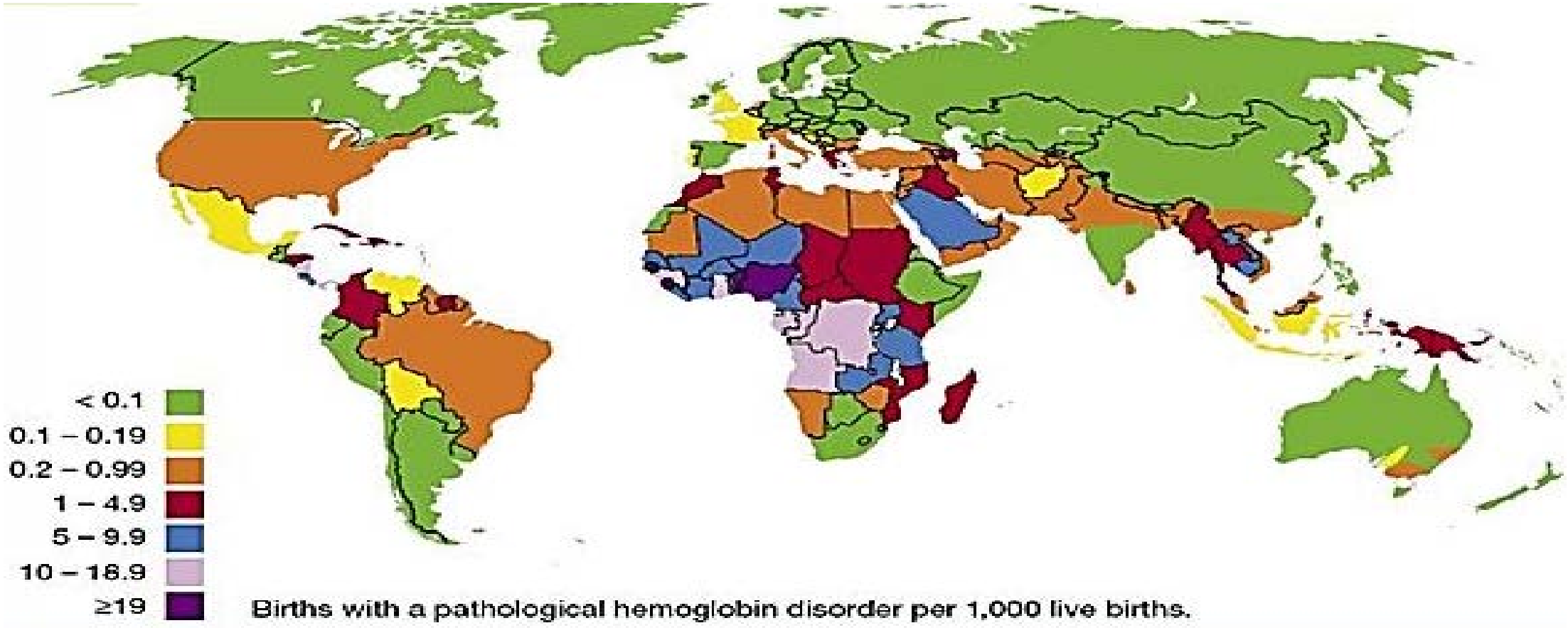
Fix β-thalassemia in Human Embryo

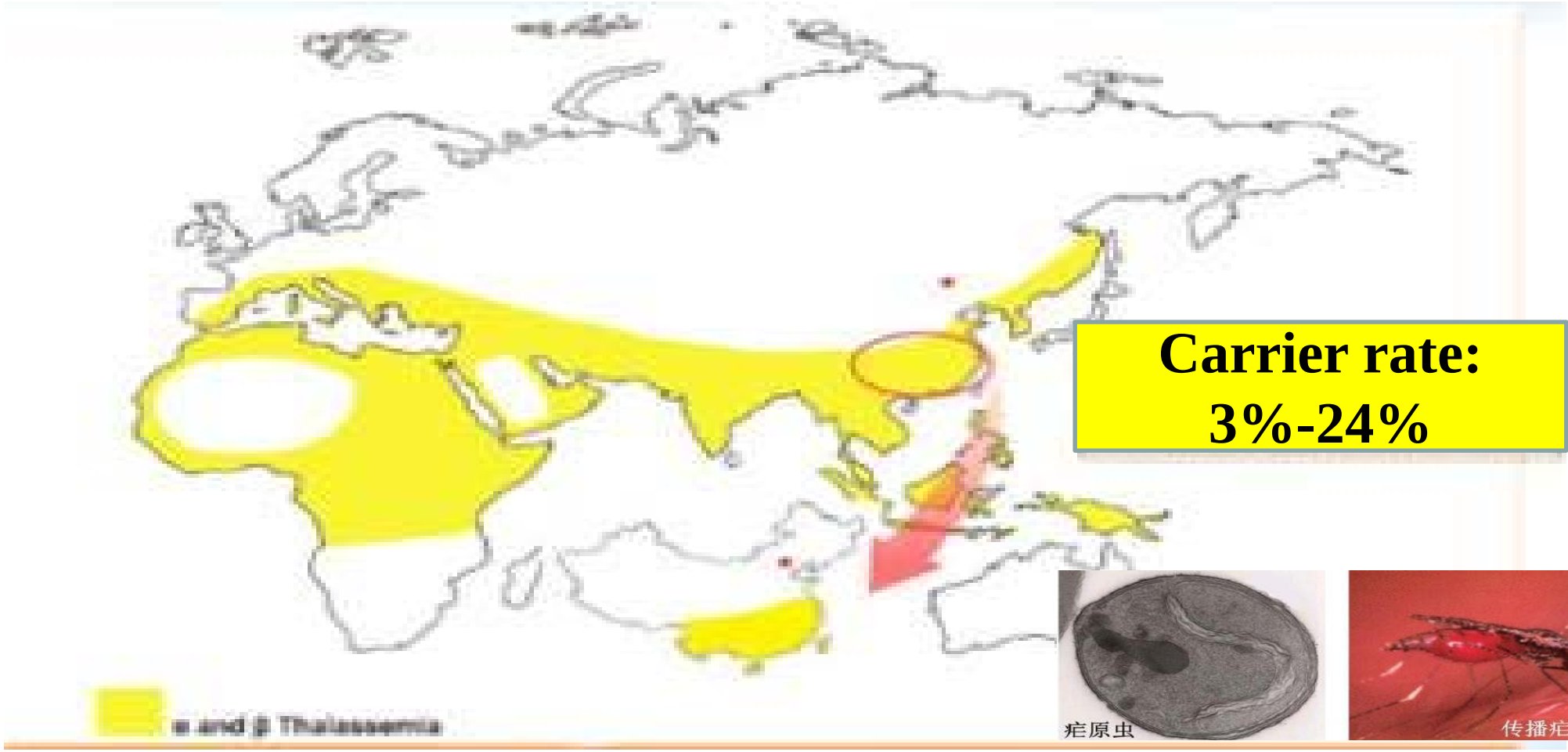
Junjiu Huang
School of Life Sciences,
Sun Yat-sen University

Thalassemia

Thalassemia is an inherited blood disorder characterized by abnormal hemoglobin production. It was found in the Mediterranean area in 1925.

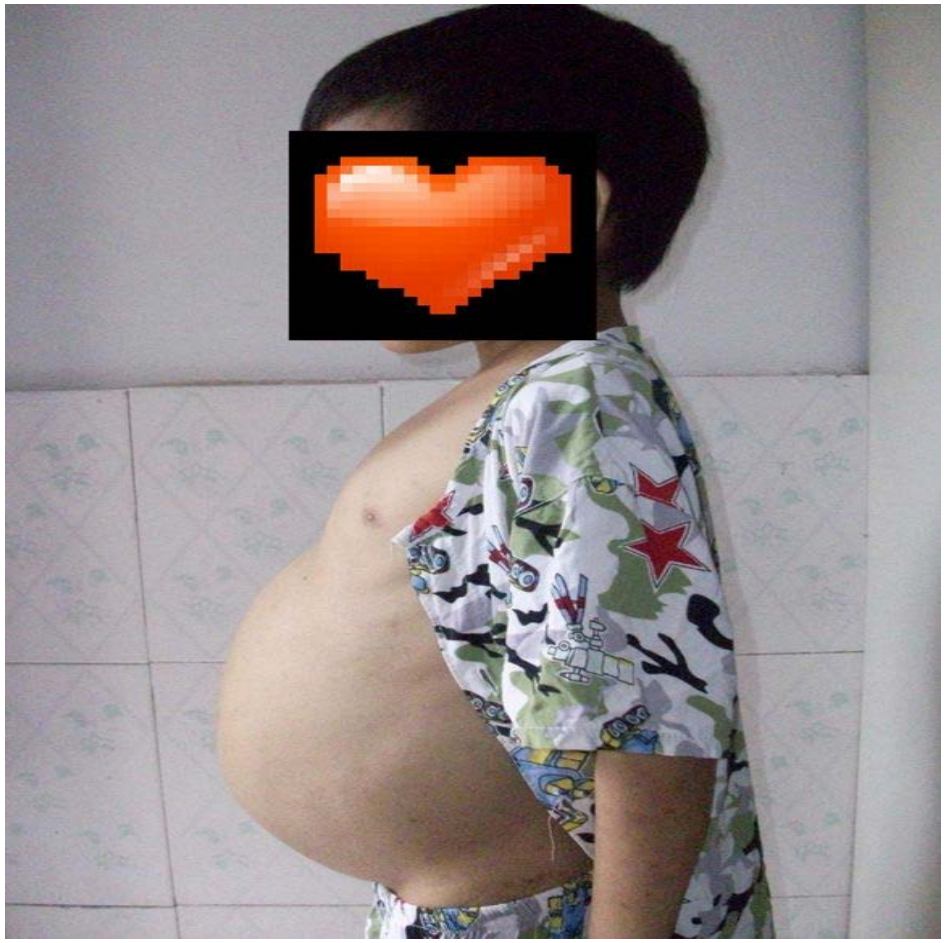


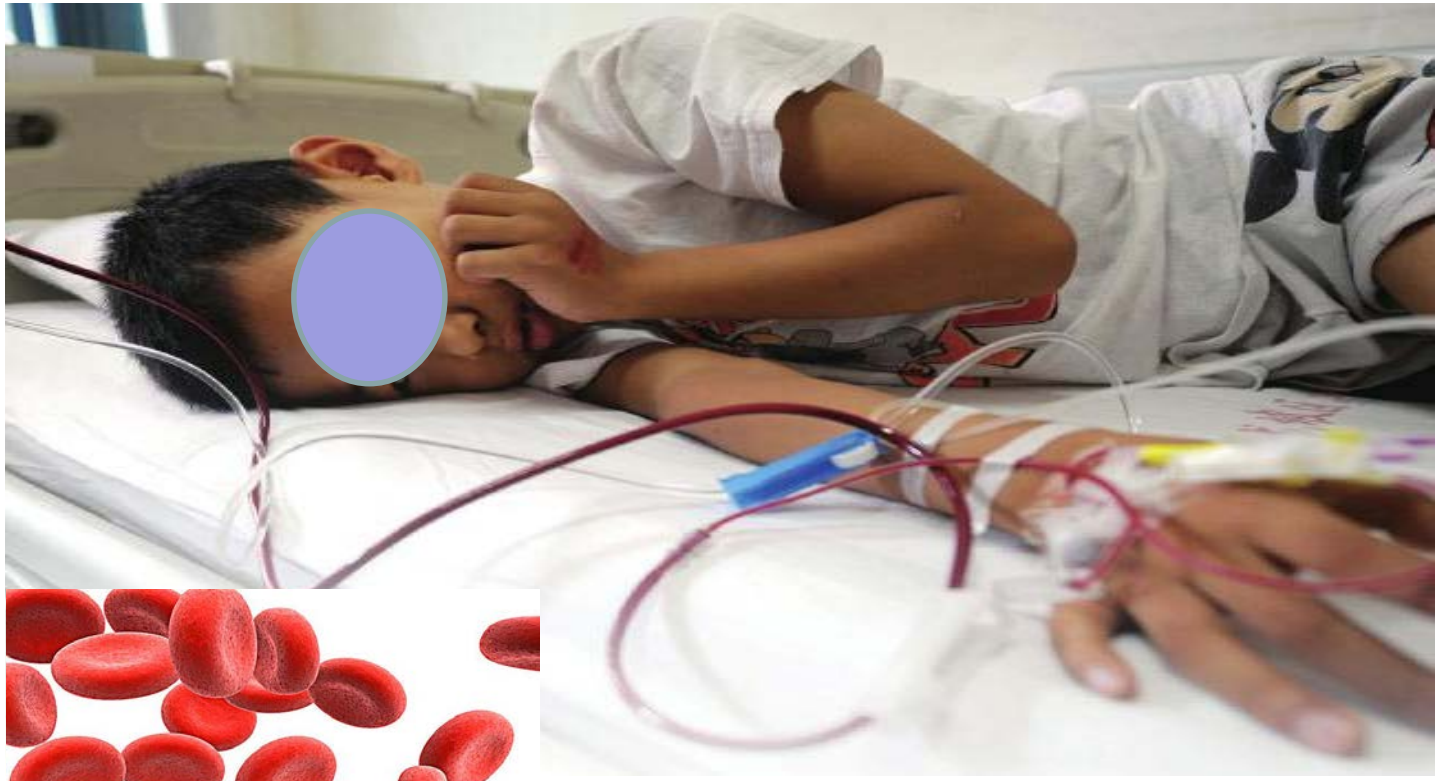
Thalassemia: High incidence of hereditary diseases in the south of China



