

Article Review

Precision Chromosomal Surgery before Birth: Allele-Specific CRIS-PR-Cas9 Editing for Trisomy 21 in Perinatal Medicine

I Nyoman Hariyasa Sanjaya,¹ Wiku Andonotopo,² Muhammad Adrianes Bachnas,³ Wisnu Prabowo,³ Eric Edwin Yuliantara,³ Efendi Lukas,⁴ Julian Dewantiningrum,⁵ Mochammad Besari Adi Pramono,⁵ Anak Agung Gede Putra Wiradnyana,¹ Ryan Saktika Mulyana,¹ Anak Agung Ngurah Jaya Kusuma,¹ Evert Solomon Pangkahila,¹ Khanisyah Erza Gumilar,⁶ Ernawati Darmawan,⁶ Muhammad Ilham Aldika Akbar,⁶ Cut Meurah Yeni,⁷ Dudy Aldiansyah,⁸ Nuswil Bernolian,⁹ Adhi Pribadi,¹⁰ Anita Deborah Anwar,¹⁰ Aloysius Suryawan,¹¹ Ridwan Abdullah Putra,¹¹ Harry Kurniawan Gondo,¹² Laksmana Adi Krista Nugraha,¹³ Waskita Ekamaheswara Kasumba Andanaputra,¹⁴ Wibisana Andika Krista Dharma,¹⁵ Dovy Djanas,¹⁶ and Milan Stanojevic¹⁷

¹Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Ngoerah General Hospital, Bali, Indonesia.

²Maternal-Fetal Medicine Division, Women Health Center, Department of Obstetrics and Gynecology, Ekahospital BSD City, Serpong, Tangerang, Banten, Indonesia.

³Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Medical Faculty of Sebelas Maret University, Dr. Moewardi Hospital, Solo, Surakarta, Indonesia.

⁴Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University of Makassar, Indonesia.

⁵Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Medical Faculty of Diponegoro University, Dr. Kariadi Hospital, Semarang, Indonesia.

⁶Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya, Indonesia.

⁷Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Syiah Kuala University, Dr. Zainoel Abidin General Hospital, Aceh, Indonesia.

⁸Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Sumatera Utara University, H. Adam Malik General Hospital, Medan, Indonesia.

⁹Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Sriwijaya University, Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia.

¹⁰Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Padjadjaran University, Hasan Sadikin General Hospital, Bandung, Indonesia.

¹¹Department of Obstetrics and Gynecology, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

¹²Department of Obstetrics and Gynecology, Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia

¹³Department of Medicine, Faculty of Medicine, Diponegoro University, Semarang, Indonesia.

¹⁴Department of Medicine, Undergraduate Program in Medical Science, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia.

¹⁵Department of Medicine, Undergraduate Program in Medical Science, Faculty of Medicine, Gajah Mada University, Yogyakarta, Indonesia.

¹⁶Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University, M.Djamil General Hospital, Padang, West Java, Indonesia

¹⁷Medical University of Warsaw, Department of Neonatology and Rare Diseases, Warsaw, Poland.

Corresponding author: Wiku Andonotopo, MD, PhD. E-mail : wiku.andonotopo@gmail.com

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Abstract

Objective: Trisomy 21 remains the most common live-born aneuploidy and a major contributor to perinatal morbidity. Although prenatal screening, particularly non-invasive prenatal testing (NIPT), has advanced substantially, clinical management offers no corrective options. Emerging allele-specific genome-editing approaches propose targeted removal or silencing of the extra chromosome 21. This review summarizes current evidence and evaluates the translational relevance of these technologies in perinatal medicine.

Methods: A narrative review was conducted following PRISMA-aligned procedures. A structured search of PubMed, Scopus, and Web of Science (January 2000–July 2025) identified 1,242 records. After duplicate removal, title/abstract screening, and full-text assessment based on predefined inclusion criteria, 54 studies met eligibility requirements. Data were synthesized across four domains: mechanistic strategies, developmental applicability, translational feasibility, and ethical–regulatory considerations.

Results: Allele-specific CRISPR-Cas9 studies demonstrated selective cleavage of the supernumerary chromosome 21 in cellular models, with partial restoration of near-euploid transcriptional patterns. Additional approaches—XIST-mediated silencing and centromere destabilization—provided alternative mechanisms with varying stability and specificity. Evidence remains limited to in vitro systems, with no validated embryo or fetal applications. Key challenges include mosaicism, delivery barriers, individualized SNP targeting, and ethical governance.

Conclusions: Allele-specific chromosome editing represents a promising but still experimental direction for future perinatal therapeutics. Current findings justify continued multidisciplinary investigation while emphasizing cautious interpretation and rigorous ethical oversight prior to any clinical translation.

Keywords: Chromosome therapy; CRISPR-Cas9; fetal genome surgery; perinatal gene editing; trisomy 21

Bedah Kromosom Presisi Sebelum Lahir: Penyuntingan Spesifik Alel CRISPR-Cas9 untuk Trisomi 21 dalam Kedokteran Perinatal

Abstrak

Tujuan: Trisomi 21 tetap menjadi aneuploidi yang paling sering ditemukan pada kelahiran hidup dan merupakan kontributor utama terhadap morbiditas perinatal. Meskipun skrining prenatal—khususnya non-invasif prenatal testing (NIPT)—telah mengalami kemajuan yang signifikan, penatalaksanaan klinis hingga kini belum menawarkan opsi korektif. Pendekatan pengeditan genom spesifik alel yang mulai berkembang mengusulkan penghilangan atau penghambatan terarah terhadap salinan ekstra kromosom 21. Tinjauan ini merangkum bukti terkini serta mengevaluasi relevansi translasional teknologi tersebut dalam kedokteran perinatal.

Metode: Tinjauan naratif dilakukan dengan mengikuti prosedur yang selaras dengan PRISMA. Pencarian terstruktur terhadap PubMed, Scopus, dan Web of Science (Januari 2000–Juli 2025) mengidentifikasi 1.242 rekaman. Setelah penghapusan duplikasi, penyaringan judul/abstrak, dan penilaian teks lengkap berdasarkan kriteria inklusi yang telah ditentukan, sebanyak 54 studi memenuhi persyaratan kelayakan. Data disintesis ke dalam empat domain: strategi mekanistik, aplikabilitas perkembangan, kelayakan translasional, serta pertimbangan etika dan regulasi.

Hasil: Studi CRISPR-Cas9 spesifik alel menunjukkan pemotongan selektif terhadap kromosom 21 supernumerari pada model seluler, dengan pemulihan parsial pola transkripsi menuju profil ekspresi gen yang menyerupai kondisi euploid. Pendekatan lain—seperti penghambatan berbasis XIST dan destabilisasi sentromer—menyediakan mekanisme alternatif dengan tingkat kestabilan dan spesifisitas yang bervariasi. Bukti saat ini terbatas pada sistem in vitro, tanpa aplikasi yang tervalidasi pada embrio maupun janin. Tantangan utama meliputi mosaikisme, hambatan pengantaran, kebutuhan penargetan SNP individual, serta tata kelola etis.

