CRISPR-mediated chromosome elimination and XIST-driven silencing in Down syndrome models: A systematic review

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Abstract. The most common chromosomal aneuploidy compatible with life is Down syndrome (DS or trisomy 21), caused by an extra copy of chromosome 21. Although symptomatic care remains the mainstay of management, new gene-editing technologies now allow the supernumerary chromosome to be directly silenced or eliminated, providing a critical background for evaluating novel chromosome-level correction techniques for trisomy 21. The present systematic review synthesized preclinical studies on two genome engineering strategies for correcting trisomy 21: X-inactive specific transcript (XIST)-mediated RNA silencing and CRISPR/CRISPR-associated protein 9 (Cas9)-mediated chromosome deletion, in cell and animal models. PubMed, Scopus and Web of Science were systematically searched according to the PRISMA 2020 and PRISMA-S guidelines (inception to March, 2025). Eligible studies applied these interventions to human or murine trisomic cells or *in vivo* models, and were assessed using the SYRCLE (animal studies) and OHAT-like (cell-based studies) risk-of-bias domains. In total, six preclinical studies met the inclusion criteria. Of these studies, three used doxycycline-inducible XIST transgenes to silence one extra chromosome 21 in DS-induced pluripotent stem cells, achieving >90% silencing while rescuing hematopoietic and neurogenic phenotypes. The other two studies used CRISPR approaches, namely multi-site chromosomal cleavage or allele-specific guide RNAs, achieving 10-30% of disomic derivatives with normalized transcriptomic readouts, while the sixth, an earlier study, used a selection-based counter-selection strategy (TKNEO/ganciclovir) that isolated rare disomic clones at markedly lower efficiency (~10^{-4}). The main limitations were off-target editing and incomplete/mosaic silencing; no study examined *in vivo* functional rescue beyond cellular systems. Overall, both XIST silencing and CRISPR-based editing effectively cleared the extra chromosome 21 *in vitro* and partially corrected gene-expression programs and cell-type functions, paving the way toward chromosome-level therapy. However, the evidence remains preliminary; further studies are warranted to enhance conversion efficiency, evaluate genomic safety (off-target and structural variants), and assess durability, safety and functional rescue in animal models in order to establish translational feasibility.

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Abbreviations: APP, amyloid precursor protein; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; FISH, fluorescence *in situ* hybridization; DS, Down syndrome; ES cells, embryonic stem cells; H3K27me3, trimethylation of lysine 27 on histone H3; IGF, insulin-like growth factor; iPSC, induced pluripotent stem cell; OHAT, Office of Health Assessment and Translation; p53, tumor protein p53; sgRNA, single-guide RNA; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation; WGS, whole-genome sequencing; XIST, X-inactive specific transcript

Key words: Down syndrome, trisomy 21, XIST, chromosome 21 silencing, CRISPR/Cas9, genome editing, induced pluripotent stem cells, epigenetic silencing

Introduction

Down syndrome (DS), also known as trisomy 21, is the most common viable human aneuploidy, occurring in ~1 in 700 live births, and is associated with intellectual disability and a wide range of associated developmental and medical conditions (1). The pathogenic basis is a dosage imbalance on supernumerary chromosome 21, which leads to the synchronized overexpression of hundreds of genes, and the global and pervasive dysregulation of cells. There are currently no therapies that correct the underlying genetic defect; therefore, treatment remains largely supportive and focuses on the management of associated complications. Previous *in vitro* approaches to the 'correction' of trisomy 21 used complex, inefficient techniques such as random loss of the extra chromosome and a selectable marker (2) or loxP-mediated targeted mitotic recombination on chromosome 21 (3), resulting in rare disomic clones and non-translatable outcomes (4,5).

One approach [X-inactive specific transcript (XIST)-mediated silencing] takes advantage of an inbuilt system of X inactivation present on the X chromosome, which is used to silence chromosome 21 (6). The long non-coding RNA XIST has a function of coating the chromosome in *cis*, by extensive RNA-DNA interaction, when it is expressed from the X-inactivation (XIC) center, the locus from which the human XIST gene was originally cloned and shown to be transcribed exclusively from the inactive X chromosome (7-9). Fluorescence *in situ* hybridization (FISH) research first demonstrated that XIST RNA physically 'paints' and coats the inactive chromosome in *cis* (10), and XIST RNA targets PRC2 to the deposition of the repressive histone mark, trimethylation of lysine 27 on histone H3 (H3K27me3), throughout the chromosome. This heterochromatin mark is then passed and maintained to the next cell division or divisions by another component of the heterochromatin called constitutive heterochromatin protein 1, which results in a stable epigenetically silenced state; XIST expression itself has been shown to be required for the initiation of X-inactivation in mouse models (11,12). The induction of such silencing in a model of DS has been shown to be effective when XIST is inserted into one of the additional copies of chromosome 21. This provides the benefit of temporal control via doxycycline-inducible expression systems for XIST, as well as theoretical reversibility, although differentiation studies indicate that X-inactivation shifts from a reversible to a largely irreversible state as cells mature, raising the question of whether the same transition applies to XIST-induced silencing of chromosome 21 (13). However, the silencing is not fully penetrant; 5-10% of the genes on chromosome 21 are not silenced (so-called 'escape genes') (14,15) and the stability of silencing after long-term differentiation protocols has yet to be investigated.

