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RESEARCH ARTICLE
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HARNESSING CRISPR-CAS TECHNOLOGIES FOR PAEDIATRIC IMMUNODEFICIENCIES: A NEW FRONTIER

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Abstract

Aim: This research examines the safety and effectiveness of CRISPR-Cas technologies in correcting genetic defects and restoring immunological function.

Method: This study reviewed CRISPR-Cas research on treating paediatric immunodeficiencies using PRISMA criteria.

Databases: The searches conducted in PubMed, Web of Science, and Embase primarily focused on scholarly papers published in English

Findings: Five of seventeen papers matched the requirements for more complete research after a thorough examination. Numerous studies have demonstrated that CRISPR-Cas may correct genetic abnormalities in SCID and Wiskott-Aldrich Syndrome. Thus, animal models have showed considerable immune function improvements and disease amelioration. Due to a lack of clinical application data, laboratory results differ from paediatric patient outcomes.

Conclusion: In conclusion, CRISPR-Cas technology allows precise genetic changes, promising therapy for paediatric immunodeficiencies. Despite promising preclinical results, longer-term clinical trials are needed to determine safety and effectiveness. To maximise the utility of CRISPR-Cas for paediatric immunodeficiency therapies, laboratory discoveries must be translated into clinical practice.

Keywords: CRISPER-Cas, Genetic defect, Paediatric, Immunodeficiencies.

INTRODUCTION

Background:

Sequence-based engineering, or genome-editing, manipulates DNA sequences and variations via insertions, deletions, integrations, and replacements (Figure 1). Selective genome editing relies on cellular DNA repair (Braff et al., 2016). Regulatory proteins and programmable sequence-specific nucleases may also modify epigenetic and genomic sites (Figure 2). The CRISPR gene editing system has been reprogrammed to fight viral incursions from its original purpose as an immune system in numerous prokaryotes and archaea. Reprogramming has worked for various animals, including humans (Lin et al., 2022). CRISPR technology uses Cas enzymes to cleave particular nucleic acid sequences. Due of its simplicity and programmability, CRISPR technology is replacing previous therapeutic gene editing technologies (Naseem et al., 2022). The CRISPR-Cas9 technique has reversed gene mutations in blood disorders, muscular degeneration, neurological disorders, cardiovascular disorders, renal disorders, genetic abnormalities, stem cell disorders, and optical abnormalities (Bhokisham et al., 2023). The CRISPR-Cas9 method allows the production of numerous disease models, which improves knowledge of these illnesses and has great therapeutic potential if the genetic reason is found. CRISPR-Cas9 genome editing reprogramming may restore gene function or reduce mutations. CRISPR-Cas9 is used in bio sensing as well as genome editing (Zakiyyah et al., 2022). Cas13, Cas12a, and Cas14 orthologues have collateral non-specific enzymatic activity that may degrade tagged nucleic acids to provide fluorescence signals for nucleic acid identification (Mandip and Steer, 2019).

