy

- Replacement of entire mt genome
- Applicable to any mtDNA mutation type
- Preclinical animal studies demonstrate safety and efficacy
- Some ESCs (15%) demonstrated loss of donor mtDNA and reversal to the maternal haplotype
- Haplotype-matching may be required
- Clinical trials ongoing in the UK; preclinical studies in U.S.
- Several births reported worldwide

OHSU

ARTICLE

doi:10.1038/nature23305

Correction of a pathogenic gene mutation in human embryos

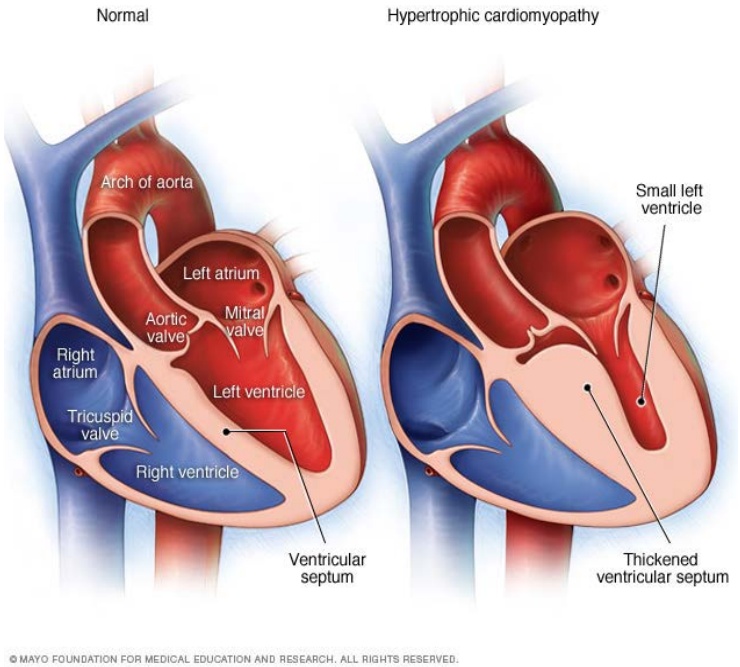
Hong Ma[1]*, Nuria Marti-Gutierrez[1]*, Sang-Wook Park[2]*, Jun Wu[3]*, Yeonmi Lee[1], Keiichiro Suzuki[3], Amy Koski[1], Dongmei Ji[1], Tomonari Hayama[1], Riffat Ahmed[1], Hayley Darby[1], Crystal Van Dyken[1], Ying Li[1], Eunju Kang[1], A.-Reum Park[2], Daesik Kim[4], Sang-Tae Kim[2], Jianhui Gong[5,6,7,8], Ying Gu[5,6,7], Xun Xu[5,6,7], David Battaglia[1,9], Sacha A. Krieg[9], David M. Lee[9], Diana H. Wu[9], Don P. Wolf[1], Stephen B. Heitner[10], Juan Carlos Izpisua Belmonte[3]§, Paula Amato[1,9]§, Jin-Soo Kim[2,4]§, Sanjiv Kaul[10]§ & Shoukhrat Mitalipov[1,10]§

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.

OHSU

MYBPC3 Mutation Causes Hypertrophic Cardiomyopathy

- Late onset, autosomal dominant disease
- Causes hypertrophic cardiomyopathy (HCM); prevalence in general population is 1:500
- Deletions in MYBPC3 account for ~35% of all cases of HCM



Patient

- Male with Hypertrophic Cardiomyopathy
- MYBPC3 mutation (4bp deletion), c.1420_1423 GAGT deletion
- Heterozygous

Wild-type allele: cgggtggagtt**gagt**gtgaagtatcggagga
Mutant allele : cgggtggagttt----gtgaagtatcggagga

