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Gene Therapy and Down Syndrome: Can the future of Medicine bring a breakthrough?

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Abstract

Down syndrome (DS), or trisomy 21, is the most common chromosomal disorder, characterized by intellectual disability, congenital anomalies, and an increased risk of neurodegenerative diseases. This review synthesizes current knowledge on the etiology, clinical presentation, and modern therapeutic strategies for DS, including early intervention, medical management, and psychosocial support. Particular emphasis is placed on the revolutionary potential of **CRISPR-Cas gene-editing technology** as a tool for future treatment strategies. Drawing upon findings from many scientific publications, the article discusses the mechanism of CRISPR, its current medical applications, and its experimental use in silencing the extra chromosome 21 or correcting DS-associated genes. Technical, ethical, and delivery-related limitations are explored, alongside speculative but promising research directions, including base editing, prime editing, and cerebral organoids. The article concludes with a reflection on whether societal perceptions of genetic diversity should evolve alongside scientific innovation, highlighting that the greatest breakthrough may come not from altering genomes, but from fostering inclusion and understanding.

Keywords:

Down syndrome; CRISPR-Cas Systems; Gene therapy; Gene Silencing; Early diagnosis; Neurodevelopment disorders;

Introduction

Down syndrome (DS), or trisomy 21, remains the most prevalent chromosomal aneuploidy diagnosed at birth, characterized by a broad spectrum of physical, cognitive, and developmental features. The growing life expectancy and societal integration of individuals with DS have prompted an increased focus on early diagnosis, targeted therapeutic interventions, and genetic research aimed at alleviating or modifying the condition's manifestations.

The objective of this review is to present a comprehensive, interdisciplinary overview of the current understanding of Down syndrome - from its genetic etiology and clinical features, through contemporary approaches in care and rehabilitation, to the emerging frontiers of gene-editing technologies, particularly CRISPR-Cas systems. A special focus is placed on evaluating the experimental and potential therapeutic applications of CRISPR in DS models, including chromosomal silencing, correction of gene dosage effects, and targeting symptom-related genes.

This article is based on an extensive review of scientific literature published between 2015 and 2025. The sources were retrieved using major academic search engines and medical databases, including **PubMed**, **Google Scholar**, **ScienceDirect**, **Scopus**, and the **Cochrane Library**. Selection criteria included peer-reviewed articles, clinical guidelines, preclinical studies on animal and cellular models, and systematic reviews addressing Down syndrome and CRISPR-related therapies. Additionally, official recommendations and epidemiological data from global health organizations were considered to support the clinical context.

Methodologically, the work employs a **narrative literature review approach**, structured thematically. The analysis synthesizes findings from 35 key academic papers, offering a consolidated perspective on diagnosis, care standards, molecular mechanisms, and genetic interventions in DS.

The results of this synthesis confirm significant progress in multidisciplinary management of DS, from early developmental support to preventive medical protocols. At the same time, it highlights how **CRISPR-Cas gene-editing systems**, though not yet clinically applied to DS, offer groundbreaking potential. Experimental studies have demonstrated the ability to silence the extra chromosome 21 or modulate expression of overactive genes such as DYRK1A and APP, restoring cellular functions in vitro and in murine models.

This work ultimately underscores the dual role of science — to innovate responsibly and to inspire deeper reflection on how we, as a society, value human diversity in the genomic era.

1.Down Syndrome – Etiology, Risk Factors, and Susceptible Groups

Down syndrome (DS), or trisomy 21, is the most prevalent chromosomal disorder among live births, characterized by a spectrum of phenotypic manifestations including intellectual disability, congenital malformations, and neurodegenerative changes. This review synthesizes current knowledge on the genetic basis, clinical features, diagnostic methods, and management strategies associated with DS.

First described by John Langdon Down in 1866, and genetically defined in 1959, Down syndrome remains a model disorder for studying aneuploidy and its systemic consequences. It affects approximately 1 in 700 live births globally [1]. While advances in prenatal diagnostics and medical care have improved outcomes, DS continues to pose lifelong challenges for individuals and caregivers. This review integrates current data to provide a detailed picture of the condition.

DS is most commonly caused by nondisjunction leading to full trisomy 21 (95% of cases), with less frequent causes including Robertsonian translocations (~4%) and mosaicism (~1%) [1]. The majority of nondisjunction events are maternal in origin and increase in frequency with maternal age [2]. Genetic testing techniques, including karyotyping and FISH, are Advanced maternal age remains the single most significant risk factor. For example, the risk increases from 1 in 1,250 at age 25 to 1 in 100 by age 40 [2] (Table 1). Other potential contributors such as paternal age, environmental exposures, and polymorphisms in meiotic genes have been studied but remain inconclusive.

Maternal Age	Risk (1 in ...)
20	1,500
25	1,250
30	1,000
35	350
40	100
45	30
>45	20

Table 1. Maternal Age and Risk of Down Syndrome *[Source: own elaboration based on 2]*

Typical physical traits include brachycephaly, upslanting palpebral fissures, epicanthal folds, flat nasal bridge, and hypotonia. Short stature and joint laxity are common, while congenital heart defects occur in ~50% of individuals, especially atrioventricular septal defects (AVSD) [1,3].

Individuals with DS display varying degrees of intellectual disability, generally in the mild to moderate range. MRI studies reveal altered brain morphology, including reduced cerebellar and hippocampal volumes [1]. By middle age, nearly all individuals show neuropathological features of Alzheimer's disease, linked to the triplication of the APP gene [1,4].