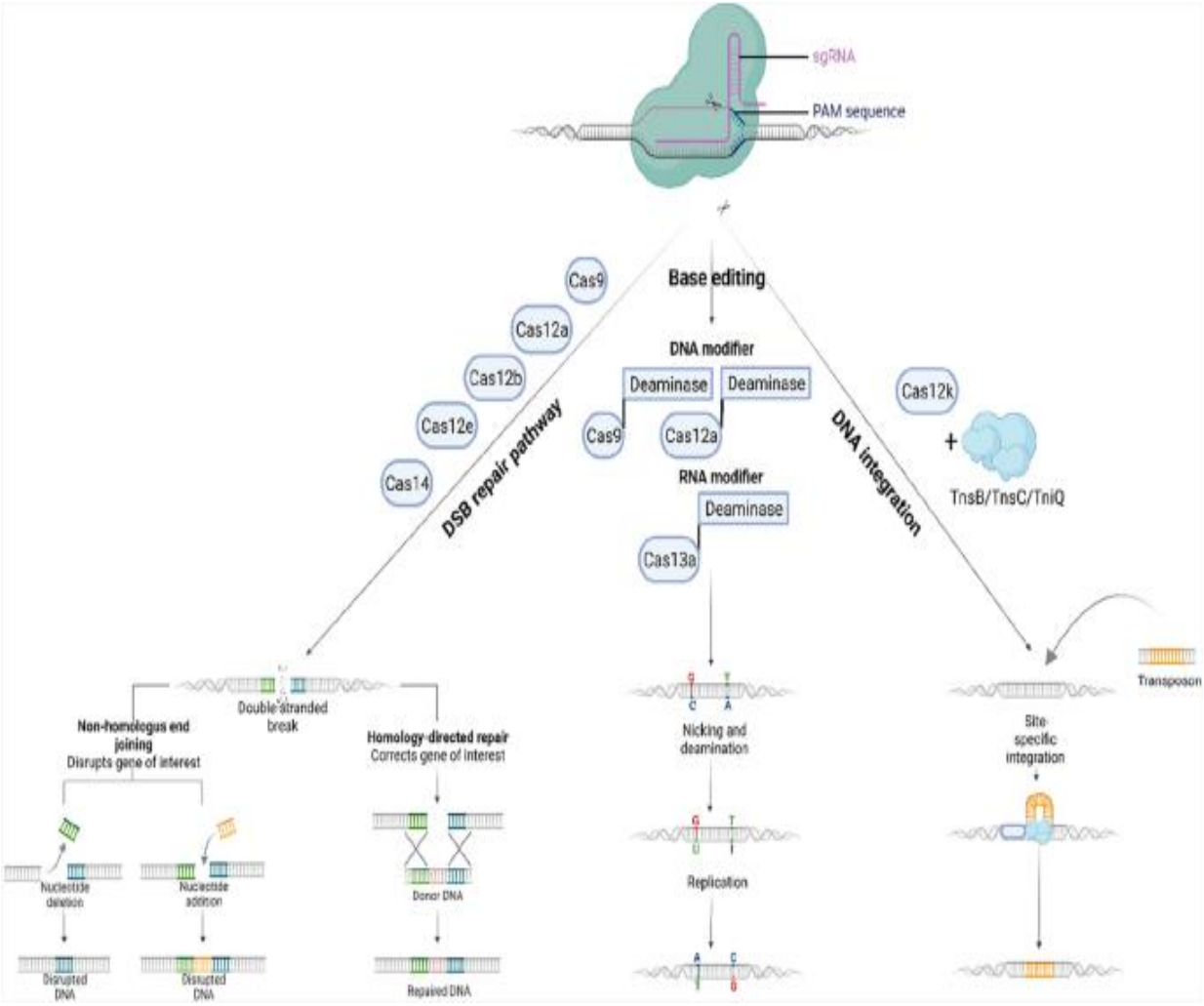


Figure 1: An Update on the Application of CRISPR Technology in Clinical Practice

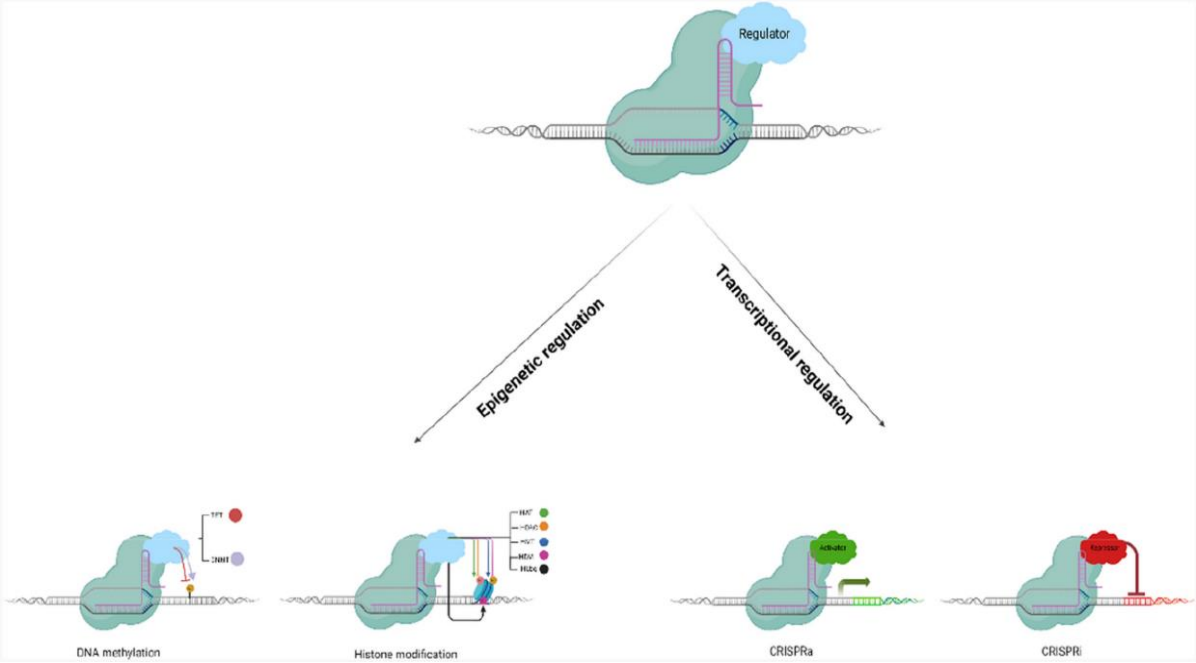


Figure 2: An Update on the Application of CRISPR Technology in Clinical Practice

The CRISPR-based gene-editing methods have different enzymatic activity and nucleic acid binding requirements (Uddin et al., 2020). Most CRISPR uses employ spCas9 from Streptococcus pyogenes. Cas9 uses a CRISPR RNA (crRNA) scaffold with a sequence it identifies and a trans-activating crRNA (tracrRNA) with a 20-nucleotide complementary sequence to target a DNA sequence (Serajian et al., 2021). CRISPR systems may fuse their crRNA and tracrRNA RNA fragments into a single-guide RNA (sgRNA) whilst still targeting and cleaving certain nucleic acids. Compared to ZFN and TALEN, CRISPR-based methods make switching gene targets easier (Liu et al., 2023). Changing the 20 nucleotides at the start of the single guide RNA (sgRNA), which directs the Cas protein to the targeted sequence, is enough to target a new genomic region in CRISPR-based systems (Wagner et al., 2022). Thus, CRISPR is fast changing medical and life science research, especially clinical trials. As demonstrated, categorising CRISPR-Cas systems helps explain their genesis and advance science. CRISPR system categorisation is achieved by sequencing and Cas protein composition modifications. Huang et al. (2022) proposed two CRISPR system groups with six major and thirty-three subtypes. Class I systems have three types, I, III, and IV, and have effector complexes with multiple subunit Cas proteins and a crRNA interference step.

Type I has seven subtypes: I-A, I-B, I-C, I-D, I-E, I-F, and I-G. Type I systems use effector complexes like the CRISPR-associated complex, an antiviral defence mechanism (Cascade complex), to occupy the interference step (Gleerup and Mogensen, 2022). This cascade complex contains Cas3, Cas5, Cas7, Cas8, and maybe more Cas proteins (subtype-dependent). Type 1 systems require Cas3, a Cascade complex component, to break foreign DNA. Type III has six subtypes: III-A, III-B, III-C, III-D, III-E, and III-F. Several type III adaptation modules include reverse transcriptase (De Ravin and Brault, 2019). Since type III systems lack a cas6 gene, most subtypes use Cas6 proteins from other CRISPR-Cas loci to cleave pre-crRNA. Subtypes III-A, III-D, III-E, and III-F employ a Csm complex with crRNA and Csm/Cas proteins, whereas III-B and III-C benefit from an effector complex with crRNA and Cmr/Cas proteins (Huang et al., 2022). The effector complex cleaves DNA/RNA hybrids in subtypes III-A, III-B, and III-C. Subtypes III-D and III-E target RNA. In subtype III-F, DNA is expected (Baddeley and Isalan, 2021).