High incidence in Guangdong, Guangxi and Guizhou……

Clinical Symptoms

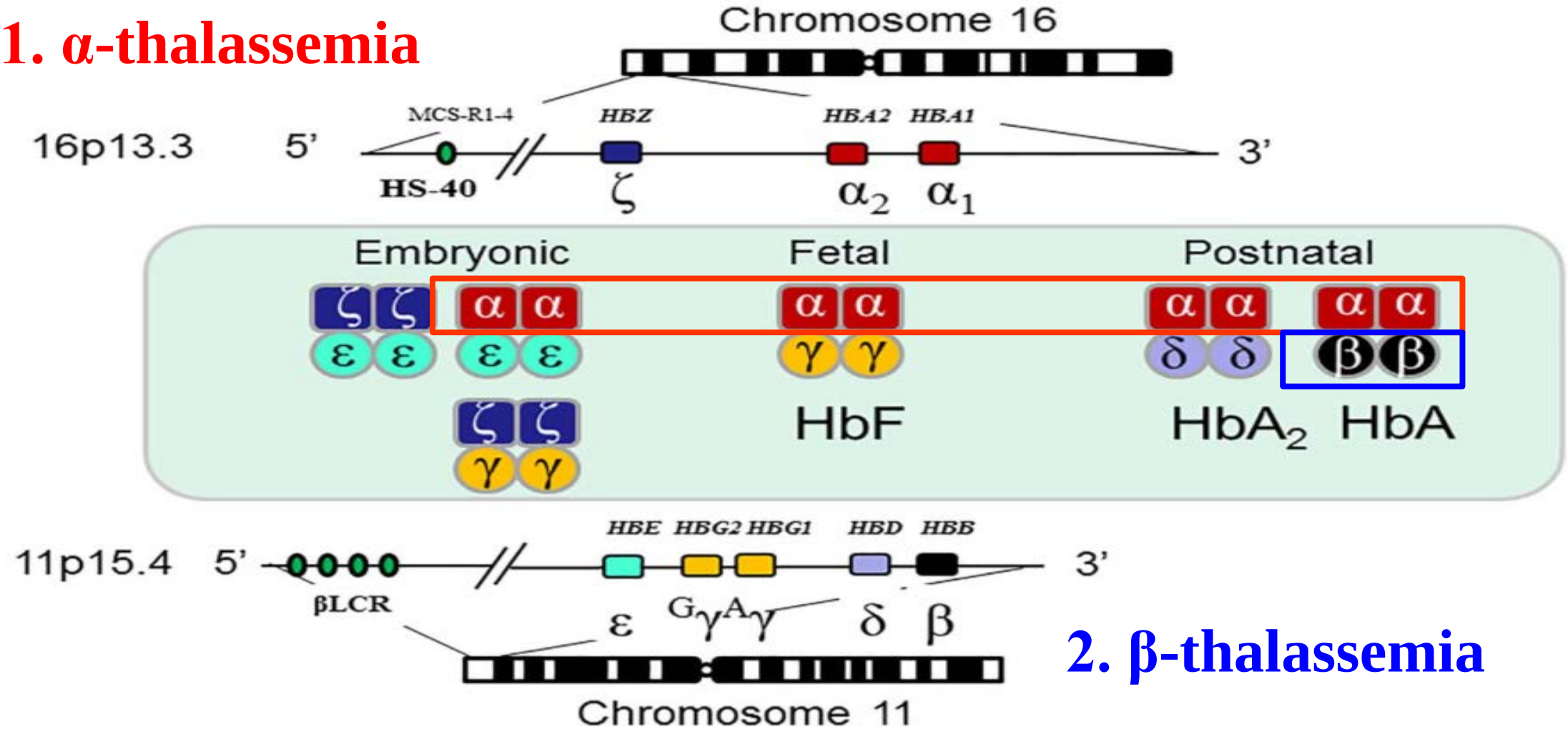




1. **Transfusion;**
2. **Allogeneic hematopoietic-cell transplantation.**

Genetic Mechanism of Thalassemia

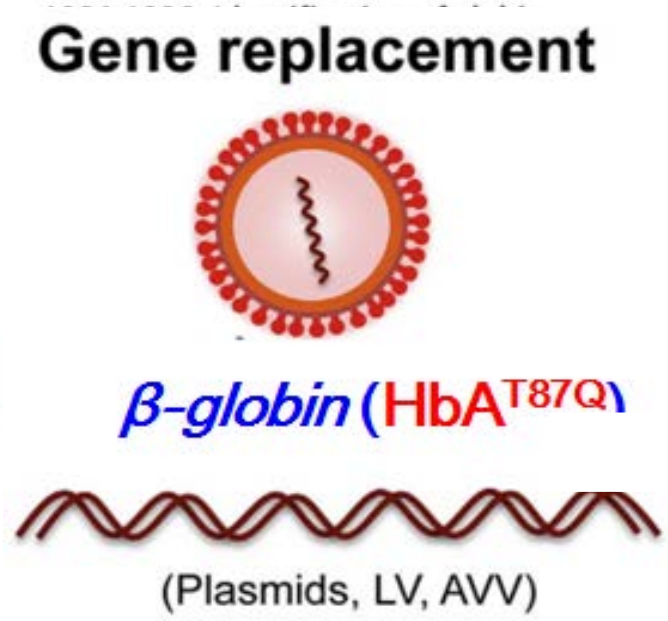
1. α-thalassemia



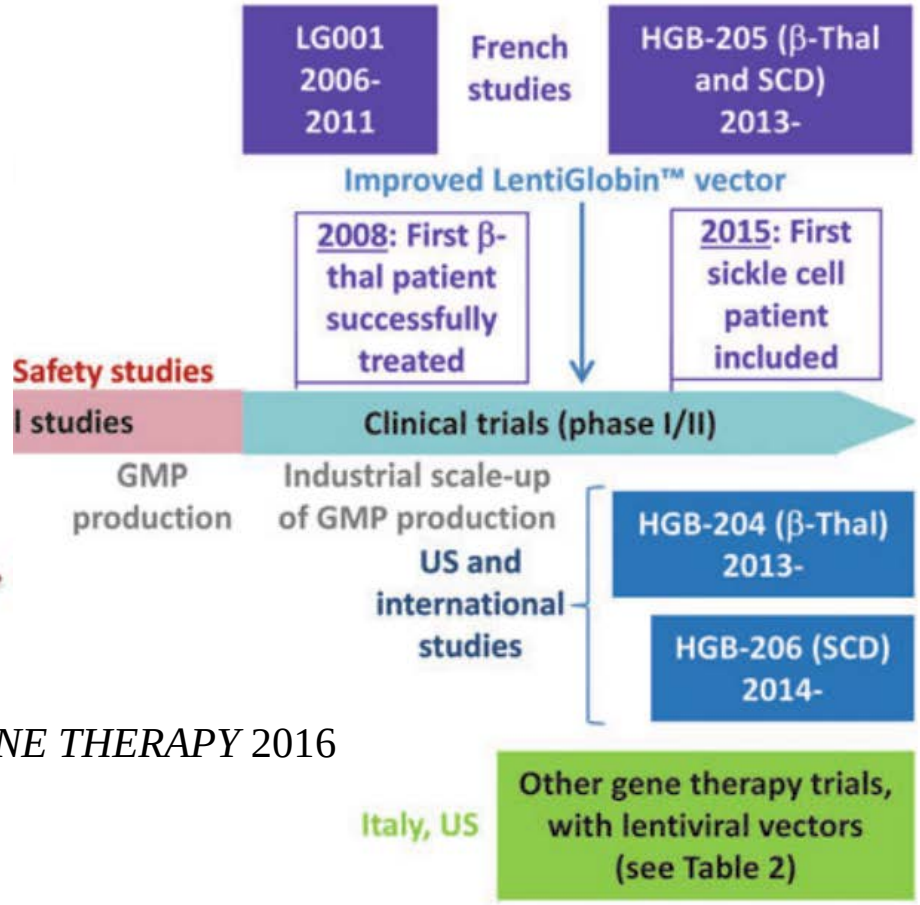
2. β-thalassemia

Cornelis L. Harteveld, Blood Cells, Molecules and Diseases 2017

Gene Therapy with LentiGlobin by Bluebird Bio



Emmanuel Payen et al. *HUMAN GENE THERAPY* 2016



2010

Vol 467|16 September 2010|doi:10.1038/nature09328

LETTERS

Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia

Marina Cavazzana-Calvo[1,2]*, Emmanuel Payen[3,4,5]*, Olivier Negre[3,4,5,6], Gary Wang[7], Kathleen Hehir[8], Floriane Fusil[3,4,5], Julian Down[8], Maria Denaro[8], Troy Brady[7], Karen Westerman[8,9], Resy Cavallesco[9], Beatrix Gillet-Legrand[6], Laure Caccavelli[1,2], Riccardo Sgarra[10], Leila Maouche-Chrétien[3,4], Françoise Bernaudin[11], Robert Girot[12], Ronald Dorazio[8], Geert-Jan Mulder[8], Axel Polack[8], Arthur Bank[13], Jean Soulier[5], Jérôme Larghero[5], Nabil Kabbara[5], Bruno Dalle[5], Bernard Gourmel[5], Gérard Socie[5], Stany Chrétien[3,4,9], Nathalie Cartier[14], Patrick Aubourg[14], Alain Fischer[1,2], Kenneth Cornetta[15], Frédéric Galacteros[16], Yves Beuzard[3,4,5], Eliane Gluckman[5], Frederick Bushman[7], Salima Hacein-Bey-Abina[1,2]* & Philippe Leboulch[3,4,9]*

2018

The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 APRIL 19, 2018 VOL. 378 NO. 16

Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia

A.A. Thompson, M.C. Walters, J. Kwiatkowski, J.E.J. Rasko, J.-A. Ribeil, S. Hongeng, E. Magrin, G.J. Schiller, E. Payen, M. Semeraro, D. Moshous, F. Lefrere, H. Puy, P. Bourget, A. Magnani, L. Caccavelli, J.-S. Diana, F. Suarez, F. Monpoux, V. Brousse, C. Poirot, C. Brouzes, J.-F. Meritet, C. Pondarré, Y. Beuzard, S. Chrétien, T. Lefebvre, D.T. Teachey, U. Anurathapan, P.J. Ho, C. von Kalle, M. Kletzel, E. Vichinsky, S. Soni, G. Veres, O. Negre, R.W. Ross, D. Davidson, A. Petrusich, L. Sandler, M. Asmal, O. Hermine, M. De Montalembert, S. Hacein-Bey-Abina, S. Blanche, P. Leboulch, and M. Cavazzana

A clinical approval for the gene therapy might come in 2019 ?!