Medical Comorbidities

DS is associated with a wide array of comorbidities:

- **Cardiovascular**: AVSD and ventricular septal defects.
- **Gastrointestinal**: Duodenal atresia, Hirschsprung disease.
- **Endocrine**: Hypothyroidism (congenital or autoimmune).
- **Hematologic**: Higher incidence of leukemia (ALL and AMKL), especially in infancy [1,4].
- **Immunologic**: Increased susceptibility to infections, autoimmune disorders.
- **Sensory**: Hearing loss (often from otitis media), visual problems including cataracts [3].

Diagnostic Advances

Prenatal Screening

Modern prenatal diagnostic approaches for Down syndrome focus on maximizing detection while minimizing invasiveness. Non-invasive prenatal testing (NIPT) based on analysis of cell-free fetal DNA in maternal plasma has emerged as the most accurate screening tool, with sensitivity and specificity exceeding 99% [5,19]. NIPT is routinely offered to pregnant individuals, especially those of advanced maternal age or with high-risk first-trimester screening results. Since June 5, 2024, it has also been offered to all pregnant women in Poland as part of standard prenatal care.

In addition to NIPT, combined first-trimester screening, which evaluates nuchal translucency via ultrasound along with serum markers (PAPP-A and free β-hCG), remains a widely used method [19]. In the second trimester, the quadruple test (AFP, hCG, estriol, and inhibin-A) offers further risk assessment. (Table 2)

Method	Invasive	Gestational Age (weeks)	Accuracy
First-trimester screening	No	11–13	~85%
Non-Invasive Prenatal Test (NIPT)	No	From 10	>99%
Amniocentesis	Yes	15–20	~100%
Chorionic Villus Sampling (CVS)	Yes	10–13	~100%

Table 2. Prenatal Diagnostic Methods for Down Syndrome [*Source: own elaboration based on 5,19,20]*

Definitive prenatal diagnosis requires invasive techniques: chorionic villus sampling (CVS) typically performed at 10–13 weeks, or amniocentesis conducted after 15 weeks of gestation. Chromosomal abnormalities are confirmed via karyotyping, fluorescence in situ hybridization (FISH), or quantitative fluorescent PCR (QF-PCR) [19,20].

Genetic Counseling and Risk Evaluation

Genetic counseling is essential following a DS diagnosis. Counseling addresses recurrence risk, options for ongoing pregnancy, and potential medical and developmental challenges. In families with Robertsonian translocations, carrier testing of parents is crucial due to increased recurrence risk in future pregnancies [5]. Prenatal screening has been revolutionized by non-invasive prenatal testing (NIPT) using cell-free fetal DNA, offering detection rates >99% [5]. Confirmation is achieved by invasive procedures such as chorionic villus sampling or amniocentesis followed by cytogenetic analysis.

Postnatal diagnosis is usually based on clinical features and confirmed by karyotype. Early diagnosis allows for prompt evaluation of organ systems and planning for multidisciplinary care.

Management and Intervention

A comprehensive, multidisciplinary approach is the cornerstone of optimal care for individuals with Down syndrome. Management should begin shortly after diagnosis and continue through all life stages.

Medical Management

Besides early surgical correction of congenital defects, standardized surveillance protocols guide follow-up of thyroid function, vision, hearing, hematologic profiles, and growth [3,21]. Immunization according to national schedules is crucial due to immunological vulnerability. Preventive care also includes annual ophthalmologic and audiologic evaluations, sleep apnea screening, and regular dental checks.

Gastrointestinal anomalies such as duodenal atresia or Hirschsprung disease require timely surgical intervention. Endocrinologic issues like hypothyroidism are managed with lifelong thyroxine replacement. Hematological surveillance is particularly important in infancy and early childhood given increased leukemia risk [20,21].

Developmental and Educational Support

Early intervention programs (starting as early as infancy) offer physical, occupational, and speech therapy to maximize developmental potential. Interdisciplinary centers coordinate therapy and evaluate progress. These services improve motor skills, communication abilities, and school readiness [4].

Educational support includes special education accommodations, inclusion strategies, and individualized education programs (IEPs). Vocational training and supported employment options help young adults with DS transition into semi-independent living.

Psychosocial and Community-Based Care

Parental support, mental health resources, and community advocacy groups play a vital role in quality of life for families. Social workers and developmental pediatricians help coordinate access to benefits, services, and inclusive education pathways. Comprehensive care recognizes not just the medical but also the emotional and social needs of individuals with DS and their families.

Medical Management

Cardiac and gastrointestinal anomalies are typically corrected surgically. Regular screening for thyroid function, hearing, vision, and hematologic abnormalities is critical. Immunization schedules should be rigorously followed [3].

Developmental and Educational Support

Early intervention programs including physical, occupational, and speech therapy significantly improve outcomes. Educational integration and individualized learning plans foster cognitive and social development [4].

Future Directions

Recent transcriptomic studies have deepened understanding of gene dosage effects on cellular homeostasis in DS. Data suggest that oxidative stress, chronic inflammation, and mitochondrial dysfunction play pivotal roles in neural and systemic phenotypes [18]. Specific dysregulation of antioxidant defense genes like SOD1 and GPX1 leads to a redox imbalance contributing to neurodegeneration and cellular senescence [18,22].

Neuroimaging and connectivity studies have identified altered brain structure and white matter pathways that correlate with cognitive decline and early Alzheimer-like pathology in DS [26,27]. These findings support a shift toward integrated diagnostic frameworks combining imaging, biomarkers, and genetic profiling.

