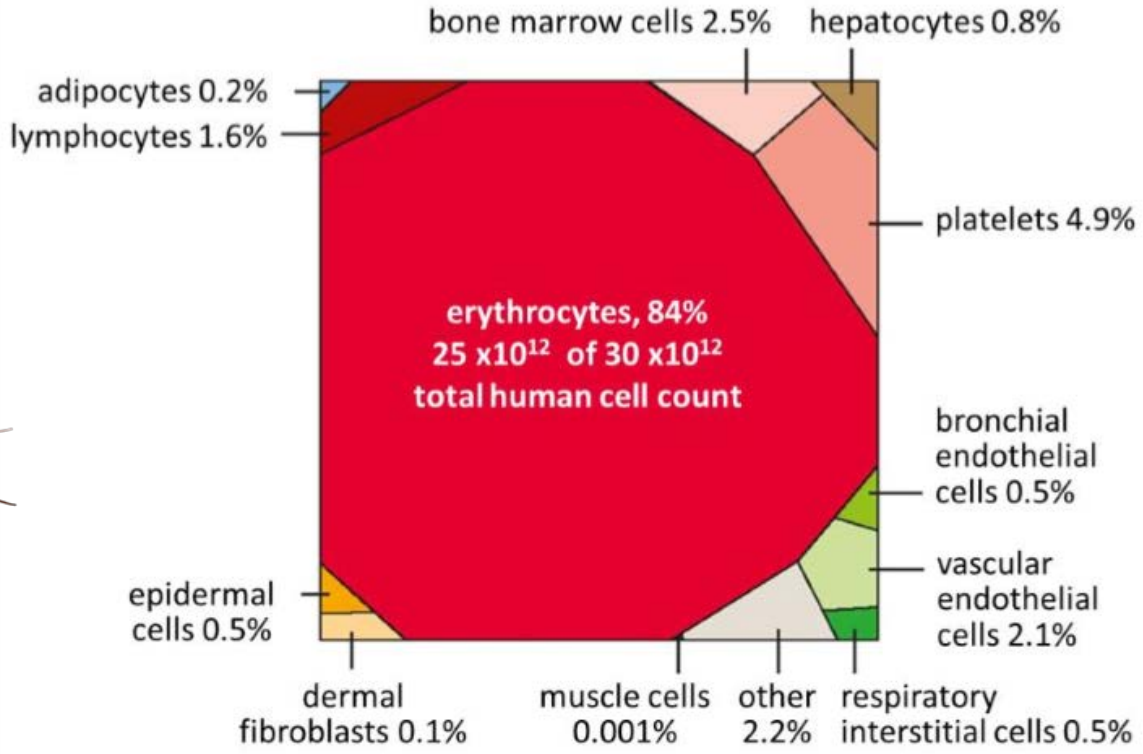
Sickle Cell Disease and related Hemoglobinopathies

Merlin Crossley, UNSW Sydney



from Sender, Fuchs, Milo PLoS Biol. 2016 Aug 19;14(8):e1002533

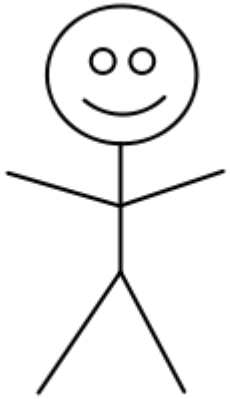
7000 years ago in Africa

Shriner D, Rotimi CN. Am J Hum Genet. 2018 Apr 5;102(4):547-556. doi: 10.1016/j.ajhg.2018.02.003. Epub 2018 Mar 8.

A mutation occurred in a human β-globin gene

Shriner D, Rotimi CN. Am J Hum Genet. 2018 Apr 5;102(4):547-556. doi: 10.1016/j.ajhg.2018.02.003. Epub 2018 Mar 8.

The resulting human showed resistance to malaria



+/-

The fortunate +/- carrier started a family

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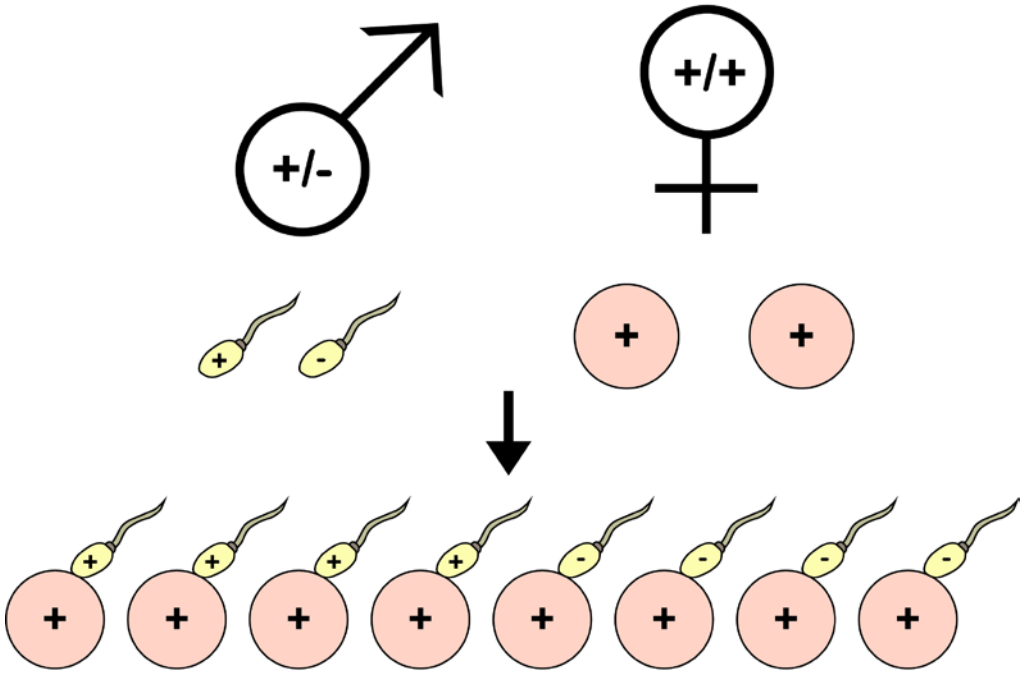
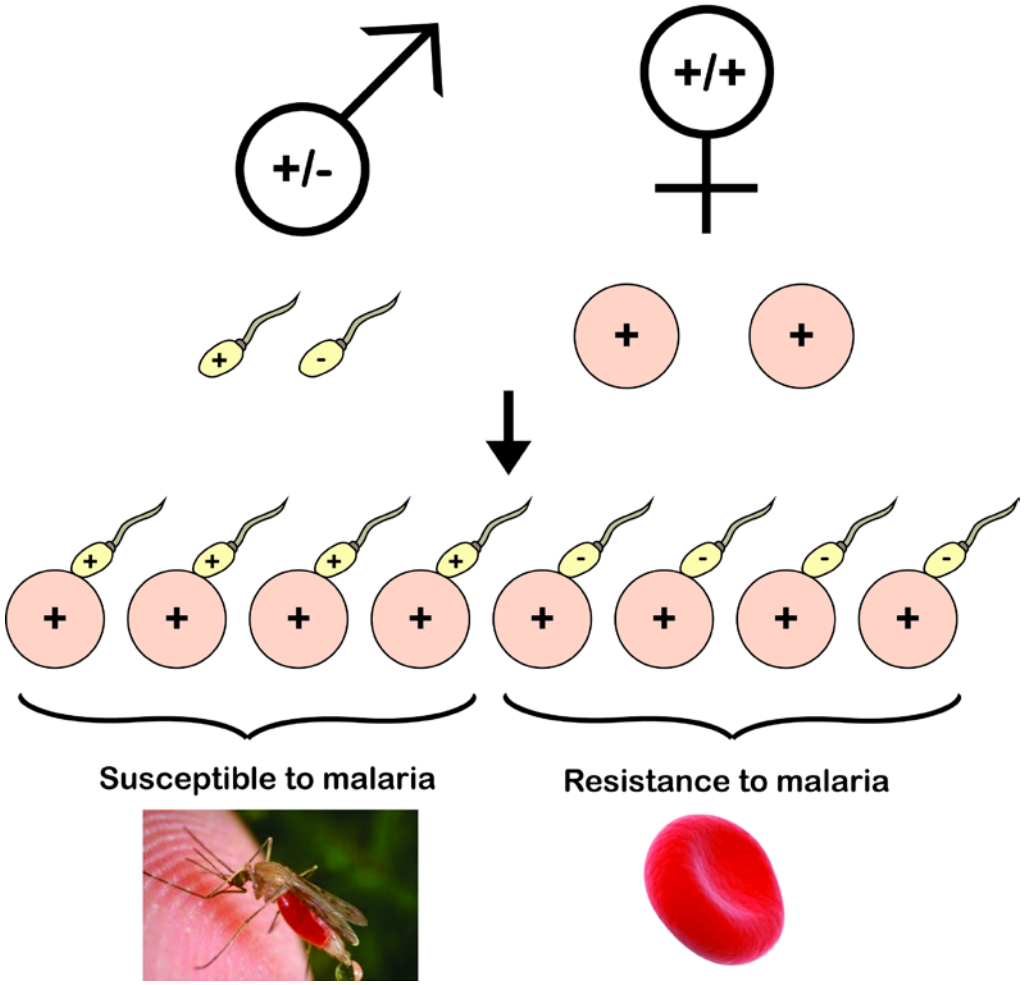
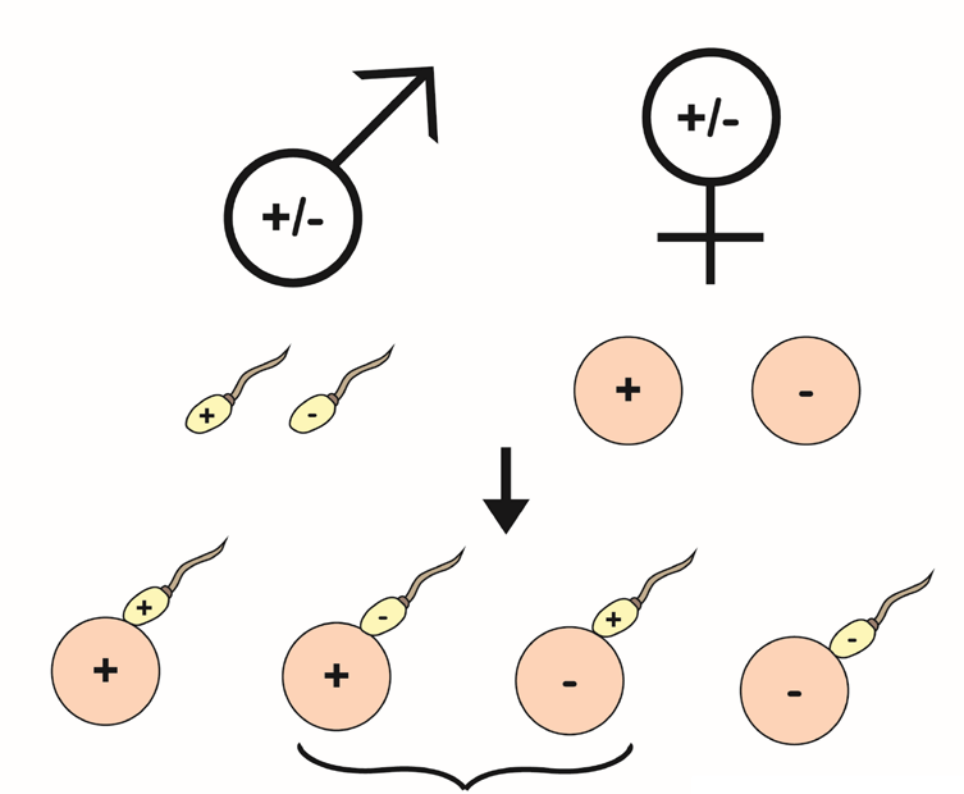


