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Genome Editing Offers Hope for Sickle Cell Disease Patients

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ABSTRACT

In a recent paper, DeWitt (2016) and colleagues optimise a gene editing approach utilising CRISPR/Cas9 in blood stem cells to correct the sickle cell disease mutation, boosting hope for new gene therapy clinical trials in the near future. In this review we discuss sickle cell disease: its cause, current treatment, and recent advances towards a gene therapy cure.

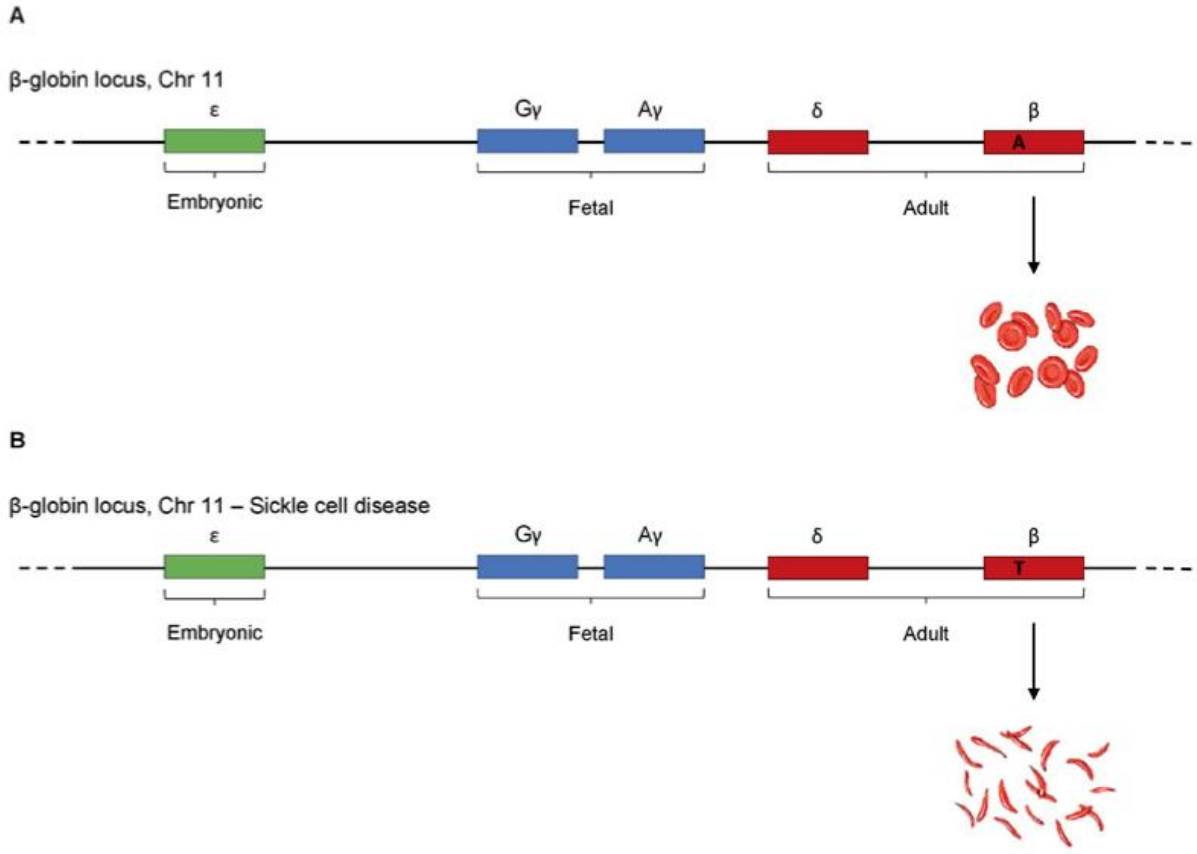
Keywords: Sickle cell disease, haemoglobin, haematopoietic stem cells, gene therapy, CRISPR/Cas9, genome editing

SICKLE CELL DISEASE AND CURRENT TREATMENT

Pain crises, anaemia, and frequent infections are but a few common symptoms of sickle cell disease (SCD). SCD is a monogenic disease caused by the autosomal-recessive inheritance of an A→T transversion in the β-globin gene (HBB; **Figure 1**; Frenette *et al.* 2007) that affects over 275,000 newborns every year worldwide, mainly of African descent. As such, it is a major cause of morbidity and mortality (Modell *et al.* 2008).

Although SCD was the first disease to be described on a molecular level, only one drug is currently approved by the FDA for its treatment — hydroxyurea. With an excellent safety profile and high effectiveness hydroxyurea is a useful treatment, but it is not a definitive cure (Platt 2008). The only cure currently available for SCD is a haematopoietic stem cell transplant from an unaffected donor, an expensive and complicated procedure that requires a perfect donor match and is not free of severe complications (eg graft vs. host disease). Therefore, the search for alternative therapies for SCD is ongoing.