Organoid-based models and iPSC-derived neurons from individuals with DS now offer unprecedented opportunities to model disease progression and screen therapeutic compounds in a patient-specific manner [24,25]. These systems can recapitulate aspects of cortical development and synaptic signaling defects seen in DS brains, facilitating discovery of neuroprotective strategies.

Advances in prenatal screening, particularly the use of cfDNA and high-throughput molecular assays, have increased diagnostic sensitivity while reducing the need for invasive procedures [19]. Molecular karyotyping and QF-PCR provide rapid confirmation and enable detection of mosaicism and translocations, which are critical for accurate prognosis and genetic counseling [19,20]. Recent advances in genomics, including transcriptomic and proteomic profiling, are enhancing understanding of DS pathophysiology. There is growing interest in targeting specific pathways, such as oxidative stress and inflammation, with pharmacological agents [5]. The potential of gene therapy and CRISPR technology, while theoretical at present, opens new avenues for research.

2. **Therapeutic Strategies and Rehabilitation in Down Syndrome**

While there is no cure for Down syndrome (DS), therapeutic and rehabilitative strategies have significantly evolved to enhance quality of life and promote functional independence across the

lifespan. This chapter presents an integrative view based on comprehensive findings from studies [1–35].

Early Intervention and Developmental Therapies

Early childhood is a critical window for neurodevelopment. Evidence consistently shows that early intervention services yield substantial cognitive, motor, and social benefits in children with DS. Key strategies include:

- **Physical therapy**: Targeting hypotonia, motor delays, and postural control.
- **Occupational therapy**: Enhancing fine motor skills and daily functional abilities.
- **Speech and language therapy**: Improving expressive/receptive communication, articulation, and feeding skills.

Early access to these services improves school readiness and supports long-term adaptive functioning.

Multisystem Medical Monitoring

Individuals with DS are prone to a wide range of medical comorbidities requiring coordinated, interdisciplinary care:

- **Cardiac care**: Congenital heart defects (especially AVSD) require early echocardiographic screening and often surgical repair.
- **Endocrine surveillance**: Regular screening for congenital and autoimmune hypothyroidism.
- **Vision and hearing checks**: Essential due to high rates of otitis media, hearing loss, strabismus, and cataracts.
- **Gastrointestinal and hematological follow-up**: Including risks of Hirschsprung disease, duodenal atresia, and childhood leukemia.

Clinical guidelines recommend life-stage-specific checklists for regular assessment and preventive care.

Educational Support and Inclusive Practices

Educational programs tailored to individual learning profiles help optimize academic outcomes. Interventions include:

- **Individualized Education Programs (IEPs)**

- **Inclusion in mainstream classrooms with support services**
- **Augmentative and alternative communication (AAC) tools**

Educational support also fosters social integration, self-esteem, and lifelong learning.

Psychosocial and Vocational Support

Psychosocial well-being is essential. Interventions focus on:

- **Parental training and family counseling**
- **Peer inclusion and community programs**
- **Behavioral therapy to manage ADHD, anxiety, or autism-like features**

As individuals with DS age, vocational training and supported employment promote autonomy and integration into adult society.

Innovations and Assistive Technologies

Emerging tools include:

- **Digital apps** for language and cognitive stimulation
- **Wearable technologies** for mobility and health tracking
- **Tele-rehabilitation** for remote service delivery, especially post-COVID-19

These tools expand access and engagement across diverse settings.

3. CRISPR Technology – Mechanism and Medical Applications

Introduction to CRISPR

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) represents a cutting-edge gene-editing system first discovered as part of the bacterial adaptive immune response. It enables precise targeting and modification of genetic sequences through the use of an RNA guide and an associated nuclease, most commonly Cas9. This system has revolutionized molecular biology by allowing scientists to directly and efficiently edit the genomes of virtually any organism [30, 31].

Mechanism of CRISPR-Cas Systems

The CRISPR-Cas9 system operates via a two-component strategy: the Cas9 endonuclease and a synthetic guide RNA (gRNA). The gRNA directs Cas9 to a specific DNA sequence, where

the enzyme creates a double-strand break (DSB). Following this, the cell's repair mechanisms take over, engaging either non-homologous end joining (NHEJ), which often introduces small insertions or deletions (indels), or homology-directed repair (HDR), which can be used for precise gene correction or insertion when a repair template is provided [30, 34].

Other enzymes such as Cas12 and Cas13 have since expanded the CRISPR toolkit. Cas12 is also used for DNA targeting but introduces staggered DSBs, while Cas13 targets RNA, enabling post-transcriptional gene regulation [32, 35]. Furthermore, novel systems like base editing and prime editing provide refined editing capabilities without requiring DSBs, reducing cytotoxicity and off-target effects [34] (Figure 1).