Taking this into account, research into non-coding RNA therapeutics is progressing rapidly in the pharmaceutical industry, underscoring the potential of long non-coding transcripts such as XIST to control gene expression and conceptually linking to the epigenetic silencing strategies used to correct trisomy in DS models (16). This led the authors of the present study to systematically analyze all published studies investigating chromosome 21 elimination or XIST-mediated chromosome silencing in DS models. The existing data on viability, effectiveness and molecular and functional results are summarized. The implications for the pathophysiology of DS and the possible advances in therapy have been considered as well.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-mediated chromosome elimination relies on a distinct, more direct mechanism. Cas9, guided by one or more single-guide RNAs (sgRNAs), introduces site-specific double-strand breaks (DSBs) in genomic DNA (17). When multiplex sgRNAs are used to generate several simultaneous DSBs across a single chromosome, the DNA repair machinery of a cell, principally non-homologous end joining, attempts to resolve these breaks; however, the resulting damage frequently overwhelms the mitotic checkpoint machinery and promotes the loss of the fragmented chromosome during subsequent cell divisions, yielding daughter cells that have lost the targeted chromosome copy entirely (18,19). As standard sgRNAs cannot easily distinguish between the two parental copies of chromosome 21, several groups have instead used allele-specific guide RNA design, exploiting single-nucleotide polymorphisms unique to the supernumerary copy, to selectively target only the extra chromosome while sparing the two normal copies (20,21). Since the original description of CRISPR/Cas9 genome editing, high-fidelity Cas9 variants and improved guide RNA design algorithms have substantially reduced off-target cleavage; these more recent, higher-fidelity platforms and other next-generation genome editing tools are introduced later in the present systematic review and discussed in greater depth in the Discussion section.

It has been demonstrated that CRISPR-based chromosome engineering approaches for trisomy 21 (22), typically in isolation from XIST-mediated silencing. To the best of our knowledge, this is the first systematic review to apply PRISMA 2020 methodology to directly compare both chromosome-level correction strategies side by side within the same evidence base, extracting harmonized data on editing/silencing efficiency, validation methods and safety signals across studies. This comparative, methodologically explicit approach allows readers to weigh the relative maturity, efficiency and risk profile of the two strategies rather than considering them separately. In addition to synthesizing existing findings, the present systematic review also considers the major translational barriers that determine the clinical feasibility for either approach, including delivery of XIST constructs or CRISPR components to relevant tissues, the challenge of mosaic correction within a treated cell population, the developmental timing required for phenotypic benefit, potential immune responses to viral or non-viral delivery vehicles, and the risk of genomic instability introduced by the editing process itself; these barriers are revisited in greater depth in the Discussion section.

Data and methods

Protocol registration. The present systematic review was carried out following the PRISMA 2020 guidelines (23). A systematic search of the PubMed, Scopus and Web of Science databases was conducted, with the last update in March, 2025, to examine the studies that addressed chromosome 21 silencing and/or elimination in DS models.

Search strategy details

PubMed search. The PubMed search included the following key words: [('Down syndrome' OR 'trisomy 21' OR 'DS' OR 'T21') AND ('XIST' OR 'X-inactivation' OR 'chromosome silencing' OR 'CRISPR'OR 'Cas9' OR 'genome editing' OR 'chromosome elimination' OR 'chromosome removal' OR 'aneuploidy correction')].

Scopus search. The following key words were used to search the Scopus database: TITLE-ABS-KEY [('Down syndrome' OR 'trisomy 21' OR 'DS' OR 'T21') AND ('XIST' OR 'X-inactivation' OR 'chromosome silencing' OR 'CRISPR' OR 'Cas9' OR 'genome editing' OR 'chromosome elimination' OR 'chromosome removal' OR 'aneuploidy correction')].

Web of Science search. The Web of Science search included the following: TS=[('Down syndrome' OR 'trisomy 21' OR 'DS' OR 'T21') AND ('XIST' OR 'X-inactivation'

OR 'chromosome silencing' OR 'CRISPR' OR 'Cas9' OR 'genome editing' OR 'chromosome elimination' OR 'chromosome removal' OR 'aneuploidy correction')].

The following Boolean operators were used: AND, OR, NOT. Search terms were truncated and phrase-searched as appropriate for each database. There were no date limitations in the preliminary search, but only publications in English were included in the final analysis. The search was carried out on March 15, 2025 which represents the latest literature update. To identify further potentially eligible studies, citation tracking and hand-searching of relevant reviews and reference lists were conducted.

Protocol registration status. The present systematic review was not prospectively registered in PROSPERO prior to the commencement of the study. The review methodology was carried out in accordance with the PRISMA 2020 guidelines; however, there was no formal pre-registration. The present systematic has been registered in PROSPERO (https://www.crd.york.ac.uk/PROSPERO/view/CRD420261480620) to increase transparency and adherence to best practices in systematic review reporting.

Eligibility for inclusion/exclusion. Original research studies (*in vitro* or *in vivo*) in which researchers specifically attempted to counteract the gene dosage effect of this trisomy either by: i) Integrating or activating XIST on chromosome 21 to induce its silencing; or ii) by using genome editing (e.g., CRISPR/Cas9) to selectively eliminate or inactivate the extra chromosome 21 were included. Studies needed to provide results on chromosome 21 copy number or expression (such as evidence of silencing, the loss of the extra chromosome) and downstream cellular or developmental phenotype.

Studies not focused on DS/trisomy 21 (i.e., addressing other aneuploidies) and studies that were conference abstracts, reviews, or commentaries without original data were excluded.

Study selection and data extraction. Systematic review software Covidence (Veritas Health Innovation Ltd.) was used for duplicate removal, which automatically identified and eliminated bibliographic duplicates from the combined database results. Following duplicate removal, two of the authors (MJRA and MEF) employed the same eligibility criteria to assess all titles and abstracts for eligibility. Cohen's kappa coefficient (κ=0.78, 95% CI, 0.70-0.86) was used to assess the inter-reviewer agreement on the screening of the titles and abstracts and the level of agreement was found to be substantial. Potentially relevant articles were then reviewed by both authors separately and full text articles were read and assessed for inclusion by both authors. There was some disagreement about the inclusion of studies in the present systematic review, which occurred in three studies when full text was read; these were settled by discussion, with a third author (NK) to arrive at a consensus. A full flow diagram, summarizing the entire study selection process, is presented in Fig. 1, and the number of records at each stage of the selection process and the reasons for discarding them at the full text review stage are stated.