Type IV includes IV subtypes A, B, and C. When these systems adapt and cleave foreign targets, Cas proteins are generally absent. Using a particular Cas6 protein to cleave pre-crRNA is common (Lin et al., 2021). Some sources propose type IV effector complexes include Cas5, Cas7, and Csf1. Insufficient data and difficulty cloning the various effector complexes into a functioning vector or producing them as a ribonucleoprotein protein (RNP) complex limit the use of class I CRISPR

systems for genome editing (Seok et al., 2021). Thus, class II systems, which may generate a variety of genetic alterations, were investigated for genome editing. Class II systems have a crRNA and a large multidomain effector complex, distinguishing them from Class I systems (Lagutina et al., 2015). Type II, V, and VI systems exist. Type II has three subcategories: II-A, II-B, and II-C. To process pre-crRNA, the type II system needs three proteins: RNase III, which matures pre-crRNA, Cas9, which identifies the PAM sequence and targets a specific strand of target DNA, and tracrRNA, which identifies the target (Wanzel et al., 2016).

Type II-C systems handle pre-crRNA differently than other subtypes. The Type V systems—V-A, V-B, V-C, V-D, V-E, V-F, V-G, V-H, V-I, and V-K—use the Cas12 protein as an effector complex (Wang, 2016). Subtype V-A of Type V uses the effector complex for pre-crRNA processing, whereas others employ RNase III. The Cas12 protein splits DNA targets into two strands, unlike Type II systems (Ottaviano et al., 2022). The Type VI systems include four subclasses: A, B, C, and D. Type VI systems utilise just the Cas13 protein as an effector complex, unlike class II systems. Pre-crRNA is processed by the effector complex. Type VI effector complexes feature two RNase-collaborating HEPN domains (Sarkar and Khan, 2021). Nucleotide binding to targets in eukaryotic and prokaryotic organisms occurs via these domains. These systems need a protospacer flanking sequence (PFS) to cleave ssRNA targets, not PAM for double-strand DNA (Table 1).

Table 1: Different types of the CRISPR–Cas systems

Class	Type	Subtypes	Target	Spacer integration	Pre-crRNA processing	Effector complex	Target cleavage
I	I	I-A	DNA	Cas1, Cas2, [Cas 4]	Cas6	Cas7, Cas5, SS*, Cas8/LS	Cas3", Cas3'
		I-B	DNA				
		I-C	DNA				
		I-D	DNA				
		I-E	DNA				
		I-F	DNA				
		I-G	DNA				
	III	III-A	DNA + RNA	Cas1, Cas2, [RT]	[Cas6]	Cas7, Cas5, SS, Cas10/LS	Cas10/LS
		III-B	DNA + RNA				
		III-C	DNA + RNA				
		III-D	RNA?				
		III-E	RNA?				
		III-F	DNA?				
	IV	IV-A	unknown	[Cas1], [Cas2]	[Cas6]	Cas7, Cas5, [SS], Csf1/LS	unknown
		IV-B	unknown				
		IV-C	DNA?				
II	II	II-A	DNA	Cas1, Cas2, [Cas 4]	RNase III	Cas9	Cas9
		II-B	DNA				
		II-C	DNA				
	V	V-A	DNA	[Cas1], [Cas2], [Cas 4]	Cas12	Cas12	Cas12
		V-B	DNA				
		V-C	DNA				
		V-D	DNA				
		V-E	DNA				
		V-F	DNA				
		V-G	RNA				
		V-H	unknown				

		V-I	DNA				
		V-K	unknown				
	VI	VI-A	RNA	Cas13	Cas13	Cas13	Cas13
		VI-B	RNA				
		VI-C	RNA?				
		VI-D	RNA				

Source: Morshedzadeh et al. (2024)

CRISPR-Cas has transformed genetic medicine, especially for child immunodeficiencies and other hereditary illnesses. These illnesses may kill people with innocuous microorganism infections owing to immune system dysfunction (Fernández-Calleja et al., 2019). HSCT, prophylactic antibiotics, and symptomatic relief are conventional therapies. The latter strategy concerns GVHD and donor shortages (Morshedzadeh et al., 2024). CRISPR-Cas can fix genetic abnormalities and produce precise genomic modifications in these youngsters (Lin et al., 2022). Neonatal immune deficits such SCID, Wiskott - Aldrich syndrome, and Chronic Granulomatous Disease affect the globe. T and B cell dysfunction makes infants with severe cumulative immune deficiency (SCID), often known as "bubble boy disease," more susceptible to infections (Naseem et al., 2022). Due of SCID's prevalence of 1 in 58,000 to 1 in 100,000 live babies, effective therapies are required (Bhokisham et al., 2023).
Using a bacterial defensive mechanism, CRISPR-Cas precisely cleaves DNA to introduce functional genes and remove defective ones (Mandip and Steer, 2019 Immunodeficient newborns may be cured by directly rectifying the gene in their cells. HSC mutations may be found and corrected outside the body using CRISPR-Cas9 (Zakiyyah et al., 2022). Reintroducing the cells restores the recipient's immune system via the repaired gene. In an X-linked SCID-X1 mouse model, CRISPR-Cas showed early results (Uddin et al., 2020). CRISPR-Cas9-corrected HSCs were implanted into mice to fix them. After this success, researchers are testing CRISPR-Cas9-modified cells on SCID and other immunodeficiency patients for safety and effectiveness (Serajian et al., 2021).

Rationale

Paediatric immunodeficiencies, which include a wide range of diseases characterised by abnormal immune system activity or absence of critical components, are difficult to treat. Lifesaving therapies include stem cell transplantation, enzyme replacement, and lifelong immunoglobulin replacement (Liu et al., 2023). Donor recruitment, graft-versus-host disease, and ongoing treatment expenses and logistics are downsides. These drugs treat symptoms, not genetic problems. CRISPR-Cas has revolutionised disease treatment. CRISPR-Cas systems are the most advanced gene editing method, providing precise, efficient, and adaptable DNA changes (Gleerup and Mogensen, 2022). Monogenic gene mutations cause several paediatric immunodeficiencies. With CRISPR-Cas, genetic causes of many diseases may be addressed. Treatment would replace management since it may restore immune systems (Wagner et al., 2022).
Despite its benefits, CRISPR-Cas in adolescents raises additional challenges. Due to immune system complexity and paediatric development, gene editing must be done cautiously and conditionally (De Ravin and Brault, 2019). Germline modifications may influence non-therapy patients, necessitating significant regulatory oversight and ethical standards. Due to therapeutic shortages, CRISPR-Cas must be studied. One in 58,000 neonates have severe combined immunodeficiency, and many have persistent, sometimes fatal infections unless they get early medical care (Huang et al., 2022). New techniques are needed for donor matching and treatment-associated morbidity. CRISPR-Cas technologies, which can directly fix genetic abnormalities, offer a natural solution.