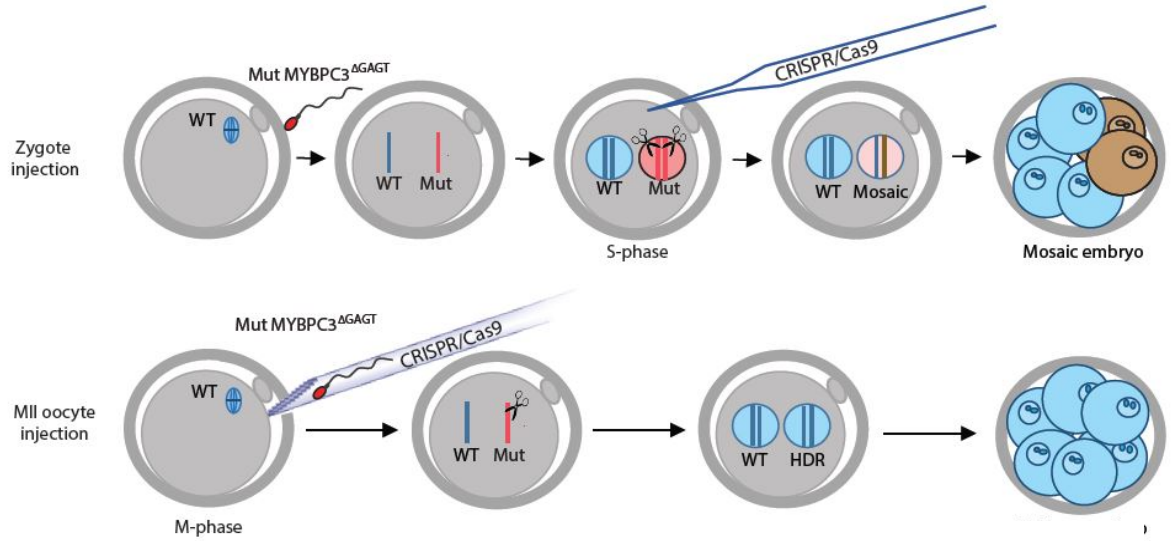
CRISPR/Cas9

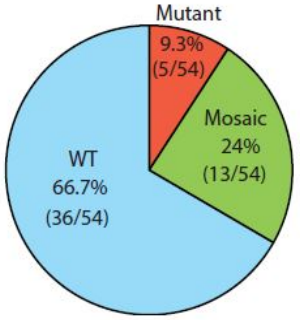
- **Cas9 nuclease**
- **Guide RNA:** 5′-ggtggagtttgtgaagtat-3′
- **DNA Template (190bp)**

agatggcctcaggggagccaaccctcatgctcaccctgcctggacagagccccctgtgctcatc
acgcgccccttggaggaccagctggtgatggtggggcagcgggtggagttt**gagt**g**c**ga**g**gtat
cggaggaggggcgcaagtcaaatggtgagttccagaagcacggggcatgggtgttgggggc

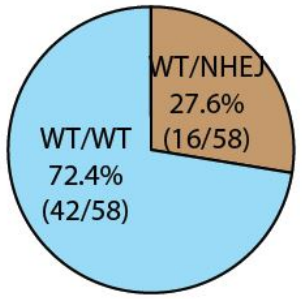
Bold: Mutation, Blue: PAM sequence, Red: marker SNPs

