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Advancing Genome Editing with CRISPR-Cas: A Review of Mechanism, Application, Recent trends and Limitations.

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Abstract:

CRISPR-Cas, that gene-editing thing that everyone is talking about, was essentially bacteria's method of switching to viruses. For scientists tinkering with DNA, it's comparable to the Swiss Army knife. We're talking about almost frightening precision and a number of doors opening up for the treatment of illnesses that people used to dismiss. This review article enlighten the specifics: mechanism of CRISPR, application, recent advertisement with future prospects and limitations.

Introduction

Gene editing is basically science's way of playing with DNA. You can snip, swap, or patch up genes with way more consequences. It's honestly fascinating how much this has shaken up biology and medicine. Suddenly we're not just talking about understanding how our genes tick, but actually cranking up crop yields and maybe—just maybe—finally getting a grip on some gnarly genetic diseases (Mushtaq et al., 2021; Rautela et al., 2021).

Ishino and the gang just kinda tripped over CRISPR chilling in some *E. coli* back in 2018. Who knew bacteria were running their own high-tech bouncer service, kicking unwanted DNA out like it's last call? That discovery was the big CRISPR-Cas tech was off to the races. Suddenly, scientists had this wild, laser-precise gene editing gadget at their fingertips. It's the kind of thing you'd expect in a Black Mirror episode, except, nope, it's totally a thing.

Genetic research is cruising at warp speed. Scientists can tweak genes with scary accuracy, snipping out the bad stuff without

making a mess (shoutout to Zhang, 2019; Verma et al., 2023). Medicine's where it gets even crazier. We're talking about actually fixing broken genes, custom-building treatments based on your personal DNA cocktail, and even coming up with new ways to slap down viruses and cancer. Personalized medicine, regenerative therapies, you name it—it's all in play now. The future's looking kinda wild, honestly.

Alright, let's cut to the chase. If you thought CRISPR was just some cool gene-editing trick from a few years ago, buckle up. Stuff's moving at breakneck speed. We're not just talking about your grandma's CRISPR anymore—now there's prime editing, CasΦ (yeah, that's a weird name), and even wild new ways to use CRISPR for diagnostics. Regulations? Don't even get me started; those are flipping constantly.

So what's the deal with all these upgrades? This review dives into the gritty details—how CRISPR's actually being used to tackle heavy-hitter diseases like Alzheimer's, Parkinson's, heart problems, nasty viruses, blood issues, eye stuff,

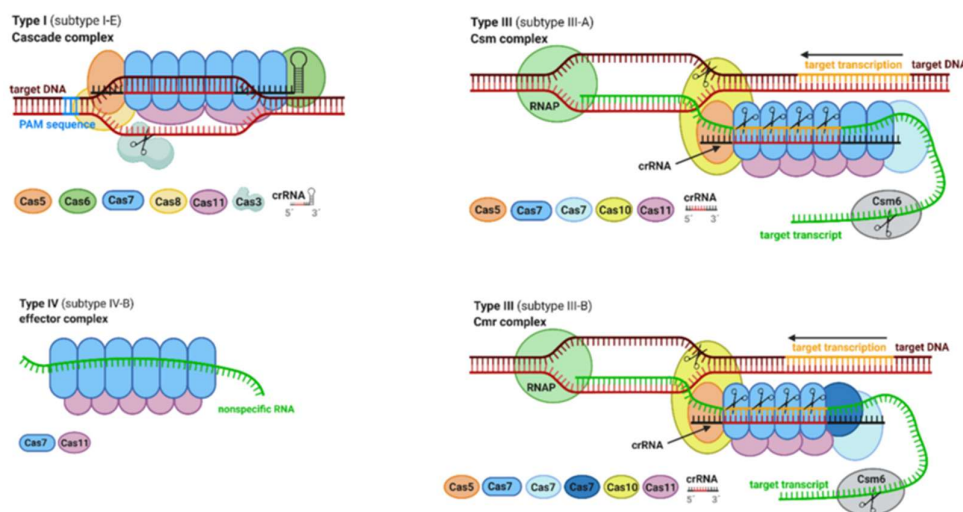
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mitochondrial weirdness, muscular dystrophy, cancer... basically, if it's bad, CRISPR's lining up to fight it. Of course, it's not all sunshine and Nobel Prizes.

