Gene Editing with CRISPR: A Novel Approach to Type 1 Diabetes Management

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Abstract

Type one diabetes, a genetic predisposition, affects roughly 8.4 million people around the globe. It is a chronic, autoimmune condition where the body's immune cells respond to and attack insulin-producing cells within the pancreas. While there is currently no known treatment for type one diabetes, there have been many proposed treatments for type 1 diabetes, the most prevalent being the CRISPR-Cas9 gene editing complex. In this study, we will be exploring various genetic sequences CRISPR-Cas9 can edit to treat type 1 diabetes, and explore the potential downstream effects of either non-homologous end joining, homology directed repair, or site directed mutagenesis of the proposed sequences.

Key words: CRISPR, type 1 diabetes, CTLA4, single nucleotide polymorphism, Akita mutation

Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease affecting roughly 9.5 million people around the globe (Ogle et al). T1D is characterized by T-cell mediated destruction of insulin-producing beta cells located in the pancreas, leading to reduced insulin production and skyrocketing blood glucose concentration (Burrack et al). Symptoms of T1D include excessive thirst, frequent urination, and unexplained weight loss (Cleveland Clinic). Further development of T1D can lead to a life-threatening complication known as diabetes-related ketoacidosis (DKA), with symptoms like nausea/vomiting, confusion, and loss of consciousness. In the absence of insulin, a vital protein in glucose oxidation, the liver undergoes fatty acid oxidation and releases ketones into the bloodstream, which lowers blood pH (Cleveland Clinic). Genetics and environmental factors play key roles in the onset and progression of the disease, with the number of T1D global cases rising rapidly in lower-income settings (Ogle et al). Furthermore, T1D is most prevalent in populations of European ancestry, though cases of T1D have occurred in those with Asian, African, and Hispanic ancestry. The cause of type 1 diabetes has been proposed to be the result of either genetic predisposition or variations in or around sequences responsible for insulin or autoimmune traits, such as the insulin (INS), human leukocyte antigen (HLA), cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), and interleukin-2 receptor alpha chain (IL2RA) genes, may result in higher type 1 diabetes risk (Redondo et al).

One example of a genetic predisposition includes modifications to the human leukocyte antigen (HLA), which are genes in the major histocompatibility complex responsible for immune proteins that distinguish between self and non-self (Nordquist et. al). Haplotypes and genotypes formed in the DRB1-DQA1-DQB1 loci have been shown to display the strongest association between T1D and the HLA region (Noble et al). Specifically, the heterozygous combination of “DR3/DR4”, was detected in 90% of European T1D cases and confers the highest risk of T1D development (McGrail et al).

Current treatment for T1D involves frequent insulin injections, providing patients with a source of insulin that they are unable to produce themselves. However, T1D patients undergoing insulin replacement therapy often experience severe hypoglycaemia episodes, a lifelong dependency on exogenous insulin, insulin resistance, mild obesity, and psychiatric conditions (Pathak et al). Additionally, efficacy of insulin replacement therapy is limited, as exogenous insulin is unable to fully replicate the biological actions of endogenous insulin (Pathak et al).

CRISPR/Cas-9, a powerful gene editing complex, offers a more targeted approach for the treatment of T1D. Originating in the *Escherichia coli* (E. coli) viral defense mechanism, CRISPR/Cas-9 works by using a designed guide RNA to locate the gene of interest. There, the Cas-9 nuclease cleaves the DNA in three different methods: non-homologous end joining, homology directed repair, and site directed mutagenesis (Asmamaw et al). These methods involve gene deletion, replacement, or single-base substitution, respectively (Figure 1). Since somatic cells are capable of duplicating rapidly, type 1 diabetes treatment can expand from lifelong insulin injections to a few administrations of gene therapy treatment.