Gene Editing

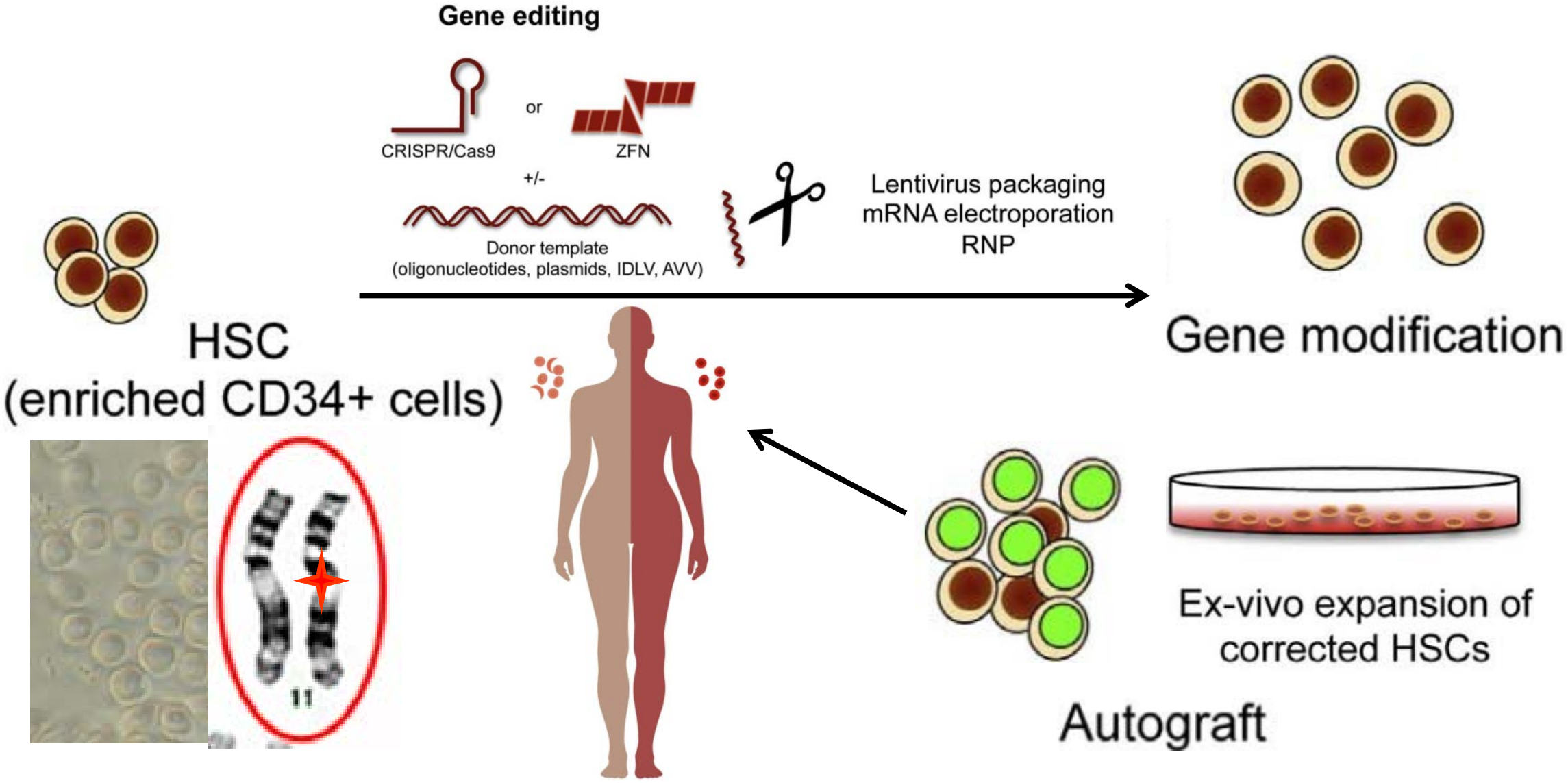
1. Science: Breakthrough of the Year 2012 （TALEN）

2. Science: Breakthrough of the Year 2013 （CRISPR/Cas9）
3. Nature: Ten people who mattered this year 2013（Feng Zhang）

4. Nature: Ten people who mattered this year 2015（Junjiu Huang）
5. Science: Breakthrough of the Year 2015（CRISPR/Cas9)
6. Nature: The science events that shaped 2015（CRISPR/Cas9)

7. Nature: Ten people who mattered this year 2017（David Liu）
8. Science: Breakthrough of the Year 2017（Pinpoint gene editing)
9. Nature: The science events that shaped 2017（Human embryo editing)

Treat β-thalassemia with Gene Editing in HSC



Cornelis L. Harteveld, Blood Cells, *Molecules and Diseases* 2017

Fix β-thalassemia in Somatic Cells

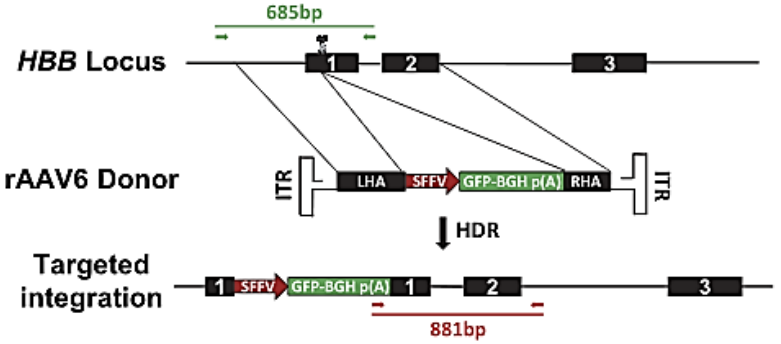
ARTICLE

2016：

doi:10.1038/nature20134

CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells

Daniel P. Dever[1]*, Rasmus O. Bak[1]*, Andreas Reinisch[2], Joab Camarena[1], Gabriel Washington[1], Carmencita E. Nicolas[1], Mara Pavel-Dinu[1], Nivi Saxena[1], Alec B. Wilkens[1], Sruthi Mantri[1], Nobuko Uchida[3]†, Ayal Hendel[1], Anupama Narla[4], Ravindra Majeti[2], Kenneth I. Weinberg[1] & Matthew H. Porteus[1]



Cell

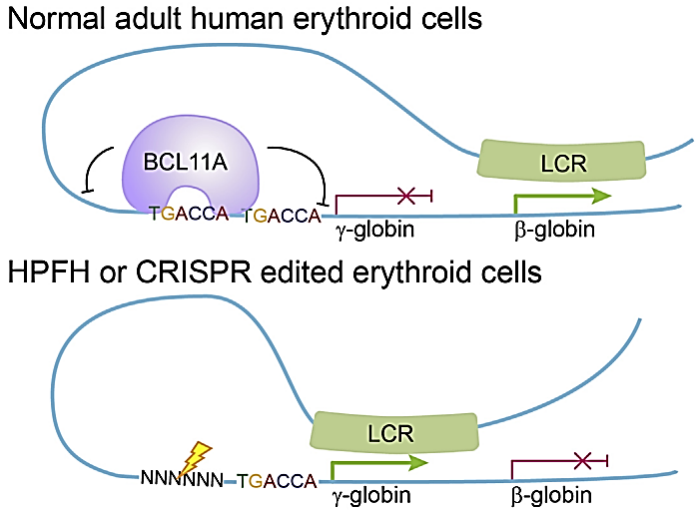
2018：

Article

Direct Promoter Repression by BCL11A Controls the Fetal to Adult Hemoglobin Switch

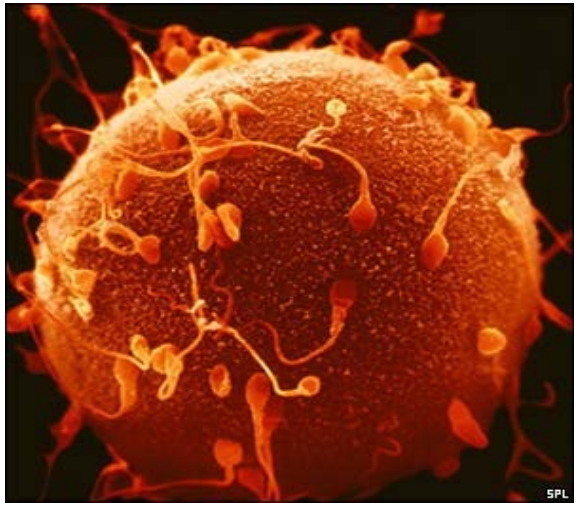
Nan Liu,[1,9] Victoria V. Hargreaves,[1,9] Qian Zhu,[2,9] Jesse V. Kurland,[3] Jiyoung Hong,[1] Woojin Kim,[1] Falak Sher,[1] Claudio Macias-Trevino,[1] Julia M. Rogers,[3,4] Ryo Kurita,[5] Yukio Nakamura,[5] Guo-Cheng Yuan,[2] Daniel E. Bauer,[1] Jian Xu,[6] Martha L. Bulyk,[3,4,7] and Stuart H. Orkin[1,8,10,*]

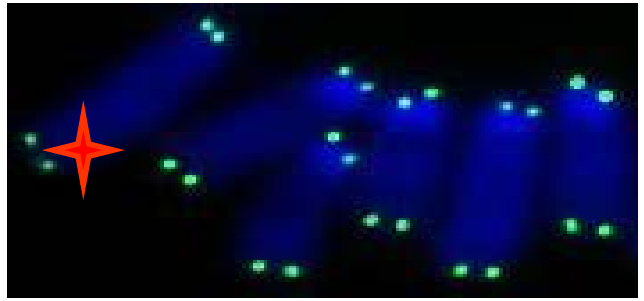
[1]Cancer and Blood Disorders Center, Dana Farber Cancer Institute and Boston Children's Hospital, Harvard Medical School, Boston, MA, USA



How to Get a Healthy Baby for Patients?

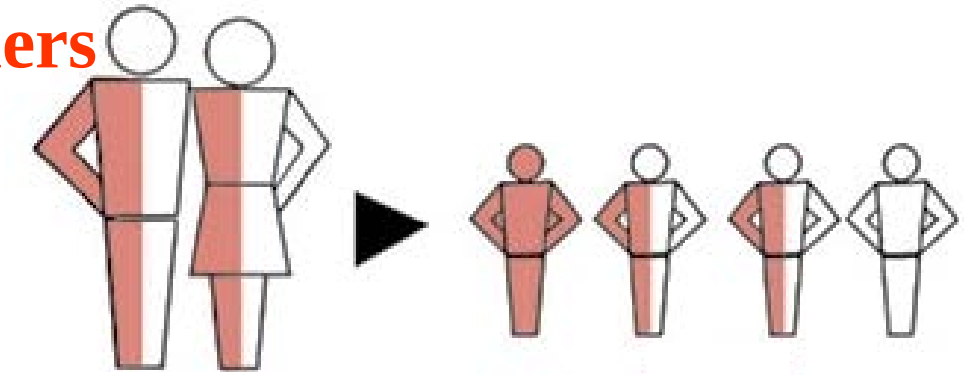
Inherited Mutations





A zygote develops to a complex human body, which is made of **60 trillion cells**.