The +/- carriers prospered



More and more carriers met other carriers



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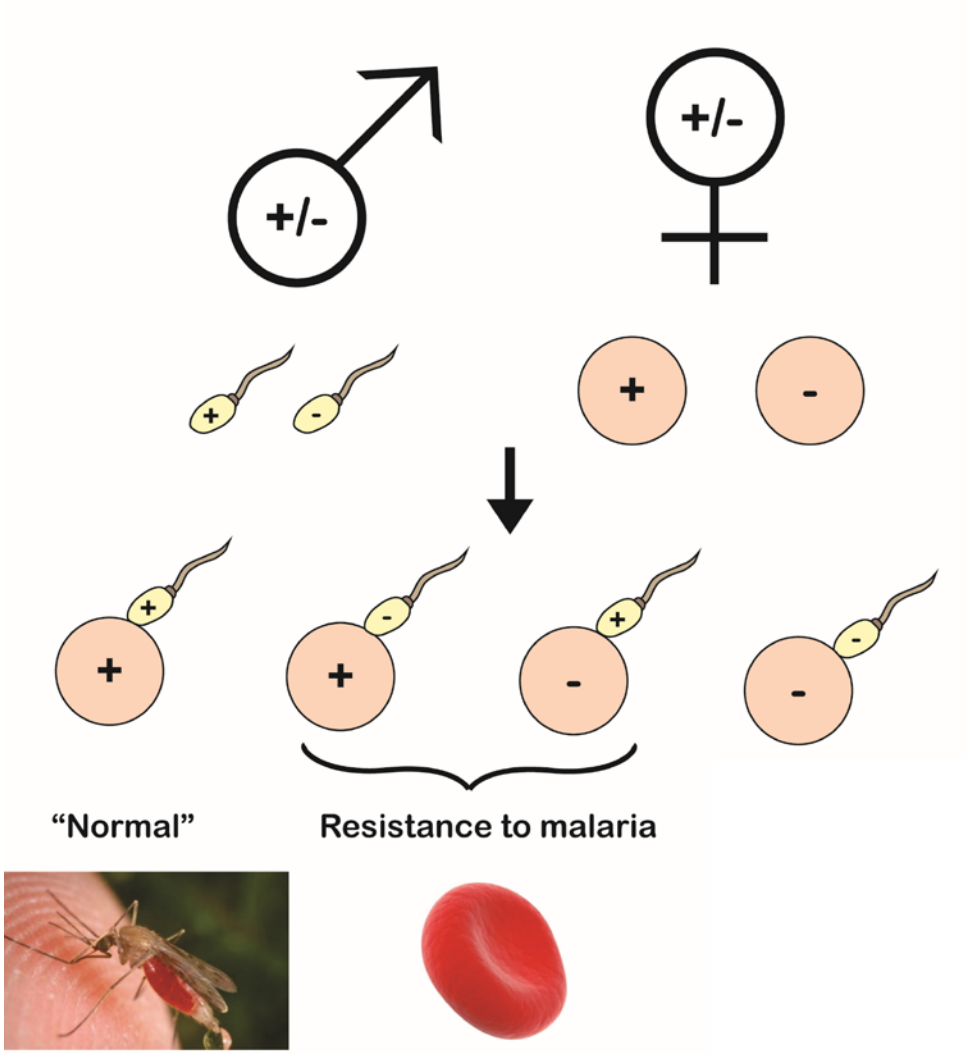

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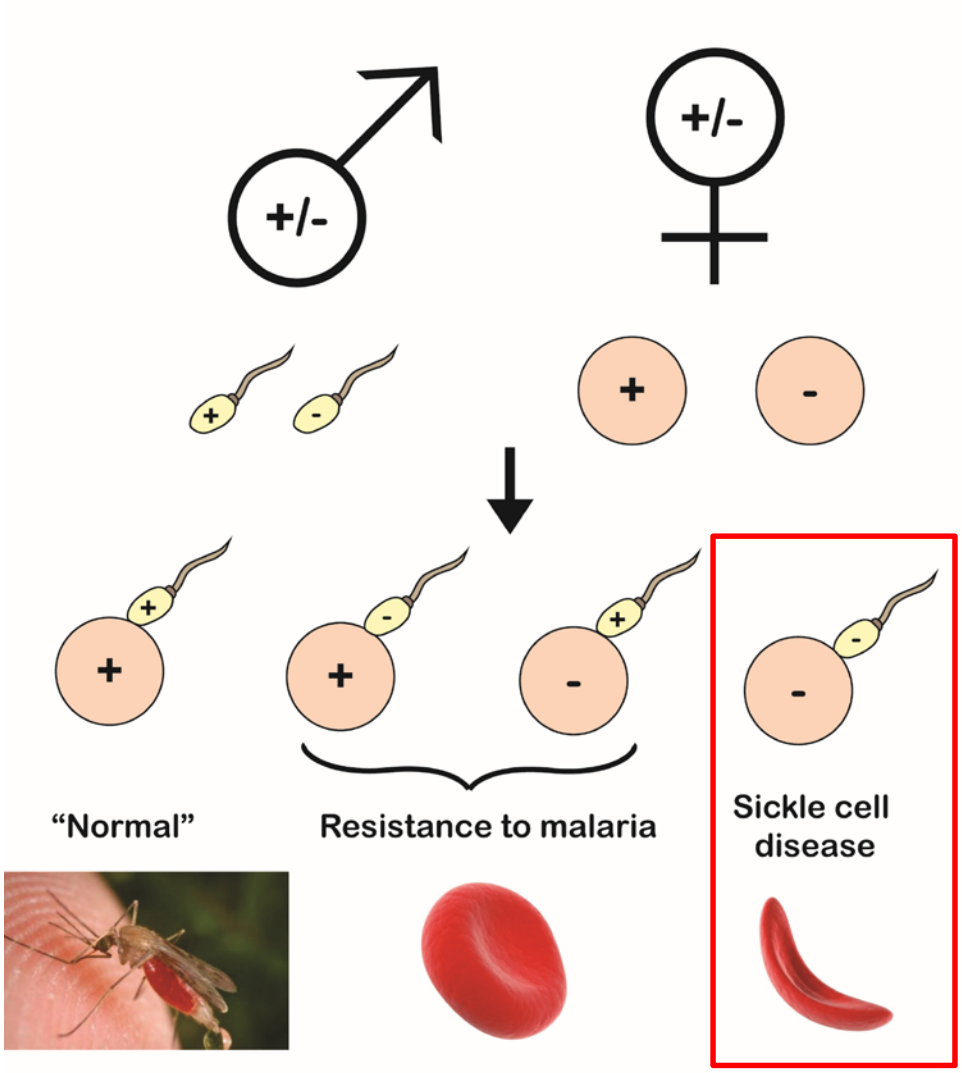