DNA Repair Outcomes and Mosaicism





S-phase injected embryos

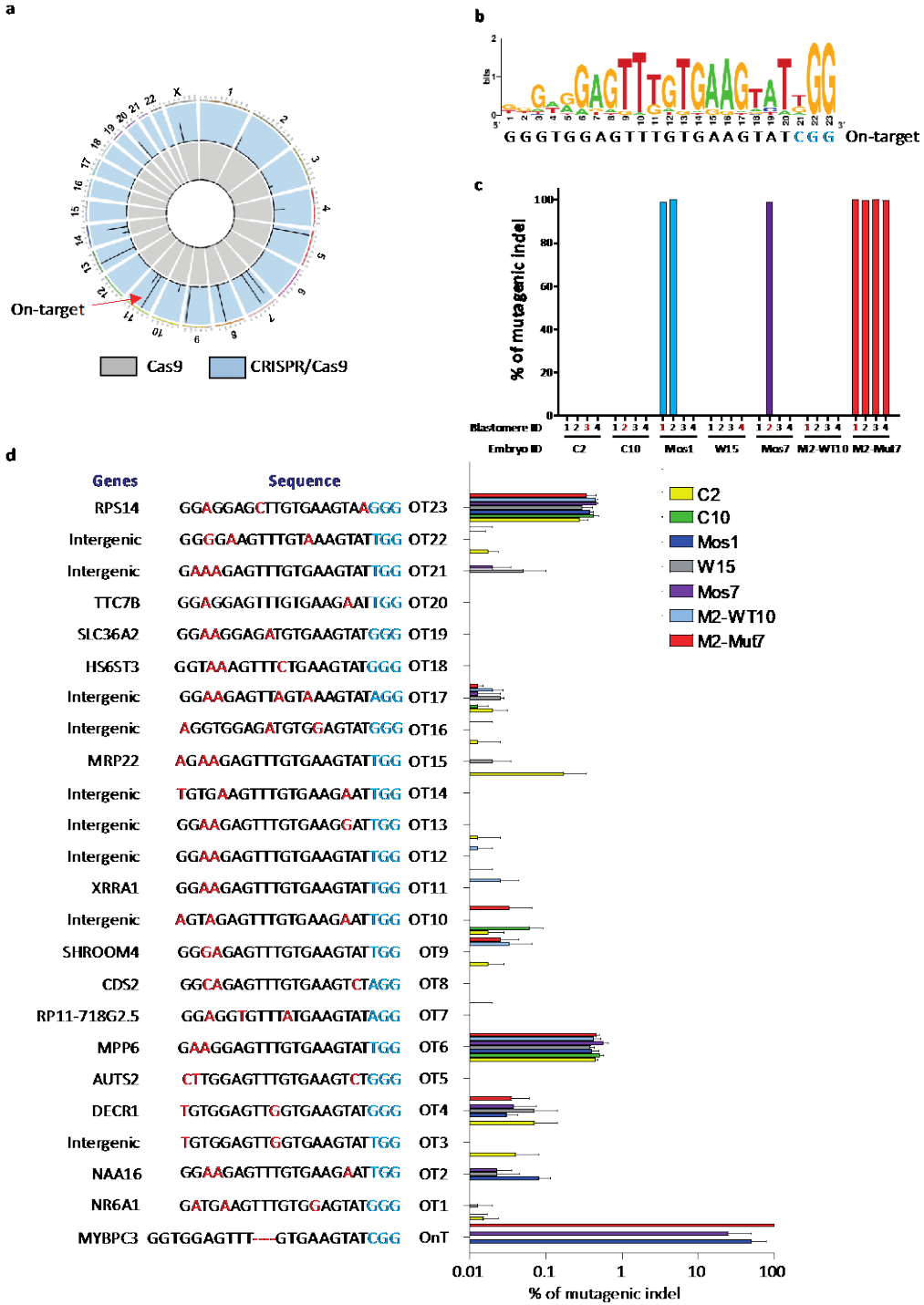


M-phase injected embryos

Ma et al., Nature 2017