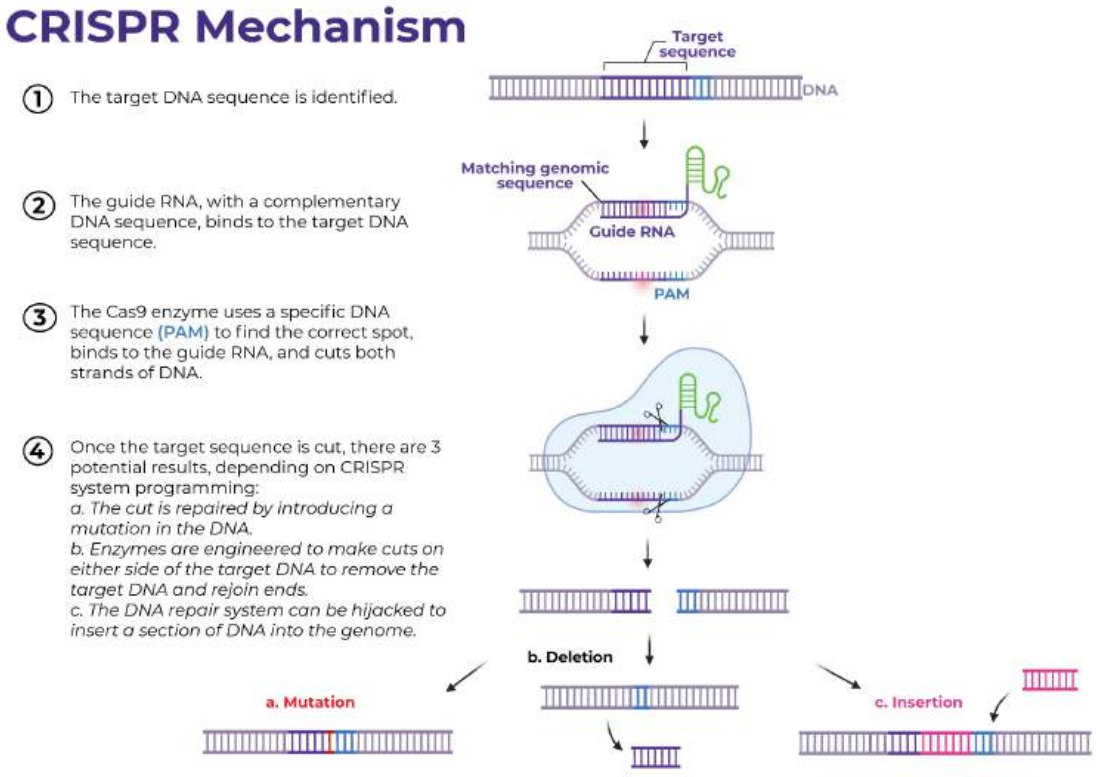

Figure 1: The mechanism of action of CRISPR. Once a target sequence is identified, a complementary oligonucleotide called the guide RNA binds to the target DNA sequence. Once binding occurs, the Cas9 protein uses a specific DNA sequence, PAM or protospacer adjacent motifs, as a signaling cascade for nucleic acid cleavage. Following the cleavage event, the damage is repaired by either non-homologous end joining, homology direct repair, or site-specific mutagenesis resulting in a mutation, deletion or insertion of the target sequence. Figure is adapted from the RNA Institute at UMass Medical School (UMass Med).

While CRISPR/Cas-9 is a powerful tool with potential and variability, ethical concerns and human germplasm cell controversies limit widespread use of the gene editing complex. The death of Jesse Gelsinger, an American teenager who underwent gene therapy in 1999, caused widespread skepticism and hesitation to use gene therapy on humans. Four days after the gene therapy administration, Gelsinger succumbed after experiencing a large-scale autoimmune response to the insertion of the viral vector carrying the ornithine transcarbamylase gene (Gostimskya). Currently, the CRISPR/Cas-9 system is being used to test various sequences responsible for type 1 diabetes, such as the protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene.

In this study, I plan to explore safe and efficient ways of using the CRISPR/Cas-9 complex to alter various genetic mutations involved in the onset of type 1 diabetes, specifically involving the human insulin gene and the CTLA-4 coding gene.

