



## National Tay-Sachs & Allied Diseases Association

### Gene Editing (CRISPR-Cas9)

#### What is gene editing?

Our genes provide specific instructions to make proteins, which carry out essential functions in our bodies. Genes are made up of a series of nucleotides, the building blocks of DNA, and are represented by four 'letters' that form the DNA code. If a gene has a harmful change in its DNA code (i.e. mutation or pathogenic variants), it may not provide correct instructions to produce functional proteins. Some genetic mutations are linked to the development of specific conditions. Tay-Sachs, Sandhoff, GM1 gangliosidosis, and Canavan disease are all genetic conditions caused by pathogenic variants in specific genes, namely the *HEXA*, *HEXB*, *GLB1* and *ASPA* genes, respectively.

**Gene editing is a type of gene therapy that involves changing genetic material to stop or slow the progression of disease. Gene editing can insert, remove, modify, or replace DNA within genes.**

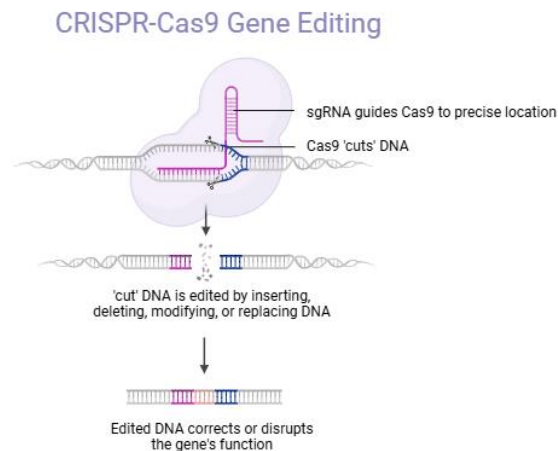
#### What is CRISPR-Cas9?

There are many gene editing technologies. Some of these include ZFNs (**z**inc **f**inger **n**ucleases), TALENs (**t**ranscription **a**ctivator-**l**ike **e**ffector **n**ucleases), and the most widely recognized CRISPR-Cas9 (**c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats, **C**RISPR-**a**ssociated protein **9**) (PMID: 27908936). The discovery of the CRISPR-Cas9 genome editing system was viewed as such an important breakthrough in science that the principal investigators, Jennifer Doudna, Ph.D. and Emmanuelle Charpentier Ph.D., were awarded the 2020 Nobel Prize in Chemistry (<https://www.nobelprize.org/uploads/2020/10/popular-chemistryprize2020.pdf>). 'Editing' a gene to alter its function creates great promise for treating genetic conditions, especially those without a definitive cure such as the GM2 gangliosidoses, GM1 gangliosidosis, and Canavan disease.

The CRISPR-Cas9 system has two main components: the guide RNA (gRNA) and the nuclease (i.e. DNA-cutter; Cas9) (PMID: 27059283). The brilliance of CRISPR and the other genome editing methods is their ability to be targeted to any site within the genome. With CRISPR, the gRNA is designed to a specific target in the genome, where it serves as a guide

for the Cas9 nuclease. Once the gRNA binds to its target site, the Cas9 enzyme will act as a scissor to 'cut' the DNA at that precise location, which then allows for targeted changes to be made to the DNA code (see figure below).

Depending on the type of disease, different gene editing approaches may be used (<https://patienteducation.asgct.org/gene-therapy-101/gene-editing>). For diseases where not enough functional protein is produced, gene integration or gene activation may be used. Gene integration corrects the DNA code to allow for functional protein production. Gene activation turns on a previously inactive gene, allowing it to produce proteins. For diseases where there is an excess of protein or a dysfunctional protein is being synthesized, a gene "knock-out" approach may be used. Gene "knock-out" edits are typically done by removing a part of the genomic sequence, resulting in the loss of that genes expression, and thus its ability to produce proteins.



Created in [BioRender.com](https://www.biorender.com) 

CRISPR-Cas9 gene editing can be accomplished either by direct delivery of the system within the body (*in vivo*) or by cell-based delivery, which involves retrieving patient's cells, editing them outside the body (*ex vivo*), and then reintroducing them into the body (PMID: 32601435). NTSAD is currently funding a grant exploring this type of research on hematopoietic stem cell-based gene editing therapy to treat GM2 gangliosidosis (<https://ntsad.org/ntsad-research/ntsad-grant-opportunities/>).

### **First FDA Approved Gene-Editing Treatment: Sickle Cell Disease**

In December 2023, the FDA-approved a gene therapy for sickle cell disease, which was the first therapy approved to use gene-editing CRISPR-Cas9 technology (PMID: 39118728).

Sickle cell disease is caused by pathogenic variants in the *HBB* gene, leading to red blood cells becoming a 'sickled' shape and impairing their function.

FDA approval of gene-editing treatments for sickle cell disease has paved the way for future therapeutic development of similar gene editing therapies to treat other genetic conditions. Research efforts have begun exploring gene editing systems to treat the GM2 gangliosidoses, GM1 gangliosidosis, and Canavan disease (PMIDs: 31896760; 38737101; 35637731)

### **What are the limitations of gene editing?**

**Off-target effects:** Sometimes, the CRISPR-Cas9 system may 'cut' in the wrong place or "off-target", which could potentially lead to unintended and unwanted changes in other parts of the DNA (PMID: 32850447).

**Unintended modifications:** If there are errors in repairing the 'cut' DNA, this may lead to harmful changes or effects on the cells being 'edited' (i.e. double-stranded break toxicity, on-site mutagenesis, chromosomal rearrangement) (PMID: 32850447).

**Immune responses:** Patients may have an immune response against systems used to deliver CRISPR-Cas9 into the body (PMID: 32850447). In cases where *in vivo* editing is used, it is also possible for some patients to have a pre-existing immunity to the Cas9 protein (PMID: 30692695).

**Accessibility:** Currently, there are no FDA approved gene editing therapies for the GM2 gangliosidoses, GM1 gangliosidosis, or Canavan disease. Gene therapy, including gene editing in general, remains expensive and inaccessible to many.

**Ethical considerations:** While somatic gene editing (which *only* affects the patient's DNA and **cannot** be passed on to future generations) is permitted, human germline editing (which affects the patient's DNA *and can* be passed on to future generations) remains controversial and is currently banned in the United States. However, because each cell of the body (with the exception of mature red blood cells) contains the same genetic material, it is theoretically possible for editing to occur in germline cells, and each proposed treatment must confirm it is not heritable following editing.

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The ASGCT (American Society of Gene & Cell Therapy) has produced a series of educational resources that can help explain both the basics of gene therapy and the various approaches used. Please visit the following links for more information:

Gene Editing: <https://patienteducation.asgct.org/gene-therapy-101/gene-editing>

Gene Therapy Basics: <https://patienteducation.asgct.org/gene-therapy-101/cell-therapy-basics>

Gene Therapy Approaches: <https://patienteducation.asgct.org/gene-therapy-101/gene-therapy-approaches>

To access the literature reference, enter the PMID number into the PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) search box or Google “PMID XXXXXXXX”, replacing the “X’s” with the appropriate number.

Data is current as of August 2024.