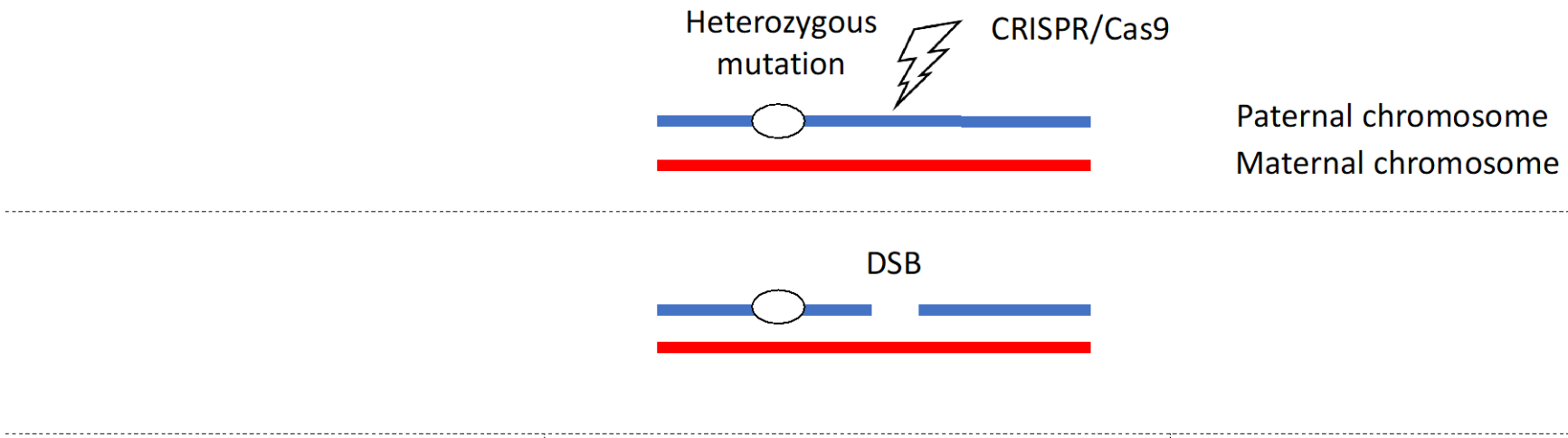
OHSU

Off-Target Effects



Ma et al., Nature 2017

GENE CONVERSION
heterozygous mutations



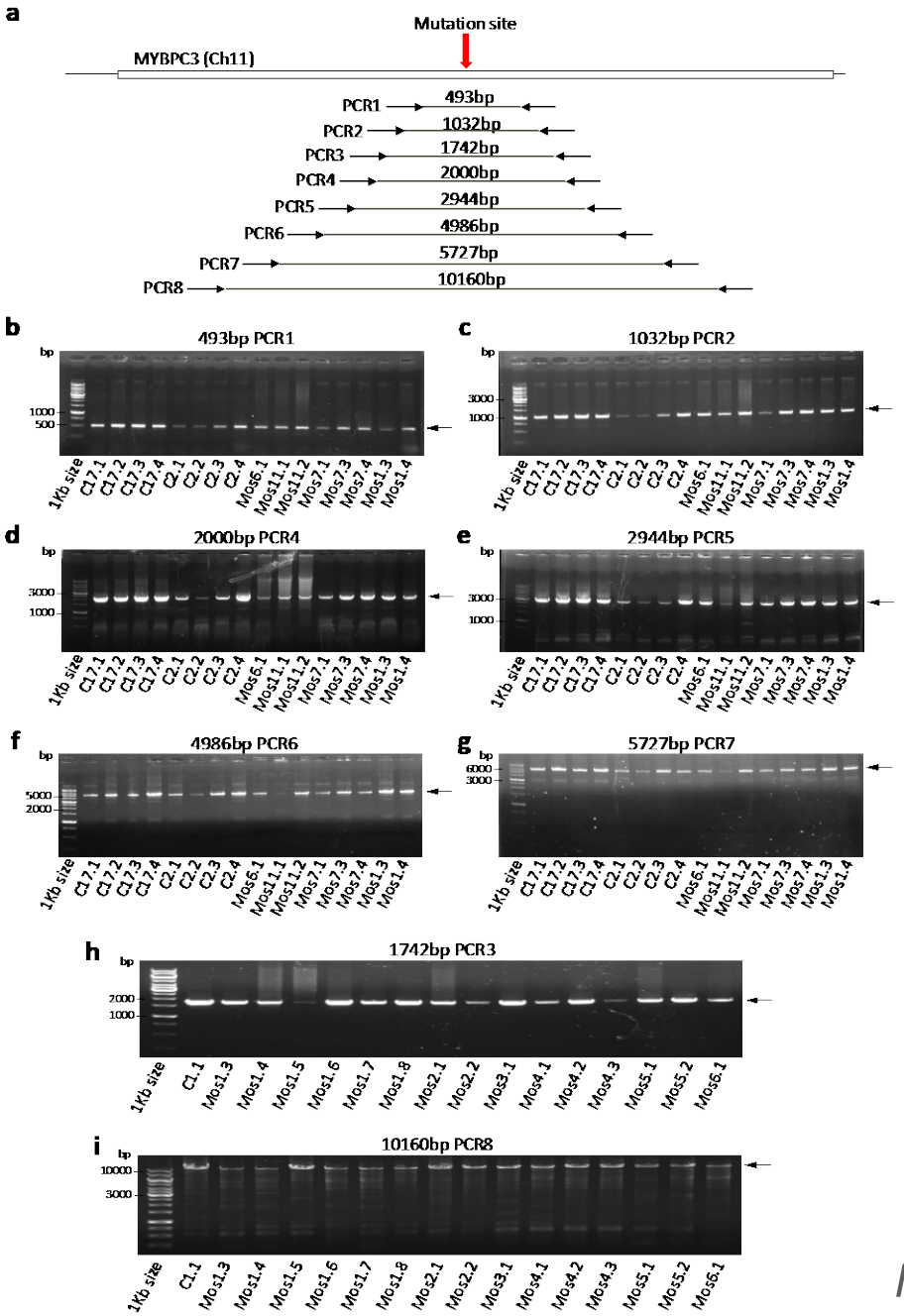
NHEJ (36%)