Discussion

INS Gene Sequence

The human insulin (INS) gene, a genetic sequence located on chromosome 11p15.5, contains instructions for creating preproinsulin, a precursor protein of insulin. It consists of three exons and two introns, with the exons coding for A, B, and C chains of the preproinsulin (Figure 2).

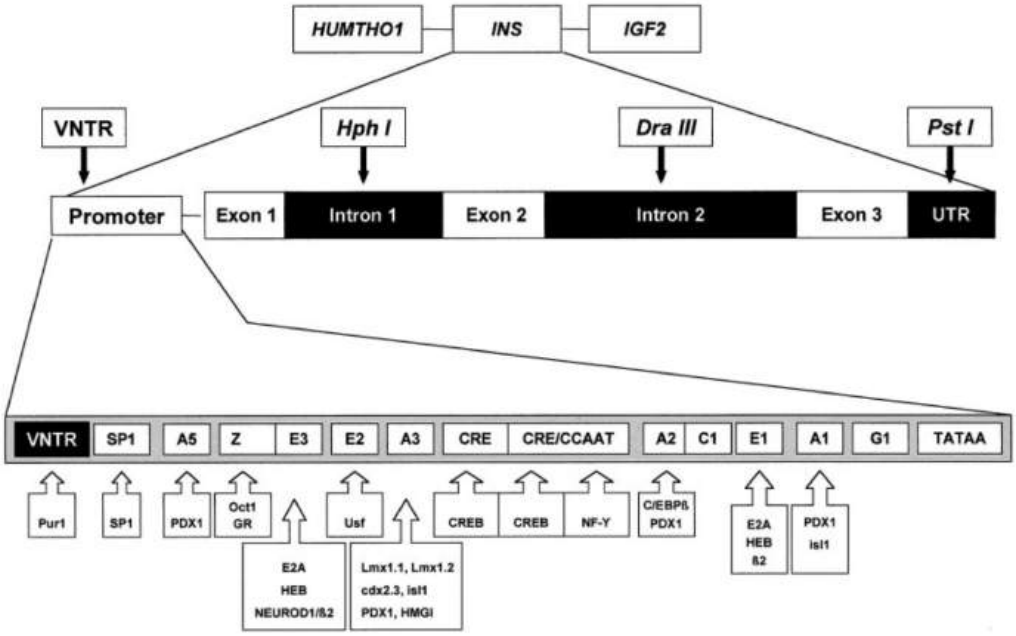

Figure 2: Schematic representation of chromosome 11p15. This region of the chromosome encodes insulin (INS) as well as tyrosine hydroxylase (HUMTHO1), and insulin-like growth factor-II (IGF-2). Figure adapted from (Pugleise et. Miceli).

Preproinsulin is initially formed in the cytoplasm of pancreatic ß-cells, and transforms into proinsulin when signal peptidase in the endoplasmic reticulum (ER) processes and removes the signal peptide. Afterwards, proinsulin undergoes oxidative folding, resulting in the formation of three disulfide bonds. This allows the proinsulin to exit the ER and be processed by the Golgi apparatus and secretory granules, leading to the formation of C-peptide and mature insulin (Liu et. al)(Figure 3). While pancreatic ß-cells mainly express this gene and produce insulin, evidence of small insulin production by the fetal central/peripheral nervous systems (CNS/PNS), adrenal gland, retina, yolk sac, gut, and mammary gland has been found. However, most cells do not express INS and pancreatic ß-cells are the only cells with the proper machinery for insulin storage and release during glucose stimulation. Nevertheless, extra-pancreatic insulin excretions may prove effective in T1D treatment, though its effects must be studied further. (Pugleise et. Miceli)