Classification and Mechanisms:

CRISPR-Cas systems fall into two main groups:

- **Class 1:** These use multi-protein complexes (e.g., Type I, III, IV).

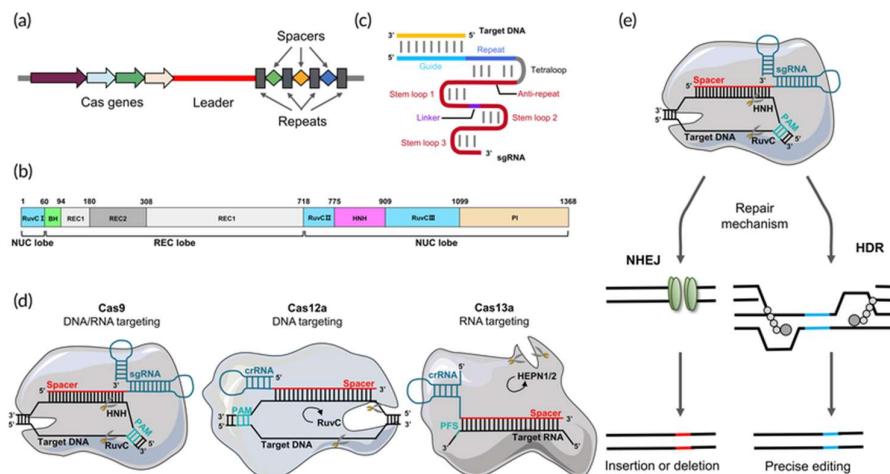


Source: ResearchGate, Class 1 systems figure [accessed 10 Jul 2025]

- **Class 2:** These rely on single-protein effectors like Cas9, Cas12, or Cas13.

These are simpler, using a single protein to handle the heavy lifting. The stars here are Cas9 (Type II, widely used for DNA editing), Cas12 (Type V, great for multiplexing), and Cas13 (Type VI, which targets RNA instead of DNA).

Their streamlined design makes them especially popular for lab and therapeutic applications.



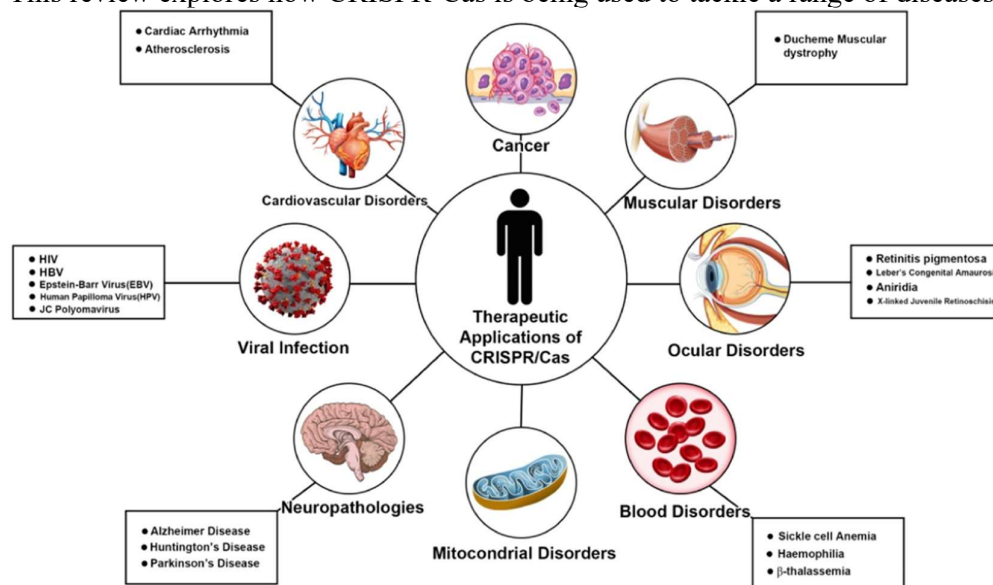
Source: ResearchGate, Class 2 systems figure [accessed 10 Jul 2025]