Interallelic Gene Conversion (64%)

HDR via external template (0%)

Ma et al., Nature 2017, 2018

OHSU



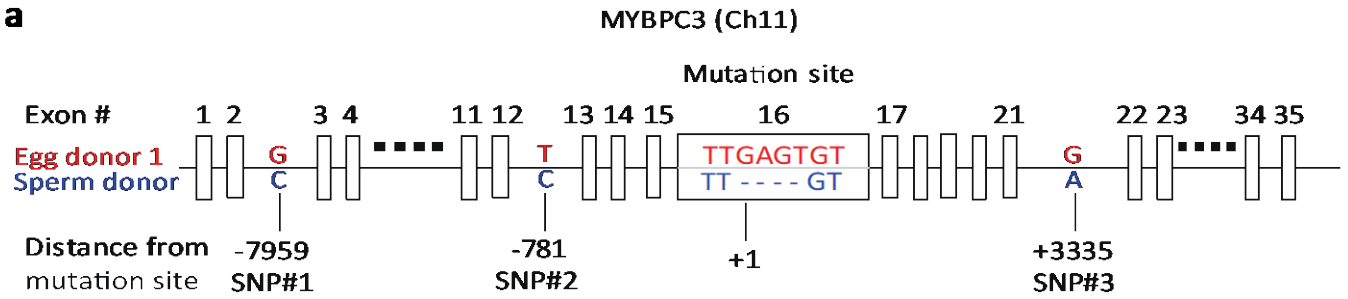
Ma et al., Nature 2018

BRIEF COMMUNICATIONS ARISING

Ma et al. reply

REPLYING TO D. Egli et al. *Nature* **560**, https://doi.org/10.1038/s41586-018-0379-5 (2018); F. Adikusuma et al. *Nature* **560**, https://doi.org/10.1038/s41586-018-0380-z (2018)