Carriers



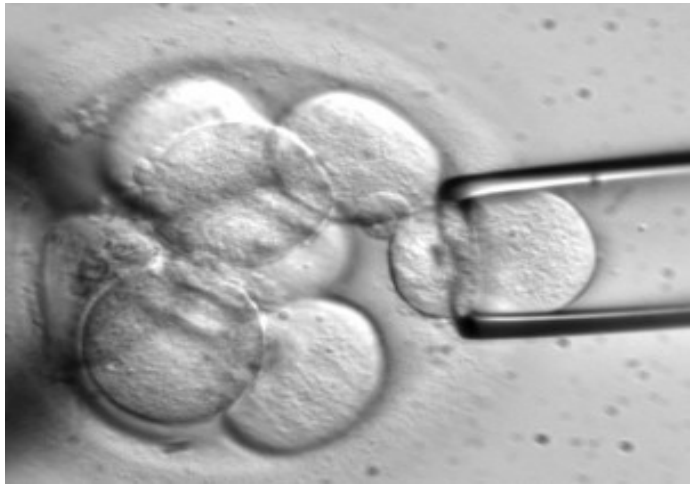
- **25% Healthy Offspring**
- **50% Carriers or Patients**
- **25% Patients**

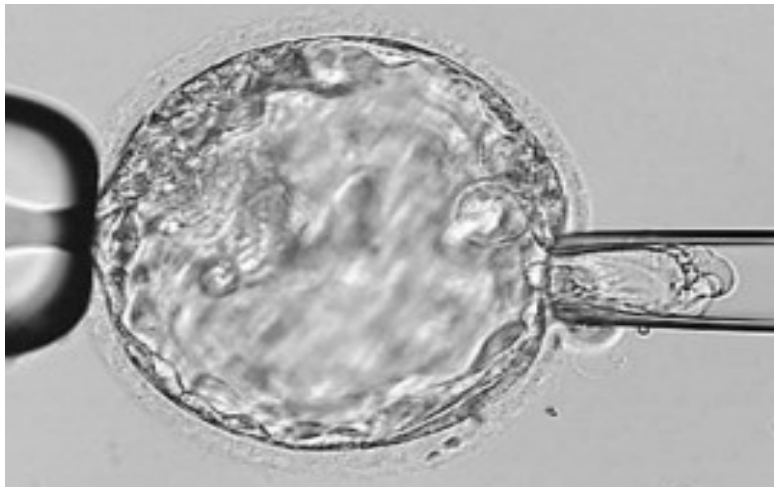
Third Generation Test-tube Baby Technology

Preimplantation Genetic Testing (PGT) : PGD and PGS

Using PGT to help β-thalassemia/DMD patients since 2000.

IVF Center in First Affiliated Hospital found in 1989.



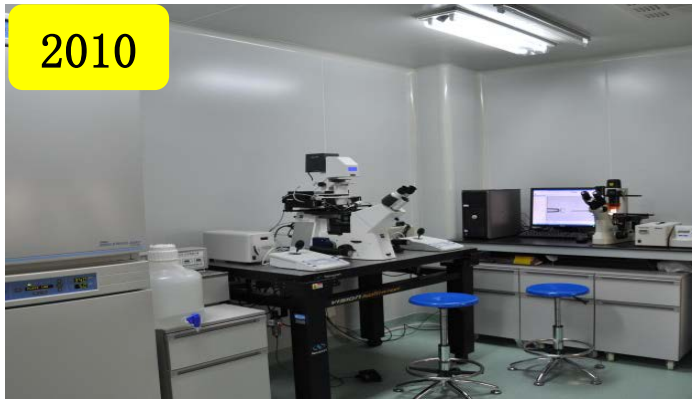


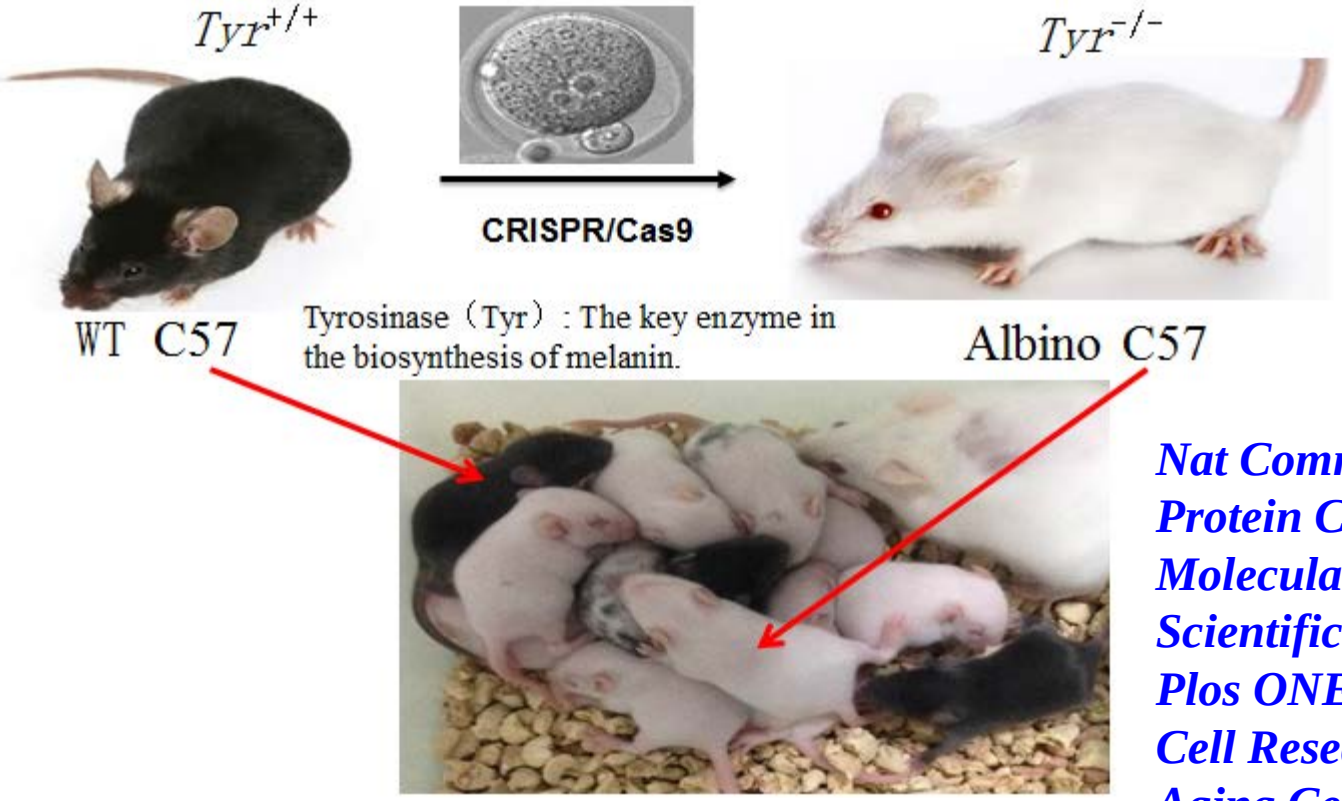
Screen Genes, Pick up Healthy Embryos!

Can We Repair or Block Inherited Mutations in Human Embryo ???

Model Mouse Research Center, Sun Yat-sen University

Gene knock out/in mice;
Conditional knock out mice





Nat Commun. 2018
Protein Cell 2017
Molecular Cell 2016
Scientific Reports 2016
Plos ONE 2015
Cell Research 2011
Aging Cell 2009
Stem Cells 2008

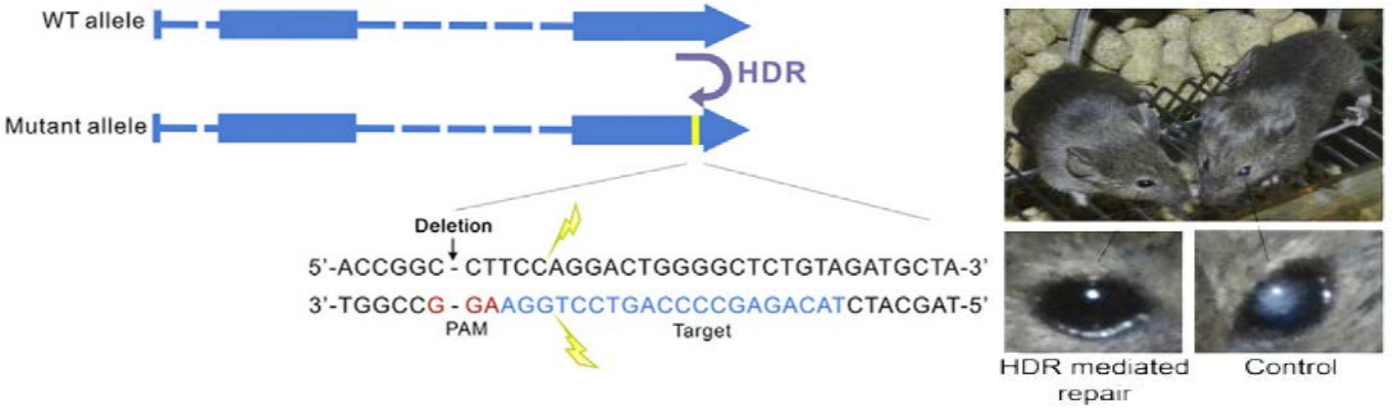
Correction of a Gene Mutation in Mouse Zygote

2013：

Cell Stem Cell
Brief Report

Correction of a Genetic Disease in Mouse via Use of CRISPR-Cas9

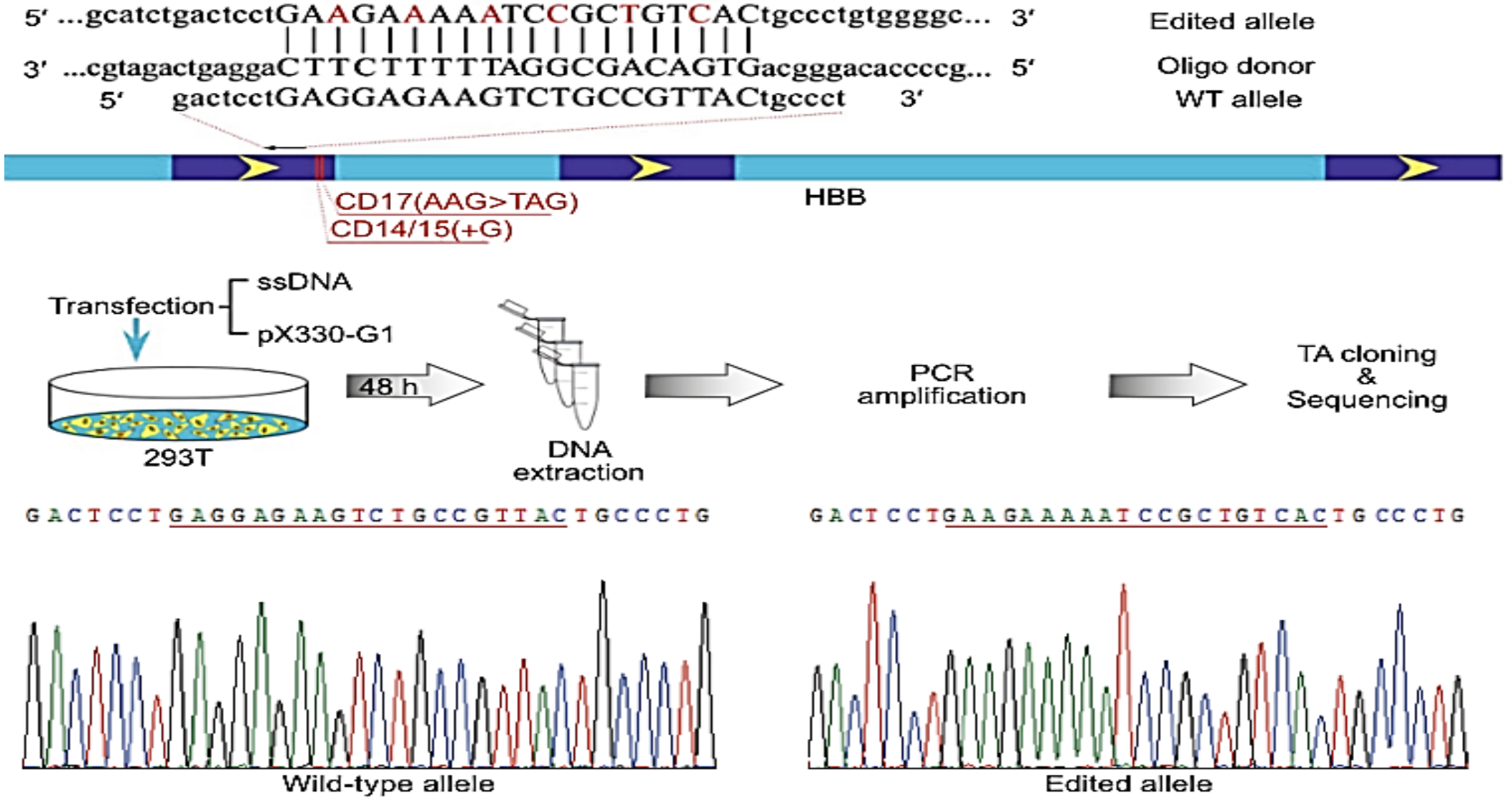
Yuxuan Wu,[1,7] Dan Liang,[1,2,7] Yinghua Wang,[1,2] Meizhu Bai,[1,3] Wei Tang,[4] Shiming Bao,[5] Zhiqiang Yan,[5] Dangsheng Li,[6] and Jinsong Li[1,3,*]
[1]Group of Epigenetic Reprogramming, State Key Laboratory of Cell Biology, Shanghai Key Laboratory of Molecular Andrology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, China