Kesimpulan: Pengeditan kromosom spesifik alel merupakan arah yang menjanjikan, namun masih bersifat eksperimental bagi terapi perinatal di masa mendatang. Temuan saat ini mendukung keberlanjutan penelitian multidisipliner, sekaligus menekankan perlunya interpretasi yang hati-hati dan pengawasan etika yang ketat sebelum penerapannya dalam praktik klinis.

Kata Kunci: Bedah genom janin; CRISPR-Cas9; penyuntingan gen perinatal; terapi kromosom; trisomi 21

Introduction

Trisomy 21, the chromosomal condition underlying Down syndrome, remains the most frequently observed aneuploidy compatible with postnatal survival and continues to impose a substantial burden on perinatal health worldwide. Population-based estimates consistently indicate that approximately one in seven hundred live births is affected, with prevalence shaped by maternal age distribution, access to prenatal screening, and sociocultural factors influencing reproductive decision-making.¹⁻³ From early gestation onward, trisomy 21 alters neurodevelopmental trajectories, disrupts metabolic homeostasis, and predisposes affected individuals to a wide spectrum of congenital and acquired conditions that persist across the lifespan.

Over the past two decades, advances in prenatal diagnostics have transformed the detection of chromosomal aneuploidies. The introduction of non-invasive prenatal testing, based on analysis of cell-free fetal DNA, has enabled highly sensitive and specific screening for trisomy 21 early in pregnancy, reducing reliance on invasive diagnostic procedures.⁴⁻⁶ These developments have been accompanied by refinements in counseling frameworks and risk stratification strategies, leading to earlier and more informed clinical discussions. Despite this progress, however, prenatal care for trisomy 21 remains fundamentally descriptive rather than interventional. Contemporary clinical pathways focus on detection, prognostication, and supportive management, without offering approaches capable of modifying the chromosomal basis of the condition itself.⁷

The notion that chromosomal abnormalities might one day be corrected rather than merely identified has long occupied a speculative space at the margins of genetics and developmental biology. Early theoretical discussions framed

aneuploidy as a quantitative problem of gene dosage, raising the possibility that transcriptional imbalance could be mitigated without altering chromosomal structure.⁸ Experimental efforts to test this idea culminated in studies demonstrating that ectopic expression of the X-inactive specific transcript could induce chromosome-wide transcriptional suppression when applied to autosomes, including chromosome 21.⁹ Subsequent refinements showed partial normalization of gene expression and improvements in neuronal differentiation in trisomic neural stem cell models, lending biological credibility to the concept of dosage compensation beyond the sex chromosomes.¹⁰ At the same time, these experiments revealed intrinsic limitations, including incomplete silencing, instability across cell divisions, and persistent mosaicism, underscoring the challenge of achieving durable correction through epigenetic means alone.¹¹

Parallel advances in genome editing began to reshape the landscape of what might be technically achievable. The development of CRISPR-Cas systems introduced a level of precision and programmability that had not previously been attainable in mammalian genomes.¹² While early applications focused on single-gene modification, attention gradually shifted toward more ambitious targets, including structural variations and chromosomal abnormalities. Conceptual work in precision gene therapy emphasized that the scalability of genome editing would ultimately depend on its ability to discriminate among highly similar genomic sequences while minimizing collateral damage.¹³ In this context, naturally occurring single-nucleotide polymorphisms emerged as a potential solution, offering molecular signatures that could distinguish one chromosomal copy from its homologs within the same nucleus.

Building on this insight, allele-specific genome-editing strategies were developed to selectively target the supernumerary

chromosome in trisomic cells. By designing guide RNAs that recognize polymorphic sequences unique to the extra chromosome 21, investigators demonstrated that targeted double-strand breaks could be induced preferentially in the surplus chromosome while sparing the normal homologous pair.¹⁴ The most compelling evidence to date showed that such an approach could result in physical elimination of the additional chromosome in human induced pluripotent stem cells derived from individuals with Down syndrome, with subsequent restoration of transcriptional patterns approaching a euploid state.¹⁵ These findings marked a conceptual shift from modulation of gene expression toward structural correction at the chromosomal level, challenging the long-held assumption that whole-chromosome abnormalities were beyond the reach of molecular intervention.¹⁶

Despite the conceptual significance of these studies, their implications for perinatal medicine remain uncertain. Experimental investigations have clarified many of the cellular consequences of trisomy 21, including chronic proteotoxic stress, altered cell-cycle dynamics, and widespread transcriptional dysregulation, which together shape the developmental phenotype of affected tissues.^{17,18} At the same time, work in developmental biology has highlighted the sensitivity of early embryonic and fetal stages to genomic perturbation, emphasizing that timing, lineage allocation, and cellular context critically influence the outcome of genome-editing interventions.¹⁹ These observations suggest that any attempt to correct aneuploidy *in vivo* would need to address not only technical feasibility but also the biological risks associated with mosaicism, incomplete correction, and unintended developmental consequences.²⁰

Developments in reproductive medicine further complicate this landscape. The widespread adoption of *in vitro* fertilization and preimplantation genetic testing has

enabled increasingly early identification of chromosomal abnormalities, creating a theoretical window in which genomic correction might occur before lineage diversification.²¹ At the same time, professional societies continue to emphasize that existing prenatal screening programs are designed to inform reproductive choices rather than to enable therapeutic intervention. Guidelines issued by obstetric and genetic organizations consistently underscore that, at present, no curative treatments exist for chromosomal aneuploidies and that counseling should reflect this reality.^{22–25} This tension between expanding diagnostic capability and the absence of corrective options has become a defining feature of contemporary perinatal genetics.

Against this background, the literature addressing chromosome-level genome editing has grown rapidly but remains fragmented. Experimental reports are dispersed across molecular genetics, stem cell biology, developmental science, and bioethics, often addressing isolated aspects of feasibility without situating them within a coherent perinatal framework. Ethical analyses raise additional questions concerning germline modification, consent, disability rights, and societal impact, yet these discussions are rarely integrated with mechanistic or translational evidence. As a result, clinicians and researchers lack a unified synthesis that evaluates whether emerging chromosome-editing technologies can be meaningfully aligned with the biological realities and ethical constraints of prenatal and perinatal care.

The present review seeks to address this gap by synthesizing available evidence on allele-specific CRISPR-Cas approaches to chromosome 21 correction within a structured, PRISMA-aligned narrative framework. Drawing on literature published between two thousand and twenty-five, this review examines the evolution of strategies from

epigenetic suppression to direct chromosomal excision, considers their developmental and translational implications, and critically evaluates the ethical and regulatory challenges that accompany chromosome-level intervention. By integrating mechanistic insights with perinatal clinical perspectives, the review introduces the concept of Precision Chromosomal Surgery as a way to frame ongoing research while acknowledging the substantial scientific and societal barriers that must be addressed before any clinical application can be contemplated.