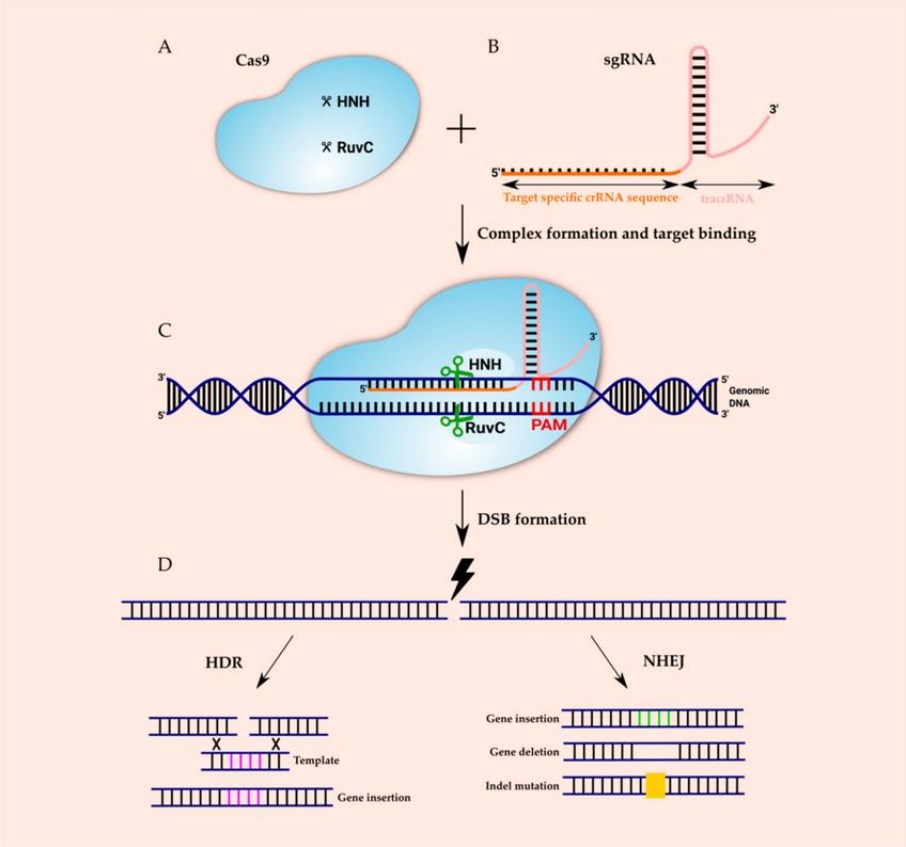

Figure 1. CRISPR-Cas9 gen editing [reprint from 34**]**

Clinical Applications of CRISPR

CRISPR has rapidly moved from laboratory discovery to therapeutic experimentation. Key areas of application include:

- **Monogenic Diseases:** CRISPR-Cas9 has shown therapeutic potential in treating disorders such as sickle cell disease and beta-thalassemia by reactivating fetal hemoglobin genes [35].
- **Cancer Immunotherapy:** Engineered immune cells, especially CAR-T cells, are enhanced with CRISPR edits for improved tumor targeting and immune evasion [32].
- **Infectious Disease Diagnostics:** SHERLOCK and DETECTR platforms utilize CRISPR for rapid, sensitive, and low-cost detection of viral RNA including SARS-CoV-2 [33].
- **Neurological Disorders:** Research is exploring the application of CRISPR in correcting mutations associated with diseases like Huntington's, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease [30, 35].
- **Cardiovascular and Metabolic Diseases:** Investigational applications are also emerging in dyslipidemia and diabetes by targeting regulatory elements of key metabolic genes.

Technical Challenges

Despite its potential, CRISPR-based therapies raise several concerns:

- **Off-target Effects:** Inaccurate DNA cleavage can result in unintended gene disruption.
- **Delivery Mechanisms:** Effective delivery to relevant tissues remains a technical hurdle.
- **Mosaicism and Immunogenicity:** Particularly in embryo editing or in vivo applications, mosaic distribution of edits and immune responses to Cas proteins must be mitigated.

Research highlights about CRISPR

CRISPR has become a symbol of precision medicine and gene innovation around the world. Its applications span far beyond the lab, and here are some expanded highlights:

- In 2018, a Chinese scientist, He Jiankui, created global controversy when he announced the birth of twin girls whose embryos had been genetically edited using CRISPR to make them resistant to HIV. This event led to international condemnation, legal repercussions, and renewed calls for global regulation of human germline editing. in 2018 by announcing the birth of the first CRISPR-edited babies, raising global ethical concerns.

- CRISPR has enabled the development of browning-resistant mushrooms by knocking out a gene involved in enzymatic browning, potentially reducing food waste in supermarkets and during transport., potentially reducing food waste in the retail industry.
- Researchers have engineered CRISPR systems to target antibiotic-resistant bacteria, offering hope against so-called "superbugs."
- Using CRISPR, scientists are exploring the possibility of de-extinction, with efforts to edit elephant DNA to closely resemble that of the woolly mammoth, aiming to resurrect traits such as cold resistance and fur growth, such as the woolly mammoth, by editing elephant DNA.
- NASA is investigating how CRISPR functions in space by testing gene-editing tools on the International Space Station. These experiments aim to understand how DNA repair mechanisms work in microgravity, which has implications for astronaut health during long-term space travel. under microgravity to study DNA repair and mutations during spaceflight.
- Scientists have recently developed CRISPR tools that can turn genes on or off without cutting the DNA, using systems like CRISPRa and CRISPRi (activation and interference).
- In 2018, CRISPR was used for the first time to treat a genetic disease (Leber congenital amaurosis) in a human patient directly inside the body.
- Researchers have created a CRISPR-based “gene drive” that can spread genetic modifications rapidly through populations — studied especially for mosquito-borne diseases like malaria.
- CRISPR has been used to create “glow-in-the-dark” mushrooms and animals in laboratory settings for educational and experimental purposes.
- A CRISPR-powered diagnostic device the size of a USB stick has been developed to detect COVID-19 in less than an hour in field conditions.
- CRISPR was first observed in the genome of bacteria as a defense mechanism against viruses — bacteria "remember" viral sequences to neutralize them upon future infections.