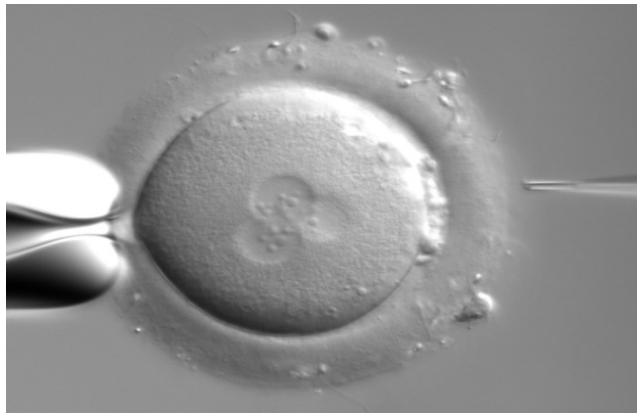
What Risks in Fixing β-thalassemia in Human Embryo with CRISPR/Cas9 ?

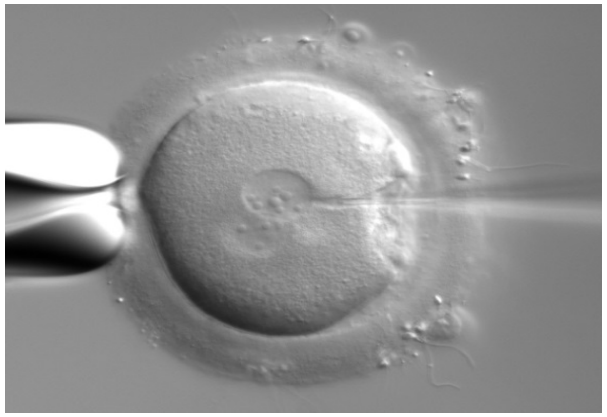
2014：

CRISPR/Cas9-mediated editing of *HBB* gene in human cells



CRISPR/Cas9-mediated editing of *HBB* gene in human tripronuclear zygotes





Unexpected repairs

Targeted editing of the *HBB* gene in human 3PN zygotes by intra-cytoplasmic injection

Group No.	Cas9/gRNA/ssDNA (ng/μL)	Survived /injected	GFP+	PCR-amplified	Cas9-cleaved	Edited with ssDNA	Recombined with HBD
1	100/20/2	10/11	6	6	4	0	1
2	100/20/20	22/29	17	17	7	1	0
3	200/40/200	12/14	12	10*	6	2	2
4	200/40/200	27/32	24	21*	11	1	4
Total	-	71/86 (82.6%)	59	54	28 (51.9%)	4 (14.3%)	7 (25.0%)

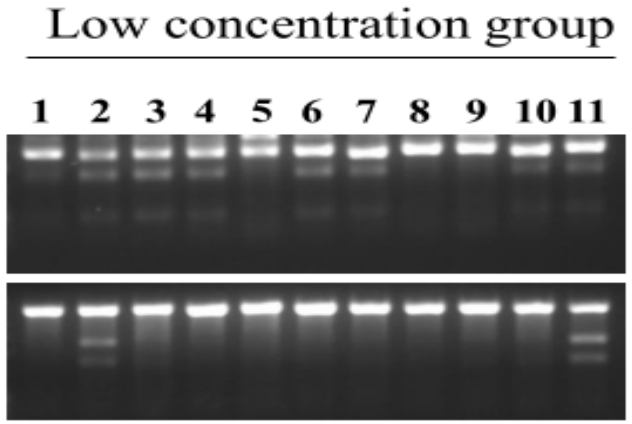
CRISPR/Cas9 has off-target effect in human tripronuclear embryos

No.16 embryo

GCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA	8/50
GCATCTGACTCCTGAAGAAAAATCCGCTGTCACTGCCCTGTGGGGCAAGGTGAA	6/50
GCATCTGACTCCTGAAGAAAAATCCGCCGTTACTGCCCTGTGGGGCAAGGTGAA	1/50
GCATCTGACTCCTGAGGAGAAGACTGCTGTCAATGCCCTGTGGGGCAAAGTGAA	21/50
GCACCTGACTCCTGAG——AAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA	14/50

CRISPR/Cas9 induced on- and off-target indels in exomes of human 3PN embryos

Cas9/gRNA (ng/μL)	100/20		
Sample No.	A	B	C
On-target indels	1	1	1
Candidate off-target sites	1	0	1
T7E1 assay confirmed off-target sites	1	0	1



2015：

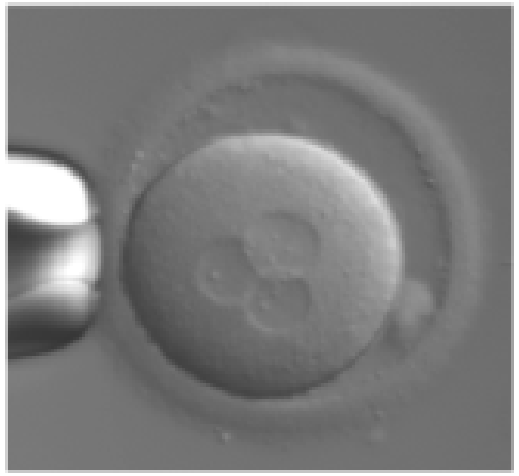
RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

The world's first report of human embryos altered by Gene Editing.

Risks!!!



1）NHEJ（nonhomologous end joining);

2）Mosaicism；

3) Off-target mutations;

4）Using endogenous genes as templates;

2017：

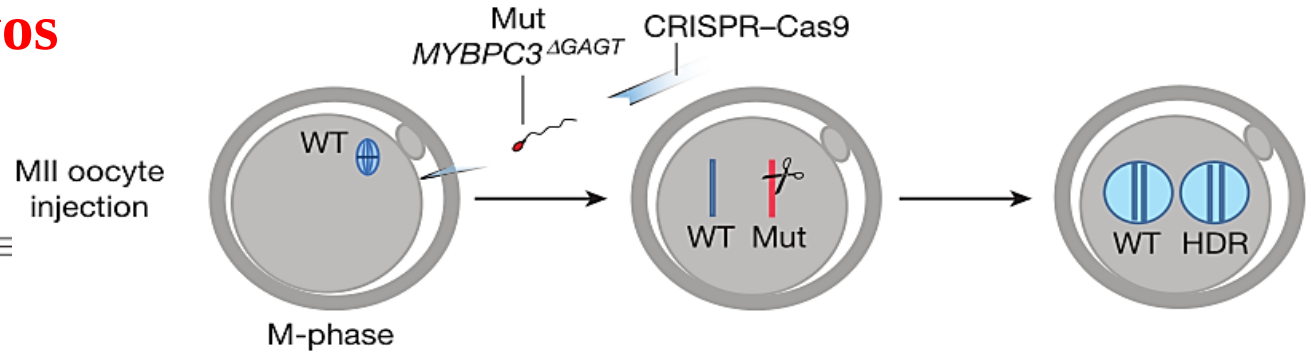
Science Home News Journals Topics Careers
Advertisement

First U.S.-based group to edit human embryos brings practice closer to clinic

By Kelly Servick | Aug. 2, 2017 , 1:00 PM

Using the endogenous WT allele as a template in normal human embryos

ARTICLE



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]§

2017/08/02

2016:

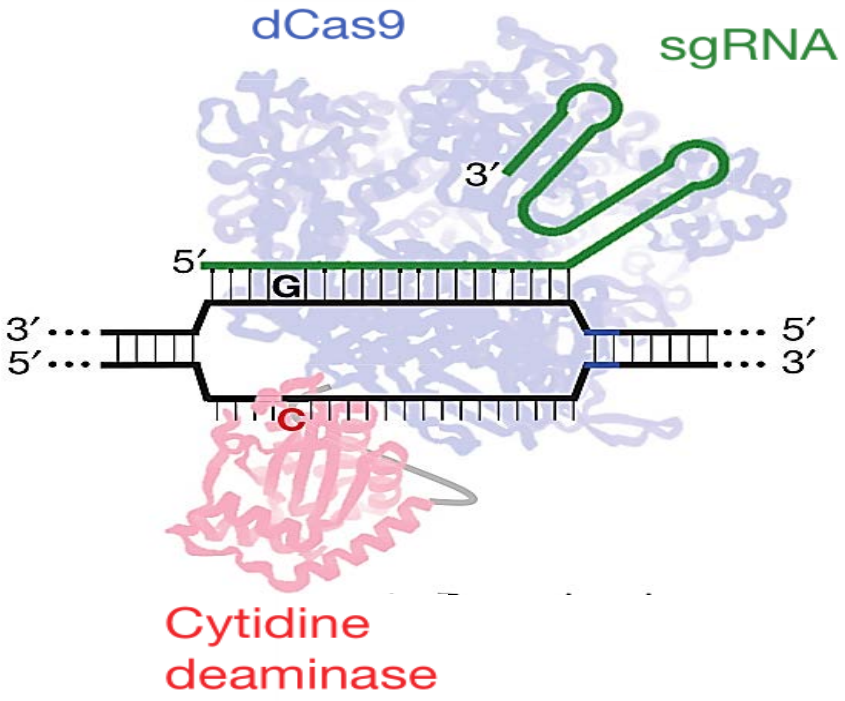
Cytidine Base Editor(CBE)

LETTER

doi:10.1038/nature17946

Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

Alexis C. Komor[1,2], Yongjoo B. Kim[1,2], Michael S. Packer[1,2], John A. Zuris[1,2] & David R. Liu[1,2]



$C \rightarrow T$ (or $G \rightarrow A$)

2017:

Gene Modified Mouse Embryo with Cytidine Base Editor

Protein Cell
DOI 10.1007/s13238-017-0418-2

CrossMark Protein & Cell

RESEARCH ARTICLE

Effective gene editing by high-fidelity base editor 2 in mouse zygotes

Puping Liang[1,2,3]✉, Hongwei Sun[1], Ying Sun[1], Xiya Zhang[1], Xiaowei Xie[1], Ji
Zhen Zhang[1,4], Yuxi Chen[1], Chenhui Ding[3], Yuanyan Xiong[1], Wenbin Ma[1], D
Junjiu Huang[1,2]✉, Zhou Songyang[1,2,3,5]✉

[1] Key Laboratory of Gene Engineering of the Ministry of Education, Guangzhou Key Lab