a



b

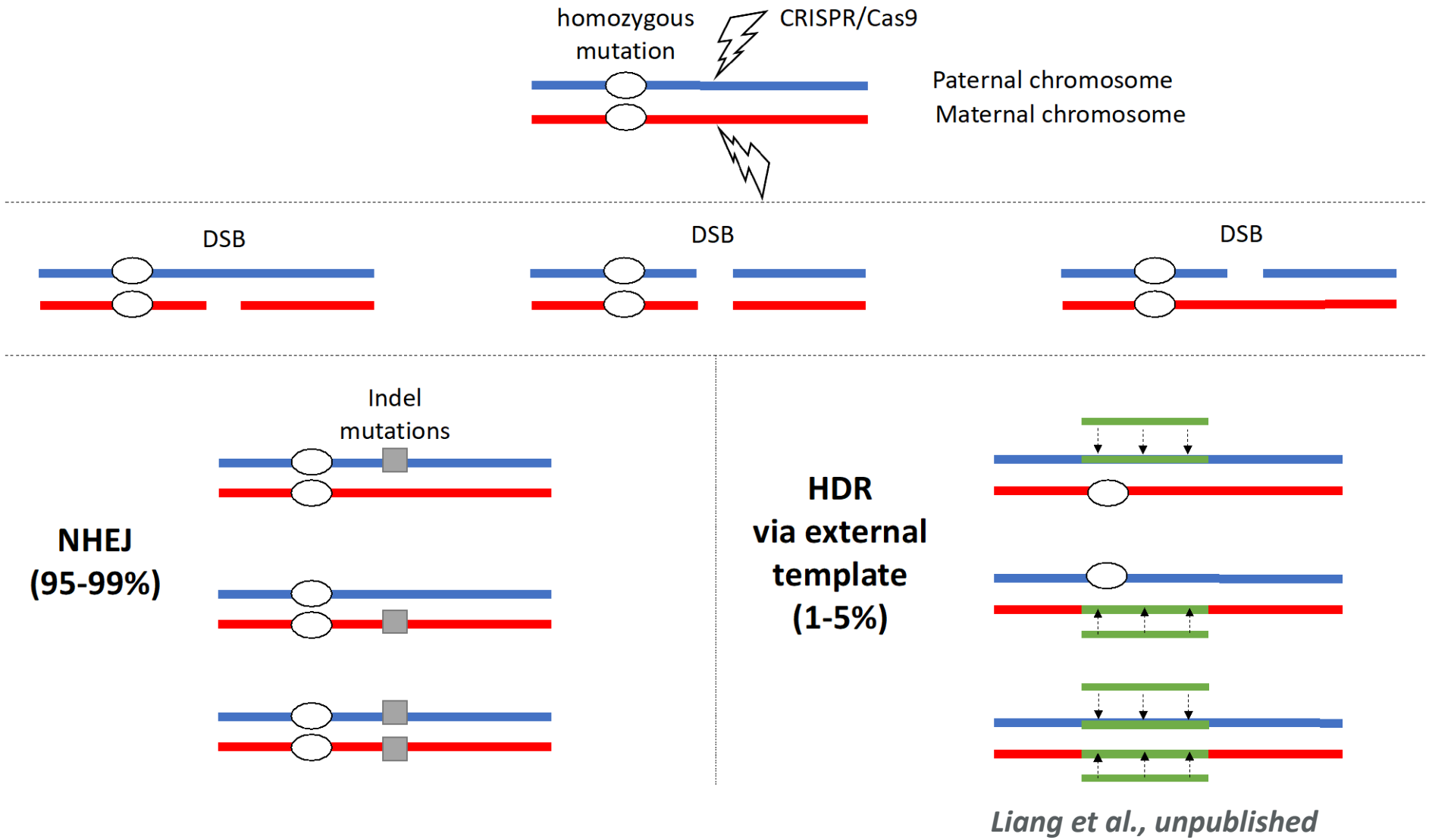
Sample	MYBPC3 genotype	SNP #1	SNP #2	SNP #3
Egg donor 1	WT/WT	G	T	G
Sperm donor	WT/Mut	C	C	A
WT 4.1	WT/WT	G/C	T/C	G/A
Mos 2.3	WT/NHEJ	G/C	T/C	G/A
Mos 3.2	WT/NHEJ	G/C	T/C	G/A
Mos 2.1	WT/HDR	G	T	G
Mos 3.1	WT/HDR	G	T	G
Mos 2.2	WT/HDR	G/C	T	G/A

Conversion tract

Ma et al., Nature 2018

OHSU

Molecular Mechanisms of DNA Repair
homozygous mutations



Liang et al., unpublished

OHSU

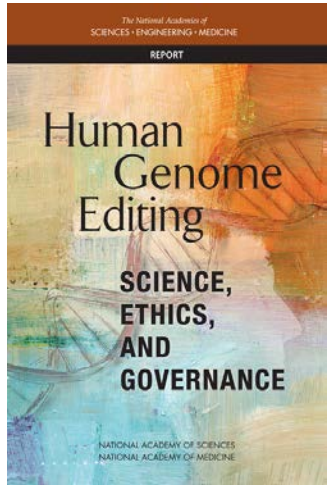
Germline Gene-Editing Summary

- ❑ High targeting efficacy
- ❑ High efficiency of repair by gene conversion
- ❑ Results have been independently replicated
- ❑ Applicable to heterozygous mutations only
- ❑ Complements conventional PGD by rescuing mutant embryos
- ❑ Advantages over conventional treatments including somatic gene therapy (more cost-effective)
- ❑ Efficiency and safety must be improved and ethical issues addressed before moving to clinical trials