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CRISPR-Cas works like a high-tech security system for bacteria. First, bacteria basically play Pokémon with DNA, collecting little bits from nasty invaders and stashing them in their own DNA as “spacers”—kind of like a scrapbook of microbial mugshots. Next up, they turn these spacers into crRNAs, which are basically molecular snitches that point the Cas proteins to their next target. Then comes the really wild part: Cas proteins, with their crRNA sidekicks, hunt down the matching DNA (or RNA) by looking for this short sequence called PAM—it’s like the postal code for DNA. Once they find it? Snip, snip. The Cas protein chops up the target, either to shut down invaders or, if you’re a scientist, to tweak genes however you want.

What’s actually nuts is how many flavors of this system there are. Type I has Cas3, **Therapeutic Applications :**

This review explores how CRISPR-Cas is being used to tackle a range of diseases:



Neurological Disorders

CRISPR-Cas9 is a game-changer for studying and treating brain disorders. For Alzheimer's, researchers have used base editing to convert the risky apoE4 gene to the safer apoE3 in mouse astrocytes. They've also explored suppressing glia maturation

which just goes full paper-shredder and chews up DNA bit by bit. Cas9 (the celebrity of the bunch) is more surgical—makes these neat double cuts, perfect for swapping DNA in or out. Cas12 is more of a wildcard; it makes these staggered cuts that are pretty useful for certain fixes. And then there's Cas13, which ignores DNA altogether and targets RNA—super handy for messing with gene expression or going after RNA viruses, all without touching the original genetic code. Basically, scientists now have this Swiss Army knife of gene-editing tools, each one with its own specialty. Wrapping your head around how all these pieces fit together? That's what's really unlocked all the crazy gene-editing stuff you hear about—from souped-up lab experiments to potentially curing hardcore diseases. Wild times.

factor (GMF), which fuels inflammation in Alzheimer's. In Huntington's disease, caused by faulty CAG repeats in the *HTT* gene, CRISPR has silenced the mutant protein in cell and animal models. For Parkinson's, linked to *SCNA* gene mutations, CRISPR has deactivated *SCNA* in neurons,



promoting healthy cell growth and reducing stress.

Cardiovascular Diseases

heart disease is a total menace. We're talking nearly 18 million people gone in 2019 alone. And a big piece of that mess? High cholesterol. Blame your genes, sometimes—especially culprits like PCSK9. This little genetic troublemaker cranks up your LDL (yep, that's the "bad" cholesterol), which does your heart absolutely no favors.

Now, here's where it gets sci-fi in the best way. Scientists have been rolling out CRISPR-Cas9 (think genetic scissors but cooler) and zapping the PCSK9 gene in mice. End result? Cholesterol levels dropped like a rock. They didn't stop there. ANGPTL3, another gene, got the CRISPR treatment too. That one's all about triglycerides, and when they targeted it, both triglycerides and cholesterol took a nosedive. The magic isn't just about cholesterol, either. CRISPR's been tinkering with genes tied to heart rhythm problems and atherosclerosis, helping animals' hearts work better overall. So, yeah, all this gene-editing wizardry could be a total game-changer for fighting heart disease. Fingers crossed the hype holds up. CRISPR is making strides in treating eye diseases. For retinitis pigmentosa, linked to *RHO* gene mutations, CRISPR has knocked out faulty genes in mice, restoring vision. In Leber congenital amaurosis, it's corrected mutations in retinal cells with lasting results. For aniridia, caused by *PAX6* mutations, CRISPR fixed defects in mouse models. It's also shown promise for X-linked retinoschisis by repairing *RS1* gene mutations, achieving about 50% correction in lab models.

Blood Disorders

CRISPR offers hope for curing blood disorders like sickle cell disease, caused by a single *HBB* gene mutation. Therapies like Casgevy boost fetal hemoglobin to ease

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symptoms. For β -thalassemia, CRISPR corrects *HBB* mutations or reactivates fetal hemoglobin. In hemophilia, CRISPR has fixed *FVIII* and *FIX* gene mutations, restoring clotting function in lab studies.