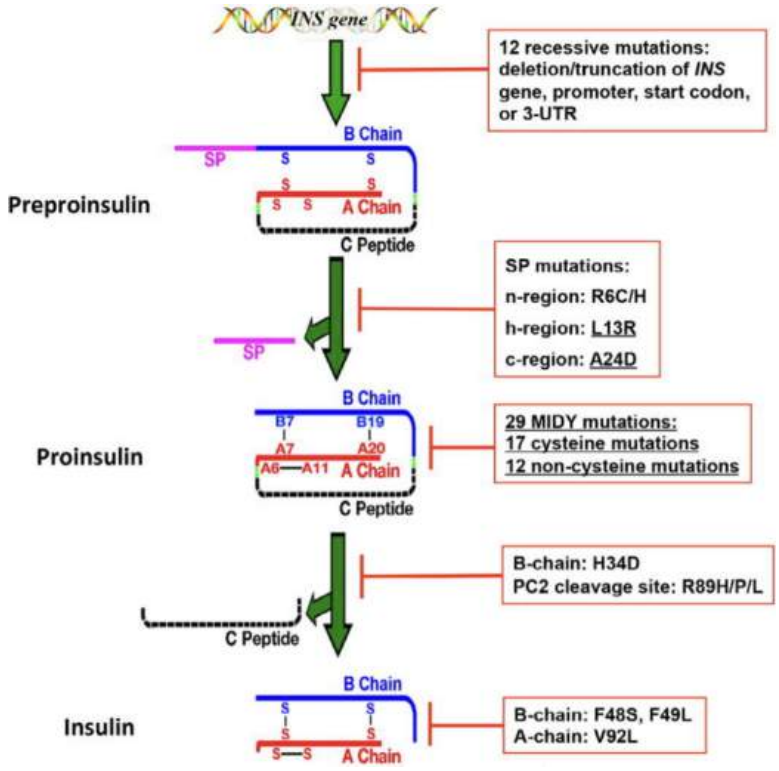

Figure 3: The molecular pathway responsible for converting preproinsulin to insulin. Along the pathway of insulin formation, several mutations pose threats for disrupting the final product, namely in the formation of preproinsulin from the INS gene, and the formation of proinsulin from preproinsulin. Figure is adapted from (Liu et. al).

Mutations in the INS gene can result in dramatic reduction of insulin production and can be split into two categories: recessive and dominant. Around 80% of insulin gene mutations are autosomal dominant, and around 77% of these mutations cause Mutant INS-gene induced Diabetes of Youth (MIDY) (Liu et. al).

Ins2 Mutation - Akita

In a 1999 case study of maturity-onset diabetes of the young (MODY) Akita mice, involved researchers discovered a missense mutation in the insulin 2 (Ins2) gene. A cysteine residue at the seventh amino acid of the A chain was replaced with tyrosine (Figure 4), leading to the formation of only one disulfide bond between the A and B chains (Wang et. al) and a faulty proinsulin-2 molecule. This mutant proinsulin-2

accumulated in the ER, causing ER stress due to a gain-of-toxic function and eventual ß-cell death (Ron). Expression of MIDY blocks wild-type proinsulin from exiting the ER, which decreases overall insulin production.

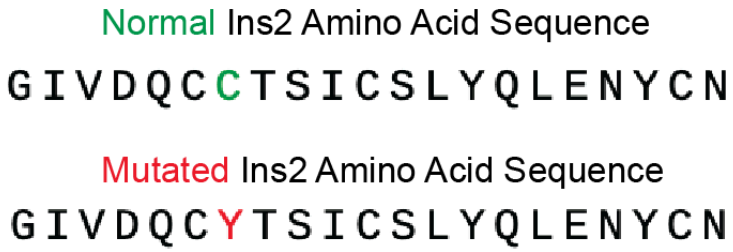

Figure 4: Ins2 Amino Acid Mutations. Mutation occurs in the 7th amino acid of the A chain from a cysteine residue to a tyrosine. This results in the formation of two less disulfide bonds, leading to misfolding and a faulty proinsulin-2 molecule.