- Jennifer Doudna and Emmanuelle Charpentier were awarded the 2020 Nobel Prize in Chemistry for their groundbreaking development of CRISPR-Cas9 gene-editing technology
- CRISPR is used not only in medicine but also in agriculture — to create crops that are resistant to diseases, drought, or pests.
- This technology has potential applications in conservation — for instance, by eliminating diseases transmitted by mosquitoes that threaten endangered species
- CRISPR is used in modern diagnostic tests, enabling fast and affordable detection of COVID-19 and other pathogens

Future Directions

Future efforts will likely focus on improving CRISPR specificity, developing safe in vivo delivery systems (e.g., lipid nanoparticles, virus-like particles), and Advances in single-cell sequencing and AI-driven design of gRNAs are expected to further enhance precision and safety. Additionally, combinatorial approaches using CRISPR with epigenetic modulators or RNA interference may open new therapeutic avenues [30–35].

4. CRISPR Potential in the Context of Down Syndrome

Silencing the Extra Chromosome 21

Down syndrome (DS) is caused by the presence of a third copy of chromosome 21. One of the most compelling and advanced areas of research using CRISPR involves the selective silencing of this extra chromosome. Using the CRISPR-Cas9 system, researchers have inserted the XIST gene—normally involved in X chromosome inactivation—into the additional chromosome 21 in induced pluripotent stem cells (iPSCs) derived from individuals with DS. The result was successful transcriptional silencing of the entire chromosome, significantly reducing the overexpression of trisomic genes and restoring more typical gene expression patterns [13, 33] (Figure 2).

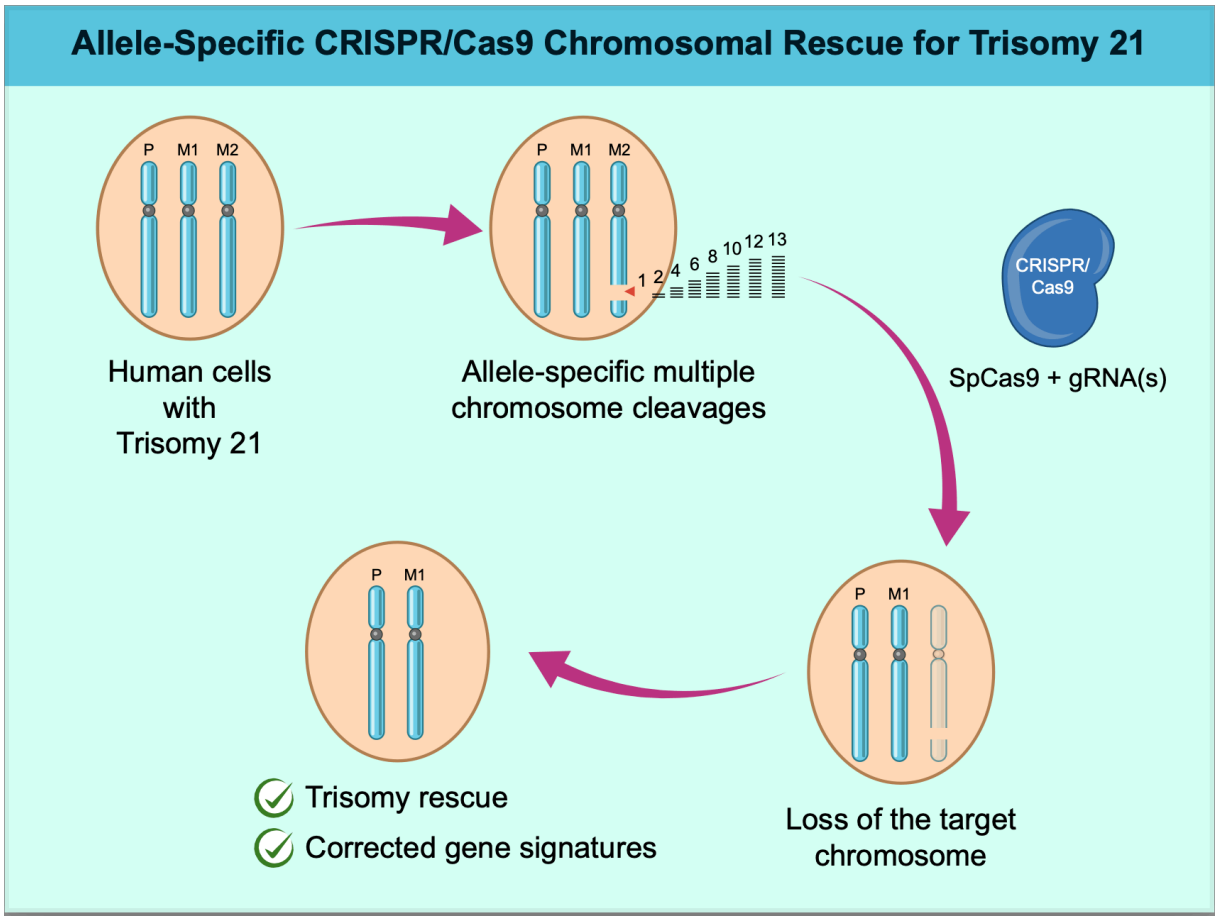

Figure 2. CRISPR-Cas 9 eliminates trisomy 21 [reprint from zespoldowna.info]

This approach is still experimental and limited to in vitro models, but it offers a powerful proof of concept that entire chromosomes may be silenced therapeutically in the future.

CRISPR in Cellular and Animal Models

Numerous experimental studies have utilized CRISPR-Cas9 in both human-derived cells and mouse models of DS, such as the Ts65Dn mouse. In these systems, specific dosage-sensitive genes—such as DYRK1A, RCAN1, APP, and SOD1—have been targeted to investigate their contribution to the DS phenotype.