Extraction of data from the data management system and quality assessment. The data elements that were abstracted from each eligible study included the following: i) Author, year of publication and journal; ii) model system [human induced pluripotent stem cell (iPSC)-derived cells, mouse embryonic stem cells (ES cells), primary cells, or *in vivo* models]; iii) specific cell type or tissue investigated (hematopoietic progenitors, neural progenitors, differentiated neurons, cardiomyocytes, etc.); iv) intervention class (XIST-mediated silencing or CRISPR-based elimination); v) delivery method and efficiency (doxycycline-inducible transgene integration strategy, viral vs. non-viral delivery for CRISPR components, guide RNA designs, etc.); vi) reported efficiencies of chromosomal silencing/elimination (in percentages and cell population measurements); vii) assays used to verify trisomy correction (FISH for chromosome enumeration, karyotyping, digital droplet PCR, whole-genome sequencing (WGS), locus-specific genomic analyses, etc.); viii) observed effects on DS-relevant cellular phenotypes (proliferation rates, differentiation capacity, activation of signaling pathways, transcriptomic profiles, etc.); ix) safety assessments performed (off-target analysis, structural variant detection, genomic instability measurement, etc.); and x) duration of study.

The risk-of-bias assessment was qualitative and adapted to the heterogeneity of the evidence base: Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) domains were used for animal studies, and Office of Health Assessment and Translation (OHAT)-like criteria were used for cell-based studies. These criteria included the use of appropriate control groups (isogenic diploid and trisomic comparisons), biological replication (number of independent experiments, independent cell lines, or independent animal cohorts), independent validation with multiple orthogonal assays, satisfactory reporting of outcome measures, and evaluation of potential selection bias. Due to the experimental heterogeneity, the formal numerical scoring of risk of bias was not used; instead, indicators of rigor and validation were qualitatively appraised to provide a context for the interpretation of the findings.

Data synthesis. The findings were analyzed and summarized in a narrative format, and then categorized by the strategy employed (XIST-based silencing vs. CRISPR-mediated elimination); comparative features were highlighted in terms of feasibility, efficiency, methods of validation, and phenotypic results. There were too few studies to conduct a meta-analysis and the outcome measures differed. A narrative synthesis and evidence tables (Table I) that outline the characteristics and findings of each study are presented.

Results

Study selection. A total of 132 records were identified in the literature search. The abstract screening and full text evaluation resulted in six studies being included in the present systematic review (Fig. 1). All studies were published over the past 13 years (2012-2025), reflecting the recent emergence of experimental approaches to correct trisomy 21 at the chromosomal level. All included studies were preclinical proof-of-concept investigations using cell models or animal-derived embryonic stem cell models, owing to ethical and technical barriers to potential clinical translation. The main features of the studies that were

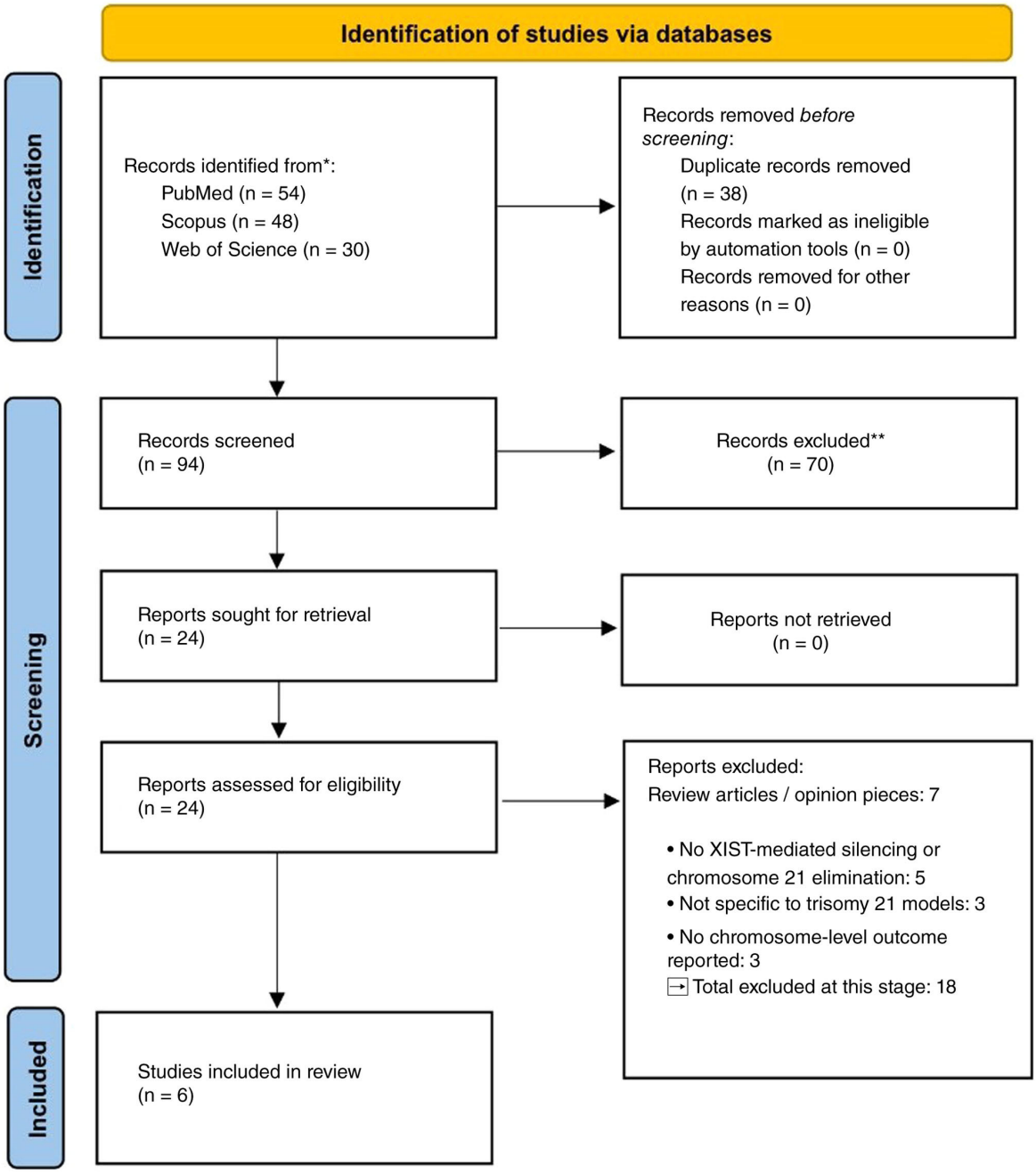


Figure 1. PRISMA flow diagram illustrating the selection, inclusion and exclusion of the studies in the present systematic review.

included, such as the experimental models and correction strategies are presented in Table I.