CTLA4-coding Gene Sequence

CTLA4, an immune checkpoint receptor located on chromosome 2q33 (chr2:203,853.888—203,873,965, GRCh38), encodes an immune checkpoint receptor that inhibits T-cell activation and counterbalances the stimulatory effects of CD28, a T-cell surface protein. The genetic sequence encoding CTLA4 contains 4 exons (Figure 5), with exon 1 encoding the leader peptide, exon 2 the ligand binding domain, exon 3 the transmembrane domain, and exon 4 the cytoplasmic tail of the protein (Gough et. al). CTLA4 mainly functions by stopping autoimmune T-cells at the initial stage of T-cell activation (Buchbinder et. Desai). Regulatory T-cells (Tregs) express CTLA4, which binds to CD80 and CD86 on antigen presenting cells (APCs). CTLA4 has a higher binding affinity to C80 and C86 than C28, enabling it to outcompete stimulatory signaling and maintain immune tolerance (Rowshanravan et. al). Polymorphisms within CTLA4, such as rs231775, have been linked to altered protein expression and function, which contributes to increased susceptibility of T1D (Chen et. al).

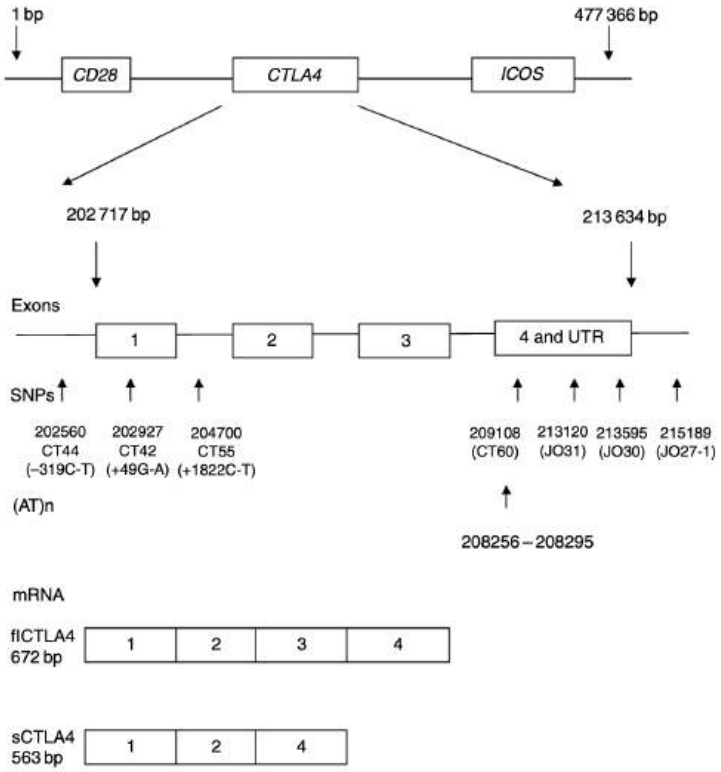

Figure 5: CTLA4 gene map with the locations of common SNPs. The 477,366 base stretch of chromosome 2, region 2q3 contains three genes, CTLA4, CD28, and ICOS, with CTLA4 containing 4 exons. Figure is adapted from (Gough et. al).

CTLA4 Signaling Pathway

While CTLA4 acts as a CD28 homolog with greater binding affinity to CD80 and CD86, it competitively reduces the costimulatory signal generally produced by CD28 + CD80/86 binding. Since the relative amount of CD28 + CD80/86 binding determines whether a T-cell undergoes activation or anergy, an increased presence of CTLA4 reduces T-cell production and survival, leading to a weaker immune response. Furthermore, Tregs utilize CTLA4 to regulate effector T cell functions and maintain peripheral tolerance (Figure 6)(Buchbinder et. Desai).