Methods

Study Design and Review Framework

This review was conceived as a structured narrative synthesis aimed at mapping emerging developments in allele-specific CRISPR-Cas9 strategies for chromosome 21 correction within a perinatal and developmental context. A narrative design was selected deliberately, reflecting the early and exploratory stage of chromosome-level genome editing research. The existing body of evidence is dominated by in vitro

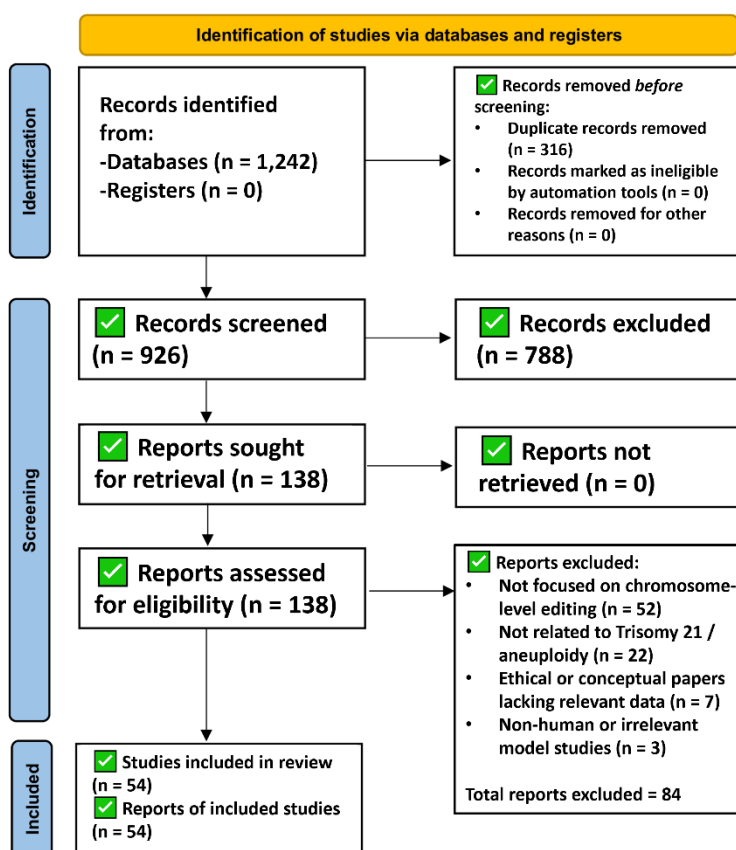


Figure 1 PRISMA 2020 Flow Diagram for Literature Screening on Allele-Specific Chromosome 21 Editing. This PRISMA 2020 flow diagram illustrates the identification, screening, eligibility assessment, and final inclusion of studies for this narrative review. A total of 1,242 records were retrieved from PubMed, Scopus, and Web of Science (2000–2025). After removal of 316 duplicates and multi-stage screening, 138 full-text articles were assessed for eligibility, and 54 studies met the inclusion criteria and were incorporated into the final synthesis.

experiments, mechanistic studies, and ethical or conceptual analyses, with no clinical trials and no animal models sufficiently analogous to human development to permit quantitative pooling or meta-analytic comparison.¹⁻⁵ In this setting, a narrative approach allows integration of mechanistic insight, developmental relevance, and translational uncertainty in a manner that would not be achievable through formal statistical synthesis.

Although the review followed established principles of transparency and reproducibility in evidence identification and selection, it does not meet the formal criteria of a systematic review. For this reason, the study was not registered in PROSPERO, and no predefined protocol was published. This decision is consistent with current guidance for narrative and scoping reviews addressing rapidly evolving fields in which the available literature is heterogeneous and largely preclinical.⁶⁻⁸

Literature Search Strategy

A comprehensive literature search was conducted across PubMed, Scopus, and Web of Science to identify relevant publications from January 2000 through July 2025. This timeframe was chosen to encompass the earliest experimental explorations of chromosome-wide dosage modulation, including XIST-mediated silencing, as well as more recent studies demonstrating allele-specific chromosome elimination using CRISPR-based platforms^[9-12]. Search terms were developed iteratively to balance sensitivity and relevance and included combinations of “Trisomy 21,” “Down syndrome,” “CRISPR-Cas9,” “allele-specific editing,” “chromosome elimination,” “preimplantation gene correction,” “fetal therapy,” and “perinatal genomics.” Boolean operators and database-specific syntax were adapted as needed to optimize retrieval.

While a full PRISMA systematic-review workflow was not applied, core PRISMA principles were followed to ensure transparent documentation of study identification, screening, eligibility assessment, and inclusion. The overall flow of records through these stages is summarized in the PRISMA 2020 flow diagram presented in Figure 1, which provides a visual account of how the final body of literature was assembled.

Study Selection and Eligibility Assessment

All records retrieved from the database searches were imported into a reference management system, and duplicate entries were removed prior to screening. Titles and abstracts were reviewed independently by two reviewers to assess relevance to allele-specific genome editing, chromosome-level correction strategies, or translational considerations related to perinatal medicine. Discrepancies at this stage were resolved through discussion, with emphasis on conceptual relevance rather than study design alone. Full-text assessment was subsequently performed for articles addressing mechanisms of chromosome elimination, epigenetic approaches to dosage compensation, allele-specific CRISPR-Cas strategies, or ethical and regulatory frameworks related to embryo or fetal genome editing^[13-16]. Only peer-reviewed articles published in English were considered eligible. Conference abstracts, opinion pieces without original scientific or analytical content, studies unrelated to trisomy 21 or genome editing, and publications lacking relevance to developmental or perinatal contexts were excluded. Following this multistage process, fifty-four studies met the predefined inclusion criteria. The progression from initial retrieval to final inclusion is detailed in Figure 1.

Data Extraction and Thematic Synthesis

Data extraction was conducted manually

using a thematic framework designed to capture the dimensions most relevant to chromosome-level intervention. Extracted elements included mechanistic principles underlying allele-specific editing, experimental strategies for chromosome removal or silencing, developmental timing considerations, translational feasibility, and ethical or regulatory implications of embryo- or fetus-directed interventions. This approach reflects the multidisciplinary nature of the field and acknowledges that technical feasibility, developmental biology, and ethical governance are tightly interwoven in discussions of chromosomal correction [17–20]. Given the marked heterogeneity of study designs, outcome measures, and experimental models, no attempt was made to perform quantitative synthesis or comparative effect estimation. Instead, evidence was integrated narratively to identify convergent themes, unresolved challenges, and areas of conceptual advancement. This qualitative synthesis aligns with prior guidance for reviews in emerging genomic technologies, where the primary objective is to clarify conceptual trajectories rather than to estimate effect sizes [21–23].

Analytical Approach and Conceptual Integration

The final synthesis integrates molecular mechanisms, experimental findings, and translational considerations into a coherent narrative aligned with the concept of Precision Chromosomal Surgery. This framework emphasizes how allele-specific genome editing might theoretically intersect with reproductive and perinatal medicine while maintaining a clear distinction between experimental proof-of-concept and clinically actionable intervention. Ethical and regulatory perspectives were incorporated throughout the analysis rather than treated as a separate domain, reflecting the inseparability of

technical feasibility and societal governance in chromosome-level genome editing.^{24,25} By adopting this structured yet flexible methodological approach, the review aims to provide a transparent account of how the literature was identified, evaluated, and synthesized, while remaining appropriately cautious about the limits of current evidence.