The mutation and others like it spread around the world

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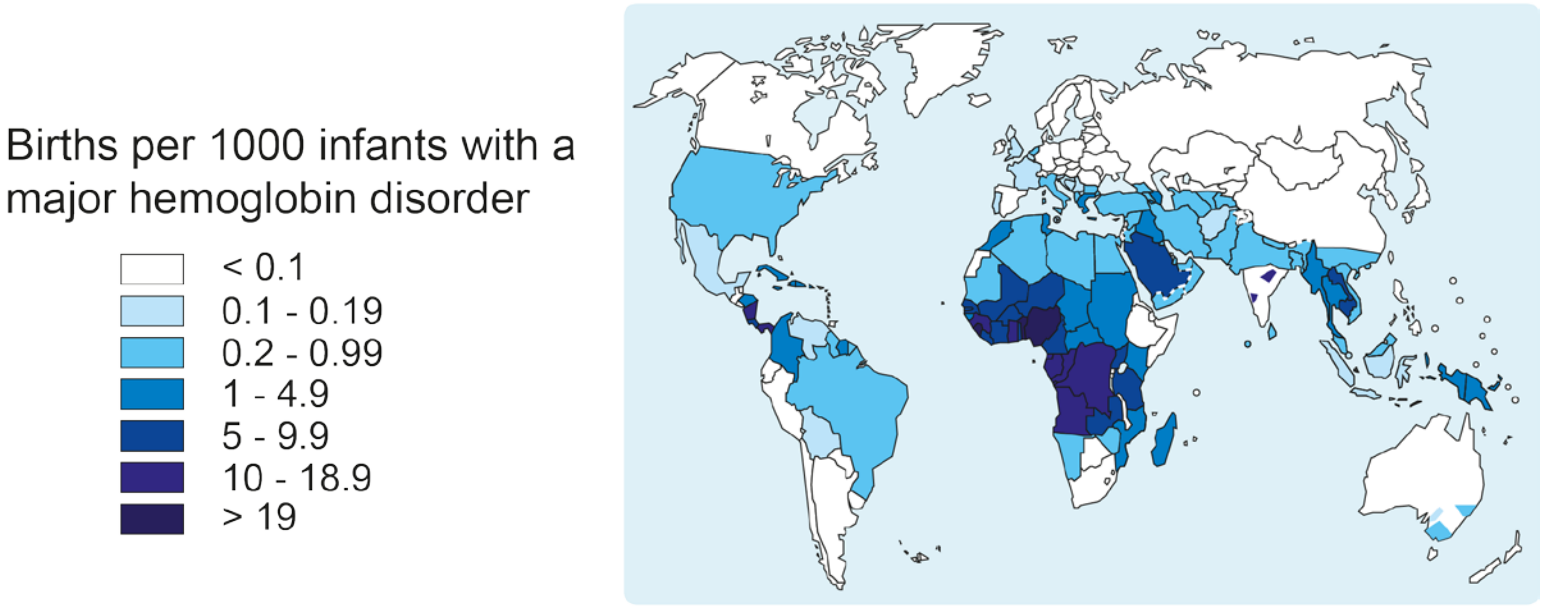
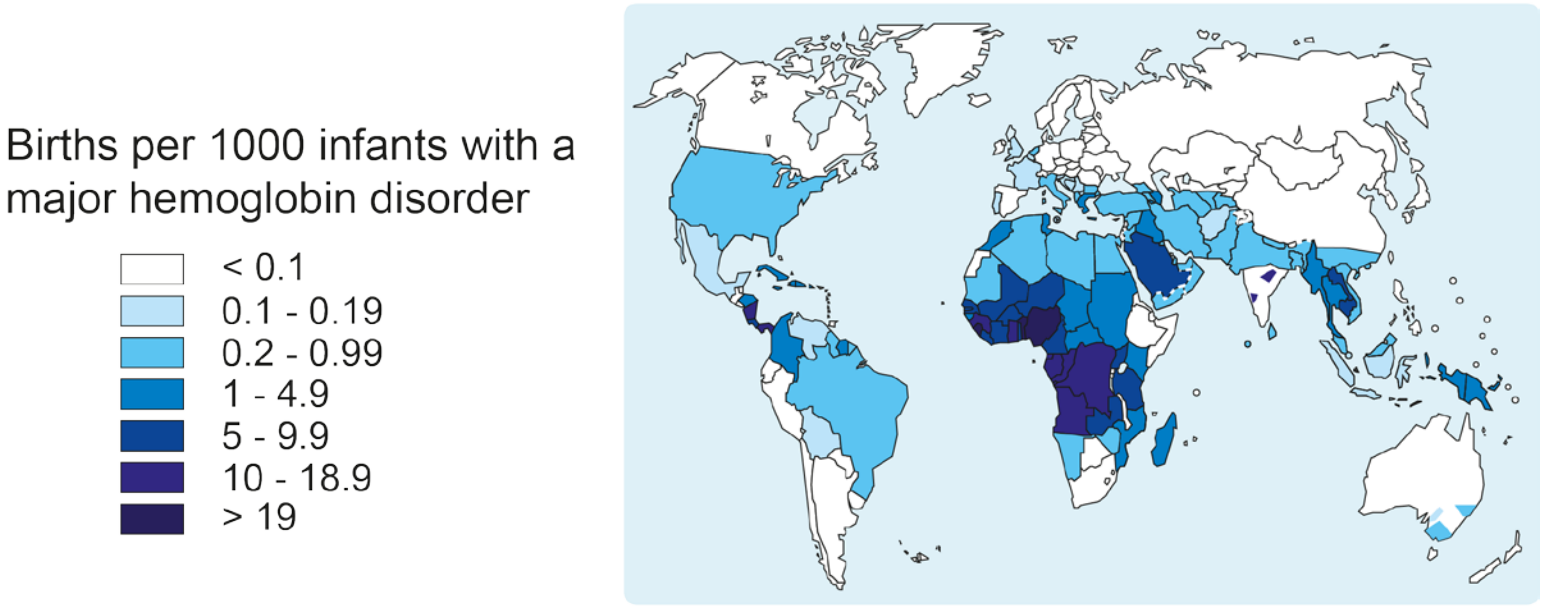
- 5% of people carry a β-globin mutation
- >300,000 -/- affected babies born each year



Modell B, Darlison M. Bull World Health Organ. 2008 86(6):480-7

The mutation and <u>others like it</u> spread around the world

- 5% of people carry a β-globin mutation
- >300,000 -/- affected babies born each year
- In developing countries most die before the age of 5
- In the US lifelong health care is imperfect and expensive



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- Add a replacement gene to blood stem cells – gene therapy
- Correct the mutation in blood stem cells, iPS cells or embryos? – CRISPR
- De-repress the fetal globin gene to compensate – CRISPR or drugs (e.g. Hydroxyurea)

Understanding is the first step to helping

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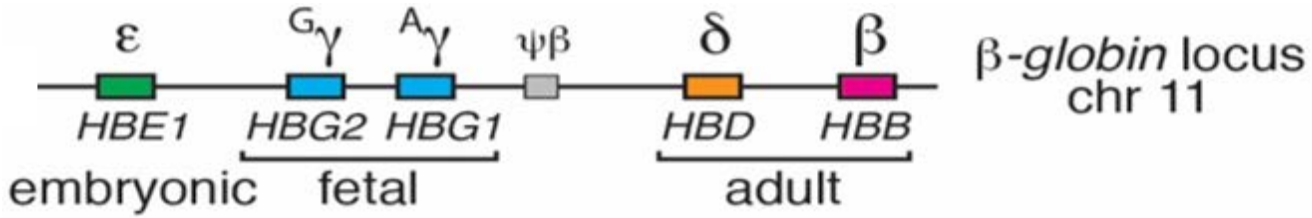

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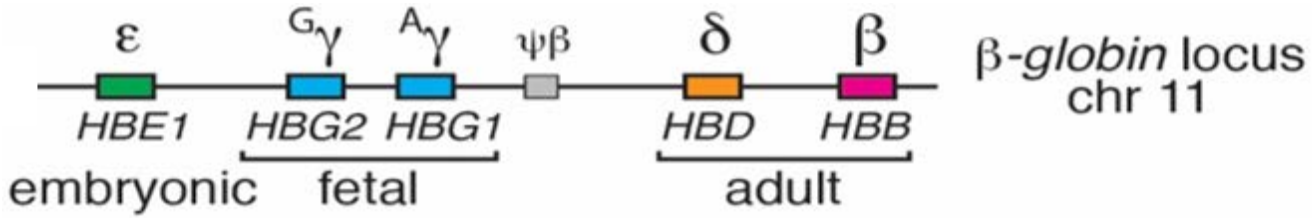

2018 ~1,000 different defects known but the Sickle mutation accounts for >50% of patients