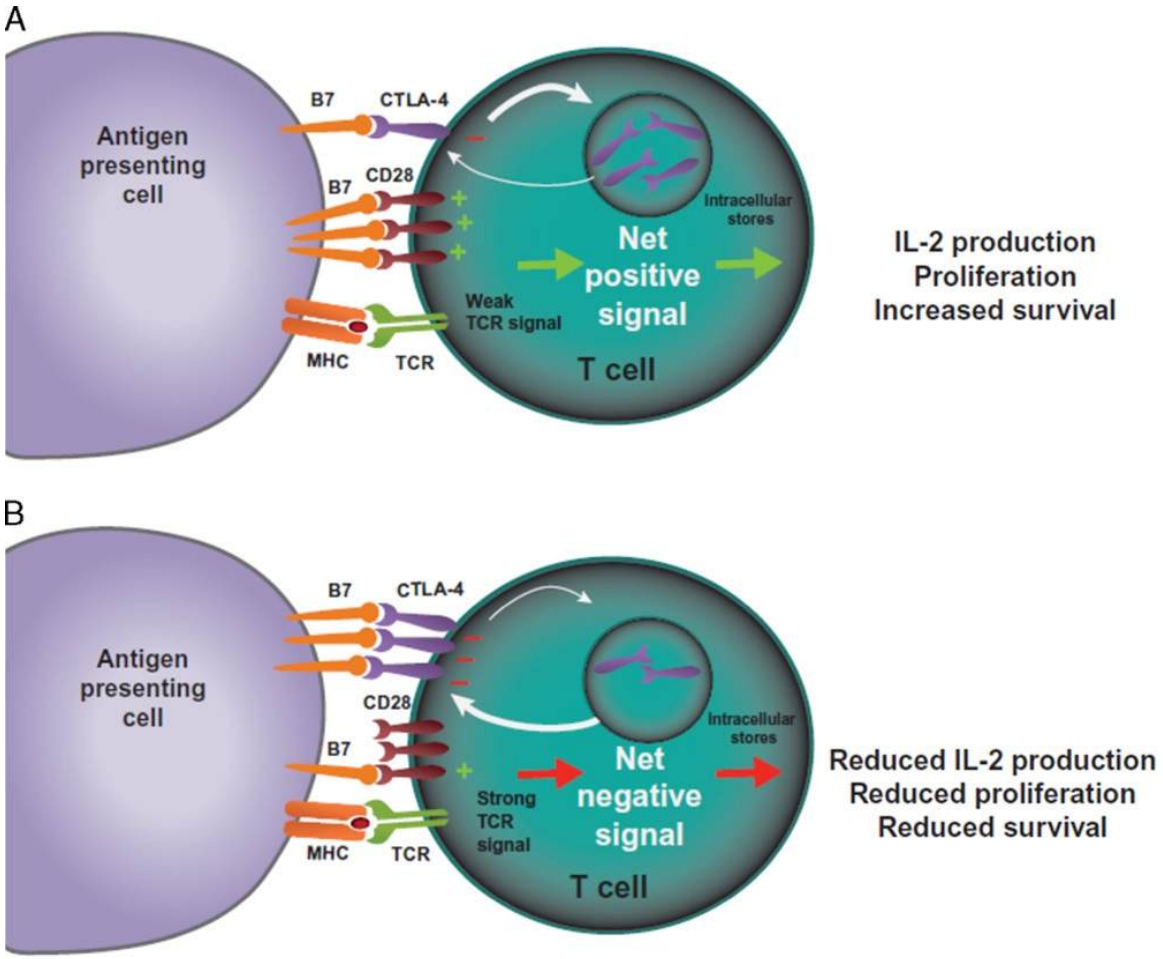

Figure 6: Signaling pathway of CTLA-4. In the presence of an antigen presenting cell, CTLA-4 works to regulate T cell production, keeping the host immune response from damaging healthy cells. Figure adapted from (Buchbinder et. Desai).