Results and Findings

Literature Screening and Evidence Mapping

The structured search across PubMed, Scopus, and Web of Science yielded 1,242 records potentially relevant to chromosome-level intervention in Trisomy 21. After duplicate removal, title and abstract screening reduced this set substantially, leading to retrieval of 138 full-text articles for eligibility assessment. Of these, fifty-four studies met the predefined inclusion criteria and were incorporated into the final synthesis. The flow of records through identification, screening, eligibility evaluation, and inclusion is documented in the PRISMA 2020 flow diagram shown in Figure 1. The resulting literature did not converge around a single experimental paradigm but instead clustered across several intersecting domains. A small number of studies addressed direct chromosome elimination through allele-specific genome editing. Earlier work explored epigenetic approaches to dosage modulation, while a broader body of publications examined developmental, translational, ethical, and policy dimensions of genome editing in reproductive and perinatal contexts. The relatively modest number of eligible studies reflects the early stage of chromosome-scale editing research and highlights an ongoing conceptual transition from prenatal detection toward theoretical correction, a progression schematically represented in Figure 2.

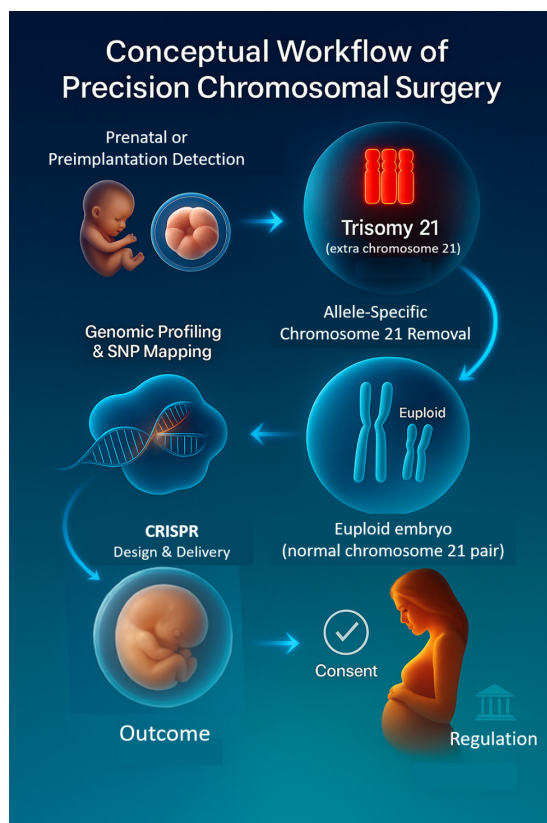


Figure 2 Conceptual Workflow of Allele-Specific Chromosome 21 Removal for Trisomy 21 Correction. Prenatal or preimplantation detection identifies the presence of Trisomy 21, characterized by an extra copy of chromosome 21. Genomic profiling and single-nucleotide polymorphism (SNP) mapping enable the design of allele-specific CRISPR-Cas systems, which selectively target and remove the extra chromosome. Following CRISPR design and delivery, chromosomal dosage is restored to the normal euploid state with two copies of chromosome 21, resulting in a corrected embryo with normalized karyotype. The workflow emphasizes ethical and regulatory checkpoints, including parental consent and adherence to clinical governance, essential for future translational applications of chromosome-level gene therapy.

Allele-Specific CRISPR Editing: A Foundational Advance

Within the reviewed literature, the most consequential experimental advance was the demonstration that naturally occurring single-nucleotide polymorphisms can be exploited to achieve allele-specific targeting of the supernumerary chromosome 21. In trisomy 21 induced pluripotent stem cells, Hashizume and colleagues showed that CRISPR-Cas9 nucleases guided by polymorphism-specific sequences could preferentially

cleave the extra chromosome, resulting in its elimination in a subset of edited cells and partial restoration of transcriptional patterns toward a euploid state.¹ Gene-expression normalization was particularly evident in pathways related to neural differentiation and metabolic regulation, both central to Down syndrome pathophysiology. This work represents a conceptual departure from earlier efforts aimed at attenuating gene dosage and instead introduces structural correction as a plausible experimental goal. The mechanistic logic underlying allele discrimination and

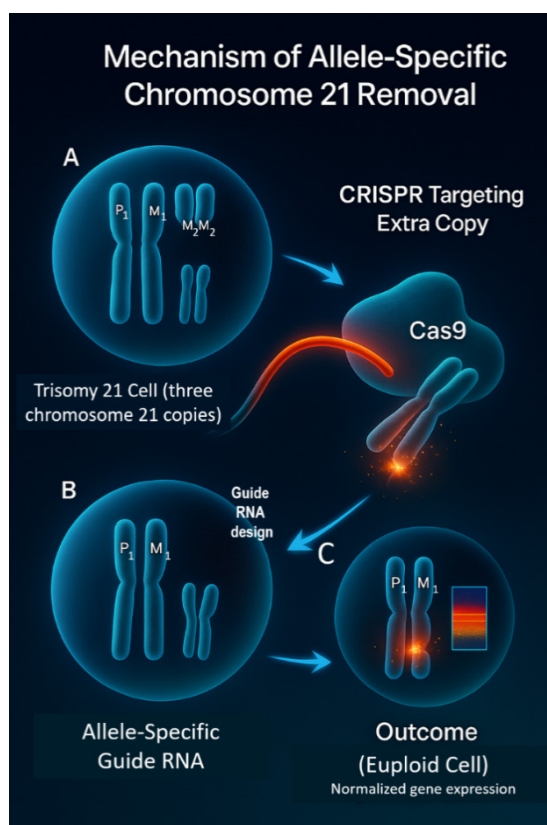


Figure 3 Mechanism of Allele-Specific Chromosome 21 Removal for Trisomy 21 Correction. (A) A Trisomy 21 cell containing three copies of chromosome 21 (one paternal and two maternal) is illustrated, representing the genomic imbalance responsible for Down syndrome pathophysiology. (B) Single-nucleotide polymorphism (SNP) differences unique to the extra chromosome enable the design of allele-specific guide RNAs, which selectively target the surplus chromosome while sparing the normal homologous pair. The CRISPR-Cas9 complex binds the unique sequence, inducing targeted double-strand breaks that promote degradation and removal of the extra chromosome. (C) Post-editing, cells exhibit the corrected euploid state with only two copies of chromosome 21, accompanied by normalized gene expression patterns, as represented by the transcriptome heatmap inset. This approach demonstrates a conceptual framework for chromosome-level gene therapy targeting aneuploid conditions.

selective chromosome targeting is illustrated in Figure 3, which depicts how guide RNAs can differentiate among homologous chromosomes on the basis of single-base variation. Although editing efficiency was incomplete and mosaic outcomes persisted, these findings provide the first empirical evidence that whole-chromosome removal is technically feasible in human cellular systems.