Viral Infections

CRISPR-Cas tech is kind of a game-changer when it comes to fighting off nasty viruses. It's like scientists finally got their hands on a cheat code. Take HIV, for example—researchers are straight-up going after the virus's LTR region (that's basically the engine room for its genes), and it's actually dialing down the virus in lab dishes and even animal test runs. Pretty wild. And get this, they're also tweaking stuff like CCR5 receptors in immune cells. So, basically, they're making these cells give HIV the middle finger, but without messing up their regular jobs. Result is HIV can't throw its usual party in those cells. The numbers coming out of these studies are no joke—HIV just can't keep up.

But it's not just HIV in the crosshairs. CRISPR's been flexing on other viruses, too—hepatitis B, herpes simplex, even those pesky coronaviruses. One flavor of this tech, Cas13, is extra cool because it hunts down RNA directly. Think of it like a heat-seeking missile for viral genetic code, just tearing through RNA-based bad-dies. Honestly, the fact that CRISPR can mess with both DNA and RNA viruses? That's huge. We're not talking sci-fi anymore—this could actually flip the script on how we treat viral diseases. The future? Yeah, it's looking a little less doomed.

Mitochondrial Disorders

Mitochondrial diseases, caused by mtDNA mutations, are tough to treat, but CRISPR's mitoCas9 has reduced faulty mtDNA in cells and zebrafish. RNA-free base editing offers a safer way to fix single-base mutations without cutting DNA.

Duchenne Muscular Dystrophy



DMD, a severe muscle disorder, stems from *DMD* gene mutations. CRISPR has corrected these in mouse models and human cells, using techniques like exon skipping and base editing to restore functional dystrophin protein.

Cancer

here's the real talk on CRISPR: it's the hot-shot of gene editing, the hype is kinda deserved. We're talking about a tool that rolls up its sleeves and actually hunts down those nasty cancer genes, patches up busted tumor suppressors, and even throws hands with stubborn drug resistance. in cancers like leukemia or lymphoma—plus a laundry list of others—scientists are flipping genetic switches back on, shrinking tumors, and making CAR-T therapy hit way harder. And check this out: they're even using CRISPR to nuke viral genes in cancers caused by stuff like HPV and hepatitis. Science fiction? Nah, just your average Tuesday in the lab.

Recent Technological Advances:

1. First FDA-Approved CRISPR Therapy: In 2023, Casgevy, a CRISPR-based therapy, was approved for sickle cell disease and β -thalassemia, helping patients stay symptom-free by boosting fetal hemoglobin. [\(https://innovativegenomics.org/news/crispr-clinical-trials-2024/\)](https://innovativegenomics.org/news/crispr-clinical-trials-2024/) [\(https://www.endocrinologyadvisor.com/features/future-of-crispr/\)](https://www.endocrinologyadvisor.com/features/future-of-crispr/)
2. CRISPR-Cas12a Trials: Editas Medicine's trials for blood disorders using Cas12a show similar success to Casgevy, with no major side effects. [\(https://innovativegenomics.org/news/crispr-clinical-trials-2024/\)](https://innovativegenomics.org/news/crispr-clinical-trials-2024/)
3. CRISPR-Cas3 for Bacteria: A 2021 trial used Cas3 to target *E. coli* in urinary infections, proving safe by 2023