CTLA4 +49 G/A (rs231775)

CTLA4 +49 G/A is a single nucleotide polymorphism (SNP) from A to G on exon 1 position 49 (Figure 7) that causes an amino acid exchange from threonine to alanine, leading to misfolding in the leader peptide and a loss of function in the CTLA4 receptor (Table 1). A study describing the polymorphism of CTLA4 +49 G/A discovered that a homogenous combination of CTLAA4 +49 GG/AA conferring a higher risk of T1D than a heterozygous combination (Hossen et. al). According to a recent study, CTLA4 +49 G/A, along with another gene variation called CT60, is significantly associated with type 1 diabetes risk in the Chinese Han Population. (Chen et al.). In fact, patients carrying

these risk alleles had a 65% increased chance of developing T1D compared to patients with normal CTLA4 alleles.

Figure 7: CTLA-4 Sequence Mutations. Mutation occurring in the 49th position of exon 1 from an alanine to guanine results in an amino acid change from threonine to alanine. This amino acid exchange leads to misfolding and loss of function in the CTLA-4 receptor.

Feature	**Normal (A allele)**	**Mutated (G allele)**
Nucleotide at cDNA position 49	A	G
Codon (position 17)	**A**CC	**G**CC
Amino Acid	Threonine (Thr)	Alanine (Ala)
Effect	Normal CTLA-4 signal peptide	Altered signal peptide, affects trafficking and expression

Table 1: Summary of changes upon CTLA-4 mutation.

Using CRISPR on CTLA4 +49 G/A (rs231775) and Akita

Prime editing would be the best method to correct SNPs using the CRISPR-Cas9 system, as it can directly insert or remove nucleotides without causing a double-strand breakage in DNA. A prime editing guide RNA (pegRNA), which consists of a spacer, scaffold, reverse transcription template containing the desired edit sequence, and a primer binding site, is used to guide a Cas9-nickase/reverse transcriptase fusion to the target site. Here, Cas9-nickase/reverse transcriptase fusion introduces a single-strand

break (or a nick) in the target DNA. Afterwards, the pegRNA initiates reverse transcriptase to replace the removed nucleotide (GenScript pegRNA).

Designing pegRNA for CTLA4 + 49 G/A (rs231775)

To correct the CTLA4 exon-1 +49G/A variant, a pegRNA sequence with Cas9-NGG was constructed using the pegFinder software (Chow et. al). The pegRNA design consists of: a spacer sequence to target the SNP, an RT template and PBS that synthesize nucleotides for correction of the coding error, and a recommended PE3b nicking sgRNA to increase editing efficiency.

Spacer (sgRNA): CTCAGCTGAACCTGGCTGCC
RT template (14 bp): GGCCAGGTCCTGGT
PBS (11 bp): AGCCAGGTTCA
Sense 3' extension: GGCCAGGTCCTGGTAGCCAGGTTCA
Full-length pegRNA:
CTCAGCTGAACCTGGCTGCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGGCCAGGTCCTGGTAG
CCAGGTTCA
PE3b nicking sgRNA: AGGGCCAGGTCCTGGTAGCC

This configuration corrects the codon from GCC (Ala) to ACC (Thr) at position 17 of CTLA4, restoring the signal peptide. However, this design has not been validated and requires in-vivo/vitro testing to confirm the reliability.

Conclusion

In this study, we examined how CRISPR-Cas9, and specifically prime editing, could be applied to correct various genetic mutations implicated in type 1 diabetes, like the Akita mutation in the mice Ins2 gene and rs231775 in CTLA4. By analyzing these sequences and proposing editing strategies, we highlighted how restoring insulin biosynthesis and reducing autoimmune reactivity at the genetic level may address the underlying factors of ß-cell destruction. While technical and ethical concerns regarding genetic editing still remain, these findings emphasize a proof-of-concept and the potential of precision gene editing to advance from insulin supplements towards disease modifying therapies. Ultimately, developing safe and effective CRISPR-based interventions could transform the treatment landscape for type 1 diabetes, offering hope to the millions worldwide who are affected by this autoimmune disease.

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