For instance, in trisomic mouse models, partial inactivation of DYRK1A has resulted in improved cognitive function, synaptic plasticity, and hippocampal neurogenesis. Similar strategies applied to APP have reduced Alzheimer-like plaque formation and neurodegeneration [14, 15, 34].

Cellular models using iPSCs from DS patients have demonstrated that CRISPR-mediated gene correction can normalize cell proliferation rates and restore differentiation pathways in neural

and cardiac lineages [12, 13]. These findings support the potential of CRISPR to reverse developmental deficits at the cellular level.

Editing Symptom-Associated Genes

Beyond whole chromosome silencing, CRISPR offers a highly specific means to target individual genes responsible for particular DS symptoms. For example:

- **DYRK1A**: Overexpression contributes to cognitive dysfunction. CRISPR knockdown improves learning and memory in mice.
- **SOD1 / GPX1**: Genes involved in oxidative stress pathways, whose regulation may reduce neuronal damage.
- **APP**: Linked to early-onset Alzheimer's pathology; its partial correction reduces plaque burden.

By focusing on key targets, CRISPR allows researchers to develop tailored interventions that may be more feasible for clinical application than global chromosomal correction.

Toward Translational Applications

While current applications remain largely experimental, recent research suggests growing feasibility in using CRISPR to correct trisomy 21 at the chromosomal level. A team of scientists has reportedly achieved allele-specific editing of the extra chromosome 21 in human stem cells, leading to its selective elimination in a subset of cells. In these edited cells, researchers observed normalization of gene expression and improvements in cellular behavior. Though these results are preliminary and not yet validated clinically, they illustrate the conceptual viability of full chromosomal correction.

Additionally, new delivery platforms are under development to facilitate CRISPR-based editing in the nervous system. One such method involves the use of lipid nanoparticles capable of transporting CRISPR components across the blood-brain barrier. This strategy, still in preclinical stages, could eventually make it possible to correct gene expression in the brains of individuals with Down syndrome.

CRISPR may not yet be ready to "cure" Down syndrome, but it provides unprecedented access to dissect and potentially rebalance the biological mechanisms that underlie it.

5. Challenges and Limitations

Challenges and Limitations of CRISPR in the Context of Down Syndrome

Safety of Gene Therapy

Despite the growing therapeutic potential of CRISPR technology, safety remains one of the main clinical concerns. In the context of Down syndrome (DS), where the targets may include entire chromosomes or specific genes, the risks of side effects are multifaceted:

- **Off-target effects:** These refer to unintended modifications in non-target areas of the genome. Such changes can disrupt gene expression and, in extreme cases, lead to tumor formation.
- **Cellular response variability:** In DS cell models, gene editing efficiency varies significantly between cells. Some cells may not undergo editing or may respond abnormally, limiting the overall effectiveness of the therapy [13–15].
- **CRISPR delivery challenges:** Accurately and efficiently delivering CRISPR tools (e.g., Cas9 and gRNA) to target tissues such as the brain remains a challenge. New methods, such as lipid nanoparticles, are under testing, but their efficacy and safety have yet to be confirmed [34, 35].

Technological Limitations

- **Therapeutic durability:** In some cases, especially with somatic editing, repeated administration of therapy may be necessary.
- **Complexity of whole-chromosome editing:** Although in vitro silencing of the extra chromosome 21 has been achieved, translating this success to in vivo applications is highly complex. Further research is needed to ensure stability and long-term efficacy of such modifications.

Ethical and Social Aspects

- **Human genome modification:** In DS, editing may affect traits deeply connected to individual identity. This raises questions about the boundaries of human genetic intervention and biological diversity.

- **Consent and autonomy:** In prenatal or early-childhood therapies, informed consent from the patient is not possible. Decisions must be made by parents or guardians, which may lead to ethical concerns.
- **Stigmatization and social pressure:** Technological advancements could create social pressure to eliminate Down syndrome, raising concerns about the acceptance of people with disabilities and the right to genetic diversity.

The Need for Regulation and Oversight

All CRISPR-related interventions in the treatment of Down syndrome should be subject to international and national bioethical regulations. Transparent oversight, ethics committee involvement, and public dialogue involving physicians, patients, scientists, and legal experts are essential.

6. The Future of Gene Therapy in Down Syndrome

Promising Research Directions and Potential Breakthroughs

Recent advances in molecular biology and genetics have opened new horizons for the application of gene therapy in Down syndrome (DS). A key focus of ongoing research is the correction of gene dosage effects—particularly those involving critical genes such as DYRK1A, APP, and SOD1—using CRISPR-based editing platforms. Preclinical studies using DS mouse models and patient-derived induced pluripotent stem cells (iPSCs) have demonstrated that modulation of these genes can lead to improvements in cognitive function, neurogenesis, and cellular oxidative balance [5–15, 30–35].

One emerging line of investigation involves the use of base editing and prime editing technologies to perform precise, single-nucleotide corrections without inducing double-strand breaks. These approaches may offer safer and more targeted alternatives to traditional CRISPR-Cas9 methods, particularly for editing genes involved in neurodevelopment and immune function in DS [31–34].

Another major breakthrough is the creation of cerebral organoids—3D brain-like structures derived from iPSCs—which can be genetically edited using CRISPR to model DS in vitro. These organoids replicate key developmental and pathological features of DS brains, offering a novel platform for drug screening and mechanistic studies [25, 27, 30].