Comparative Models of Chromosome Dosage Correction

Prior to the emergence of SNP-guided chromosome excision, strategies for addressing trisomy 21 focused largely on reducing functional gene dosage without altering chromosomal structure. XIST-mediated silencing approaches demonstrated that ectopic activation of X-inactivation–like mechanisms could suppress transcription

Table 1 Comparative Strategies for Trisomy 21 Chromosome Correction

Approach / Technology	Mechanism of Action	Key Insight	Key Outcome	Advantages / Strengths	Limitations / Risks	Current Stage of Development	Quality Score (AMSTAR / ROBIS / NOS)
XIST-mediated silencing [16,17,18,19]	Ectopic expression of the XIST long non-coding RNA to inactivate one copy of chromosome 21 epigenetically	Repurposes natural X chromosome inactivation for autosome dosage correction	Partial suppression of trisomic gene overexpression in neural stem cells; promoted neuronal differentiation	Reversible, avoids DNA double-strand breaks, uses endogenous epigenetic pathways	Incomplete silencing, mosaicism risk, uncertain developmental stability	In vitro proof-of-concept in iPSC-derived neural stem cells	NOS: Moderate
Centromere destabilization [25]	Targeted disruption of centromere-specific proteins or transcriptional destabilization leading to mis-segregation and loss of the extra chromosome 21	Exploits chromosome mis-segregation to induce aneuploid rescue	Chromosome loss events in cultured cells but low efficiency and specificity	Conceptually simple; may not require allele-specific guides	High off-target risk, unpredictable chromosomal consequences, low efficiency	Conceptual / early in vitro	ROBIS: High risk of bias
Allele-specific CRISPR-Cas9 chromosomal excision [1]	CRISPR-Cas9 nucleases guided to unique SNP polymorphisms on the supernumerary chromosome 21, inducing double-strand breaks and elimination of that chromosome	First demonstration of targeted removal of an entire chromosome using allele specificity	Up to 37.5% efficiency in human iPSCs, restoration of near-euploid transcriptome in neural and metabolic pathways	High specificity (SNP-discriminating), durable correction, transcriptional normalization	Personalized SNP mapping required, mosaicism risk, off-target edits, germline editing ethics	In vitro proof-of-concept (2025)	NOS: High (low risk of bias)

Footnote: AMSTAR = A Measurement Tool to Assess Systematic Reviews; ROBIS = Risk of Bias in Systematic Reviews; NOS = Newcastle-Ottawa Scale for observational studies.

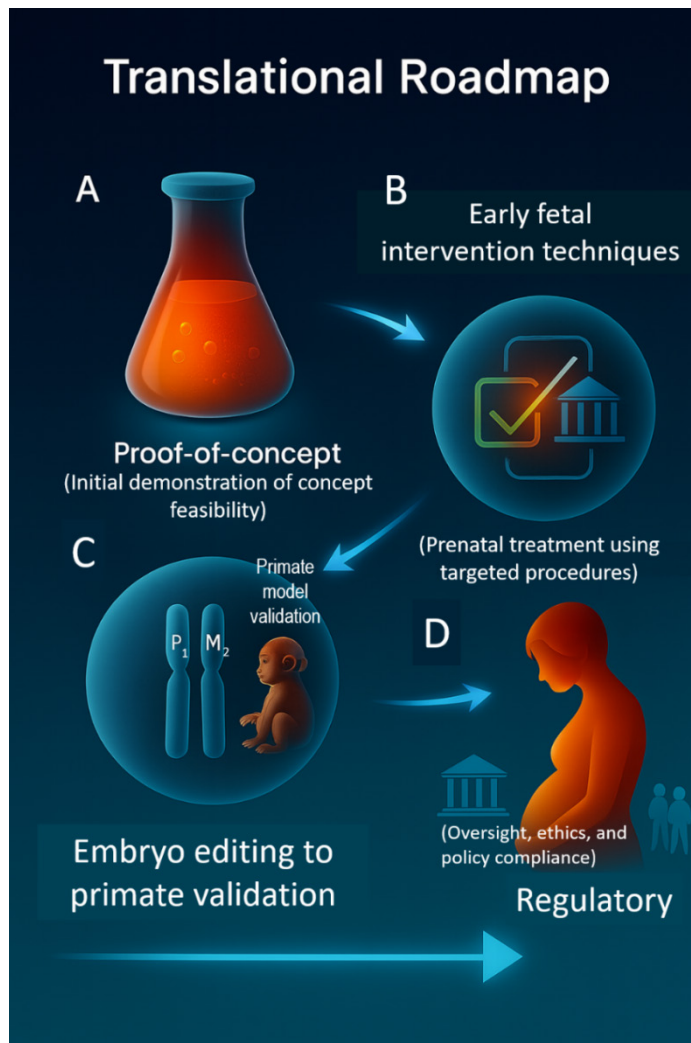


Figure 4 Translational Roadmap for Chromosome 21 Precision Surgery. (A) **Proof-of-concept:** Early-stage feasibility studies including in vitro assays, organoid systems, and initial small-animal models to establish experimental validity. (B) **Early fetal intervention techniques:** Preimplantation or early gestational approaches such as in vitro fertilization (IVF) embryo editing, ultrasound-guided fetal delivery, and mosaicism screening to evaluate safety and efficacy in early developmental stages. (C) **Embryo editing to primate validation:** Progression from small-animal studies to non-human primate models, providing critical data on feasibility, long-term effects, and cross-species translation of chromosomal surgery. (D) **Regulatory and ethical oversight:** Frameworks ensuring compliance with bioethics, safety regulations, and public engagement, addressing societal and policy considerations necessary for future clinical application. Together, these steps outline the integrated scientific, translational, and regulatory workflow required to advance chromosome-level therapeutic interventions for aneuploidy disorders toward clinical use.

across chromosome 21 in neural stem cell models, leading to partial phenotypic improvement.^{16–19} While these studies established biological plausibility for chromosome-wide modulation, they also revealed important constraints, including incomplete silencing, epigenetic instability, and persistent mosaicism across cell divisions. Alternative approaches aimed at inducing loss of the extra chromosome through centromere destabilization or mis-segregation were explored conceptually and experimentally, but these methods were characterized by low efficiency and substantial risk of unintended genomic disruption^[20]. When viewed collectively, these strategies illustrate a progression from functional attenuation toward structural intervention. A comparative synthesis of the principal chromosome-modulating approaches, including their mechanisms, advantages, and limitations, is presented in Table 1, which situates allele-specific CRISPR editing within the broader landscape of trisomy 21 correction strategies.