Aim and Objectives

The aim of this study was to assess the efficacy and safety of CRISPR-Cas technologies in treating paediatric immunodeficiencies, which will pave way for innovative therapeutic options.

1. To assess CRISPR-Cas technologies' potential to fix genetic abnormalities that cause paediatric immunodeficiencies.

2. To investigate the clinical outcomes and immune function restoration in paediatric patients treated with CRISPR-Cas technology.
3. To determine the short- and long-term safety profiles of CRISPR-Cas-based treatments in paediatric populations.

Research Question

RQ: What impact do CRISPR-Cas technologies have on treatment outcomes and safety in immunodeficient paediatric, considering into account the efficacy of genetic correction and immune function restoration?

PICO Framework

Table 2: PICO (Population, Intervention, Comparison, Outcome) Framework

Element	Description
Population	Children and adolescents (aged 0-18 years) diagnosed with primary immunodeficiencies
Intervention	Use of CRISPR-Cas9 or other CRISPR systems for gene editing to correct genetic defects
Comparison	Current standard treatments (e.g., stem cell transplantation, enzyme replacement therapy, immunoglobulin replacement)
Outcome	Efficacy (genetic mutation correction, immune function restoration), safety (adverse effects, long-term outcomes), and clinical outcomes (infection rates, survival rates)

METHODS

Study Design

The systematic study examined CRISPR-Cas technology's safety and efficacy in paediatric immunodeficiency treatment. CRISPR-Cas was chosen as a therapeutic alternative because of its methodological rigour, which allows for extensive and impartial data collection given its novelty. All available data is systematically evaluated in evidence-based healthcare research (Pati and Lorusso, 2018). PRISMA-compliant reviews are open and reproducible. CRISPR-Cas therapy trial designs, results, and patient demographics for paediatric immunodeficiencies required systematic study. This method allowed to compare CRISPR-Cas to conventional treatment and uncover research gaps. The therapeutic effects and safety of CRISPR-Cas on paediatric immunodeficiencies were widely studied. All relevant databases were searched for literature. Several reviewers selected publications, extracted data, and graded quality to reduce bias and enhance reliability (Snyder, 2019).

Eligibility Criteria

The systematic study included direct CRISPR-Cas applications in paediatric hereditary immunodeficiency patients. This study collects high-quality, relevant data from peer-reviewed English-language scholarly papers. These articles should mention efficacy and safety. Based on criteria, non-assessment studies are removed. These include adult population studies, reviews and comments, non-English literature, and studies lacking effectiveness or safety results.

Inclusion Criteria

- Studies using CRISPR-Cas technologies for genetic editing in paediatric patients.
- Clear outcomes on efficacy and safety reported.
- Studies focusing on genetic immunodeficiencies and related genetic disorders.
- Peer-reviewed original research articles published in English.

Exclusion Criteria

- Studies focusing on adults or non-paediatric populations.
- Reviews, commentaries, and editorial pieces.
- Studies not utilising CRISPR-Cas technologies.
- Non-English language articles.
- Studies without clear outcomes on efficacy or safety.

Search Strategy and Databases

A well-defined search method was used to evaluate CRISPR-Cas technologies for treating paediatric immunodeficiencies. MeSH phrases and free-text keywords included "CRISPR-Cas9," "gene editing," "paediatric immunodeficiencies," and uncommon children disorders including "SCID" and "Wiskott-Aldrich Syndrome." Multiple terms were blended using Boolean operators (AND, OR) to extend the search. We searched PubMed, Web of Science, and Embase since they cover biomedical and life sciences literature effectively. The search was limited to English papers over the last decade to keep current on research and technology. This timeline was selected due to the rapid development of CRISPR-Cas technologies and their application in paediatric medicine. The iterative search strategy allowed for first-discovery adjustments to complete the literature investigation (Linares-Espinós et al., 2018).

Keyword/Phrase	Search Strategy
CRISPR-Cas9	"CRISPR-Cas9"
gene editing	"CRISPR-Cas9" OR "gene editing"
pediatric immunodeficiencies	("CRISPR-Cas9" OR "gene editing") AND "paediatric immunodeficiencies"
Severe Combined Immunodeficiency	("CRISPR-Cas9" OR "gene editing") AND ("paediatric immunodeficiencies" OR "Severe Combined Immunodeficiency" OR "SCID")
Wiskott-Aldrich Syndrome	("CRISPR-Cas9" OR "gene editing") AND ("paediatric immunodeficiencies" OR "Severe Combined Immunodeficiency" OR "SCID" OR "Wiskott-Aldrich Syndrome")

Risk of Bias

A detailed method was used to uncover bias in systematic review articles to ensure accuracy. Research quality was assessed using the Cochrane Risk of Bias Tool for randomised trials and ROBINS-I for non-randomised studies. Thorough reviews in all areas reduced bias (Newman and Gough, 2020). Performance, reporting, attrition, detection, and selection biases were discovered. Randomised studies examined participant and staff blinding, random sequences, allocation concealment, outcome assessment blinding, inadequate outcome data, and selective reporting. Non-randomised trials were investigated for confounding factor and non-protocol treatment biases.

Each study project's bias assessment results were merged to determine bias. Each area's studies were bias-rated "low," "high," or "unclear". This allowed a narrative synthesis of bias's effects on the review's outcomes (Snyder, 2019). Data synthesis included the evaluation's findings, focusing on low-bias research. Researchers thoroughly analysed bias-prone research and their outcomes. The systematic review selected dependable data with comprehensive bias risk assessment. This study lays the groundwork for paediatric immunodeficiency CRISPR-Cas research and clinical guidelines.