Future Perspectives for CRISPR Applications (including speculative insights)

Looking ahead, CRISPR's potential in treating DS lies in a combination of technological innovations and interdisciplinary research. Key areas expected to drive future progress include:

- **In vivo CRISPR delivery systems:** Advances in non-viral delivery, such as lipid nanoparticles and virus-like particles, may enable safe and efficient targeting of brain and cardiac tissues.
- **Cell-type specific editing:** Tissue-specific promoters and engineered Cas variants could allow CRISPR to act only in trisomy-affected cells, minimizing off-target effects.
- **Epigenome editing:** Beyond genetic correction, CRISPR can also modulate epigenetic states. This opens the door to reversible therapies that adjust gene expression without altering DNA sequences.
- **Integration with transcriptomics and AI:** Combining CRISPR with high-throughput gene expression profiling and artificial intelligence can accelerate identification of key therapeutic targets.

Although a clinical application for CRISPR in Down syndrome is not imminent, several experimental approaches are emerging that may redefine what is possible in the near future. Preliminary reports from recent research indicate that allele-specific CRISPR strategies have been able to eliminate the extra chromosome 21 in up to 30% of treated cells in vitro. These corrected cells exhibited restored gene expression profiles and improved cellular behavior. While these results have not yet progressed to clinical trials, they offer an early glimpse of what targeted aneuploidy correction might look like in practice.

In parallel, the development of novel lipid-based delivery systems that can cross the blood-brain barrier is opening the possibility of targeting neural tissues directly—potentially correcting gene expression in brain regions implicated in DS. These insights, though unofficial and not yet peer-reviewed in all cases, highlight a research field that is both dynamic and full of potential.

7. Conclusions

Down syndrome (DS) remains one of the most complex genetic disorders, and its treatment continues to rely on multidisciplinary support and individualized care. Gene therapy, particularly CRISPR-based approaches, presents an exciting future path for therapeutic innovation.

While numerous technological, ethical, and biological challenges remain, current studies using iPSCs, animal models, and new delivery platforms highlight CRISPR's transformative potential. From editing individual genes to silencing entire chromosomes, CRISPR allows researchers to explore genetic modulation in unprecedented ways.

Further investment in research, transparent ethical oversight, and open public dialogue will be essential. As science advances, the continued pursuit of safe, precise, and effective interventions holds the promise of a brighter future for individuals with Down syndrome.

Yet, amid this pursuit of innovation, a profound question lingers: Should our goal be to change individuals with Down syndrome, or to change how society embraces their diversity? Perhaps the greatest breakthrough will not come from editing DNA, but from editing perspectives — seeing value not only in correction, but in inclusion.

Disclosure

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Antonarakis, S. E., et al. (2021). *Down syndrome*. Nature Reviews Disease Primers, 6(1):9. DOI: https://doi.org/10.1038/s41572-019-0143-7 PMID:32029743
2. Blanco-Montaño, A., et al. (2023). *Factores de riesgo en el origen del síndrome de Down*. Revista Médica del IMSS, 61(5), 638–644. DOI: https://doi.org/10.5281/zenodo.8316459 PMID:3779135
3. Asim, A., et al. (2015). *Down syndrome: an insight of the disease*. Journal of Biomedical Science, 22, 41. DOI: https://doi.org/10.1186/s12929-015-0138-y PMID:26062604
4. Lorenzon, N., et al. (2023). *State-of-the-art therapy for Down syndrome*. Dev. Med. Child Neurol., 65(7), 870–884. DOI: https://doi.org/10.1111/dmcn.15517 PMID:36692980
5. Congting, G., et al. (2023). *Off-target effects in CRISPR/Cas9 gene editing*. Frontiers of Bioengineering and Biotechnology. DOI: https://doi.org/10.3389/fbioe.2023.1143157 PMID:36970624
6. Tyumentseva, M., et al. (2023). *CRISPR/Cas9 Landscape: Current State and Future Perspectives*. International Journal of Molecular Science., 24,16077 DOI: https://doi.org/10.3390/ijms242216077 PMID:38003266

7. Sharma, G., et al. (2021). *CRISPR-Cas9: A Preclinical and Clinical Perspective for the Treatment of Human Diseases*. 29(2).Molecular Therapy. DOI: https://doi.org/10.1016/j.ymthe.2020.09.028 PMID:33238136

8. Bhowmik, R., et al. (2022). *CRISPR/Cas9: a tool to eradicate HIV-1*. AIDS Research and Therapy. DOI: https://doi.org/10.1186/s12981-022-00483-y PMID:36457057

9. Du, Y., et al. (2023). *CRISPR/Cas9 systems: Delivery technologies and biomedical applications.* Asian Journal of Pharmaceutical Sciences. 18(2023)100854 DOI: https://doi.org/10.1016/j.ajps.2023.100854 PMID:38089835

10. Xu, X., et al. (2021). *Nanotechnology-based delivery of CRISPR/Cas9 for cancer treatment.* Advanced Drug Delivery Reviews.176(2021)113891 DOI: https://doi.org/10.1016/j.addr.2021.113891 PMID: 34324887

11. Laurent, M., et al. (2024). *CRISPR-Based Gene Therapies: From Preclinical to Clinical Treatments.* Cells. DOI: https://doi.org/10.3390/cells13100800 PMID:38786024

12. Hilary, V. Edwin, et al. (2023). *A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/ Cas13/Cas14 Proteins Utilized for Genome Engineering.* Molecular Biotechnology (2023) 65:311–325. DOI: https://doi.org/10.1007/s12033-022-00567-0 PMID:36163606