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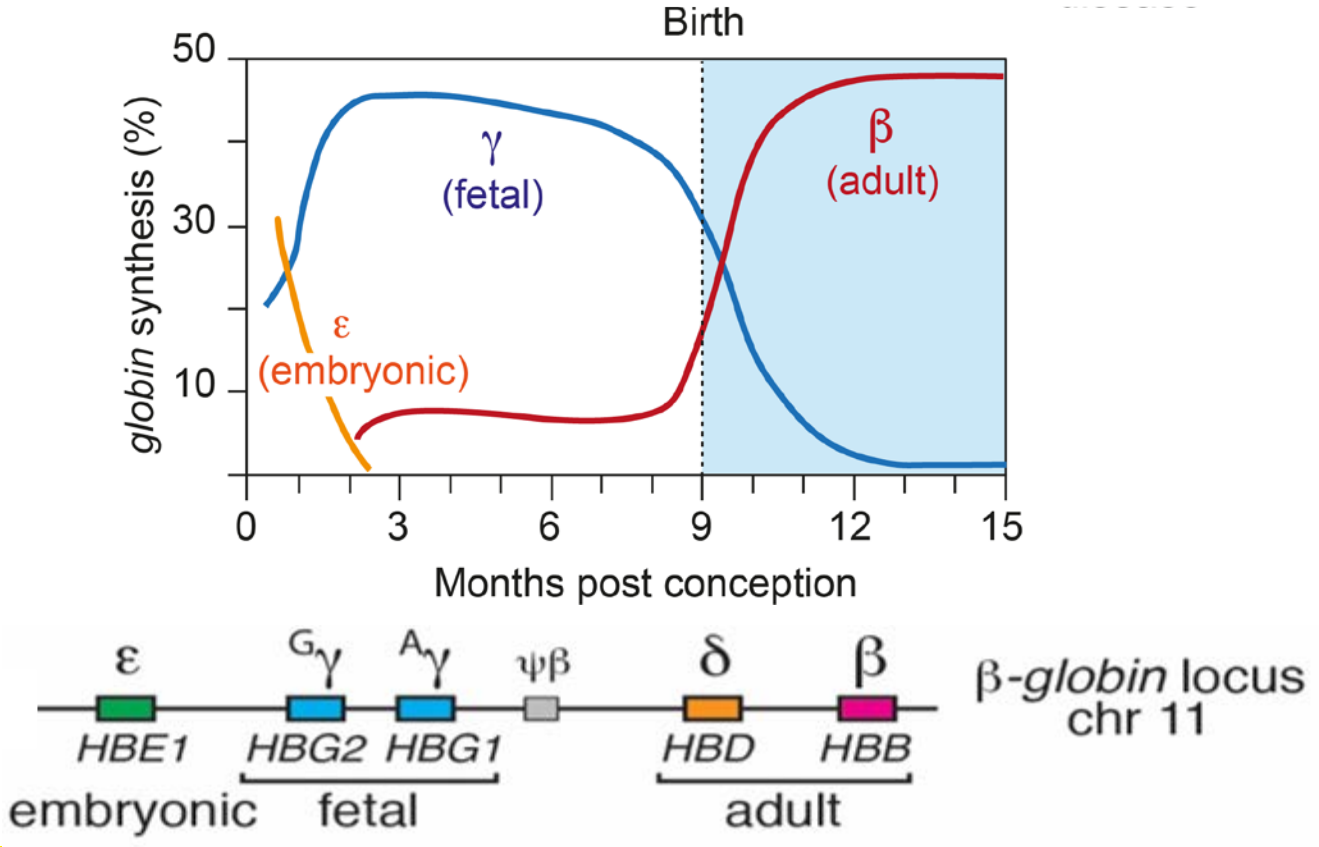
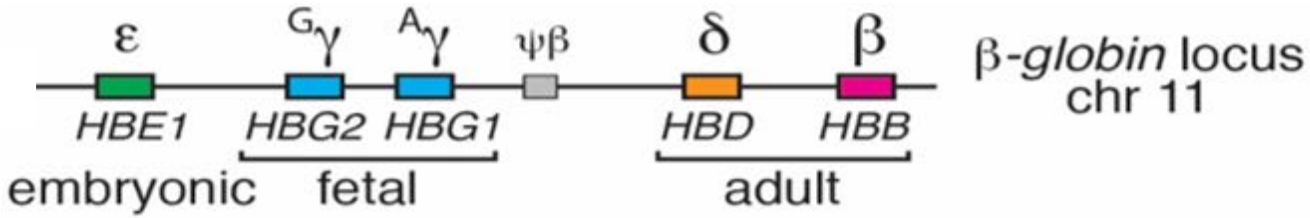
- Humans express ε-globin early, then γ-globin, then β-globin

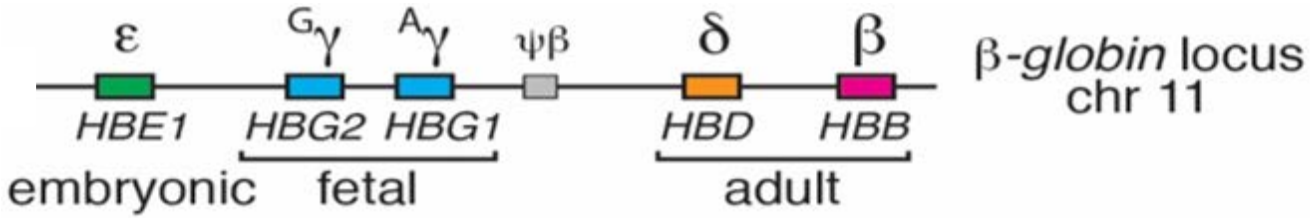


Fetal globin as a compensatory gene



Fetal globin as a compensatory gene

1948 Janet Watson notes children have fewer symptoms, attributes this to residual fetal globin expression



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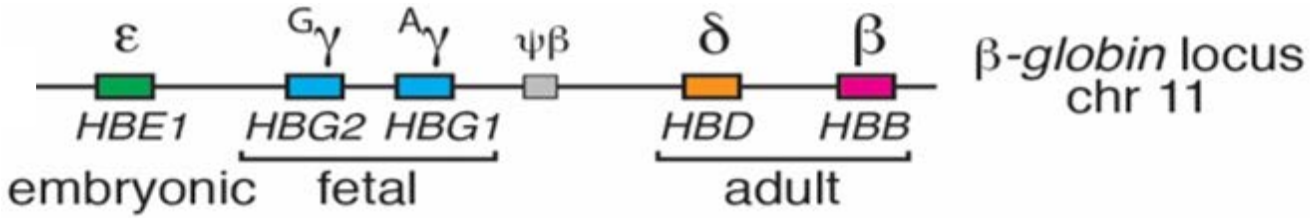