Protein Cell
DOI 10.1007/s13238-017-0432-4

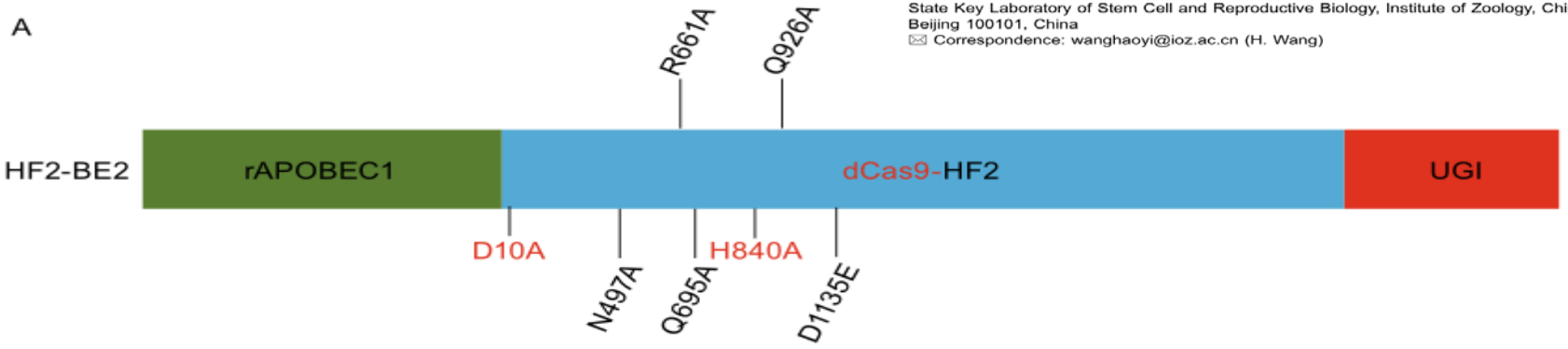
CrossMark Protein & Cell

HIGHLIGHT

Editing base in mouse model

Haoyi Wang✉

State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
✉ Correspondence: wanghaoyi@ioz.ac.cn (H. Wang)

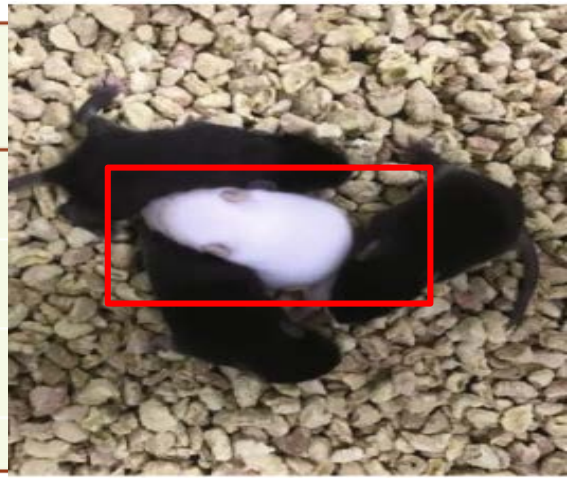


Production of Homozygous F0 mouse with CBE

Table 1. Summary of base editing by HF2-BE2 in founder mice

Group	Survived/Injected embryos (%)	Pups/ Transferred (%)	Albino pups (%)	Mosaic pups (%)	Mutant black pups (%)
gRNA-1 + HF2-BE2 mRNA*	120/162 (74.1)	13/120 (10.8)	0	0	2 (18.2) *
gRNA-2 + HF2-BE2 mRNA	106/145 (73.1)	11/106 (10.4)	1 (9.1)	4 (36.4)	2 (18.2)
HF2-BE2 mRNA#	108/142 (76.1)	14/108 (13.0)	0	0	0
H_2O	103/146 (70.5)	9/103 (8.7)	0	0	0

*, # Pups were cannibalized by the mother (2 for * and 1 for #).



β-thalassemia:

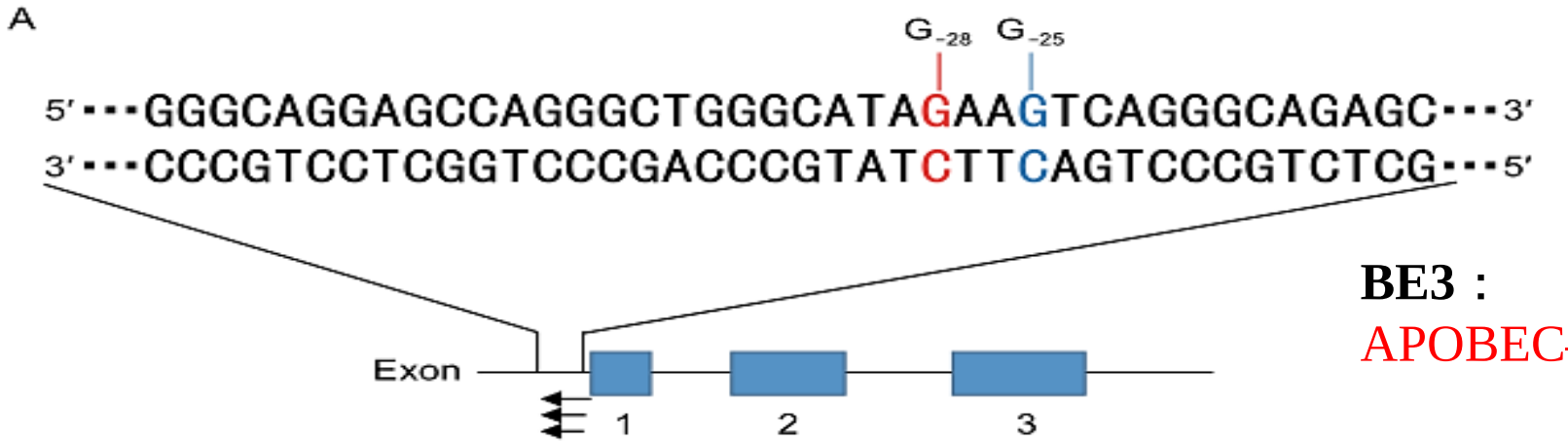
Mostly caused by **point mutations**, there are more than 100 mutations in *HBB* gene.

Can we repair β-thalassemia in Human Embryo with CBE ?

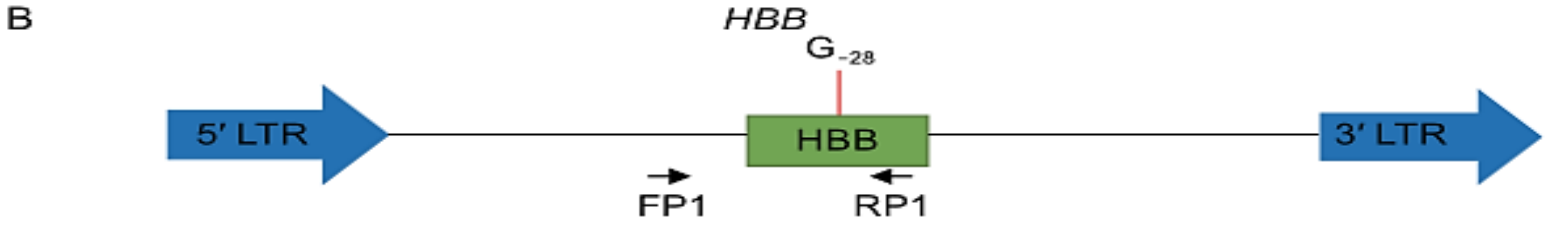
β-thalassemia in Memorial Hospital, Sun Yat-sen University

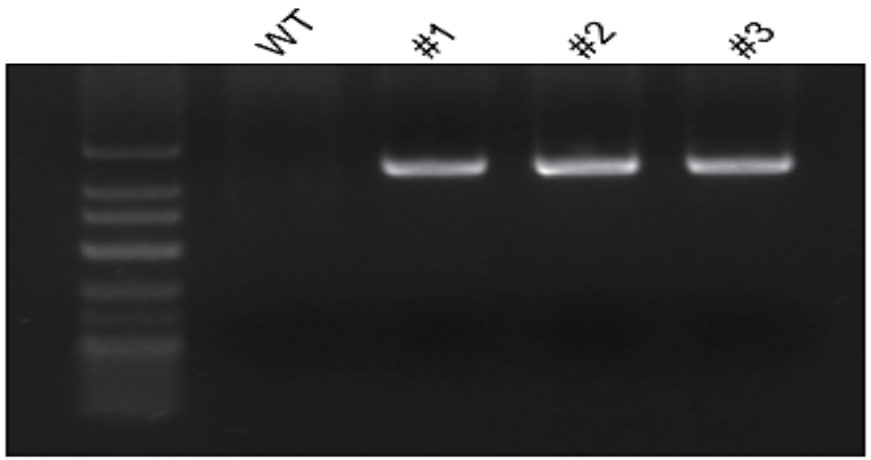
HBB Gene	No. Patiens	Ratio（%）
41-42M/N	151	6.7
654M/N	103	4.6
-28M/N(A>G)	**49**	**2.2**
17M/N	30	1.3
CD17M/N	12	0.5
βEM/N	9	0.4
41-42M/41-42M	8	0.4
71-72M/N	5	0.2
27/28M/N	4	0.2
-29M/N	4	0.2
14-15M/N	2	0.1
41-42M/654M	2	0.1
43M/N	2	0.1
654M/17M	2	0.1

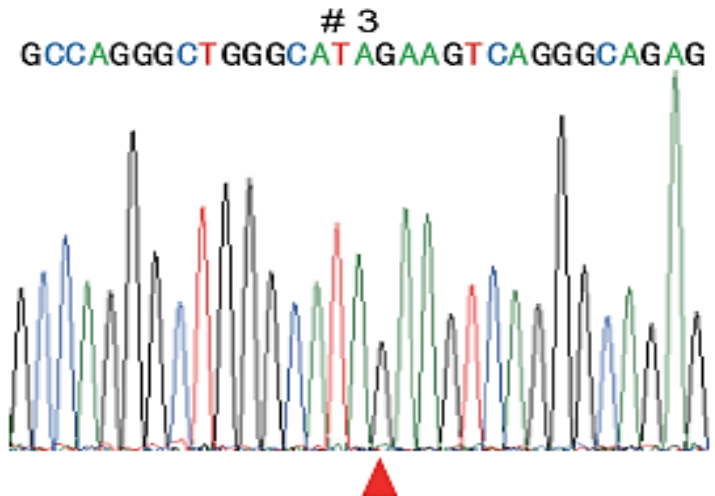
Correcting *HBB* −28 (A>G) mutation in human cell line.