The relatively small number of included studies reflects the genuine novelty and narrowness of this specific research area rather than an overly restrictive search strategy. Of the 132 records identified, the large majority were excluded as they addressed XIST biology, CRISPR/Cas9 mechanisms, or chromosome engineering in contexts unrelated to trisomy 21 correction (e.g., cancer cytogenetics, X-inactivation biology in non-DS contexts, or CRISPR applications to other monogenic disorders), were conference abstracts or narrative reviews without original data, or did not report a direct measure of chromosome 21 copy number or expression change. Broadening the eligibility criteria to include indirect or purely mechanistic studies would have compromised the focus of the present systematic review on direct evidence of trisomy correction. The authors consider it acceptable that only six studies meeting these criteria exist at present, consistent with the field being at a very early, proof-of-concept stage, rather than that relevant studies being missed; citation tracking and hand-searching of reference lists (described above) did not identify additional eligible primary studies beyond those retrieved by the database searches.

Overview of the included studies. Of note, three of the studies used epigenetic silencing of the supernumerary chromosome 21 with XIST and two used genome-editing techniques with CRISPR/Cas9 to remove or truncate the extra chromosome (6,14,15,20,24). In addition, one earlier study used a selection-based counter-selection strategy

Table I. Characteristics and findings of the selected studies included in the present systematic review.

Study (year of publication)	Strategy	Model/system	Delivery/targeting	Confirmation of trisomy correction	Key outcomes	Notes/limitations	(Refs.)
Li *et al*, 2012	Selection-mediated loss of the extra Chr21 (TKNEO counter-selection)	Human DS iPSCs	Targeted insertion of TKNEO into one Chr21 allele (APP locus); ganciclovir selection enriched rare clones that had lost the targeted Chr21	Southern/qPCR copy-number assays; FISH; karyotype	Generated disomic subclones; disomic cells proliferated faster and formed more endothelium *in vivo*; hematopoietic differentiation effects were inconsistent	Low frequency (~10^{-4}); relies on spontaneous chromosome loss rather than precise targeting; *in vitro* only.	(5)
Jiang *et al*, 2013	XIST-mediated chromosome silencing (DOX-inducible transgene)	Human DS iPSCs	ZFN-mediated insertion of a large inducible XIST cassette into DYRK1A on Chr21	RNA/DNA FISH; heterochromatin marks (H3K27me3, UbH2A); genome-wide expression; 'Chr21 Barr body'	Near-chromosome-wide transcriptional repression; rescue of proliferation and neural rosette formation; dose rebalancing towards disomy	Some genes escape silencing; cell culture proof-of-concept only.	(6)
Chiang *et al*, 2018	XIST silencing during hematopoietic differentiation	DS iPSC-derived hematopoietic models	DOX-inducible XIST; compared ±XIST across matched clones	RNA-seq; colony assays; pathway analyses	Normalized over-production of megakaryocyte/erythroid colonies; reduced hyperactive IGF signaling; functional rescue *in vitro*	Hematopoietic context; no *in vivo* testing.	(14)
Czermiński and Lawrence, 2020	XIST silencing in neural stem cells and neurons	DS iPSC-derived neural stem cells/neurons	Inducible XIST activated at various neurogenic stages	XIST RNA FISH; H3K27me3 and H2AK119ub; bulk and single-cell transcriptomics	Restored neuronal differentiation; reduced progenitor over-cycling; Notch pathway normalization	Requires transgene delivery; effectiveness depends on developmental stage.	(15)
Zuo *et al*, 2017	CRISPR/Cas9 multi-site cleavage to eliminate target chromosomes	Mouse ES cells and embryos; human DS iPSCs	sgRNAs to repeated/unique sites across target chromosome; also cocktails of sgRNAs; tested DNA-repair inhibition to boost efficiency	DNA-FISH; karyotype; loss of hChr21 in DS iPSCs; loss of Y chromosome *in vitro*/*in vivo*	Demonstrated selective chromosome loss (Y and autosomes, incl. hChr21) using CRISPR; laid groundwork for chromosome-level editing	Occasional partial deletions/rearrangements; off-target risks must be assessed; mainly proof-of-concept.	(24)

Table I. Continued.

Study (year of publication)	Strategy	Model/system	Delivery/targeting	Confirmation of trisomy correction	Key outcomes	Notes/limitations	(Refs.)
Hashizume *et al*, 2025	Allele-specific CRISPR cleavage to remove the duplicated Chr21	Human DS iPSCs and fibroblasts	gRNAs designed to allele-specific sites (phased by WGS); transient knockdown of DNA damage-response genes increased loss rate	FISH; STR genotyping; CN assays; transcriptomic signatures	Efficient elimination of the supernumerary Chr21; restoration toward disomic gene expression; editing effective even in differentiated cells	Requires individualized allele phasing; potential off-target cutting if allele specificity imperfect; safety profiling needed.	(20)

TKNEO, thymidine kinase-neomycin resistance cassette; DS, Down syndrome; iPSC, induced pluripotent stem cell; APP, amyloid precursor protein; qPCR, quantitative polymerase chain reaction; FISH, fluorescence *in situ* hybridization; XIST, X-inactive specific transcript; DOX, doxycycline; H3K27me3, trimethylation of lysine 27 on histone H3; UbH2A, ubiquitinated histone H2A (lysine 119); Cas9, CRISPR-associated protein 9; CN, copy number, CRISPR, clustered regularly interspaced short palindromic repeats; STR, short tandem repeat; WGS, whole-genome sequencing.