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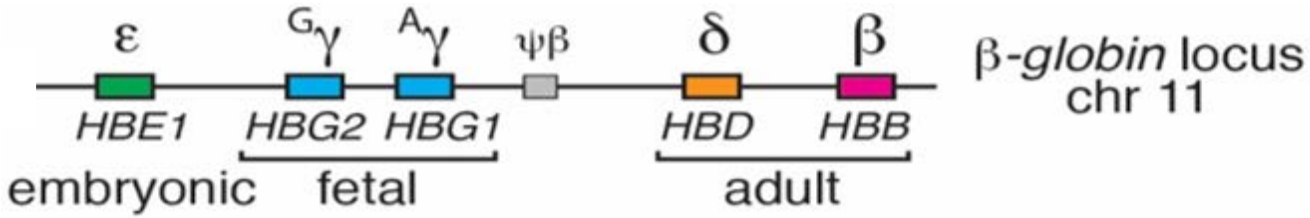
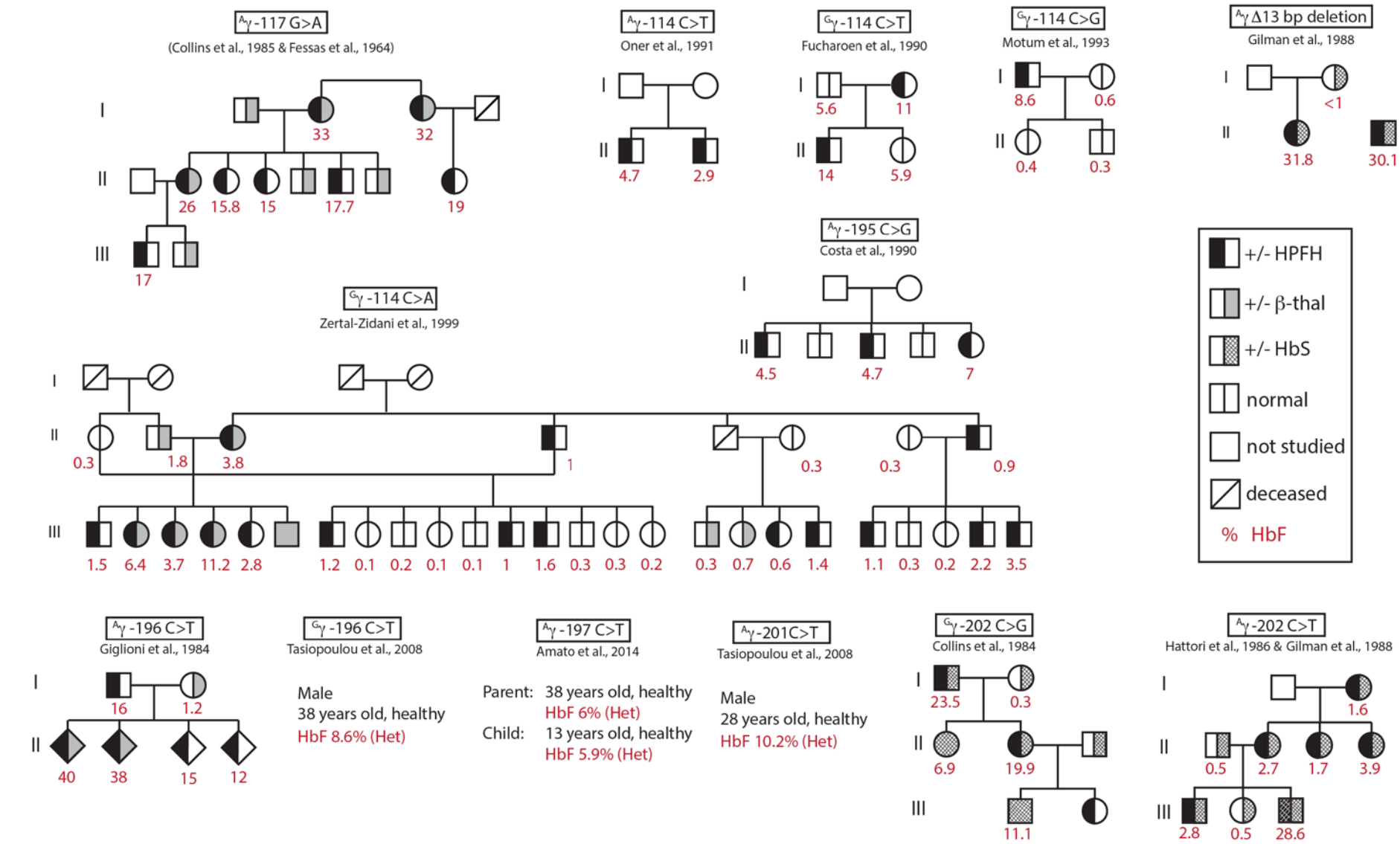
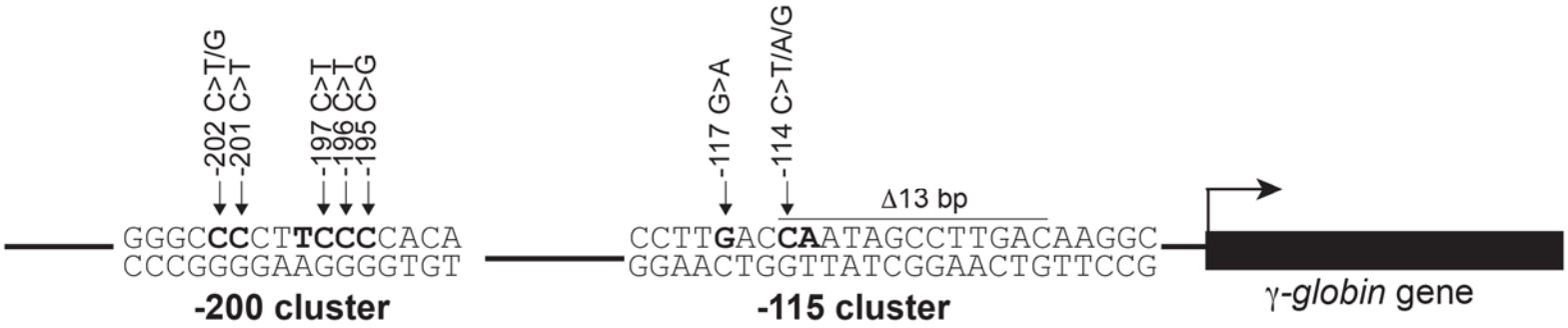
1984 Francis Collins shows a fetal globin promoter mutation at -117 causes HPFH



Several different HPFH families were discovered



How do the regulatory mutations in the fetal globin promoter alleviate repression?



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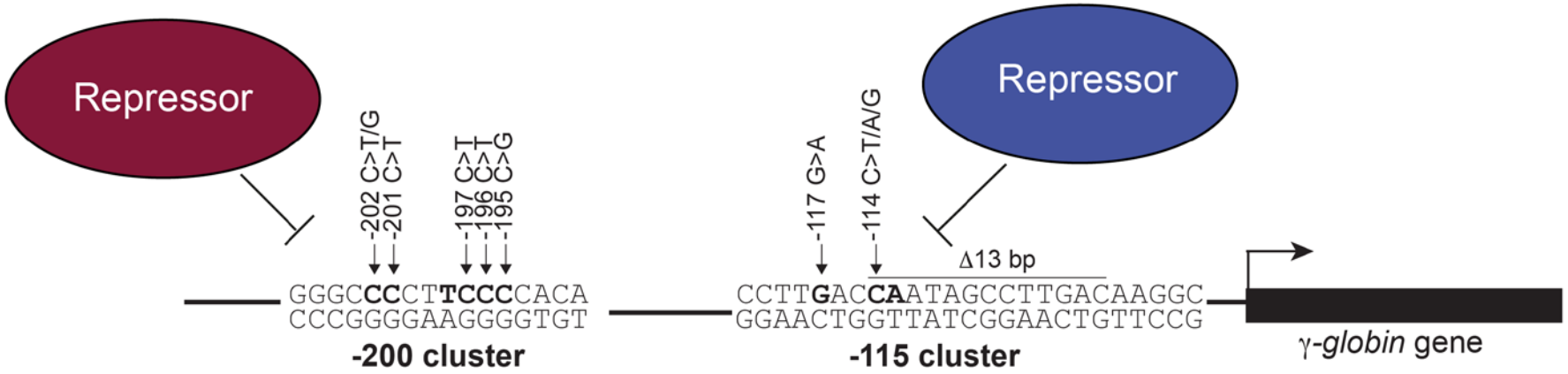

Identifying the -115 site transcriptional repressor

Gabriella Martyn

Performed a candidate screen on several key erythroid transcription factors

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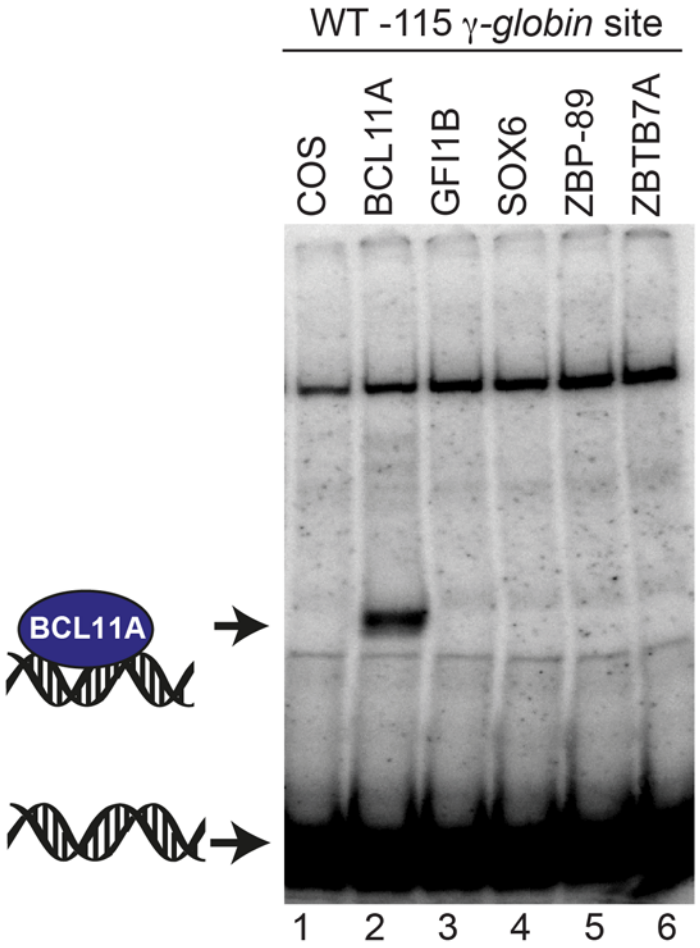