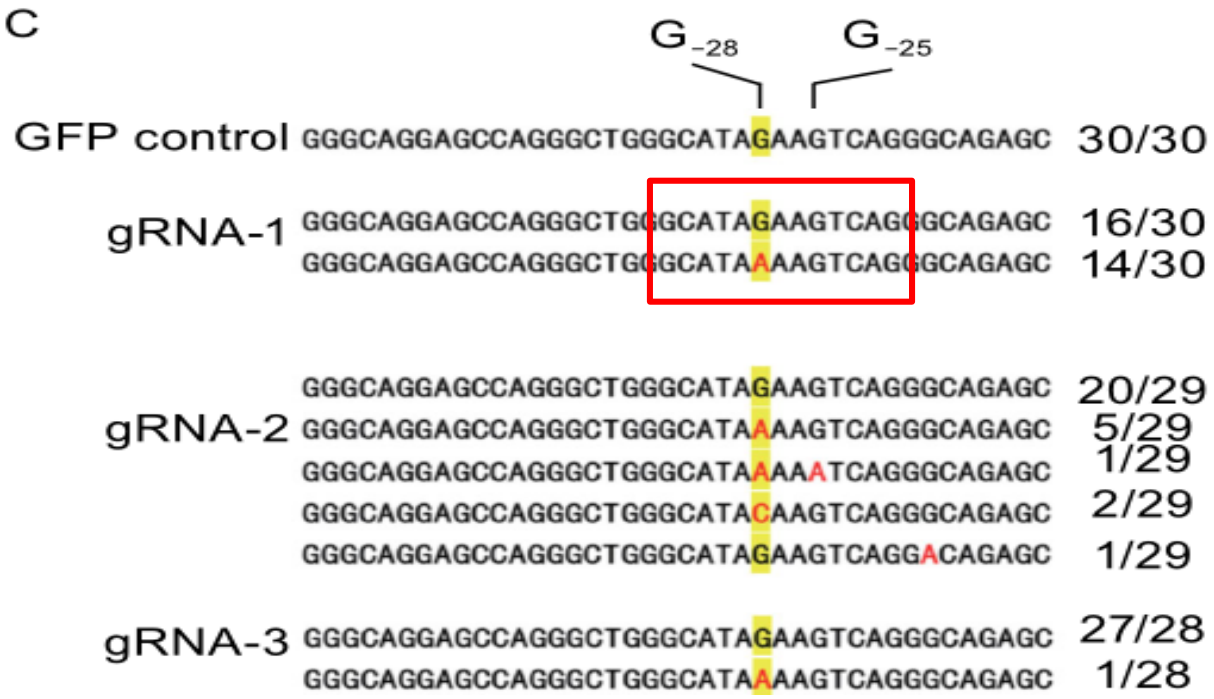
BE3 :
APOBEC–XTEN–dCas9(A840H)–UGI

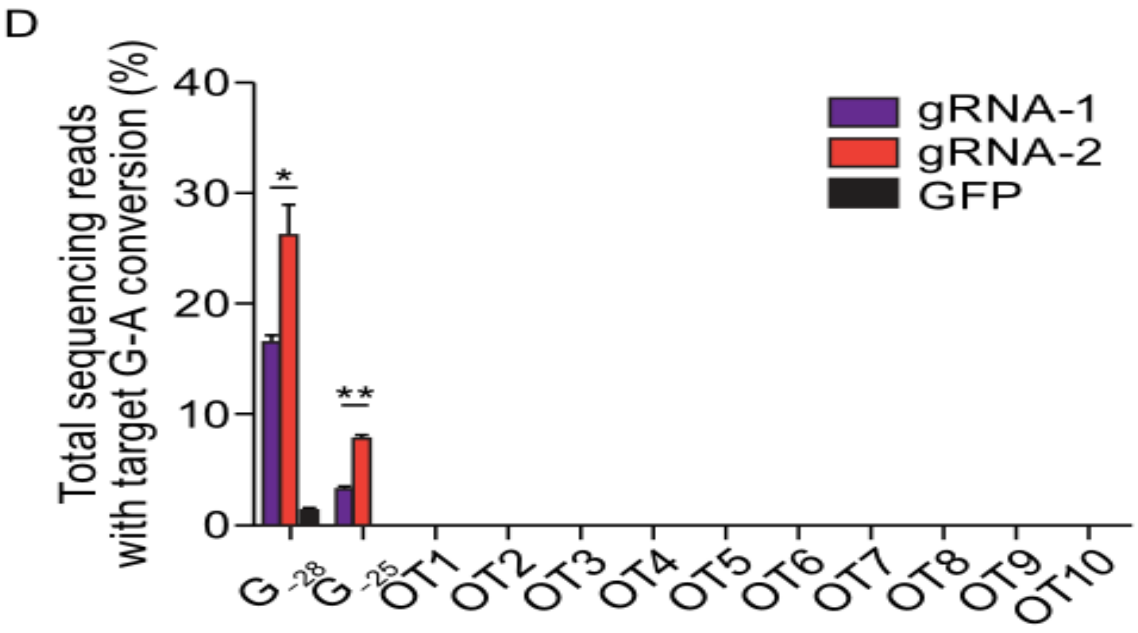




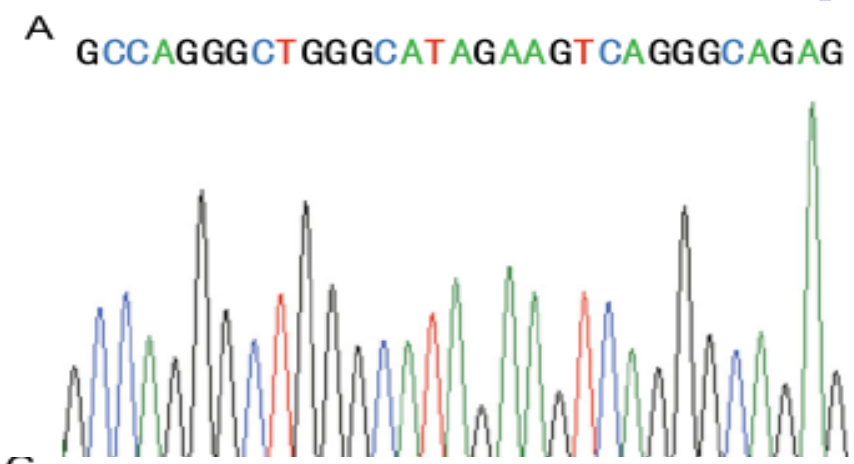


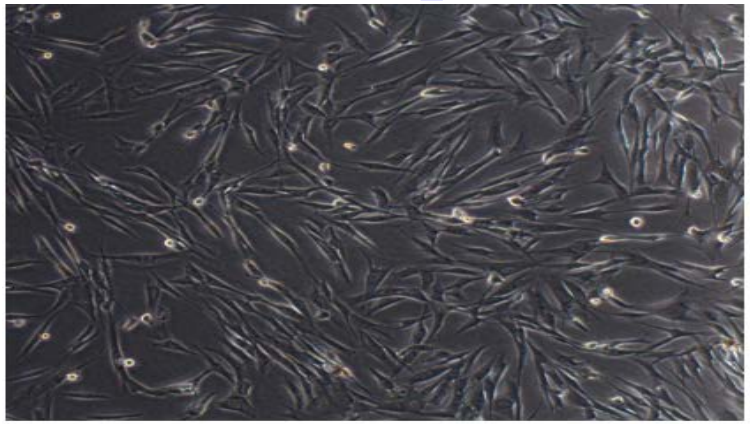
Precise repairing of *HBB* −28 (A>G) mutation



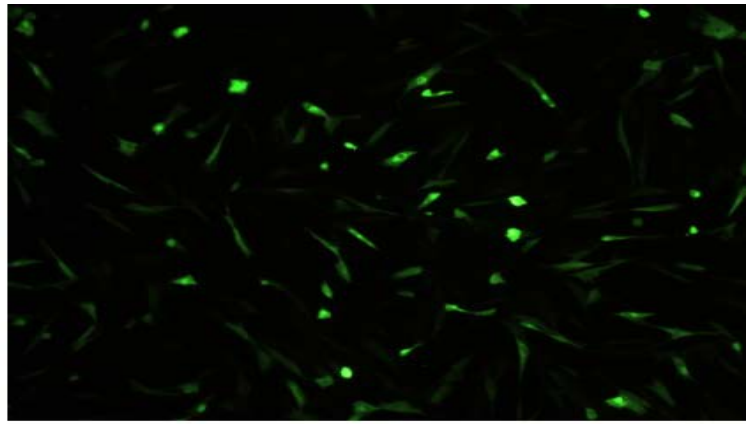


Correcting −28 mutation in primary skin fibroblast cells of a β-thalassemia patient

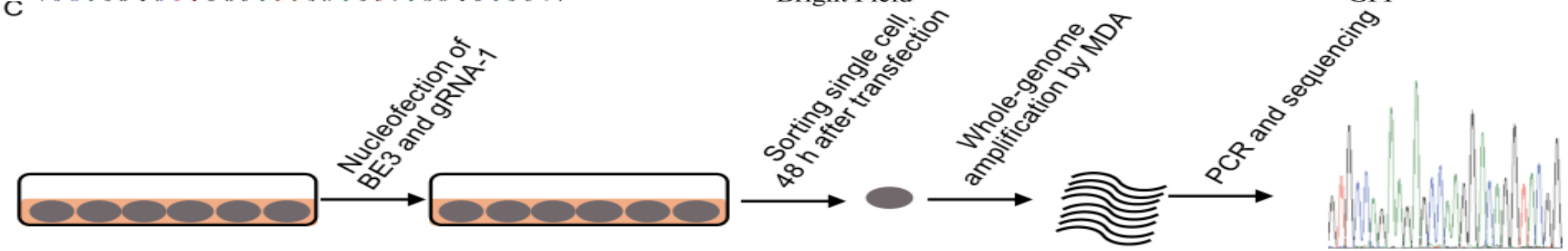




Bright Field



GFP



MDA-amplified single cell No.	PCR-amplified single cell No.	<Homozygous> $G_{-28}G_{-25}/G_{-28}G_{-25}$ cell NO. (%)	<Heterozygous> $A_{-28}G_{-25}/G_{-28}G_{-25}$ cell NO. (%)	<Wild-type> $A_{-28}G_{-25}/A_{-28}G_{-25}$ cell NO. (%)	$G_{-28}G_{-25}/A_{-28}A_{-25}$ cell NO. (%)	$A_{-28}A_{-25}/A_{-28}A_{-25}$ cell NO. (%)	$G_{-28}A_{-25}/G_{-28}A_{-25}$ cell NO. (%)
30	28*	20 (71.4)	3 (10.7)	2 (7.1)	1 (3.6)	1 (3.6)	1 (3.6)

2017:

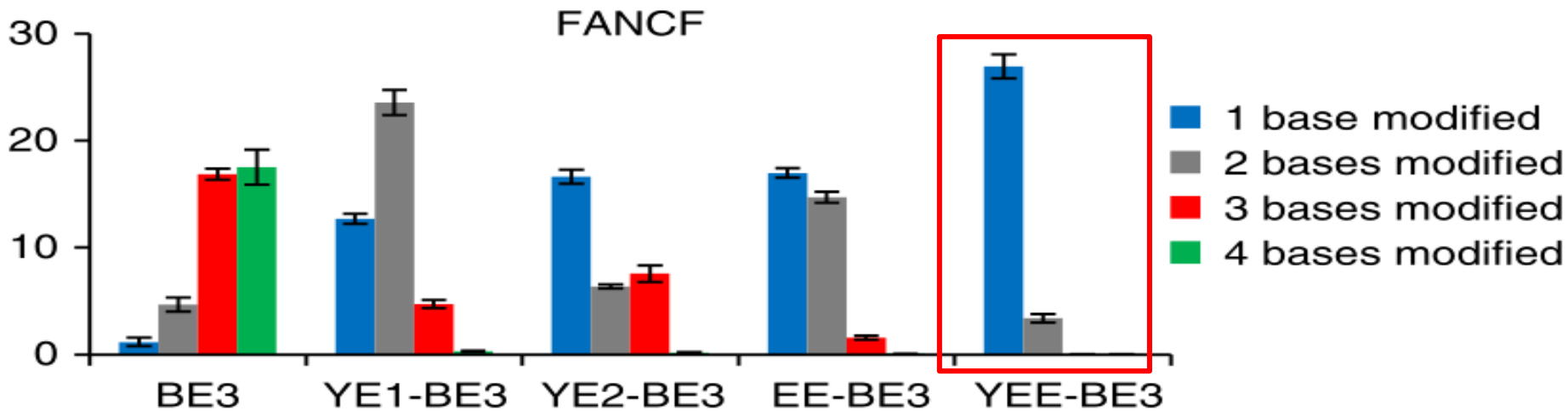
Precise Editing a Single-base with YEE-BE3

LETTERS

nature biotechnology

Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions

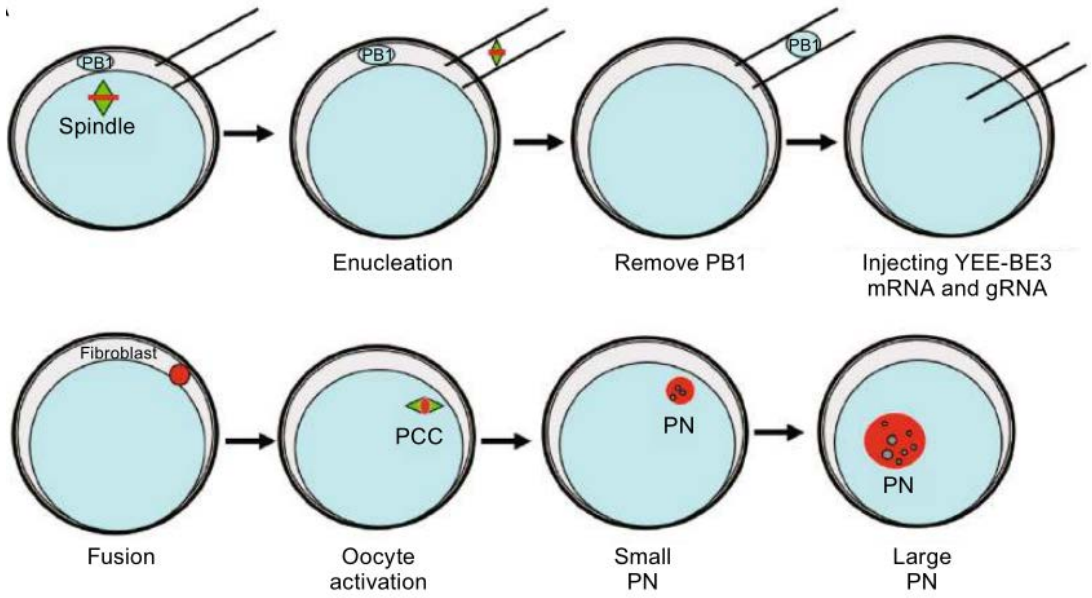
Y Bill Kim[1,2], Alexis C Komor[1,2], Jonathan M Levy[1,2], Michael S Packer[1,2], Kevin T Zhao[1,2] & David R Liu[1–3]