(TKNEO/ganciclovir) to isolate rare disomic clones that had spontaneously lost the extra chromosome, representing a conceptual precursor to the genome-editing approaches described above (5). Each study confirmed that at least some of the treated cells were corrected and assessed the effects of these treatments on chromosome 21 gene expression and cellular phenotypes. Collectively, these studies illustrate key approaches that can be exploited to correct trisomy 21, including selection-based trisomy rescue in iPSCs; XIST-mediated chromosome silencing; and chromosome elimination and/or truncation using CRISPR (5,6,14,15,20,24) (Fig. 2).

In each included study, the correction of trisomy 21 was achieved *in vitro* by one of two approaches, namely epigenetic silencing or genome editing, within a restricted population of corrected cells, leading to the normalization of chromosome 21 gene dosage in a proportion of treated cells. The XIST-based approach involved generating a doxycycline-inducible XIST transgene in pluripotent stem cells from individuals with DS, resulting in genome-wide RNA coating, the formation of a condensed heterochromatic domain resembling an inactive X chromosome and the transcriptional repression of >90% of genes on the targeted chromosome. The recruitment of canonical heterochromatin modifications (e.g., H3K27me3 and ubiquitinated H2A) was also observed. A small number of genes on chromosome 21 are never silenced by XIST, and silencing is not complete in all cells (14,15,25).

Functionally, XIST-mediated silencing reversed the overexpression of chromosome 21 genes and partially corrected DS-related cellular anomalies in lineage-specific *in vitro* model systems. In hematopoietic differentiation systems, trisomic progenitors exhibited a growth defect in both megakaryocytes and erythrocytes, as well as hyperactivation of insulin-like growth factor (IGF) signaling pathways, which were both normalized to disomic levels following the induction of XIST. In neural progenitor models, the activation of XIST reduced the overproduction of neural progenitors, promoted neuronal differentiation and normalized abnormal Notch pathway activation, all of which were confirmed by transcriptomic analyses (14,15).

CRISPR/Cas9-based strategies aimed to permanently correct the disorder by directly removing the extra chromosome 21. The techniques used included multiplex guide RNA-mediated cleavage throughout chromosome 21 to promote the loss of the chromosomes during mitosis, as well as allele-specific targeting based on informative single-nucleotide polymorphisms and megabase-scale interstitial deletions that include most of the long arm of chromosome 21. These approaches resulted in the restoration of chromosomal disomy to a range of 10-30%, which represents a considerable increase over the rates of spontaneous chromosome loss. Trisomy was corrected by FISH, karyotyping and locus-specific genomic analyses. The corrected cells exhibited gene expression restored to normal levels on chromosome 21, and their short-term growth potential was the same as that of normal cells (20,24).

Despite the successes of CRISPR-based studies, certain issues still exist, including segmental deletions, chromosomal rearrangements, the incomplete loss of the whole chromosome and occasional off-target cleavage, particularly when allele discrimination was imperfect. There was also concern about

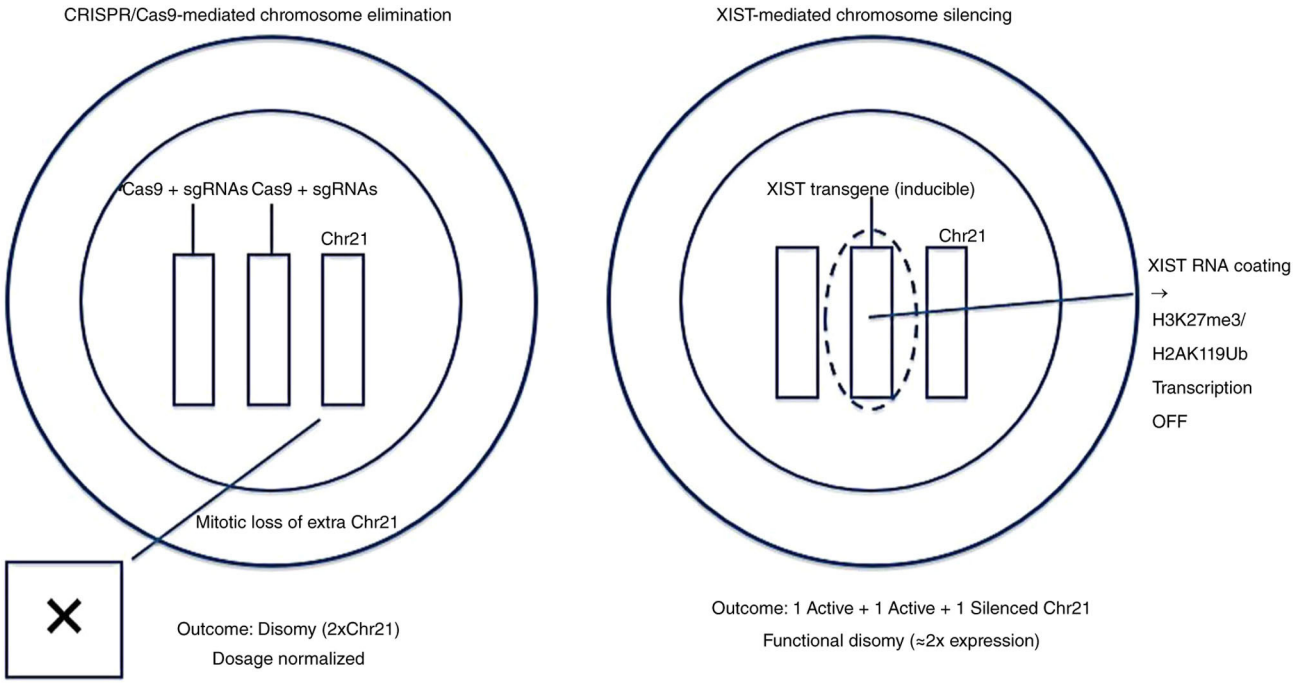


Figure 2. Schematic comparison of XIST-mediated chromosome 21 silencing and CRISPR/Cas9-based chromosome elimination. The image was created by the authors based on information from previous studies (5,6,14,15,20,24). XIST, X-inactive specific transcript; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; H3K27me3, trimethylation of lysine 27 on histone H3.

genomic instability that may occur if DNA repair pathways are only temporarily disrupted. None of the included studies performed complete sequencing of the corrected cells. Long-term outcomes remain a critical research gap: None of the studies presented included a systematic evaluation of the longevity of XIST-induced silencing after long-term differentiation, through multiple passages, and/or through directed maturation.