Applications in Embryo and Fetal Therapy

None of the studies meeting inclusion criteria reported successful chromosome-level editing in human embryos or fetuses. Nevertheless, several publications addressed the theoretical relevance of extending allele-specific editing approaches into reproductive and perinatal settings. Advances in *in vitro* fertilization and preimplantation genetic testing have enabled increasingly early identification of aneuploidy, raising the possibility that correction might occur before extensive lineage commitment.²¹ Intervention at this stage is conceptually appealing because earlier editing could reduce the likelihood of mosaicism, a recurrent limitation of post-zygotic genome modification. Contextual insight is also provided by experimental work in fetal gene therapy for monogenic disorders, which has

demonstrated the technical feasibility of *in utero* delivery under carefully controlled conditions.^{22–24} However, extrapolation to chromosome-scale editing introduces additional complexity related to delivery efficiency, genomic stability, and long-term developmental consequences. These translational considerations are integrated into the developmental trajectory outlined in Figure 4, which frames chromosome correction as a multistage process extending from experimental proof-of-concept toward hypothetical clinical application.

Technical, Biological, and Ethical Barriers

Across the reviewed literature, several persistent barriers to translation were identified. Allele-specific chromosome editing depends on the presence and accurate mapping of informative polymorphisms, necessitating individualized guide RNA design and limiting scalability across diverse populations.¹ Whole-chromosome removal amplifies concerns regarding off-target effects and genomic instability, given the magnitude of structural alteration involved. Mosaicism remains a significant biological challenge, particularly when editing occurs after early embryonic cleavage stages. Ethical and regulatory analyses emphasized that chromosome-level intervention raises questions extending beyond technical feasibility. Issues related to germline modification, consent on behalf of future individuals, societal perceptions of disability, and equitable access to advanced therapies have been examined extensively, with no consensus regarding acceptable thresholds for clinical use.^{7–11,21–25} The major ethical and regulatory dimensions identified across these discussions are summarized in Table 2, underscoring the need for governance frameworks that evolve in parallel with scientific capability.

Table 2 Ethical, Regulatory, and Societal Considerations for Chromosome-Level Editing

Domain	Critical Question / Ethical Dilemma	Relevance to Chromosomal Editing	Regulatory Context / Global Guidance	Proposed Safeguards / Solutions	Implementation Challenges	Impact Level
Germline Implications [7,8,9]	Will edits be heritable and passed to future generations?	Preimplantation and early fetal editing can modify the germline permanently, impacting descendants.	WHO, Nuffield Council, and NAS permit research but prohibit clinical germline editing outside strict frameworks.	Restrict use to severe, untreatable conditions; independent ethics review; heritability risk counseling.	Variability in national policies; unpredictable multigenerational effects; public resistance.	High
Safety and Risk Thresholds [1,12]	What levels of off-target edits and mosaicism are acceptable?	Whole-chromosome editing poses greater genomic stability risks than single-gene edits.	No explicit thresholds exist; safety frameworks evolving via WHO Genome Editing Advisory Committee.	Use high-fidelity CRISPR tools, multi-omics off-target screening, validated preclinical animal models, long-term follow-up.	Detection of rare genomic rearrangements is challenging; embryonic models are limited.	High
Consent and Autonomy [10,11]	How to obtain meaningful informed consent for embryonic or fetal interventions?	Future patients cannot consent; decisions rest entirely with parents or guardians.	Ethics bodies emphasize enhanced informed consent protocols and ethics committee oversight.	Develop specialized consent frameworks, parental counseling, independent patient advocates.	Complexity of genomic data; emotional context of prenatal decision-making.	High
Equity and Access [13]	Will chromosome editing widen healthcare disparities?	High cost and technical specialization could make therapy available only to wealthy populations.	Global bioethics frameworks emphasize equitable access to transformative health technologies.	Public funding, sliding-scale pricing models, equitable distribution policies.	Economic inequality and limited capacity in low-resource settings.	Medium
Societal Impact and Disability Rights [14,15]	Will editing stigmatize individuals living with Down syndrome or promote eugenic misuse?	The technology could shift social attitudes toward disability and normalcy.	Professional societies call for public engagement and disability advocacy inclusion.	Establish strict therapeutic criteria; public education campaigns; inclusion of advocacy groups in policymaking.	Potential stigma and distrust from disability rights communities.	High
Intellectual Property & Data Governance [16,17]	Who owns the editing technology and genomic data?	Patents and proprietary algorithms could limit open access and increase costs.	WIPO and WHO encourage open-access frameworks and fair licensing.	Open-source licensing for CRISPR tools; data-sharing agreements with privacy safeguards.	Balancing innovation incentives with global equity goals.	Medium

Domain	Critical Question / Ethical Dilemma	Relevance to Chromosomal Editing	Regulatory Context / Global Guidance	Proposed Safeguards / Solutions	Implementation Challenges	Impact Level
Clinical Transition Barriers [18,19]	What regulatory and logistical hurdles exist for clinical trials?	No approved pathway for in utero chromosome-scale editing trials.	FDA, EMA, and ISPD have no current category for whole-chromosome therapy.	Establish stepwise clinical trial design with transparent risk-benefit analysis.	Uncharted regulatory territory; high liability risk.	High
Public Engagement and Trust [20,21]	How to maintain public trust and prevent misinformation?	Genome editing is controversial; public perception can influence adoption.	WHO, NASEM, and other global panels emphasize early public engagement and transparency.	Regular public consultations, transparent trial reporting, proactive media engagement.	Misinformation spread, polarized ethical debates.	High

Footnote: WHO = World Health Organization; NASEM = National Academies of Sciences, Engineering, and Medicine; WIPO = World Intellectual Property Organization; ISPD = International Society for Prenatal Diagnosis. Impact Level reflects relative ethical/regulatory importance (High, Medium, Low).

Summary of Core Mechanistic and Translational Insights

Taken together, the reviewed evidence indicates that allele-specific CRISPR-Cas9-mediated chromosome elimination has advanced from theoretical speculation to experimental proof-of-concept in human cellular models.¹ Restoration of near-euploid transcriptional balance following removal of the extra chromosome provides a compelling biological signal that structural correction may be achievable under constrained conditions. At the same time, the absence of *in vivo* data, the persistence of mosaic outcomes, and unresolved ethical challenges reinforce that these findings represent an early developmental stage rather than a clinically actionable pathway. When considered alongside established prenatal screening practices, the emerging literature reflects a gradual shift from exclusive reliance on detection and counseling toward exploration of corrective strategies. Table 1 contextualizes these experimental advances within the broader diagnostic and interventional landscape, while Table 2 highlights the ethical and regulatory considerations that will shape any future translational trajectory. Collectively, these findings define both the promise and the present limits of precision chromosomal intervention in Trisomy 21.

Discussion

Redefining the Therapeutic Horizon of Trisomy 21

The emergence of allele-specific CRISPR-Cas9 strategies has begun to reshape long-standing assumptions about the immutability of chromosomal aneuploidy. For decades, trisomy 21 has been managed almost exclusively through prenatal detection, risk stratification, and postnatal supportive care, a paradigm reinforced by population-based

studies and clinical screening guidelines developed by professional societies in obstetrics and maternal–fetal medicine.^{21–25} Even as screening technologies have advanced and diagnostic precision has improved, as contextualized within the comparative landscape summarized in Table 1, the prevailing clinical framework has remained fundamentally non-interventional.