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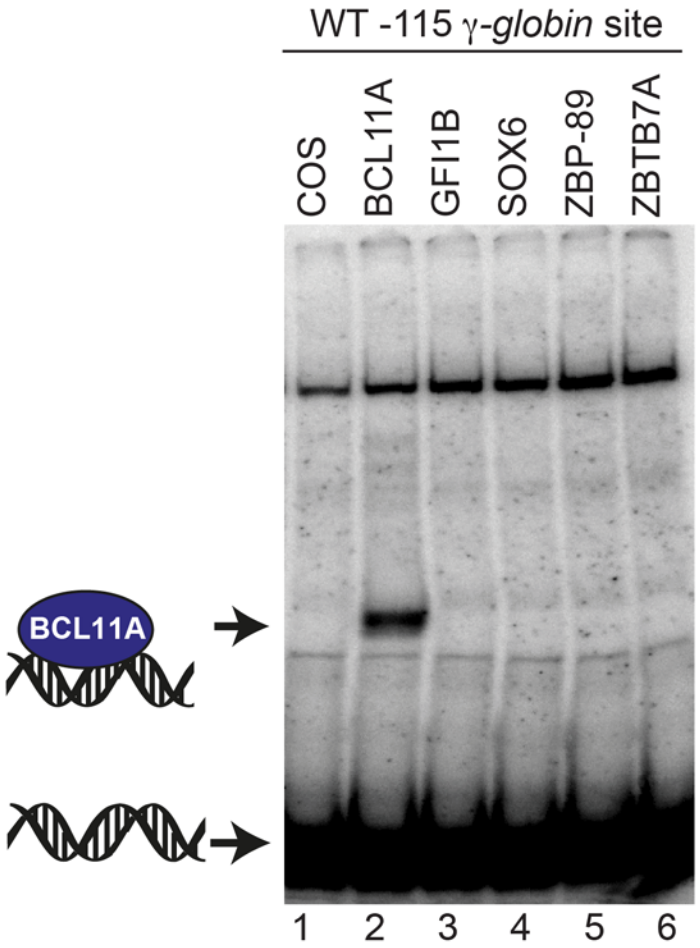
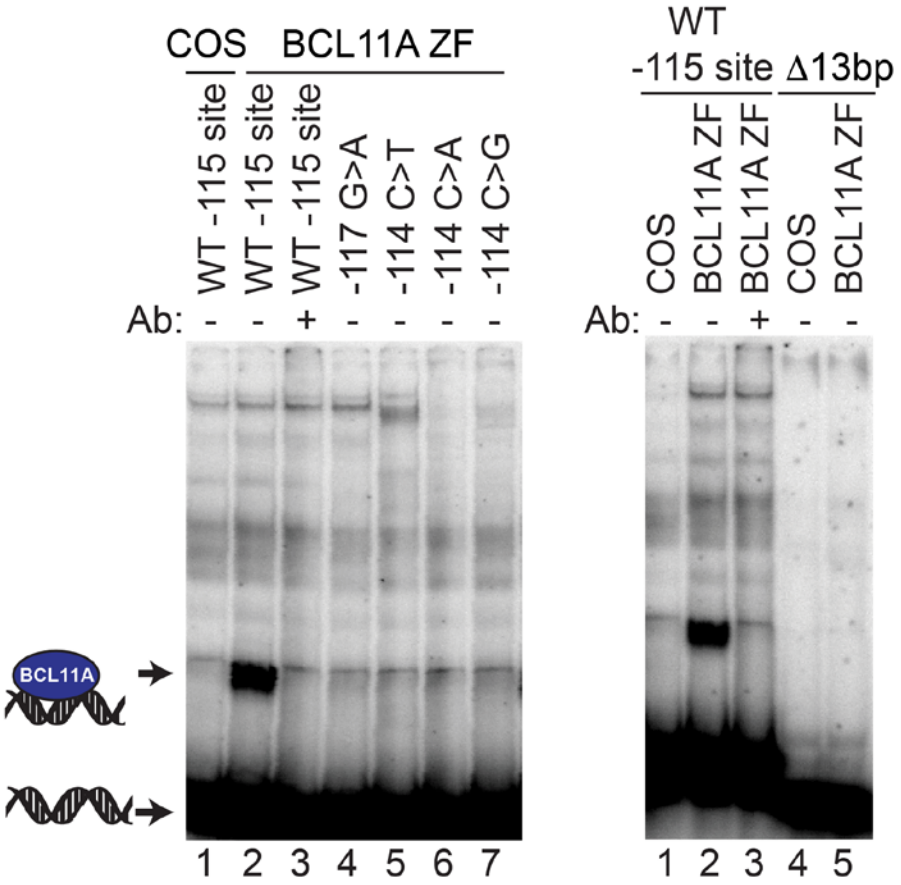


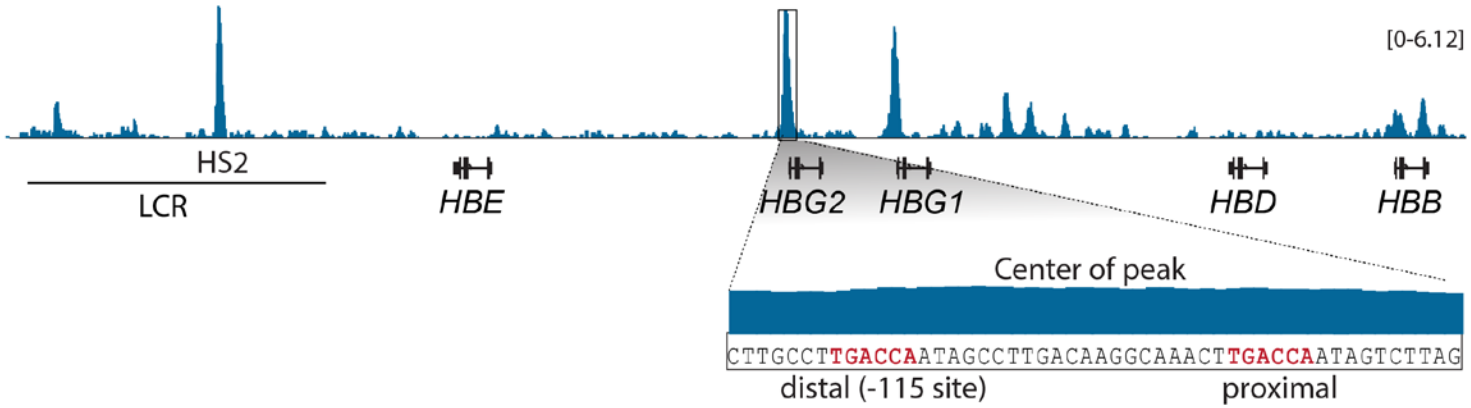
What do we know about BCL11A?

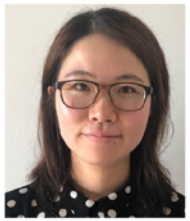
Previously shown by genome wide association studies (GWAS) to be a repressor of fetal globin