Synthesis of Results

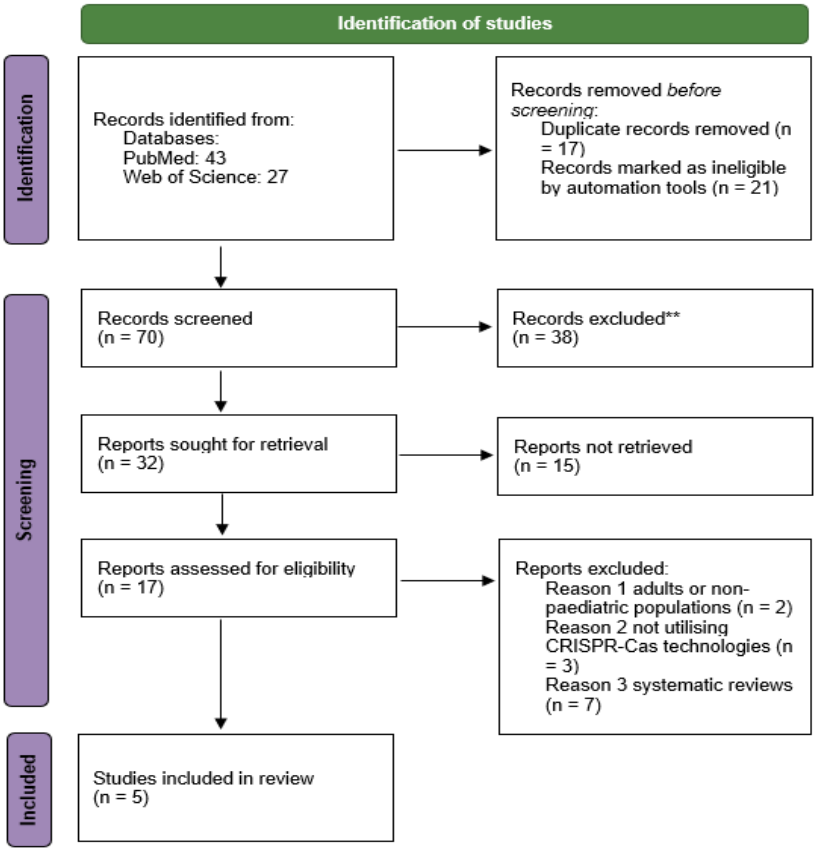
When available and derived from studies, the systematic review employed effect sizes like odds ratios, risk ratios, and mean differences as summary metrics (Pati and Lorusso, 2018). Methodology, treatments, and results varied of the included studies, which makes meta-analysis unfeasible. Instead, a narrative synthesis was utilised to combine results from all the research into a descriptive summary that could be used to other data and study methods. Throughout the synthesis process, studies were categorised by treatment and result similarities. Patterns were identified and conclusions drawn based on effect direction and amplitude. The story's inconsistencies and consistency provided a complete picture of CRISPR-Cas technologies' safety and effectiveness in paediatric immunodeficiencies. The review primarily followed the PRISMA checklist. Systematically and transparently documenting research finding, evaluation, and integration is essential (Linares-Espinós et al., 2018). The PRISMA checklist's primary components, 'Risk of bias across studies' and 'extra analyses,' were scored as negative since the data was unsuitable for quantitative synthesis or subgroup analysis.

RESULTS AND DISCUSSION

Study Selection

The research selection process used an exhaustive PRISMA protocol. Initially, the databases included 70 entries. After removing duplicates and ineligible studies, 32 data were retrievable. Twelve papers

were removed from the eligibility evaluation owing to their lack of paediatric relevance, CRISPR-Cas technology, or systematic review status. Seventeen articles were evaluated. Five papers met systematic review inclusion criteria after extensive screening. This shows that this research is relevant and meet CRISPR-Cas technology assessment criteria for paediatric immunodeficiencies (Newman and Gough, 2020).



Characteristics of Included Studies

Study Reference	Focus of Study	Key Findings	Model System	Gene(s) Edited	CRISPR System Used	Relevance to Paediatric Immunodeficiencies
Lagutina et al. (2015)	Modelling of human alveolar rhabdomyosarcoma Pax3-Foxo1 chromosome translocation	Successful modelling of Pax3-Foxo1 fusion in mice, highlighting CRISPR-Cas9's utility in precise genetic modifications.	Mouse myoblasts	Pax3, Foxo1	CRISPR-Cas9	Using CRISPR-Cas9 to simulate genetic illnesses may help investigate hereditary immunodeficiencies.
Wanzel et al. (2016)	CRISPR-Cas9–based target validation for p53-reactivating model compounds	CRISPR-Cas9 was used to establish that cancer cells require functional p53 for nutlin (which targets Mdm2) to operate and to compare it to DNA damage-inducing RITA. Nutlin's anticancer benefits rely on functioning p53, whereas RITA's efficacy is linked to DNA damage and resistance mechanisms, specifically FancD2's involvement in RITA resistance. Drugs targeting the p53 pathway have varied modes of action and resistance mechanisms, according to studies.	HCT116 colorectal and H460 lung cancer cells	TP53	CRISPR-Cas9	The work illuminates the complicated processes of medication action and resistance in reactivating tumour suppressors, which might influence therapeutic methods for paediatric malignancies with intact p53 signalling or for resistance-specific medicines.
Fernández-Calleja et al. (2019)	CRISPR/Cas9-mediated deletion of the Wiskott-Aldrich syndrome locus in murine erythroleukemia cells	Eliminating the Was gene using CRISPR/Cas9 in murine erythroleukemia (MEL) cells caused actin cytoskeleton disorganisation and changed monomeric G-actin to polymeric F-actin ratio. Was-deficient cells'	Murine erythroleukemia (MEL) cells	WAS (Was gene)	CRISPR/Cas9	Demonstrate that CRISPR-Cas9 may fix actin cytoskeleton genetic abnormalities in hematopoietic cells, helpful for treating paediatric

		actin cytoskeleton organisation and Btk activation were restored by WASp expression.				immunodeficiencies such Wiskott-Aldrich syndrome.
Seok et al. (2021)	Application of CRISPR-Cas9 in congenital heart disease (CHD)	Reviewing CRISPR-Cas9 applications for CHD showed its potential to fix genetic abnormalities that cause illness.	Various, including hiPSCs and animal models	Multiple genes related to CHD	CRISPR-Cas9	Demonstrates CRISPR-Cas9's extensive use in genetic mutation correction for hereditary immunodeficiencies
Ottaviano et al. (2022)	CRISPR-engineered CAR19 universal T cells for treatment of children with refractory B cell leukaemia	TT52CAR19 T cells modified by CRISPR-Cas9 to knock out TRAC and CD52 and express CAR19 were given to six children with relapsed/refractory CD19-positive B cell acute lymphoblastic leuk Four patients had allogeneic stem cell transplants following remission. Two patients had grade II cytokine release syndrome, one brief grade IV neurotoxicity, and one post-transplant cutaneous GVHD clearance. The research demonstrated CRISPR-engineered immunotherapy's safety, efficacy, and potential.	Children with relapsed/refractory B cell acute lymphoblastic leukaemia	TRAC and CD52	CRISPR-Cas9	This study shows that CRISPR-Cas9 may provide "off-the-shelf" CAR T cell treatments for paediatric patients with B cell leukaemia immunodeficiencies, an alternative to autologous therapy.