The efficiency of chromosome correction can vary among different iPSC lines, although the degree of this variability has not been extensively analyzed, despite its potential to significantly affect clinical feasibility. The structural variants that remain after chromosome elimination have not been extensively studied and have not yet been fully explored, as the majority of studies did not conduct a WGS analysis to comprehensively identify segmental deletions, duplications or translocations outside of the target regions. The majority of studies lacked single-cell analysis to detect the mosaic nature of corrections and cell-to-cell heterogeneity of silencing or elimination efficiency. Finally, there were no head-to-head studies of the comparative efficacy of XIST-mediated silencing and CRISPR-based elimination in the same cellular models using the same donor, restricting the ability to assess comparative efficacy. These methodological limitations significantly limit any conclusions that can be reached regarding the relative superiority of either strategy.

Comparative outcomes

Reproducibility and cell line variability. A critical gap in the evidence base is the absence of reproducibility testing across independent laboratories. All XIST-mediated silencing studies (n=3; refs. 6,14,15) were conducted by overlapping groups of researchers, and CRISPR studies (n=3; refs. 5,20,24) were performed by different independent groups; no study undertaken by one group was independently replicated by another. This represents a critical limitation as preclinical genome engineering results frequently exhibit substantial laboratory-dependent variation.

iPSC line variability was incompletely characterized across studies. The six included studies (5,6,14,15,20,24) utilized iPSC lines derived from different patients with DS and control individuals. However, no systematic assessment was conducted comparing editing efficiency, silencing durability, or phenotypic rescue across multiple isogenic iPSC lines from the same donor(s). This represents a critical gap as individual genetic background and epigenetic state can substantially influence CRISPR efficiency and XIST silencing penetrance. The three CRISPR studies (5,20,24) demonstrated correction efficiencies ranging from 10-30%; however, it remains unclear whether this variation reflects intrinsic differences in CRISPR methodology or differences in iPSC line genetic backgrounds.

Long-term clonal stability and WGS. None of the included studies comprehensively assessed durability of chromosome 21 silencing (XIST) or karyotype stability (CRISPR) through prolonged culture beyond 20-30 passages. XIST-silencing stability through extended neural or hematopoietic differentiation protocols (typically 30-60 days) was documented in only one study (15). For CRISPR-edited cell populations, WGS validation was performed in only one of the two CRISPR studies (24), and WGS analysis was typically limited to detecting the primary target chromosome elimination rather than comprehensively identifying structural variants genome-wide. This represents a critical limitation as off-target DSBs and chromosome fragmentation-induced structural variants could accumulate at substantial frequencies without detection by conventional karyotyping.

Correction for single-cell heterogeneity and mosaic correction. The vast majority of the studies (5,6,14,20,24) presented population-level data (percentage of trisomic/disomic cells) without single-cell analysis in any study to characterize heterogeneity in correction efficiency. Single-cell RNA sequencing (scRNAseq) or single-cell clonal analysis was performed in only one of the three XIST studies (15) and in none of the CRISPR studies. This reflects a lack of characterization of mosaic phenotypes, which refers to the coexistence of fully corrected, partially corrected and uncorrected cells within the same treated population. The 10-30% disomic frequency reported in studies, specifically for CRISPR approaches, is a population-level measurement and may underestimate the variability of editing that can occur from one cell to another.

Discussion

Implications for clinical practice. There has been tremendous progress made in modeling potential treatments for DS. Of note, two different approaches to cellular models of DS have been utilized to demonstrate that the extra chromosome 21 can be neutralized and the downstream molecular effects reduced by using two distinct methods: CRISPR/Cas9-mediated deletion of chromosome 21 and the use of XIST to silence the extra chromosome 21. These models provide experimental approaches for studying DS disease mechanisms and provide directions for possible therapy. CRISPR/Cas9 has also been applied successfully across a wide range of other genomic contexts, illustrating the general versatility of the platform beyond the trisomy 21-specific applications discussed here (17-19). The evidence base is limited, as few studies have been undertaken in a proof-of-concept fashion in laboratory settings, which limits the strength, validity and generalizability of the findings. For clinical translation, it will be important to make critical improvements in delivery, stability and safety as well as efficacy.

Trisomy 21 can be experimentally 'switched on' or 'switched off' *in vitro*, allowing causal inferences to be made. Isogenic trisomic and corrected (disomic) derivatives can be used to directly correlate cellular characteristics with chromosome 21 dosage. Induced XIST expression in iPSCs has been shown to correct DS-related defects in hematopoiesis and neurogenesis via the IGF and Notch signaling pathways, respectively, providing a molecular basis for phenotype-targeted therapy (14,15). In theory, the *in vitro* modulation of patients' cells followed by their reintroduction into the body could be considered a form of chromosomal medicine aimed at reducing DS-related complications such as immunologic or hematologic dysfunction. While it remains extremely challenging to target sufficient tissue with such interventions and to overcome the effects of developmental abnormalities, the administration of the therapeutic construct to neural progenitors, for example, may in theory enhance cognitive function (15).

Although the present review focuses on trisomy 21, other aneuploidy disorders could potentially benefit from similar chromosome-targeting techniques. For example, inducible XIST can help silence the extra chromosome in trisomy 13 or 18 in cell culture models, while CRISPR/Cas9 has been used to remove excess chromosomes from hybrid mouse ES cells and cancer cell lines. This is a plausible test case given its relative survivability, and other chromosomal imbalance disorders may benefit from lessons learned during treatment development (26,27).