The known HPFH mutations disrupt BCL11A binding



Detecting BCL11A binding *in vivo*

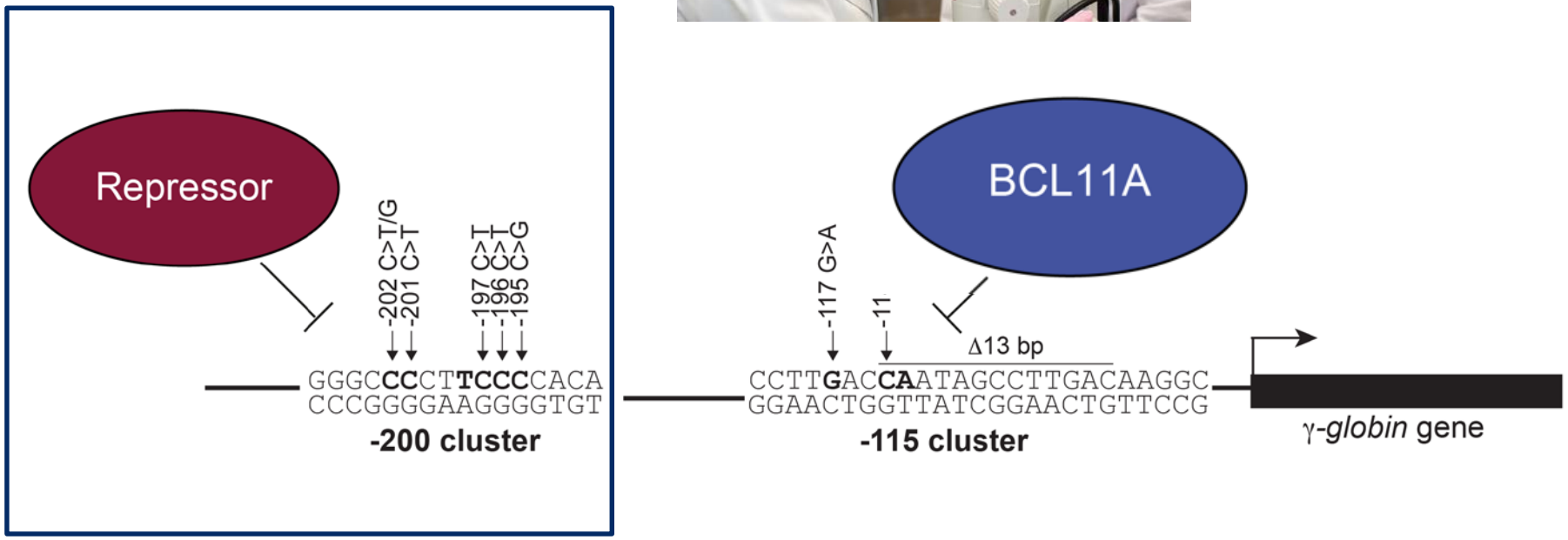




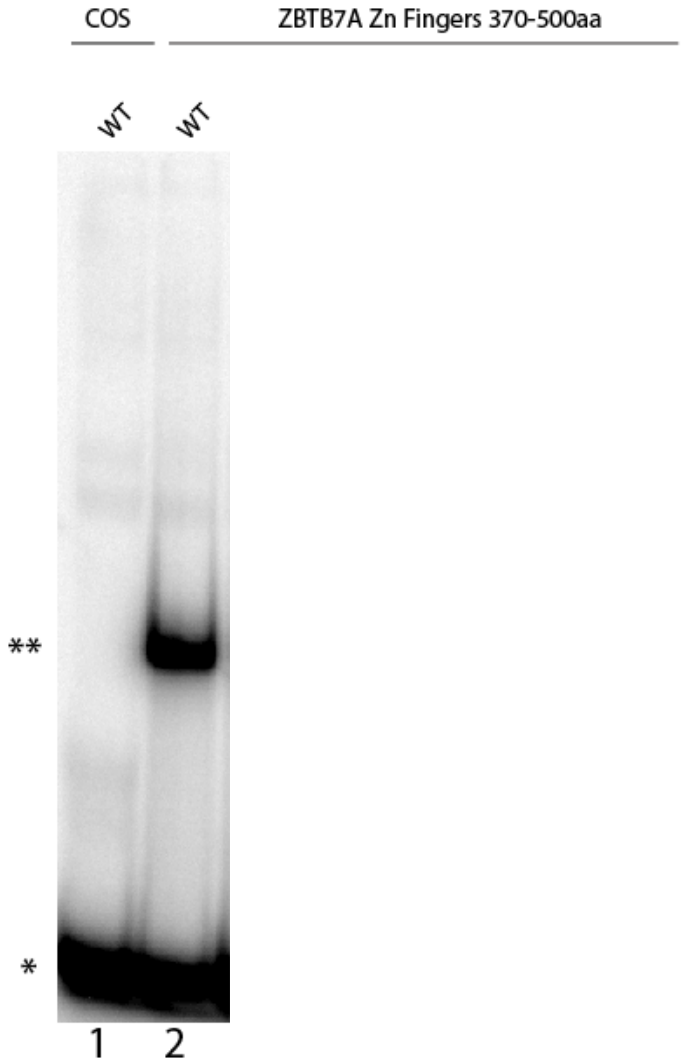
Lu Yang
(ChIP-seq)

Manan Shah
(ChIP-seq
analysis)

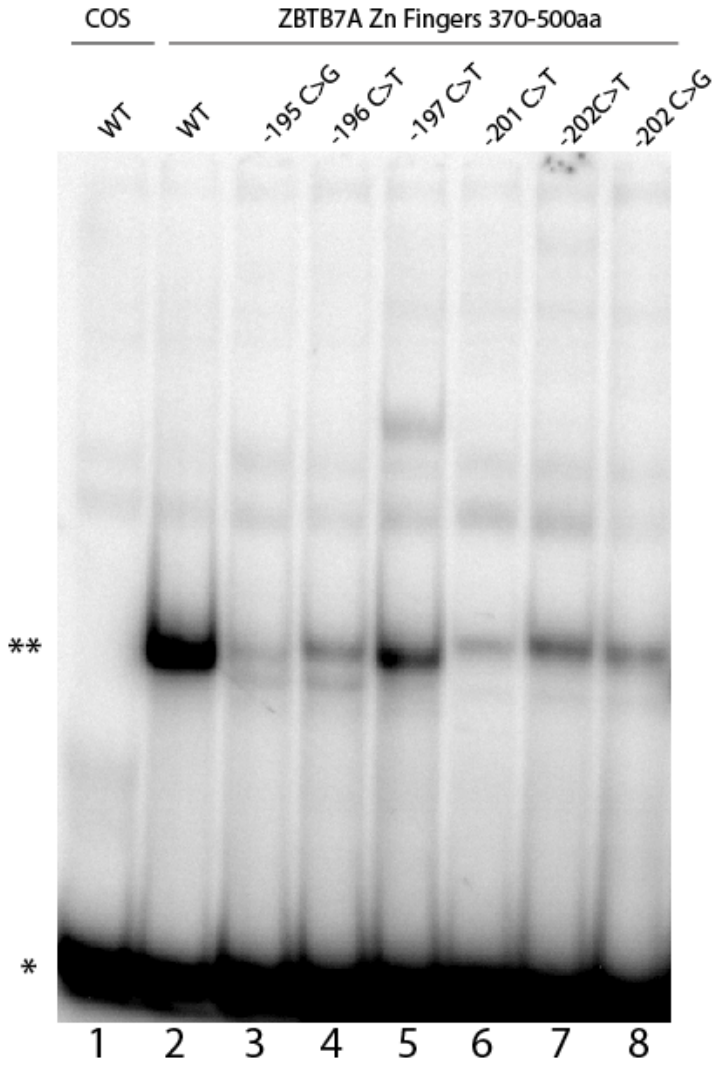
Beeke Wienert worked on the -200 cluster



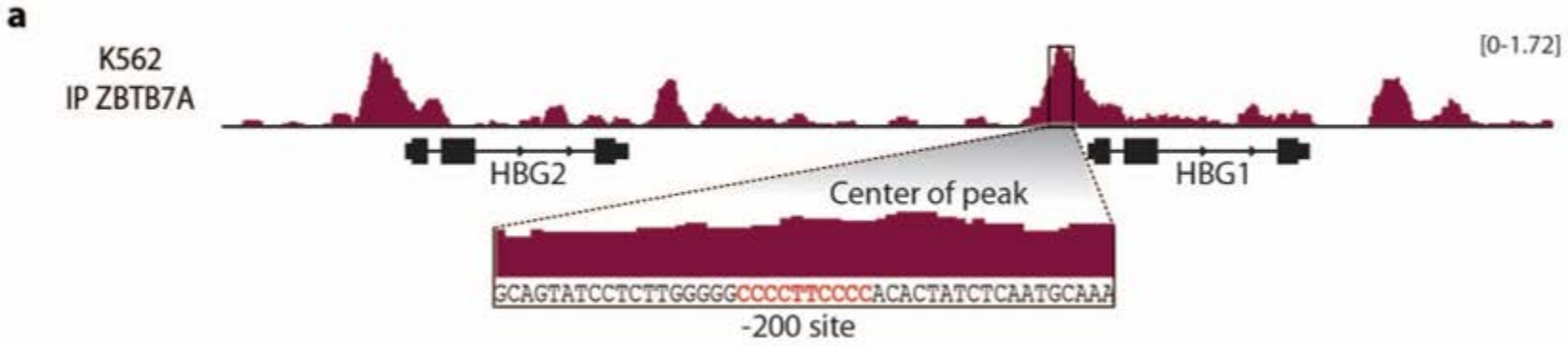
ZBTB7A/LRF binds the -200 region in vitro



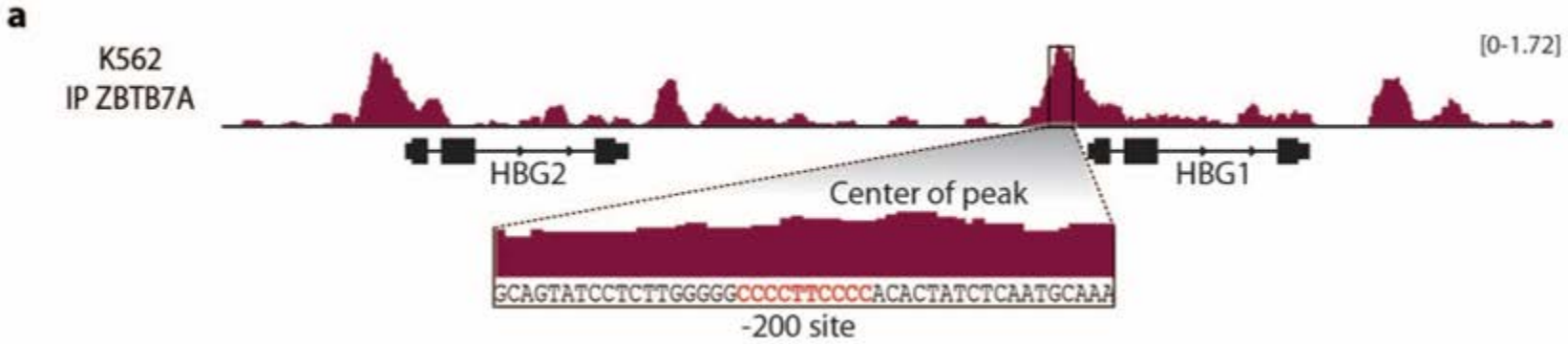
The -200 mutations disrupt ZBTB7A/LRF binding