Risk of Bias within Studies

Lagutina et al. (2015) reproduce the Pax3-Foxo1 chromosomal rearrangement in human alveolar rhabdomyosarcoma using a new method. This work shows CRISPR-Cas9's genome editing precision and applicability. Consider bias when applying mouse myoblast model results to humans. Mouse-human disease pathophysiology and immunological responses are major elements limiting our results' generalisability. Genetic models benefit child immunodeficiencies instead of treatment.

Wanzel et al. (2016) use CRISPR-Cas9 to show p53's complex function in cancer cell sensitivity to model drugs and their mechanisms of action and resistance. Details of cancer cell resistance mechanisms and drug-specific routes are the study's strength. The cancer cell lines HCT116 and H460 may misrepresent paediatric cancers and p53 status. Due to genetic differences across lineages. This research may not apply to other child cancers due to its restricted focus.

CRISPR-Cas9-mediated deletion may restore actin cytoskeleton structure, improving Wiskott-Aldrich syndrome studies, according to Fernández-Calleja et al. (2019). This work addresses a hereditary immune system problem, although the mouse erythroleukemia cell model may not adequately represent young Wiskott-Aldrich syndrome patients' complicated immune systems. Differences in actin dynamics and cellular responses between human hematopoietic cells and the model system may create bias.

Seok et al. (2021) study CRISPR-Cas9 in congenital heart disease using several models and genes. The work shows CRISPR-Cas9's adaptability, although its broad model and genetic target testing may bias it. CRISPR-Cas9 therapies may vary in effectiveness, efficiency, and safety because to the wide range of model systems (from human pluripotent stem cells to animal models) and the large number of genes modified for coronary heart disease. Generalising about paediatric immunodeficiency technology utilisation is difficult because to the multiple variations.

Ottaviano et al. (2022) found CRISPR-engineered CAR19 T cells may cure childhood B-cell leukaemia. Small sample size and patient variability increase bias, but study shows safety and effectiveness. Cytokine release syndrome and graft-versus-host disease demonstrate that patient characteristics affect therapeutic success. Thus, generalising child perspectives is harder.

Results of Individual Studies

Lagutina et al. (2015) improved cancer chromosomal translocation models. In mouse myoblasts, CRISPR-Cas9 replicated the t (2;13) (q36.1; q14.1) translocation, creating a PAX3-FOXO1 fusion gene in A-RMS, a form of human alveolar rhabdomyosarcoma. To construct the Pax3-Foxo1 fusion gene, the mouse model inverted 4.9 Mb of chromosome 3 containing the Foxo1 gene. This

engineering endeavour is more important since mice have Pax3 and Foxo1 on distinct chromosomes than humans. The study found that translocation frequency is strongly influenced by Pax3 and Foxo1 gene proximity. The higher rate of Pax3-Foxo1 fusion gene synthesis in forelimb myoblasts (1:150 vs. 1:200 in hindlimbs) was connected to Pax3 expression and locus co-localisation. The CRISPR-Cas9 approach created the fusion gene by targeting Pax3 and Foxo1 DNA double-strand breaks (DSBs). A chromosomal translocation was helped. The CRISPR-Cas9 technology achieved reciprocal translocation in 64% of targeted cells, according to FISH. Pax3-Foxo1 fusion protein was validated by western blotting. Translocated cells up- or down-regulated 50% of PAX3-FOXO1's target genes, according to RNA sequencing. Results demonstrate gene placement and temporal expression facilitate chromosomal translocation. A mouse model that accurately replicates the human A-RMS translocation expands cancer research and therapy assessment. The work shows that CRISPR-Cas9 can develop precise mouse cancer models, which aids cancer research and therapy.

Wanzel et al. (2016) investigated nutlin and RITA, two p53-targeting drugs, for CRISPR-Cas9 genome editing. Cellular defence system p53 revives tumor-suppressing molecules to fight cancer. Nutlin blocks Mdm2 to restore p53 function. Although similar, RITA's mechanism was unknown. Results reveal these drugs' mechanisms. Researchers revealed nutlin needs p53. P53-disrupted cells were produced using CRISPR-Cas9. These cells became resistant to nutlin's antiproliferative activities, suggesting the p53-nutlin relationship. The number of cells with TP53 indels increased after nutlin therapy, proving that p53 is necessary for the response. RITA works without p53, according to studies. Even after cell p53 was inhibited, RITA worked. RITA may damage DNA because H2A.X, a marker for double-strand breaks, is phosphorylated. The discovery prompted additional inquiry. The study detailed RITA resistance mechanisms. Resistance to RITA and cisplatin implies a non-p53 DNA repair pathway. The resistance movement targeted DNA crosslink repair component FancD2. The medication's potency was restored after FancD2 suppression by RNA interference, proving DNA repair and RITA resistance are linked. They also connected RITA resistance to mTOR signalling. Anti-mTOR medications like AZD8055 reduced FancD2 expression and overcame RITA resistance. Decreased mTOR signalling may boost RITA responsiveness, supporting the connection between cellular signalling pathways and DNA repair mechanisms.

