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CARDIOVASCULAR RESEARCH Onlife

Therapeutic gene editing, making a point

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Commentary on 'Treatment of a metabolic liver disease by *in vivo* genome base editing in adult mice' by Villiger *et al.*, *Nat Med* 2018 and '*In utero* CRISPR-mediated therapeutic editing of metabolic genes' by Rossidis *et al.*, *Nat Med* 2018.

Gene editing tools like clustered regularly interspaced short palindromic repeat (CRISPR)-CRISPR associated 9 (Cas9) have given us a new perspective on a potential treatment of genetic disorders, for instance by correcting disease-causing mutations or by inducing disease-supressing alterations in the genome. Given its immense potential, it comes as no surprise that the field of gene editing is developing at extremely high speed, even though the safety and side-effects are not yet fully established.[1] Although the discussion regarding the (ethical) boundaries of gene editing technology in humans has recently been sparked by the report of CRISPR-edited babies, it remains ever so important to assess the progress that is being made towards clinical applications.

Two recent papers published in *Nature Medicine* by Rossidis *et al.* and Villiger *et al.* are indicative of the advances in repairing potentially life-threatening mutations using specific and more efficient approaches than the classical CRISPR-Cas system. Both research groups apply an adapted form of CRISPR-Cas technology in combination with Base Editing enzymes to specifically induce point mutations *in vivo* leading to reversal of metabolic liver diseases in the adult,[2,3] or even as early as *in utero*.[3]

The classic CRISPR-Cas system relies on a cell's intrinsic propensity to repair double-stranded (ds) DNA breaks. Targeting an exact location of a dsDNA break can be achieved by directing a bacterial-derived nuclease (such as Cas9) to a specific chromosomal locus using a short guideRNA. Binding of this complex will result in a dsDNA break. This will kick the cell's DNA repair machinery into gear in an attempt to re-join the two edges, either via non-homologous end-joining (NHEJ) or through homology directed repair (HDR). NHEJ is the primary repair mechanism, and is achieved by re-ligating the broken ends, often resulting in the alteration several nucleotides from each strand thereby leading to random inserts or deletions (indels). HDR, which only occurs in certain phases of the cell cycle, depends on the availability of homologous regions of DNA that can serve as a template for DNA repair. A desired genetic modification in the genome can be achieved by providing exogenous partially homologous stretches of DNA as a template for HDR, but this process is extremely inefficient.

To correct most monogenetic diseases, a modification of a point mutation rather than a random disruption of the gene would be required. Research has therefore focussed on optimizing HDR efficiency, but it remains low in therapeutically relevant settings. Therefore, other approaches could be more promising, including the recently developed CRISPR-Cas associated base editors. This elegant system relies on a catalytically inactive-Cas9 fused to a cytidine deaminase that can change C-G base pairs into T-A.[4] Using a guideRNA, the base editing complex can be targeted to a specific locus, where it edits the sequence within a window of approximately five nucleotides without inducing ds breaks, and independent of HDR.[5]

In the two recent manuscripts, the therapeutic potential of base editing has been explored in the setting of metabolic diseases. Villiger *et al.*[2] investigated its applications in a model for the human autosomal liver disease phenylketonuria (PKU) which causes microcephaly, severe retardation, and seizures in humans. Phenylalanine hydroxylase (Pah)^{enu} mice harbour a point mutation in the *Pah* gene on exon 7, where a T is replaced by a C (c.835T>C), thereby inactivating the enzyme leading to a raise in L-Phe blood levels and consequent hyperphenylalaninaemia. Using a cleverly designed split Cas9 adeno-associated virus (AAV)-8 for *in vivo* delivery,[6] a cytidine deaminase base editor was guided to the mutated locus in liver cells, resulting in correction of the mutation. Within 4 weeks after tail vein injection of the virus, Pah^{enu} mice displayed a dose-dependent drop of phenylalanine to baseline levels leading to reversal of PKU, induction of weight gain, and even resulting in a change of coat colour as shown previously.[7] Strikingly, *Pah*-mRNA correction rates were ~43% after 14 weeks, with even 63% reported at very high virus dosages, and no obvious off-target effects. Interestingly, there are AAV vectors available (e.g. AAV9) that specifically target the heart, together with the fact that base editing can occur in non-dividing cells like cardiomyocytes, this approach holds great promise to treat genetic cardiac diseases.