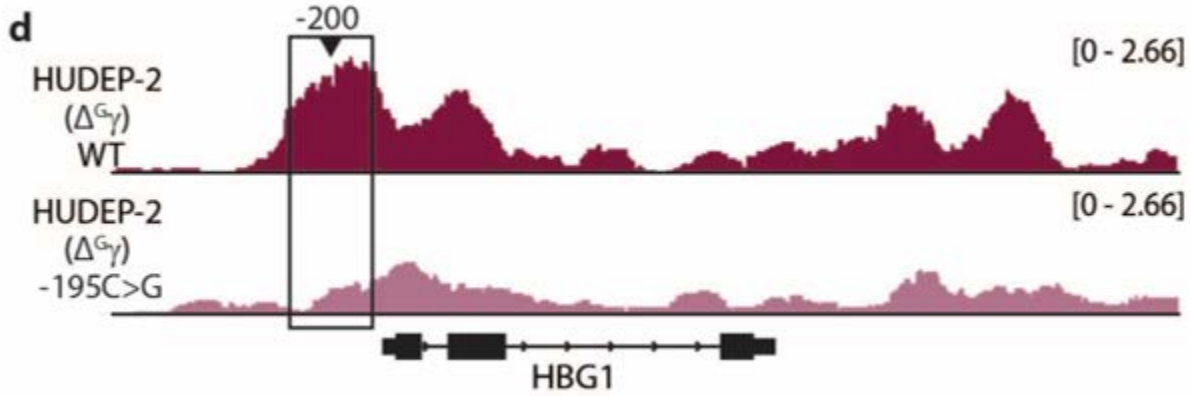


ZBTB7A/LRF binds the -200 site *in vivo*

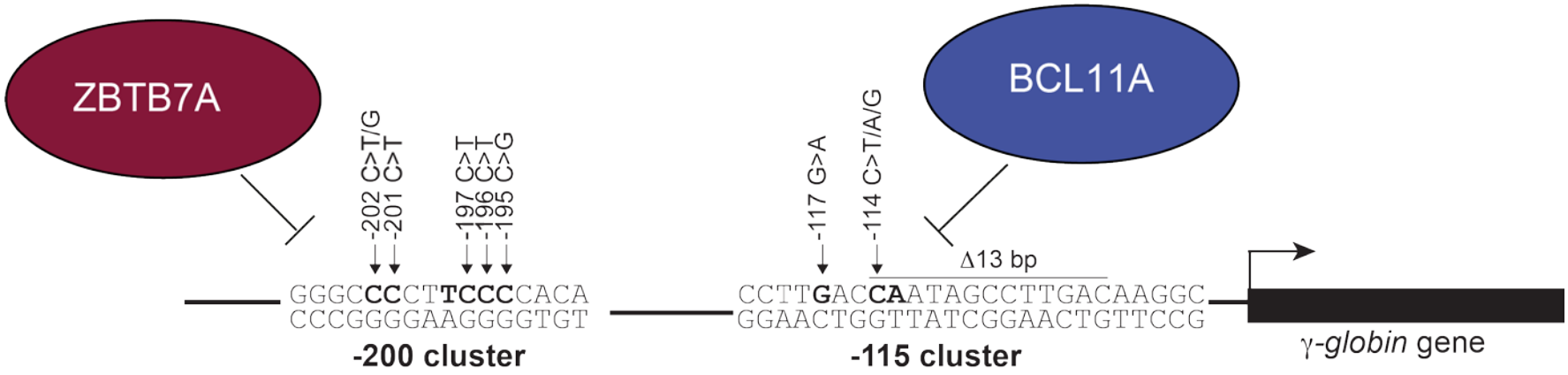


The -195 HPFH mutation disrupts binding





The mechanism of the HPFH mutations is solved



nature genetics

April 2018

Gabriella Martyn Dr Beeke Wienert

Co-first authors

UNSW SYDNEY

Current and future therapies

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- Making CRISPR-mediated deletions to disrupt fetal globin repression and boost its expression

Wienert…Matt Porteus…Merlin Crossley, Nat. Comm. 2015

Wienert…Merlin Crossley, Blood 2016

Ye … Y.W. Kan, Proc. Natl Acad. Sci. USA 2016

Traxler … Mitch Weiss, Nat. Medicine, 2016

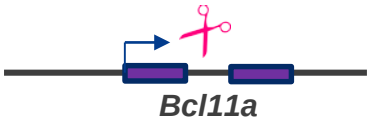
Antoniani…Matt Porteus…Anarita Miccio, Blood, 2018

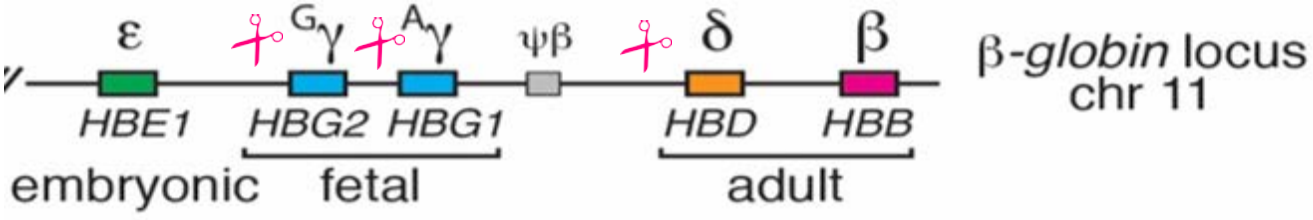
Li…André Lieber, Blood, 2018

Liu…Stuart Orkin, Cell, 2018

Martyn … Merlin Crossley, Nat. Genetics, 2018

Psatha … Thalia Papayannopoulou, Mol. Ther. Methods Clin. Dev. 2018

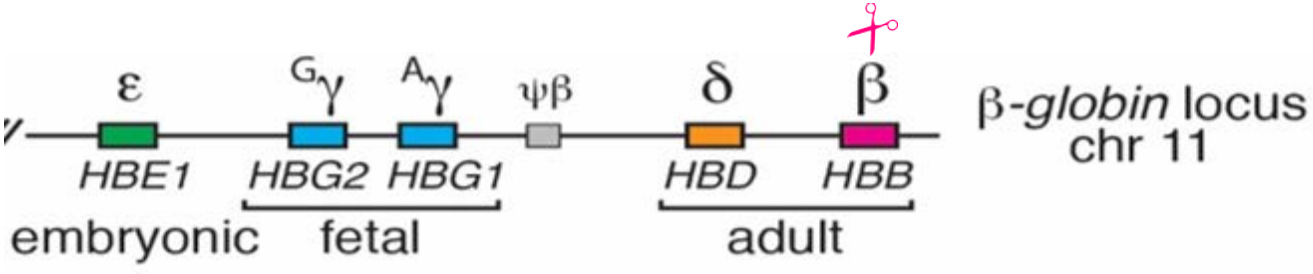




Current and future therapies

- Editing and Homology Directed Repair to correct the Sickle Cell mutation in blood stem cells

Dever … Matt Porteus, Nature 2016



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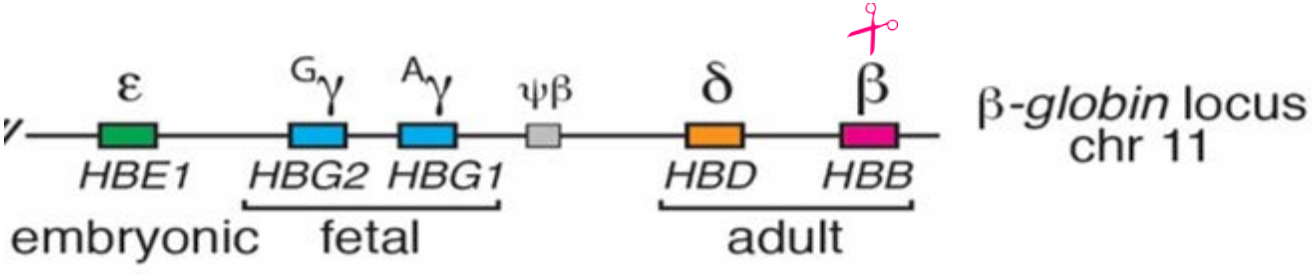
Dever … Matt Porteus, Nature 2016

- Or in Human Induced Pluripotent Stem Cells (hiPS)

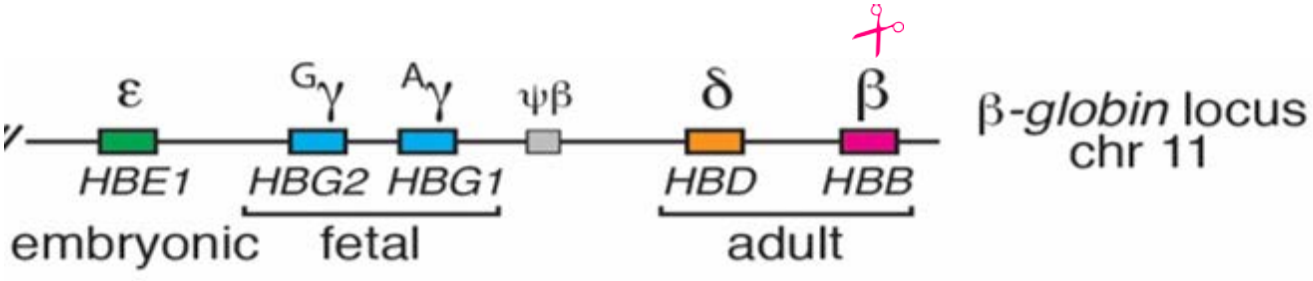
Ramalingam…Sivaprakash Ramalingam, Curr. Gene Therapy 2014



Current and future therapies

- CRISPR base editors to correct the mutation without any cutting

Liang … Junjiu Huang, Protein Cell 2017



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- Life-threatening, painful, lifelong, widespread and economically costly disorder
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- CRISPR therapies build on solid prior knowledge – of the disorder, the gene architecture, gene replacement gene therapy, stem cell transplants, etc
- Blood is well-understood and can be genetically manipulated and transplanted

Acknowledgements

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Australian Research Council

Australian Government
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NHMRC

UNSW AUSTRALIA

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Alister Funnell, UNSW (now Altius, Seattle)

Gabriella Martyn, UNSW

Beeke Wienert, UNSW

(now Berkeley, USA)

Lu Yang, UNSW

Manan Shah, UNSW

Kate Quinlan, UNSW

Merlin Crossley, UNSW

UNSW SYDNEY