Figure 1. The β-globin gene locus on chromosome 11 produces healthy adult haemoglobin (A), or sickled adult haemoglobin in SCD patients (B). ε globin (in green) is expressed in the first 3 months of embryonic gestation, after which it is silenced and is replaced by the fetal ^{G}γ and ^{A}γ globins (in blue), which dominate until about 6 months of age. From then on the adult δ and β globins (in red) make up about 97% of blood haemoglobin content, the rest being residual amounts of fetal haemoglobin. SCD is caused by an A→T transversion in the β-globin gene, and results in the production of sickled red blood cells.



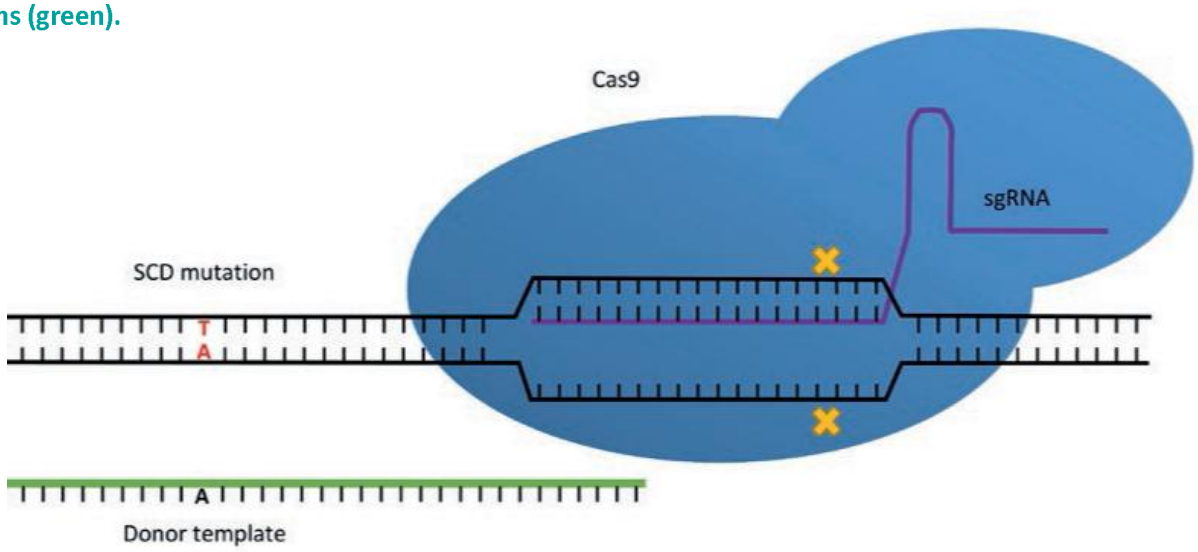
GENE THERAPY HERALDS NEW HOPE

Cue gene therapy, a lucrative approach for treating a monogenic disease caused by the lack of a functional copy of a gene. Ongoing clinical trials utilise lentiviral vectors carrying the *HBB* gene applied to patient-derived bone marrow stem cells *ex vivo*, which are later used for an autologous transplant (Makis *et al.* 2016). The procedure circumvents the need for a matching donor as well as the risk of rejection, making it a more practical strategy. Alas, the use of semi-randomly integrating lentiviruses poses the risk of insertional mutagenesis, which could potentially lead to malignant transformation as in the case of a 2000 clinical trial for X-linked severe combined immunodeficiency (SCID X-1; Hacein-Bey-Abina *et al.* 2008).

Genome editing, the modification of a patient's genome in select cells, holds promise to revolutionise medicine. It sidesteps the need for integrating vectors by the transient expression of targeted effector nucleases. The correction of the SCD mutation in haematopoietic stem cells (HSC) has been a much sought after solution to the aforementioned risks and problems. However, progress has been hindered by inefficient editing as well as difficulty targeting true HSCs capable of providing lifelong production of red blood cells carrying functional haemoglobin (Genovese *et al.* 2014).

The CRISPR/Cas9 gene editing system has revolutionised biomedical research. From functional gene screens, through disease modelling, to targeted editing for therapy, CRISPR allows a new level of control over the genome (Barrangou *et al.* 2016). Comprised of a nuclease (Cas9) directed to a 20 nucleotide target DNA sequence by a single guide RNA (sgRNA), CRISPR/Cas9 is a convenient two-component, programmable system for cleaving the genome. When combined with a DNA oligomer bearing homology to the targeted locus (termed

Figure 2. Schematic representation of a CRISPR/Cas9 ribonucleoprotein (blue) with a single guide RNA (sgRNA; purple) targeting the sickle cell disease (SCD) mutation (red), which introduces double stranded breaks (yellow) in the HBB gene (black); alongside a donor template containing the wildtype sequence with homology arms (green).



"donor template"), one can take use of one of the cell's own DNA damage repair mechanism, homology directed repair (HDR), in which it uses a similar template to rewrite the cleaved site in its own genome (**Figure 2**).