OHSU

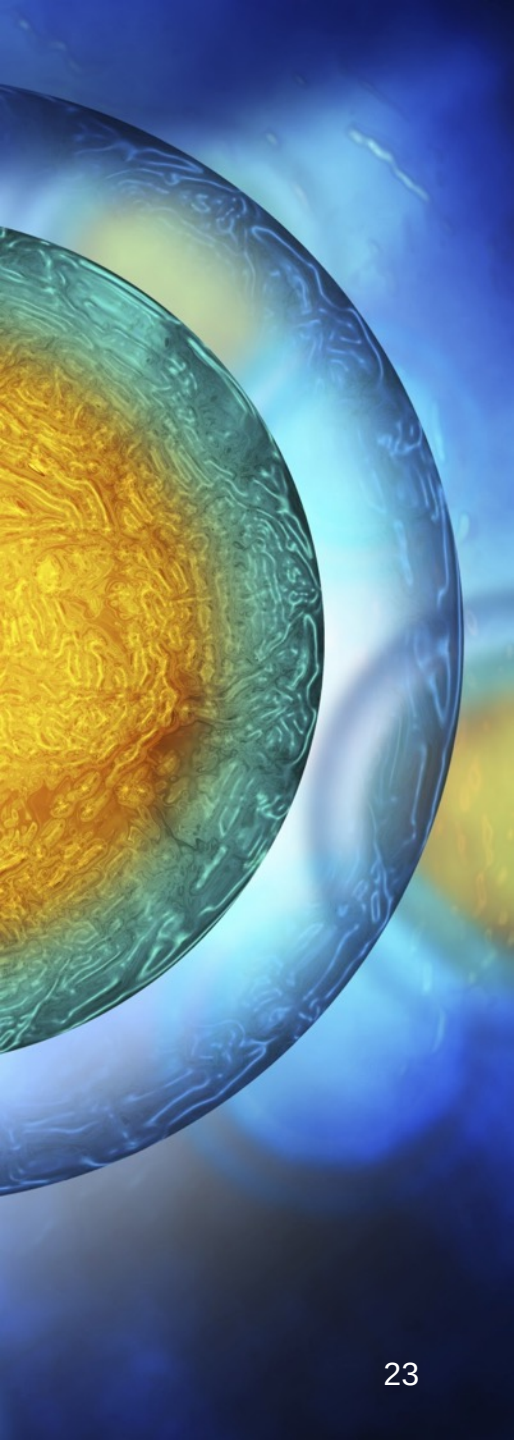
Human Genome Editing

Science, Ethics, and Governance



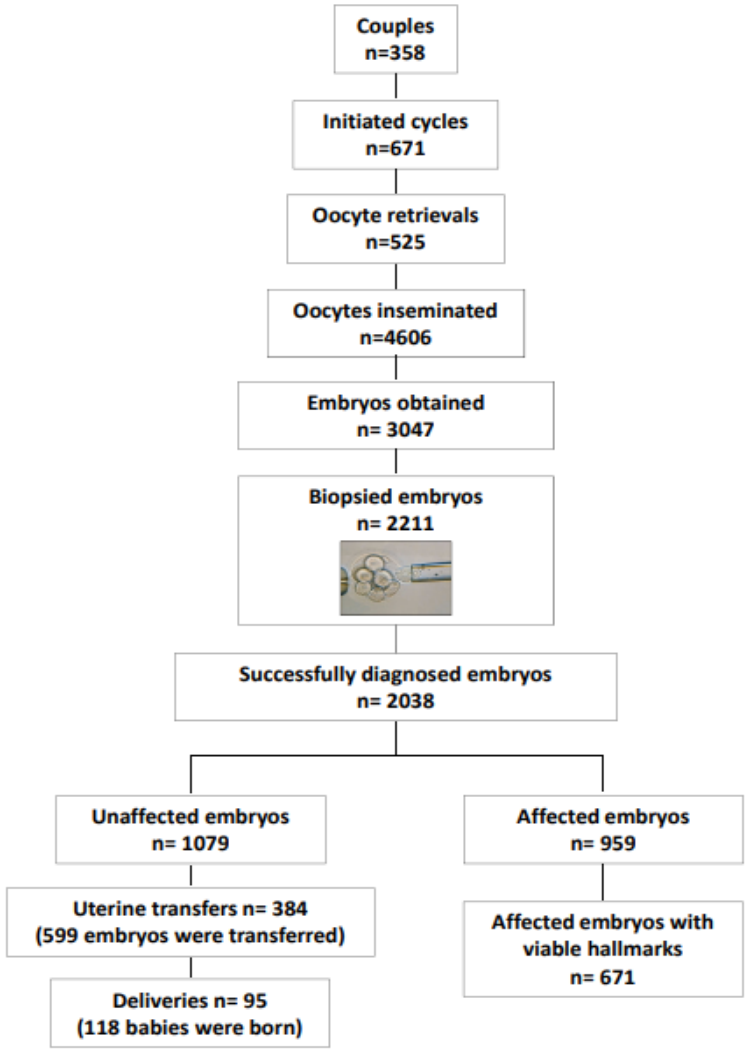
- Absence of reasonable alternatives
- Restriction to preventing a serious disease or condition
- Restriction to editing genes that have been convincingly demonstrated to cause or to strongly predispose to the disease or condition
- Restriction to converting such genes to versions that are prevalent 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
- Ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of research participants
- Comprehensive plans for long-term, multigenerational follow-up while still respecting personal autonomy
- 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
- Reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition

OHSU

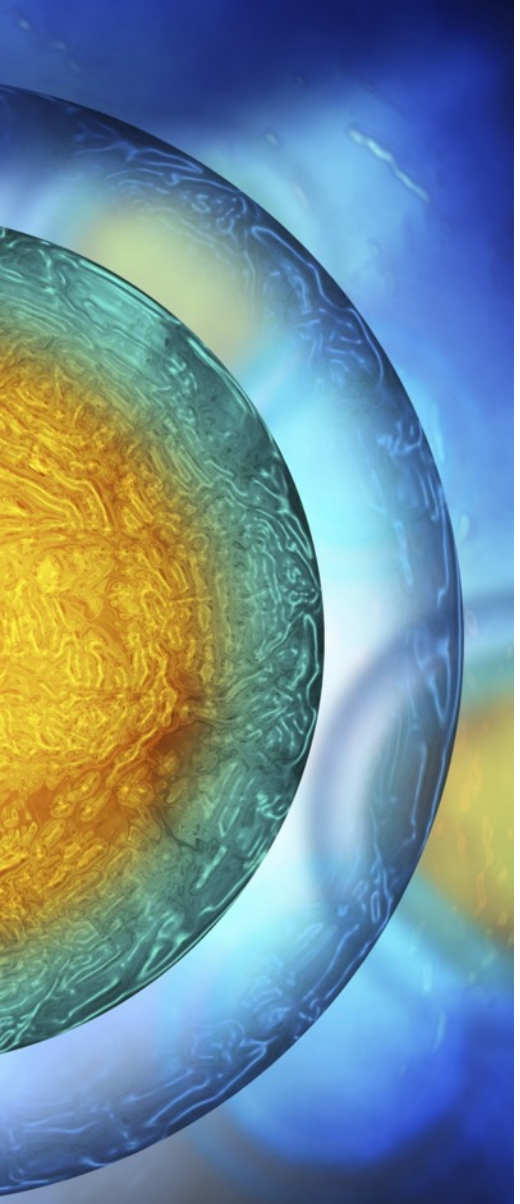


Could Failure in Preimplantation Genetic Diagnosis Justify Editing the Human Embryo Genome?

Steffann et al, Cell Stem Cell, 2018



OHSU



Future Directions

- MRT clinical trials
- MRT for other indications eg age-related infertility
- Germline gene editing targeting other gene mutations eg BRCA
- Insights into novel DNA repair mechanisms in human embryo → somatic gene therapy applications for chronic degenerative diseases of aging and cancer?

OHSU

Center for Embryonic Cell and Gene Therapy

Hong Ma
Nuria-Marti Gutierrez
Dan Liang
Amy Koski
Tomonari Hayama
Ying Li
Riffat Ahmed
Crystal Van Dyken
Hayley Darby
Don Wolf
Masahito Tachibana
Eunju Kang
Yeonmi Lee
Dongmei Ji

The Salk Institute

Juan Carlos Belmonte
Jun Wu
Keiichiro Suzuki

REI-OBGYN

Paula Amato
Diana Wu
David Lee
Sacha Krieg
David Battaglia
Tom O'Leary

KCVI

Sanjiv Kaul
Stephen Heitner

BGI

Jianhui Gong
Ying Gu
Xun Xu

Center for Genome Engineering Institute for Basic Science

Jin-Soo Kim
Sang-Wook Park
A.-Reum Park
Daesik Kim
Sang-Tae Kim

Cincinnati Children's Hospital

Taosheng Huang
Xinjian Wang
Shiyu Luo

Rowan University

Dmitry Temiakov
Karen Agaronyan

Thank You