YEE = W90Y + R126E + R132E

Effective HBB −28 (A>G) mutation repair in cloned human embryos by YEE-BE3

Cloned homozygous HBB −28 (A>G) human embryos



Survived embryo No. (Injected embryo No.)	Activated embryo No.	Harvested embryo No.	Total blastomere No.	MDA-amplified blastomere No.	PCR-amplified blastomere No.	<Homozygous> $G_{-28}G_{-25}/G_{-28}G_{-25}$ blastomere No. (%)	<Heterozygous> $A_{-28}G_{-25}/G_{-28}G_{-25}$ blastomere No. (%)	<Wild-type> $A_{-28}G_{-25}/A_{-28}G_{-25}$ blastomere No. (%)
28 (35)	24	20#	73	73	48*	37 (77.1)	3 (6.3)	8 (16.7)

2017：

The world’s first report of human embryos altered by Base Editor.

Protein Cell
DOI 10.1007/s13238-017-0475-6

CrossMark Protein & Cell

SHORT ARTICLE

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

Puping Liang[1,2], Chenhui Ding[2], Hongwei Sun[1], Xiaowei Xie[1], Yanwen Xu[2], Xiya Zhang[1], Ying Sun[1], Yuanyan Xiong[1], Wenbin Ma[1], Yongxiang Liu[2], Yali Wang[2], Jianpei Fang[3], Dan Liu[4], Zhou Songyang[1,2,4]✉, Canquan Zhou[2]✉, Junjiu Huang[1,2]✉

[1] Key Laboratory of Gene Engineering of the Ministry of Education, Guangzhou Key Laboratory of Healthy Aging Research

This first study was a successful proof of principle that the base-editing technique can be used to correct a disease mutation in a human embryo.

--*Nature* 2017

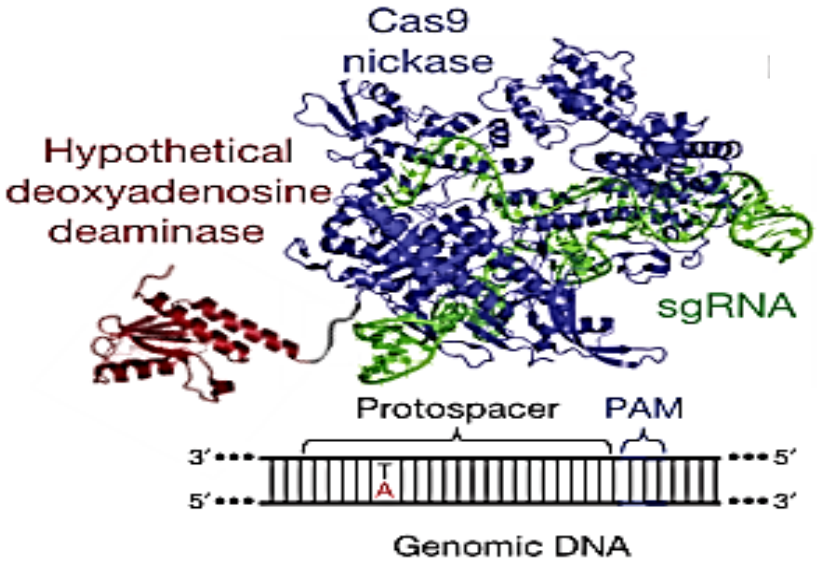
Adenosine Base Editor (ABE)

ARTICLE

doi:10.1038/nature24644

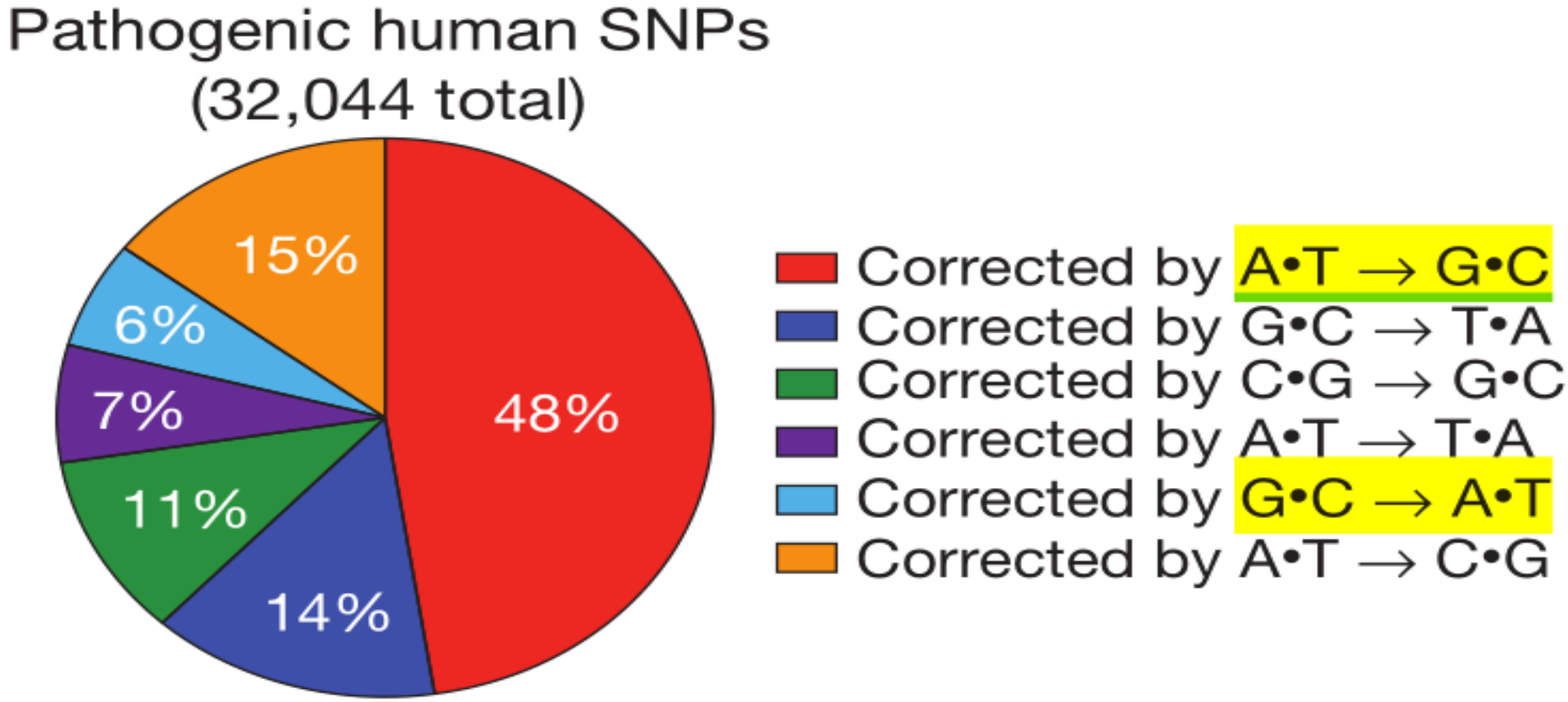
Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage

Nicole M. Gaudelli[1,2,3], Alexis C. Komor[1,2,3]†, Holly A. Rees[1,2,3], Michael S. Packer[1,2,3]†, Ahmed H. Badran[1,2,3], David I. Bryson[1,2,3]† & David R. Liu[1,2,3]



A·T to G·C

To be continue……



Nicole m. Gaudelli et al. Nature 2017

2017：

Human Genome Editing: Science, Ethics, and Governance

The National Academies of SCIENCES ENGINEERING MEDICINE

HUMAN

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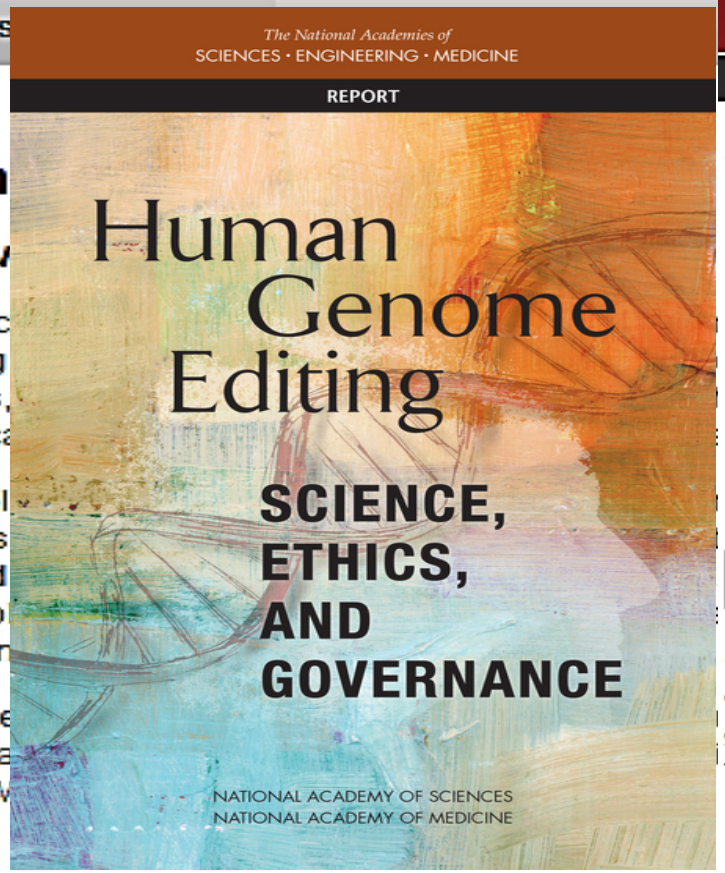

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News & Comment › News › 2017 › February › Article

NATURE | NEWS

US science advisers outline path to genetically modified babies

Modified human embryos should be allowed if researchers meet strict criteria, says long-awaited National Academies report.

Sara Reardon

14 February 2017 | Updated: 14 February 2017

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Scientists should be permitted to modify human embryos destined for implantation in the womb to eliminate devastating genetic diseases such as sickle-cell anaemia or cystic fibrosis — once gene-editing techniques advance sufficiently for use in people and proper restrictions are in place. That's the conclusion of a 14 February report from the US National Academies of Science, Engineering, and Medicine.

Condemning the reproductive application of gene editing on human germline

Committee of Genome Editing, Genetics Society of China
Chinese Society for Stem Cell Research

Nov. 27th, 2018

On Nov. 26th, 2018, Jiankui He, associate professor from Southern University of Science and Technology, announced that two babies with edited *CCR5* gene have been born in China, and he believed that this genetic modification would render these babies immune to HIV infection. While more solid facts of this experiment remain to be disclosed and the veracity of such claims are yet to be ascertained, we strongly condemn any application of gene editing on human embryos for reproductive purposes. Such intervention is against the law, regulation, and medical ethics of China. Moreover, it violates internationally accepted ethical principles regulating human experimentation and human rights law.

Thanks!

周灿权	教授	梁普平	副教授
松阳洲	教授	丁晨晖	副研究员
徐艳文	教授	张曦亚	博士
方建培	教授	孙宏伟	博士生等