13. Liu, N., et al. (2022). *CRISPR Modeling and Correction of Cardiovascular Disease*. HHS Circ Res. DOI: https://doi.org/10.1161/CIRCRESAHA.122.320496. PMID:35679361

14. Nambiar, T.S., et al. (2022). *CRISPR-Based Genome Editing Through the Lens of DNA Repair*. HHS Mol Cell 82(2): 348–388. DOI: https://doi.org/10.1016/j.molcel.2021.12.026. PMID:35063100

15. Hendriks, D., (2020). *CRISPR-Cas Tools and Their Application in Genetic Engineering of Human Stem Cells and Organoids*. Stem Cell Stem. 27(5). DOI: https://doi.org/10.1016/j.stem.2020.10.014 PMID:33157047

16. Kagan, K.O., et al. (2022). *Antenatal screening for chromosomal abnormalities.* Archives of Gynecology and Obstetrics. 305:825–835 DOI: https://doi.org/10.1007/s00404-022-06477-5 PMID:35279726

17. Baruchel, A., et al. (2023). *Down syndrome and leukemia: from basic mechanisms to clinical advances.* Haematologica. Vol.108 No.10 DOI: https://doi.org/10.3324/haematol.2023.283225 PMID:37439336

18. Abedalthagafi, M., et al. (2023). *Non-invasive prenatal testing: a revolutionary journey in prenatal testing.* Frontiers in Medicine. Vol.10. DOI: https://doi.org/10.3389/fmed.2023.1265090 PMID:38020177

19. Lanoo, L., et al. (2022). *Rare autosomal trisomies detected by non-invasive prenatal testing: an overview of current knowledge*. European Journal of Human Genetics (2022) 30:1323–1330. DOI: https://doi.org/10.1038/s41431-022-01147-1 PMID:35896702

20. Sperling, K., et al. (2023). *Population monitoring of trisomy 21: problems and approaches.* Molecular Cytogenetics. 16:6 DOI: https://doi.org/10.1186/s13039-023-00637-1 PMID: 37183244

21. Deborah, K.S., et al. (2024). *Alzheimer's Drugs APPlication for Down syndrome?* Ageing Research Reviews. 96: 102281. DOI: https://doi.org/10.1016/j.arr.2024.102281. PMID:38513771

22. Torres, E.M, (2023). *Consequences of gaining an extra chromosome*. Chromosome Research 31:24 DOI: https://doi.org/10.1007/s10577-023-09732-w PMID:37620607

23. Buczyńska , A., et al. (2023). *The Role of Oxidative Stress in Trisomy 21 Phenotype*. Cellular and Molecular Neurobiology 43(8):3943–3963 DOI: https://doi.org/10.1007/s10571-023-01417-6 PMID:37819608

24. Laurent, A.P., et al. (2020). *Gain of chromosome 21 in hematological malignancies: lessons from studying leukemia in children with Down syndrome*. Leukemia (2020) 34(8):1984–1999 DOI: https://doi.org/10.1038/s41375-020-0854-5 PMID:32433508

25. Levin, J., et al. (2023). *Diseases Affecting Middle-Aged and Elderly Individuals With Trisomy 21*. Dtsch Arztebl Int 2023; 120: 14–24. DOI: https://doi.org/10.3238/arztebl.m2022.0371 PMID: 36468261

26. Buczyńska, A., et al. (2021). *Novel Approaches to an Integrated Route for Trisomy 21 Evaluation*. Biomolecules. DOI: https://doi.org/10.3390/biom11091328 PMID:34572541

27. Wu, Y., et al. (2022). *Cell models for Down syndrome-Alzheimer's disease research.* Neuronal Signaling, 6(1) NS20210054 DOI: https://doi.org/10.1042/NS20210054 PMID: 35449591

28. Di Matei, V., et al. (2021). *Decision-making factors in prenatal testing: A systematic review.* Health Psychol Open. DOI: https://doi.org/10.1177/2055102920987455 PMID:33489303

29. Saini, F., et al. (2022). *Structural Connectivity in Down Syndrome and Alzeimer's Disease.* Frontiers in Neuroscience. DOI: https://doi.org/10.3389/fnins.2022.908413 PMID:35937882

30. Krivega, M., et al. (2022). *Consequences of chromosome gain: A new view on trisomy syndromes.* The American Journal of Human Genetics 109, 2126–2140, December 1, 2022. DOI: https://doi.org/10.1016/j.ajhg.2022.10.014. PMID: 36459979

31. Giono L.E, (2017). Crispr/Cas9 Y La Terapia Génica. Medicina (B Aires) - Volumen 77 - Nº 5, 2017 DOI: N/A PMID: 29044017

32. Exposito, J., et al. (2023). TERAPIA GENICA: ¿DÓNDE ESTAMOS?, ¿A DÓNDE VAMOS?. Medicina (B Aires). DOI: n/a PMID: 37714117

33. Shinwari, Z. K., et al. (2018). *Ethical Issues Regarding CRISPR-mediated Genome Editing.* Current Issues Of Molecular Biology. DOI: https://doi.org/10.21775/cimb.026.103 PMID:28879860

34. Janik, E., et al. (2020). *Various Aspects of a Gene Editing System—CRISPR–Cas9.* International Journal of Molecular Sciences. DOI: https://doi.org/10.3390/ijms21249604 PMID: 33339441

35. Redman, M., et al. (2016). *What is CRISPR/Cas9*?. Arch Dis Child Educ Pract Ed 2016;101:213–215. DOI: https://doi.org/10.1136/archdischild-2016-310459 PMID:27059283