A similar approach was used by Rossidis *et al.*[3] to provide proof-of-concept that base editing can be applied *in utero*, offering new possibilities to cure peri- and neonatal lethal diseases. For their experiments, adenoviral particles containing the CRISPR-base editing system were injected into the vitelline vein, which drains the blood from the yolk sac, of developmental day 16 mouse embryo's. In the first approach they targeted murine *Pcsk9* which is involved in regulation of the low-density lipoprotein (LDL)-receptor protein. Previous studies have shown that loss-of-function mutations in PCSK9 can reduce plasma cholesterol levels and reduce the risk of coronary heart disease[8] making it an interesting target to explore for cardiac disease prevention. Interestingly, *in utero* gene editing resulted in ~10% base-edited alleles which was sufficient to lower cholesterol levels. Moreover, a very low rate of indels was

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observed compared to previous post-natal studies targeting PCSK9.[9] While the editing rates declined in post-natally treated animals, it persisted in prenatally treated animals.

In their most exciting experiment, Rossidis *et al.*[3] showed the possibility of *in utero* base editing to tackle a neonatally lethal disease via inducing a disease-supressing mutation. Hereditary tyrosinaemia type 1 (HT1) is caused by a mutation in the *Fah* gene that blocks the tyrosine catabolic pathway. Ultimately, this will lead to accumulation of toxic metabolites and subsequent liver failure. Accumulation can be prevented temporarily by inhibiting the upstream HPD enzyme with the drug nitisinone. $Fah^{-/-}$ mice are a model for HT1 and display neonatal lethality within 20 days after birth. By administering a base editing system targeting *Hpd* to day 16 mouse embryo's it was possible to rescue the lethal the phenotype, with pups showing a survival similar to nitisinone treated animals up to at least 3 months.

These two studies are amongst those that pave the way for treating genetic disorders in the adult or even before birth. While both studies targeted metabolic diseases, it is easy to imagine these methods to be applied for cardiovascular diseases. Importantly, it may provide a therapy for genetic diseases that currently remain without a cure. Moreover, the ability to make point mutations in the genome is an excellent tool to mimic disease inducing mutations and to study variants of unknown significance *in vitro*. For example, human induced pluripotent stem cells differentiated into cardiomyocytes provide a great model to investigate the effects on mutations found in relation to cardiac disease.[10] In the future, we may have the ability to cure or prevent the onset or progression of cardiomyopathies due to mutations in sarcomeric genes, sodium channels,[11] or mitochondrial proteins; or to influence monogenic diseases with cardiac traits like Duchenne's Muscular Dystrophy. Hopefully, after establishing its safety and efficiency, gene editing will definitively enter the clinical arena.

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Biography: Dr Anke Smits obtained her PhD in Cardiovascular Cell Biology at the Department of Cardiology, University Medical Center in Utrecht (the Netherlands). She focused on cell-based therapy to repair the injured heart. After securing a Rubicon fellowship (2011–13) for mobility of young talent from the Netherlands Organisation for Scientific Research (NWO), she moved to the University College London (UCL), and Oxford University to study the epicardium as a source for cardiac repair. She was able to continue her work at the Department of Cell and Chemical Biology at Leiden University Medical Center (LUMC, the Netherlands) after being granted a Veni fellowship from NWO (2014–17), and a fellowship from the LUMC. Dr Smits will continue to work on influencing repair after cardiac injury via cell-mediated mechanisms at the LUMC funded by a Dekker fellowship from the Dutch Heart foundation which enables her to build her own research group.