The conceptual link between CRISPR-based genome editing and non-coding RNA-based epigenetic regulation, both discussed herein as chromosome-level correction strategies for trisomy 21, is consistent with the broader precision-medicine literature on programmable gene and chromatin regulation (16,22). Foundational mechanistic work on CRISPR/Cas9 genome editing and on long non-coding RNA-mediated gene silencing remains the primary evidentiary basis for the strategies discussed in the present systematic review; citations were selected for direct methodological or mechanistic relevance to chromosome 21 correction rather than by journal of publication.

Challenges and safety concerns. Even though it is possible to use XIST-mediated chromosomal silencing and CRISPR/Cas9-mediated chromosome elimination *in vitro*, these technologies still face significant hurdles prior to their clinical application.

The XIST transcript is large (>17 kb) and needs to be efficiently targeted in *cis* to the supernumerary chromosome 21 to achieve silencing, which is technically challenging. Random genomic integration or episomal expression can lead to unintended effects at off-target sites or poor silencing efficiency. It is difficult to achieve the uniform and stable expression of XIST in cells, and some genes on chromosome 21 are inherently difficult to silence, resulting in residual dosage imbalance. It also remains to be determined whether XIST-mediated silencing can be effectively induced postnatally or in adult tissue, and how effective it is when induced during the early developmental period (13). Further technical challenges exist for CRISPR/Cas9-based chromosome elimination as well: Multiplex genome editing increases the risk of off-target cleavage, structural chromosomal anomalies and the activation of DNA damage responses.

Following partial chromosome removal, segmental deletions, translocations or mosaic cell populations can occur. However, allele-specific techniques have limitations in that informative polymorphisms are required and the guide RNA needs to be custom-designed for each allele (20,21). *In vivo* administration is limited by vector capacity, immunological reactions and the inability to target tissues. While technically challenging, achieving a therapeutically beneficial degree of mosaicism (i.e., partial correction sufficient for clinical benefit) may be theoretically possible. The use of CRISPR/Cas9 to edit the genome causes numerous DSBs, which represent a major genomic safety risk. These risks include chromothripsis (catastrophic, localized shattering and disordered reassembly of one or more chromosomes), large structural rearrangements brought about by aberrant repair, the formation of micronuclei due to chromosome fragmentation, copy-number changes outside the target region and chromosomal instability during extended culture. This is an additional concern, as intense editing stress may result in selection for cells with an impaired DNA damage response, which could confer an oncogenic selective advantage (28).

These genomic safety concerns are significant in the realm of genome editing, and require extensive scrutiny

in the context of trisomy 21 correction. In addition to these technical issues, delivery and developmental timing represent additional barriers to translation. One of the largest hurdles for the clinical use of XIST constructs or CRISPR components is their efficient delivery to the tissues of interest, particularly the fetal brain, neural progenitors, hematopoietic stem cells and peripheral tissues. Current delivery systems cannot reliably deliver these components to a sufficient proportion of cells within developing organs. Another challenging aspect is developmental timing, since a number of DS-associated phenotypes arise during embryogenesis and early fetal development, when the majority of organs are still forming. Once developmental pathways are set, postnatal interventions may not be able to correct the established phenotypes. This time constraint would make it technically difficult and complex, ethically challenging, and legally complicated to achieve *in utero* treatment at the chromosome level. *In vivo* functional rescue following chromosome-level correction has never been demonstrated in an animal model, at least to the best of our knowledge; yet, this is an essential prerequisite for consideration of clinical translation (29).

Finally, virtually all current evidence is derived from *in vitro* models. Additional technological, safety, and ethical issues are raised when these approaches are attempted for organism-level therapies, particularly for neurodevelopmental tissues, where treatment would have to occur very early in development. Selection for cells with an impaired p53-mediated DNA damage response could confer a survival advantage during editing, highlighting the need for comprehensive preclinical safety testing (27).

Advanced genome editing strategies beyond conventional CRISPR/Cas9: Recent progress in next-generation genome editing technologies. The present systematic review focused primarily on conventional Cas9 systems because as represent the most thoroughly studied approaches for chromosome 21 correction. However, substantial progress in genome engineering technology has occurred that may provide safer, more efficient alternatives for future chromosome correction strategies in DS:

Base editing (adenine base editors and cytosine base editors). Base editors perform the direct conversion of individual DNA bases (A↔G or C↔T) without creating DSBs. For chromosome 21 correction applications, base editing could theoretically disrupt specific genes on chromosome 21 (e.g., inactivating dual specificity tyrosine-phosphorylation-regulated kinase 1A or other dosage-sensitive genes) without requiring chromosome elimination. Unlike conventional CRISPR/Cas9, base editing avoids DSB-induced chromothripsis, off-target DSBs and structural variant risks. However, achieving whole-chromosome silencing or elimination via base editing would be technically challenging, as individual gene inactivation differs substantially from chromosome-level correction (28,30).

Prime editing. As regards prime editing, prime editors achieve precise DNA modifications using Cas9-reverse transcriptase fusion proteins that write new DNA sequences at targeted loci without creating DSBs. This approach preserves the target DNA in its original state and substantially reduces off-target activity and structural variant risk compared to conventional Cas9. Prime editing could enable precise modifications to chromosome 21 genes without the genomic instability risks of CRISPR/Cas9-based elimination (31).

CRISPR-associated transposases. Newly discovered CRISPR-associated transposase systems enable large-scale DNA insertions (potentially 15-50 kb) without DSB induction. These systems could theoretically insert corrective genetic material or regulatory elements without the structural variant risks inherent to multi-site DSB approaches (32).

CRISPRi (CRISPR interference) and epigenome editing. CRISPRi using catalytically inactive Cas9 (dCas9) fused to transcriptional repressor domains enables reversible, targeted gene silencing without permanent DNA modification. Epigenome editing using dCas9 fused to epigenetic regulators (e.g., KRAB, p65, VP64) enables the dynamic chromatin remodeling of specific genomic regions. For chromosome 21 correction, dCas9-KRAB targeting multiple chromosome 21 genes could achieve dosage compensation through reversible silencing, providing advantages over irreversible XIST silencing (33,34).