This paradigm was directly challenged by the work of Hashizume, whose group demonstrated that allele-specific CRISPR-Cas9 targeting could selectively cleave and eliminate the supernumerary chromosome 21 in human induced pluripotent stem cells^[1]. Situated within the broader evidence base identified through the PRISMA-aligned screening process illustrated in Figure 1, this finding represents a conceptual transition from descriptive genomics toward structural intervention. The broader shift from detection toward correction is captured schematically in Figure 2, which reflects how advances in genomic profiling and editing specificity have narrowed the conceptual distance between diagnosis and hypothetical therapy.

Earlier efforts to address trisomy 21 largely focused on attenuating gene dosage rather than altering chromosomal structure. XIST-mediated silencing approaches, first demonstrated by Jiang and subsequently extended by Gupta and Czermiński, showed that chromosome-wide transcriptional suppression could partially normalize cellular phenotypes in neural models.^{16–19} Although biologically elegant, these strategies retained the physical presence of the extra chromosome and were limited by instability, incomplete silencing, and mosaic reactivation. Conceptual discussions of chromosomal correction have also drawn on insights from early embryo genome-editing studies reported by Ma, although these investigations addressed single-gene correction rather than whole-chromosome elimination.²⁰ Against this background,

allele-specific chromosomal excision represents a qualitative departure, reframing trisomy 21 as a potentially addressable structural abnormality rather than a fixed genomic state. The mechanistic distinction underlying this shift is illustrated in Figure 3, which emphasizes how naturally occurring polymorphisms enable discrimination between homologous chromosomes.

Translational Context in Perinatal Medicine

The translational relevance of chromosome-level editing is inseparable from developmental timing. Conceptual discussions have increasingly focused on preimplantation intervention during assisted reproductive cycles, where embryos are accessible for manipulation and consist of relatively few cells, theoretically reducing post-editing mosaicism. While work by Ma on embryo-stage genome editing for monogenic disease provides an important technical precedent, the application of similar approaches to chromosome-scale correction introduces substantially greater complexity.²⁰ Integrating chromosomal correction into preimplantation workflows, as framed within the translational pathway depicted in Figure 4, raises unresolved questions regarding heritability, safety thresholds, and regulatory permissibility.

Early fetal intervention represents a second, more speculative pathway. Experience from fetal gene-therapy studies in animal models suggests that in utero delivery is technically feasible under constrained conditions, yet extrapolation to chromosome-level editing amplifies concerns related to delivery efficiency, immune response, genomic stability, and tissue-specific mosaicism.²²⁻²⁴ The rapid lineage diversification characteristic of fetal development further complicates the prospect of achieving uniform chromosomal correction.

As illustrated in Figure 4, the translational trajectory from cellular proof-of-concept to any conceivable clinical application remains prolonged and discontinuous, shaped as much by developmental biology as by molecular precision.

Ethical and Regulatory Considerations

Any discussion of chromosome-level intervention must be situated within the ethical and regulatory frameworks governing human genome editing. Reports issued by the National Academies of Sciences, Engineering, and Medicine have consistently emphasized that germline modification occupies a distinct ethical category and remains broadly prohibited outside narrowly defined research contexts.⁷ The possibility of heritable alteration following chromosomal excision places allele-specific editing squarely within this contested domain.

Ethical analyses by Ormond and by Gabel have further underscored the societal implications of framing genomic intervention as corrective, particularly for conditions such as Down syndrome that are accompanied by established disability identities and advocacy communities.^{8,9} Related perspectives articulated by Lawrence highlight how epigenetic and genomic narratives intersect with social understandings of disability and normalcy.¹⁰ Questions of informed consent acquire additional complexity when decisions are made on behalf of future individuals who cannot participate in the deliberative process. These ethical dimensions, together with concerns regarding equity, access, and public trust, are synthesized in Table 2, which reflects the breadth of considerations that must accompany any movement toward translational application.

Strengths of the Current Evidence Base

The principal strength of the current literature

lies in the clear demonstration of technical feasibility at the cellular level. The work of Hashizume provides compelling proof-of-concept that allele-specific chromosomal targeting can achieve structural correction with partial restoration of near-euploid transcriptional patterns.¹ When contextualized alongside earlier dosage-modulation strategies, this advance clarifies how the field has moved from conceptual exploration toward tangible molecular intervention. A further strength is the growing integration of ethical, developmental, and clinical perspectives, which has helped prevent premature translational claims despite rapid technological progress.

Limitations and Unresolved Challenges

At the same time, the limitations of the current evidence are substantial. All experimental data supporting chromosome-level correction remain confined to in vitro systems, with no validated in vivo, embryonic, or fetal applications. Editing efficiency remains variable, mosaic outcomes persist, and individualized SNP mapping limits scalability across populations. Methodological heterogeneity across studies further complicates reproducibility and comparative interpretation. Ethical uncertainty surrounding germline modification and long-term developmental consequences continues to constrain translational momentum.

Future Directions and Clinical Perspective

Future progress will require advances in molecular precision, delivery systems compatible with early development, and model systems capable of capturing long-term developmental outcomes. Parallel evolution of ethical governance and regulatory frameworks will be essential to ensure responsible progress. From a clinical standpoint, conversations with families

affected by trisomy 21 often reveal a careful balance between hope for transformative innovation and a desire for clarity, safety, and respect for lived experience, a tension that continues to shape perinatal counseling in daily practice.

Conclusion

Allele-specific CRISPR-Cas9 editing has begun to reshape the long-standing view that chromosomal aneuploidies are immutable. The recent demonstration of targeted removal of chromosome 21 in trisomic human cells represents a significant conceptual advance, suggesting that correction of trisomy at its genomic origin may one day be feasible. This early work provides a scientific foundation for re-imagining the therapeutic landscape of Down syndrome, which has historically centered on detection rather than intervention. Despite the promise of these findings, the current evidence remains confined to in vitro studies, and substantial scientific, ethical, and regulatory barriers must be addressed before clinical translation can be considered. Progress will depend on improvements in editing specificity, the development of embryo- and fetus-compatible delivery systems, and rigorous evaluation of potential off-target or developmental effects. Equally essential is the establishment of ethical and governance frameworks capable of guiding responsible application of chromosome-level interventions. If future research demonstrates safety, efficacy, and ethical acceptability, allele-specific chromosomal editing may offer an additional option beyond the long-standing dichotomy of pregnancy continuation or termination: the possibility of genomic restoration. Achieving this vision will require careful, measured advancement and sustained dialogue among scientists, clinicians, ethicists, and society at large.

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Conflict of Interest

The authors declare no conflicts of interest related to the publication of this manuscript.