Fernández-Calleja et al. (2019) explored how WASp arranges MEL cell actin cytoskeletons. WAS is a severe X-linked immunodeficiency. WASp helps hematopoietic cells polymerise actin. The researchers removed 9.5 kb around the Was gene using CRISPR/Cas9. Poor actin cytoskeleton structure in MEL cells suggested significant cytoskeletal dysfunction from loss. There was no difference in total actin protein levels between wild-type and Was knockout cells. A noteworthy difference was the monomeric G-actin/polymeric F-actin ratio. Was-deficient cells showed more G-actin than F-actin, suggesting actin dynamics imbalance that impacts cell motility and stability. The study found that WASp expression in Was-deficient cells organised actin cytoskeleton. The rescue experiment indicated that WASp modulates actin polymerisation, suggesting cytoskeletal dysfunction treatment. Research examined WASp's connections with other cytoskeletal regulators. Actin cytoskeleton component Btk was activated by excess WASp. This interaction revealed the complex signalling networks that regulate cytoskeletal motions and hematopoietic cell growth. HMBA showed that Was gene lack altered cell differentiation. Was-deficient cells developed similarly to wild-type MEL, indicating that the loss did not impact erythroid precursor development. They found that CRISPR/Cas9 knockdown did not influence MEL cell proliferation since wild-type and mutant cells grew similarly. This study shows that therapeutically targeting the Was gene in erythroid cells does not affect cell growth or survival, a remarkable breakthrough.

Seok et al. (2021) illuminates coronary heart disease (CHD), a congenital defect that kills 261,247 worldwide. Mutations may cause CHD and other abnormalities. In addressing these genetic foundations, CRISPR-Cas9's precise genome editing is crucial. CRISPR-Cas9 corrects or replicates CHD mutations by targeting DNA areas using RNA molecules. The system was originally a defensive mechanism in bacteria, but current work suggests it may be utilised for genetic manipulation in larger animals. Many applications prove CRISPR-Cas9's efficacy. It regenerates patient-derived human pluripotent stem cells (hiPSCs) and creates animal models of coronary heart disease (CHD) that

mimic human CHD. CRISPR-Cas9's Barth syndrome-related TAZ gene mutation correction indicates its therapeutic promise. IPSC-derived cardiomyocytes were restored to normal function to cure dilated cardiomyopathy. These advancements show CRISPR-Cas9's promise in personalised medicine, needing genetic testing to choose the optimum therapy. CRISPR-Cas9 clinical use faces several challenges. The study suggests examining off-target consequences, gene editing efficacy, and Cas9 enzyme immunological reactivity. BRILLIANCE, a clinical pioneer, targets Leber's congenital amaurosis 10 (LCA10) using CRISPR-Cas9 technology despite these hurdles. This experiment compares CRISPR-Cas9's therapeutic benefits and drawbacks.

Ottaviano et al. (2022) conducted a groundbreaking phase 1 clinical trial utilising CRISPR-Cas9-engineered CAR19 T cells for adolescent refractory B cell leukaemia. The investigation included 11-month-to-11-year-olds with relapsed or refractory CD19-positive B-ALL. After lymphodepletion, they had TT52CAR19 T cells. TT52CAR19 T cells were produced by altering TRAC and CD52 genes using CRISPR-Cas9 and generating CAR19 in a lentiviral vector. This novel Cas9-based method selectively grows altered T cells. To help modified CD52-negative CAR19 T cells survive lymphodepletion, fludarabine, cyclophosphamide, and alemtuzumab were administered. CAR19 expression was 97%, TRAC knockout was 1% or below, and CD52 depletion exceeded 70% in TT52CAR19 T cells. At TRAC and CD52 loci, CRISPR editing demonstrated obvious on-target effects and low off-target activity. Four of six individuals with cell proliferation following therapy obtained morphological complete remission without MRD. Participants underwent allogeneic stem cell transplants. TT52CAR19 T cell therapy is safe with no acute side effects. Two patients suffered cytokine release syndrome, one had grade IV neurotoxicity, and one had cutaneous graft-versus-host disease (GVHD) resolved after transplantation. This discovery gives promise for tailored CAR T cell treatment without logistical and manufacturing difficulties. This is achieved by demonstrating that CRISPR-engineered T cells are a viable therapeutic option. The early findings suggest that universal TT52CAR19 T cells may be a novel paediatric B-ALL therapy that accelerates allo-SCT via anti-leukaemia. This study suggests genome editing might aid immunotherapy development. The experiment's promising findings were limited by its phase 1 status, small sample size, and short follow-up. Since two patients did not grow modified T cells, further research is required to investigate response variability and enhance therapeutic outcome prediction.

DISCUSSION

Lagutina et al. (2015) and Wanzel et al. (2016) simulated illnesses and studied therapeutic paths using CRISPR-Cas9. Wanzel et al. examine treatment resistance in cancer cells, whereas Lagutina et al. replicate disease genetics. Lagutina et al. want to create realistic illness models. Wanzel et al. (2016) translational research improves therapies using genetic data. The biggest difference between groups is this (Sarkar and Khan, 2021). CRISPR-Cas9 aids essential scientific and therapeutic studies across the research-to-treatment route. Fernández-Calleja et al. (2019) and Seok et al. (2021) explore CRISPR-Cas9 in congenital cardiac disease and Wiskott-Aldrich syndrome. Direct illness healing is their goal. Fernández-Calleja et al. (2019) study one immune system gene, whereas Seok et al. study many cardiovascular genetic disorders. Seok et al. (2021) explain CRISPR-Cas9's issues and potential usefulness in congenital cardiac diseases, whereas Fernández-Calleja et al. (2019) demonstrate precise genetic corrections. Polygenic illnesses are harder to manage than monogenic diseases, which can be addressed genetically (Baddeley and Isalan, 2021).

Ottaviano et al. (2022) report that CRISPR-engineered cellular therapies for paediatric leukaemia have moved beyond modelling and correction. This study suggests that CRISPR-Cas9 might build cheap medicinal therapies and progress them to clinical trials. Unlike prior investigations, Ottaviano et al. (2022) is pioneering patient CRISPR-Cas9 applications. This comparison links genetics and medicine by showing how CRISPR-Cas9 progressed from research to therapy. CRISPR-Cas9 is versatile in fundamental research, disease models, direct genomic repair, and medication development. Fernández-Calleja et al. (2019) and Seok et al. (2021) study direct sickness intervention. Wanzel et al. (2019) and Lagutina et al. (2015) present a theoretical framework for inherited disorders and pharmaceutical responses. Ottaviano et al.'s CRISPR-Cas9 therapy is pioneering. Genetic models and therapeutic use in paediatric research demonstrate how technology

may transform biomedical sciences (Lin et al., 2021). Each study's approach and emphasis determine its strengths and drawbacks, highlighting CRISPR-Cas9's complexity as a paediatric healthcare tool. Fernández-Calleja et al. (2019) indicate the major target matches CRISPR-Cas9 genome editing accuracy. This study shows the method can fix genetic defects, including the Was gene linked to Wiskott-Aldrich syndrome. Repairing actin cytoskeleton structure may restore normal cellular function and genetic corrections, as seen below. Lagutina et al. (2015) model the Pax3-Foxo1 fusion in alveolar rhabdomyosarcoma, offering crucial insights. This research may help understand and cure immunodeficiencies caused by genetic abnormalities (Morshedzadeh et al., 2024). Ottaviano et al. (2022) achieved the second target using CRISPR-engineered CAR T cells. This study reveals that modified T cells may restore immunological function in refractory B-cell leukaemia children. Additionally, CRISPR-Cas treatment is addressed. Remission and manageable side effects imply the medicine promotes health (Lin et al., 2022).