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4. Systemic Delivery: Intellia's NTLA-2001 uses nanoparticles to deliver CRISPR to the liver, cutting toxic protein levels in amyloidosis trials. [\(https://innovativegenomics.org/news/crispr-clinical-trials-2024/\)](https://innovativegenomics.org/news/crispr-clinical-trials-2024/) [\(https://www.endocrinologyadvisor.com/features/future-of-crispr/\)](https://www.endocrinologyadvisor.com/features/future-of-crispr/)
5. Base and Prime Editing: These methods allow precise edits without DNA breaks, with trials like Verve-101 lowering cholesterol. [\[\(https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-023-00447-x\)\]](https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-023-00447-x) [\[\(https://www.frontiersin.org/journals/genome-editing/articles/10.3389/fgeed.2024.1509924/full\)\]](https://www.frontiersin.org/journals/genome-editing/articles/10.3389/fgeed.2024.1509924/full) [\(https://www.endocrinologyadvisor.com/features/future-of-crispr/\)](https://www.endocrinologyadvisor.com/features/future-of-crispr/)
6. CRISPR in Agriculture: Seedless blackberries and safer potatoes were developed in 2024 using CRISPR. [\(https://innovativegenomics.org/news/crispr-in-agriculture-2024/\)](https://innovativegenomics.org/news/crispr-in-agriculture-2024/)
7. HIV and Huntington's Progress: Lab studies in 2024 and 2025 showed CRISPR eliminating HIV and editing neurons for Huntington's.
8. Improved Delivery: New Cas9 variants and delivery methods like virus-like particles reduce side effects. [\[\(https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-023-00447-x\)\]](https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-023-00447-x) [\[\(https://www.frontiersin.org/journals/genome-editing/articles/10.3389/fgeed.2024.1509924/full\)\]](https://www.frontiersin.org/journals/genome-editing/articles/10.3389/fgeed.2024.1509924/full)



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- [editing/articles/10.3389/fgeed.2024.1509924/full](#))
9. Cancer Therapies: CRISPR enhances CAR-T therapies for leukemia, with safer editing techniques. [.\[\]\(https://pmc.ncbi.nlm.nih.gov/articles/PMC11486967/\)](https://pmc.ncbi.nlm.nih.gov/articles/PMC11486967/)
 10. Ethical Advances: Efforts are underway to create fair guidelines for CRISPR's use, especially in germline editing. [.\[\]\(https://medlineplus.gov/genetics/understanding/genomicresearch/genomeediting/\)](https://medlineplus.gov/genetics/understanding/genomicresearch/genomeediting/)[.\[\]\(https://innovativegenomics.org/what-is-crispr/\)](https://innovativegenomics.org/what-is-crispr/)

Future Perspectives

CRISPR's potential is vast:

- Developing therapies for rare diseases, cancers, and neurological conditions by 2030.

- Exploring cures for HIV and hepatitis within a decade.
- Advancing personalized medicine with AI-driven gene edits.
- Creating hardier crops with new Cas variants.
- Using gene drives to control disease-carrying pests.
- Improving delivery with non-viral systems.
- Establishing global ethical standards for equitable access.
- Expanding diagnostics with tools like SHERLOCK.
- Building synthetic organisms for drug testing or bioenergy.
- Integrating AI and quantum computing to refine CRISPR workflow.

Limitations

Challenge	Description	Solution
Off-target effects	Unintended DNA changes can cause harm.	Use high-fidelity Cas9, better guide RNAs, and predictive tools.
Delivery challenges	Getting CRISPR to specific cells is tricky.	Use viral vectors, nanoparticles, or tissue-specific promoters.
Large DNA insertions	Adding big DNA pieces is inefficient.	Improve HDR and explore new delivery methods.
Immune reactions	Viral vectors may trigger immune responses.	Use non-viral systems and rigorous safety testing.
Tissue targeting	Reaching specific organs is tough.	Develop targeted vectors and promoters.
Ethical concerns	Germline editing raises ethical questions.	Create clear, global ethical guidelines.
Detecting off-targets	Spotting unintended edits is critical.	Use advanced sequencing and detection tools.
Hard-to-edit cells	Neurons and stem cells resist editing.	Design cell-specific delivery systems.
Gene expression control	Timing and location of edits need precision.	Use inducible CRISPR or optogenetic tools.
Non-dividing cells	These cells are harder to edit.	Use NHEJ or specialized CRISPR variants.

Conclusion

CRISPR-Cas started out as bacteria's secret weapon. Scientists are obsessed. You can



snip, swap, or totally silence genes like you're editing text on your phone. It's wild. Researchers use it to poke at genes and figure out what they actually do, which is way harder than it sounds. And the medical world? CRISPR's got them hyped about fixing stuff like sickle cell and even some cancers. Kinda feels like sci-fi, honestly. It's also helping build better models for diseases, which means faster drug discoveries and fewer shots in the dark. What really blows my mind is how CRISPR's so easy to use well, for scientists anyway and so flexible. The potential's huge, but yeah, people do need to chill and make sure we don't mess things up along the way.

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