Programmable chromatin regulators and chromatin remodeling. Engineered DNA-binding protein systems (TALEs, zinc fingers) fused to chromatin remodeling domains enable precise, targeted alterations in chromatin state at specific loci. These approaches could enable targeted heterochromatin formation at chromosome 21 without requiring viral vectors or XIST transgenes (34).

Comparative safety advantages. Compared to conventional Cas9 systems, base editing, prime editing and epigenome editing approaches substantially reduce the risks of chromothripsis, structural variants, off-target DSBs and genomic instability. However, each technology brings distinct advantages and limitations (28).

The field is rapidly advancing, and the clinical translation of these newer approaches remains in early stages. For DS chromosome 21 correction specifically, the most promising near-term approach may involve combination strategies; for example, using base editing or CRISPRi to inactivate individual dosage-sensitive genes, while accepting the presence of the extra chromosome, rather than pursuing complete chromosome elimination with its attendant structural variant risks (30,33).

Ethical considerations. There are serious ethical issues with proposals to change the number of human chromosomes. However, due to the long-term effects, the ethics of manipulation of the heritable genome, as well as the possibility of transmitting unwanted changes to future generations, several groups have been hesitant to allow germ-line genome editing, or even the modification of embryos to prevent trisomy 21 (35). Attempts to 'reverse' DS at the organismic level would be ethically dubious, while somatic interventions on individuals with DS, e.g. to reduce severe and life-threatening coexisting conditions (e.g., increased risk of childhood leukemia) may appear more socially acceptable in certain contexts.

The views and opinions of individuals with DS, their families and support groups are also critical. A number of individuals affected by trisomy 21 view the condition as linked to their identity and prioritize inclusion, support and quality of life over a cure. Goals of gene-editing strategies may not be in

line with the values of the community and may be interpreted and resisted as 'curative' (21). All future development should therefore be carried out transparently, with strong stakeholder engagement and mechanisms focused on autonomy and proportionality of benefit to risk and social justice, with a focus on research aims that reflect the needs and values of the key stakeholders of the research. Moreover, laws and regulatory frameworks governing genome editing vary across countries. Currently, heritable (germline) genome editing is prohibited in the majority of countries, but may be used under certain conditions with appropriate regulation for somatic interventions (35). Policy development should take account of the need to harmonize policies internationally, while respecting regional values. In addition, governance arrangements need to ensure that chromosome-level interventions in DS are treated neither as a 'societal expectation' nor as a 'treatment obligation', but rather as an option within supportive healthcare environments which recognize the autonomy and values of individuals and families affected by DS. The language used to frame interventions is critical: 'Cure' or 'correction' language can inadvertently exclude individuals with DS and their community and may be less ethically defensible, whereas language that focuses on managing a specific medical problem, such as an increased risk of leukemia in children with DS, is more ethically defensible and may reflect community values.

Future directions. Future research is required to focus on improving the precision of chromosome targeting and adapting these methods for more complex biological systems. XIST-based approaches are exploring minimal functional XIST domains, direct RNA delivery strategies, and *in vivo* DS model systems. In order to effectively deliver CRISPR *in vivo*, tissue-targeted nanoparticles, advanced viral vectors or advanced hybrid nanotechnology-CRISPR platforms are necessary. A related CRISPR/Cas9-mediated interstitial megabase-deletion methodology has recently been demonstrated for generating partial monosomy 21q in euploid human iPSCs; although this approach has not yet been applied to correct trisomy 21 directly, it illustrates the feasibility of large-scale, selection-free targeted deletions on chromosome 21 and could potentially be adapted for future DS-specific applications (36). Future CRISPR editing platforms, however, may carry reduced genotoxicity risk (37,38).

The durability of the functional rescue, clonal dynamics, off-target effects and genetic stability needs to be assessed in long-term studies. Preliminary *in vitro* results suggesting that trisomy dosage can be neutralized challenge the assumption that aneuploidy is inherently permanent (38). These technologies have contributed to the understanding of the effects of chromosome 21 dosage and will help inform possible treatments for DS, although clinical application remains distant (37-39).

In conclusion, limitations to clinical translation have been identified: Incomplete or variable repair (in some CRISPR studies, 10-30% of cells are still not corrected), mosaic populations with a mixture of corrected and uncorrected cells (70-90% of cells are still trisomic in some CRISPR approaches), variable long-term stability (through extended differentiation protocols), cell-type selectivity (phenotypic rescue is reported mainly in hematopoietic and neural contexts, but not thoroughly in other tissues), genomic safety issues (including chromothripsis from multiplex breaks, formation of structural variants, chromosomal instability during culture and potential p53-pathway selection), and severe delivery limitations for *in vivo* application. The combined effect of these factors indicates that while significant technical optimization of editing efficiency may increase the speed of editing, there are critical biological and practical hurdles to be overcome before clinical application is possible. At present, there is evidence of proof-of-concept, but no clear evidence of superiority of either approach; long-term *in vivo* safety profiles remain unknown.

Mechanistic modeling of the disease using these technologies can be beneficial in the short-term for identifying dosage-sensitive pathways and for phenotype-based drug discovery. More accurate and stringent genome-wide safety evaluation, the robust verification of phenotypic correction across tissues and effective ethical oversight are required to advance clinical translation. The concept of chromosome dosage correction through chromosome silencing and chromosome elimination is logical, important and indeed necessary for a gradual and careful approach toward therapeutic application.

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Authors' contributions

MJRA designed the study, performed the analysis of data from the literature and wrote the manuscript. MJRA, MEF and NK collected data and assisted with the writing of the manuscript. Data collection and analysis were performed by AAEE and VPS. VPS assisted with data interpretation. AAEE provided technical assistance and edited the manuscript. NK supervised the study and reviewed the manuscript. MJRA oversaw the study and edited the manuscript. NK and MJRA confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, ChatGPT was used to improve the readability and language of the manuscript and to generate the schematic figure image, and subsequently, the authors revised and edited the content produced by ChatGPT as necessary, taking full responsibility for the ultimate content of the present manuscript.

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