Early findings suggest CRISPR-Cas9 safety profiles are beneficial for children. Ottaviano et al. (2022) report safe, temporary side effects. These studies show no long-term safety. CRISPR-Cas may cure children immunodeficiencies with additional research and monitoring. Safety across settings and methods is important for genetic repair for single-gene defects and other therapeutic interventions. This research suggests that CRISPR-Cas9 may correct genetic defects, restore immunological function, and be safe for immunodeficient youngsters. The long-term effects of CRISPR-Cas must be studied, especially in young people, where developmental determinants are critical (Morshedzadeh et al., 2024). Cellular model gene editing and clinical trials of modified cell therapies demonstrate CRISPR-Cas9's intricacy. This stresses medical and scientific debate about gene editing's ethical and technological issues.

CONCLUSION

Limitations

Current research is limited by its preclinical focus and lack of human trials. Thus, the results are difficult to apply to clinical situations. CRISPR-Cas technology's safety and efficacy in children need rigorous clinical investigations due to a lack of evidence. Safety issues arise when CRISPR-Cas technologies alter genomic areas off-target. Since these alterations' long-term consequences are unknown, extensive monitoring and investigation are required to ensure lifelong safety. Germline changes may affect human DNA in hereditary ways, creating an ethical problem. Consider genetic editing's moral implications and set explicit ethical norms. Access to cutting-edge therapies is inconsistent, and financial and logistical hurdles may increase health inequities. International cooperation and government effort are required to remove this difference and expand CRISPR-Cas use.

Many research and policy initiatives are recommended to address these constraints. Comprehensive clinical trials on CRISPR-Cas' long-term safety and efficacy are required. These studies may enhance CRISPR-Cas use in paediatric immunodeficiencies. These studies must include several demographic groups to ensure generalisability and appropriate global genetic diversity representation. To increase CRISPR-Cas system accuracy and reduce off-target effects, future research should leverage bioinformatics for target selection and validation. Therapeutic CRISPR-Cas use would increase confidence and safety. Alternative CRISPR ingredient delivery strategies may increase gene editing accuracy and reduce systemic side effects. CRISPR-Cas technology needs an ethical global consensus, especially for germline editing. Regulatory and ethical frameworks that balance innovation and ethics are essential. This framework must be flexible and inclusive to incorporate diverse stakeholders in discourse.

Conclusion

CRISPR-Cas technology may help cure hereditary diseases by treating immunodeficiencies in youngsters. This systematic review analyses ongoing research on using CRISPR-Cas to address genetic defects in paediatric immunodeficiencies. This review assessed the merits and downsides of these novel therapies. This study reveals how CRISPR-Cas may heal paediatric immunodeficiency

by precisely changing genetics to treat the cause. Numerous studies have shown that CRISPR-Cas may repair genetic abnormalities and immune systems in injured youngsters. These advancements provide novel genetic disease pathways and therapies, expanding research opportunities. Most CRISPR-Cas research is preclinical, with a few early clinical trials. Please note that this field is evolving. From lab research to clinical usage, scientific, ethical, and regulatory challenges must be managed. CRISPR-Cas technologies may modify genomic regions off-target, endangering patients' health and genetics. These powerful technologies need robust ethical frameworks and regulatory control because to genome editing in children's consent and germline changes. Widespread CRISPR-Cas use has societal and economic consequences. These medications are expensive and rare, thus low- and middle-income countries often misdiagnose or untreated paediatric immunodeficiencies. Ethics need government, healthcare, and international cooperation to deliver life-saving therapies to everyone.

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APPENDICES

Section A – Risk of Bias

Randomised Studies (Cochrane Risk of Bias Tool)

Study	Random Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Bias
Lagutina et al. (2015)	Low risk	Low risk	Low risk	High risk	Low risk	Low risk	Low risk
Seok et al. (2021)	Unclear risk	Unclear risk	High risk	High risk	Low risk	Low risk	Unclear risk

Non-Randomised Studies (ROBINS-I Tool)

Study	Confounding	Selection of Participants	Classification of Interventions	Deviations from Intended Interventions	Missing Data	Measurement of Outcomes	Selection of Reported Result
Fernández-Calleja et al. (2019)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Ottaviano et al. (2022)	High risk	High risk	Low risk	High risk	Low risk	Low risk	Low risk
Wanzel et al. (2016)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

Section B – Critical Appraisal

CASP Appraisal for RCTs

CASP Checklist	Lagutina et al. (2015)	Seok et al. (2021)
Study	Yes	Yes
Clear Aim	Yes	Yes
Appropriate Methodology	Yes	Yes
Design Appropriate to Aim	Yes	Yes
Properly Recruited	Yes	No
Participants' Groups Equal	Yes	Yes
Staff/Participant Blinding	No	No
Appropriate Outcomes	Yes	Yes
Follow-Up Complete	Yes	No
Clear Results	Yes	Yes
Precision of Results	Yes	Yes
Relevance of Results	Yes	Yes
Bias Consideration	Yes	No
Ethical Issues Addressed	Yes	Yes

Newcastle-Ottawa Appraisal for Non-Randomised Studies

Study	Selection Bias	Study Design	Confounding Factors	Blinding	Data Collection Methods	Withdrawals and Dropouts	Total
Fernández-Calleja et al. (2019)	★★★	★★★	★★	★	★★★	★★★	14/18
Ottaviano et al. (2022)	★★	★★	★	★	★★★	★★★	12/18
Wanzel et al. (2016)	★★★	★★★	★★★	★★	★★★	★★	16/18