A CRISPR APPROACH TO TREATING SCD

In a recent study published in Science Translational Medicine, DeWitt — working at Jacob E. Corn's lab at the University of California, Berkeley — and colleagues (2016) optimised the gRNA and donor template combination, allowing them to achieve correction of up to 25% of alleles in SCD patient HSCs. Differentiation of these cells to erythrocytes produced red blood cells with significant amounts of wild type (WT) haemoglobin. Interestingly, the resulting erythrocytes also showed higher quantities of fetal haemoglobin, a haemoglobin variant comprised of the β-like γ globin that is expressed throughout the majority of gestation and is not affected by the SCD mutation. A similar approach previously reported by researchers at the University of California, Los Angeles, in collaboration with Sangamo Biosciences, achieved comparable results using different targeted nuclease (zinc finger nuclease) and donor template delivery method (integrase-defective lentivirus; Hoban *et al.* 2015).

One caveat to the approach taken by the researchers is the creation of unwanted mutations caused by Cas9 cleavage. Other DNA damage repair mechanisms, notably non-homologous end joining (NHEJ), are more commonly used by the cell to correct double-stranded breaks, such as the ones introduced by Cas9. NHEJ can result in small insertions or deletions (indels) that are not corrected based on the donor template (Ciccia *et al.* 2010), and the formation of indels in the *HBB* gene may disrupt its translation even further. Another potential risk is off-target cleavage by Cas9, ie cleavage of sites not exactly homologous to the intended target sequence. This could result in harmful mutations in important genes, such as cancer genes, but is exceedingly rare (Kim *et al.* 2016).

To minimise this risk, the researchers elected to use ribonucleoproteins (RNPs), pre-synthesised Cas9 proteins complexed with sgRNAs, targeting the SCD mutation on the *HBB* gene. The use of RNPs is preferable to DNA vectors (eg plasmids), as they do not make use of transgenic DNA. Moreover, RNPs have been shown to have lower off-target activity and cytotoxicity than DNA vectors (Kim *et al.* 2014). Overall, off-target activity in predicted sites showed minimal cleavage, mostly in non-coding regions, demonstrating the safety of the method employed in this study (DeWitt *et al.* 2016).

Perhaps the biggest obstacle to efficient, long-term treatment using autologous HSC transplants is the engraftment of edited, true HSCs, as opposed to more differentiated progenitors (Genovese *et al.* 2014). These progenitors do not self-renew over time, thus preventing the production of sufficient

amounts of red blood cells carrying WT haemoglobin. DeWitt *et al.*'s (2016) *in vivo* experiments showed promising results with long term engraftment of the edited human cells in the mouse bone marrow, spleen, and blood.

ALTERNATIVE STRATEGIES

Whilst the current study by DeWitt *et al.* (2016) provides a solid foundation for a gene editing approach to treating SCD, other methods are currently being developed. Of particular interest are two strategies recapitulating naturally occurring hereditary persistence of fetal haemoglobin (HPFH) mutations, which reverse the silencing of fetal haemoglobin and allow its production into adulthood (Lettre *et al.* 2016). The Orkin and Bauer research laboratories are currently pursuing the deletion of an erythroid-specific enhancer of a transcription factor necessary for fetal haemoglobin silencing known as BCL11A (Canver *et al.* 2015). Somewhat similarly, the Weiss lab is working on a deletion of a repressor binding site in the promoters of the fetal haemoglobin genes: *HBG1* and *HBG2* (Traxler *et al.* 2016). These approaches are advantageous as they do not rely on the arduous HDR, reduce the risk of unwanted mutations in the *HBB* gene, and could potentially be used *in vivo* utilising non-integrating viruses, such as the adeno-associated virus (AAV), to transiently introduce the gene-editing machinery into the bone marrow (Hoban *et al.* 2016). All aforementioned methods are expected to begin clinical trials within the next 5 years, alongside current studies focusing on gene transfer.

In summary, DeWitt (2016) and colleagues demonstrated that a homology-directed repair-based method using CRISPR/Cas9 ribonucleoproteins and donor template is a viable approach for the correction of the sickle cell mutation in sickle cell disease patient haematopoietic stem cells *ex vivo*. Furthermore, they showed that corrected cells can be successfully transplanted into mice, where they engraft and provide long-term reconstitution of the blood system. Together with similar gene editing methods, it is expected that gene therapy trials, and perhaps clinical use for SCD treatment, will become available within the not-so-distant future — a long awaited new treatment for an old disease.

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GENOMIEDITOINTI TUO TOIVOA SIRPPISOLUANEMIAPOTILAILLE

Solujen geenimuokkaus sirppisoluanemiassa on eräs mahdollinen tulevaisuuden hoitomuoto. DeWittin (2016) työryhmän uusi tutkimus kuvaa erään CRISPR/Cas9-tekniikan käyttömahdollisuuden. Kuvaamme taudin taustaa, hoitoa sekä tulevaisuuden mahdollisuuksia uusiin terapioihin, jotka perustuvat geenien käyttöön ja muokkaukseen.

Avainsanat: sirppisoluanemia, hemoglobiini, hematopoieettinen kantasolu, geeniterapia, CRISPR/Cas9, genomin muokkaus

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