Author Contributions

INHS, WA, EL and HKG conceptualized and supervised the review. MAB, WP, EEY, JD and MBAP conducted literature collection and data extraction. RSM, ESP, AAGPW, AANJK, KEG, ED and MIIA performed data analysis and contributed to critical content review. CMY, DA, NB, ADA, AS, RAP, and AP reviewed data interpretation. LAKN, WEKA, WAKD, and MS provided methodological and clinical guidance. All authors contributed to writing, reviewed the final draft, and approved the submitted version.

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References

1. Hashizume R, Wakita S, Sawada H, Takebayashi SI, Kitabatake Y, Miyagawa Y, Hirokawa YS, Imai H, Kurahashi H. Trisomic rescue via allele-specific multiple chromosome cleavage using CRISPR-Cas9 in trisomy 21 cells. *PNAS Nexus*. 2025;4:pgaf022. <https://doi.org/10.1093/pnasnexus/pgaf022>
2. Schambach A, Buchholz CJ, Torres-Ruiz R, Cichutek K, Morgan M, Trapani I, et al. A new age of precision gene therapy. *Lancet*. 2024;403:568-582. [https://doi.org/10.1016/S0140-6736\(23\)01952-9](https://doi.org/10.1016/S0140-6736(23)01952-9)
3. Gostimskaya I. CRISPR-Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Mosc)*. 2022;87:777-788. <https://doi.org/10.1134/S0006297922080090>
4. Barrangou R. Thinking About CRISPR: The Ethics of Human Genome Editing. *CRISPR J*. 2019;2:247-248. <https://doi.org/10.1089/crispr.2019.29072.rba>
5. Shinwari ZK, Tanveer F, Khalil AT. Ethical Issues Regarding CRISPR Mediated Genome Editing. *Curr Issues Mol Biol*. 2018;26:103-110. <https://doi.org/10.21775/cimb.026.103>
6. de Graeff N, Jongsma KR, Johnston J, Hartley S, Bredenoord AL. The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature. *Philos Trans R Soc Lond B Biol Sci*. 2019;374:20180106. <https://doi.org/10.1098/rstb.2018.0106>. Erratum in: *Philos Trans R Soc Lond B Biol Sci*. 2023;378:20230202. <https://doi.org/10.1098/rstb.2023.0202>
7. National Academies of Sciences, Engineering, and Medicine; National Academy of Medicine; National Academy of Sciences; Committee on Human Gene Editing: Scientific, Medical, and Ethical Considerations. *Human genome editing: science, ethics, and governance*. Washington (DC): National Academies Press (US); 2017. <https://doi.org/10.17226/24623>
8. Ormond KE, Mortlock DP, Scholes DT,

- Bombard Y, Brody LC, Faucett WA, et al. Human Germline Genome Editing. *Am J Hum Genet.* 2017;101:167-176. <https://doi.org/10.1016/j.ajhg.2017.06.012>
9. Gabel I, Moreno J. Genome Editing, Ethics, and Politics. *AMA J Ethics.* 2019;21:E1105-1110. <https://doi.org/10.1001/amajethics.2019.1105>
 10. Lawrence J, Telfer C. Interview: from Down's syndrome to basic epigenetics and back again. *Epigenomics.* 2013;5:611-4. <https://doi.org/10.2217/epi.13.71>
 11. Coller BS. Ethics of Human Genome Editing. *Annu Rev Med.* 2019;70:289-305. <https://doi.org/10.1146/annurev-med-112717-094629>
 12. Doudna JA. The promise and challenge of therapeutic genome editing. *Nature.* 2020;578:229-236. <https://doi.org/10.1038/s41586-020-1978-5>
 13. Mai CT, Isenburg JL, Canfield MA, Meyer RE, Correa A, Alverson CJ, et al. National Birth Defects Prevention Network. National population-based estimates for major birth defects, 2010-2014. *Birth Defects Res.* 2019;111:1420-1435. <https://doi.org/10.1002/bdr2.1589>
 14. de Graaf G, Buckley F, Skotko BG. Estimates of the live births, natural losses, and elective terminations with Down syndrome in the United States. *Am J Med Genet A.* 2015;167A:756-67. <https://doi.org/10.1002/ajmg.a.37001>
 15. de Graaf G, Buckley F, Skotko BG. Live births, natural losses, and elective terminations with Down syndrome in Massachusetts. *Genet Med.* 2016;18:459-66. <https://doi.org/10.1038/gim.2016.15>
 16. Jiang J, Jing Y, Cost GJ, Chiang JC, Kolpa HJ, Cotton AM, et al. Translating dosage compensation to trisomy 21. *Nature.* 2013;500:296-300. <https://doi.org/10.1038/nature12394>
 17. Gupta K, Czerminski JT, Lawrence JB. Trisomy silencing by XIST: translational prospects and challenges. *Hum Genet.* 2024;143:843-855. <https://doi.org/10.1007/s00439-024-02651-8>
 18. Hwang S, Cavaliere P, Li R, Zhu LJ, Dephoure N, Torres EM. Consequences of aneuploidy in human fibroblasts with trisomy 21. *Proc Natl Acad Sci U S A.* 2021;118:e2014723118. <https://doi.org/10.1073/pnas.2014723118>
 19. Czerminski JT, Lawrence JB. Silencing Trisomy 21 with XIST in Neural Stem Cells Promotes Neuronal Differentiation. *Dev Cell.* 2020;52:294-308.e3. <https://doi.org/10.1016/j.devcel.2019.12.015>
 20. Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. *Nature.* 2017;548:413-419. <https://doi.org/10.1038/nature23305>
 21. Andonotopo W, Bachnas MA, Pribadi A, Alamsyah Azis M, Aldika Akbar MI, Ernawati, et al. Integrating NIPT and ultrasound for detecting fetal aneuploidies and abnormalities. *J Perinat Med.* 2025. <https://doi.org/10.1515/jpm-2025-0005>
 22. American College of Obstetricians and Gynecologists. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. *Obstet Gynecol.* 2016;127:e123-e137. <https://doi.org/10.1097/AOG.0000000000001406>
 23. Chitayat D, Langlois S, Wilson RD. No. 261-Prenatal Screening for Fetal Aneuploidy in Singleton Pregnancies. *J Obstet Gynaecol Can.* 2017;39:e380-e394. <https://doi.org/10.1016/j.jogc.2017.06.013>
 24. Audibert F, Wou K, Okun N, De Bie I, Wilson RD. Guideline No. 456: Prenatal Screening for Fetal Chromosomal Anomalies. *J Obstet Gynaecol Can.* 2024;46:102694. <https://doi.org/10.1016/j.jogc.2024.102694>
 25. Audibert F, De Bie I, Johnson JA, Okun N, Wilson RD, Armour C, et al. No. 348-Joint SOGC-CCMG Guideline: Update on Prenatal Screening for Fetal

Aneuploidy, Fetal Anomalies, and Adverse Pregnancy Outcomes. *J Obstet Gynaecol Can.* 2017;39:805-817. <https://doi.org/10.1016/j.jogc.2017.01.032>.
Erratum in: *J Obstet Gynaecol Can.* 2018;40:1109. <https://doi.org/10.1016/j.jogc.2018.05.039>