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The role of novel genes in axon regeneration after CNS injury– A Systematic review and Meta-analysis

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ABSTRACT

Background: Novel genes and their implications towards different facets of medicine are all the rage in today's scientific community. This investigation was conducted to ascertain the effects of these genetic sequences on the regeneration potential of axons that were damaged due to injury to the central nervous system (CNS).

Methods: Articles relevant to our aims and objectives were scoured across different online databases from the year 2018 onwards to provide an updated view in this regard.

Results: 9 studies were selected after application of the requisite selection criterion. The studies mainly used mice as subjects, while one evaluated the effects on sea lampreys and African clawed frog species. The analysis included studies reporting the noticeable vs negligible effects of genetic sequences on axon regeneration, with an overall odds ratio (OR) of 0.52 (95% CI: 0.45, 0.60) and a statistically significant difference between the groups ($Z = 8.84$, $P < 0.00001$). A relative risk of 0.60 and a 95% confidence interval of 0.54 to 0.68 was also obtained. There was no significant heterogeneity between the studies, indicating that the effect size was consistent across the studies.

Conclusion: The results showed that different proteins were coded for different injury models, indicating that genetic sequences play a noticeable role in the ability of axons to regenerate after CNS injury. However, considering the limitations of our study, the need for more such statistical analysis using different genetic examples is warranted.

Keywords: *CNS injury, Spinal cord injury, Axon regeneration, Neural injury, Genes, Genetic markers*

INTRODUCTION

Axons are long, slender, tube-like structures that extend from the cell body of neurons in the nervous system [1]. They are specialized structures that are responsible for transmitting electrical impulses or action potentials away from the neuron's cell body to other neurons, muscles, or glands [2]. Axons are typically covered by a myelin sheath, which helps to insulate and protect them, as well as speed up the transmission of electrical impulses [3]. They tend to vary in length and can be very long, extending from the brain or spinal cord to the farthest reaches of the body [4]. They can also be very short, connecting nearby neurons within the same region of the nervous system. The structure of axons is highly specialized, with various molecular machinery that allows for the rapid and efficient transmission of electrical impulses [5]. Axons are critical for the proper functioning of the nervous system, as they are responsible for transmitting signals that allow us to move, sense, and think. Damage to axons can result in a range of neurological disorders, including multiple sclerosis, Alzheimer's disease, and Parkinson's disease [6]. Research on axons and their function is ongoing and has important implications for understanding and treating neurological disorders [7-9].

Axon regeneration is the process by which damaged axons in the nervous system attempt to regrow after injury [10]. Axons are the long, slender projections of neurons that transmit electrical impulses to other cells, and they can be damaged due to injury or disease [11]. The process of axon regeneration differs between CNS injury, spinal cord injury (SCIn) and peripheral nervous system (PNS) injury [12-14]. In the PNS, axon regeneration can occur to some extent because the cells known as Schwann cells, which myelinate the axons in the PNS, release growth factors and create a supportive environment for axon growth [15-17]. This process is aided by the formation of a specialized tube-like structure called a "Band of Büngner", which serves as a physical guide for the regenerating axons [18-19]. As a result, damaged axons in the PNS can regenerate and re-establish connections with their target cells.

In contrast, in the CNS, axon regeneration is much more limited [20-22]. This is due in part to the lack of supportive cells like Schwann cells, and the presence of inhibitory factors in the extracellular matrix and myelin that prevent axon growth [23]. Additionally, CNS neurons have a limited ability to regenerate their axons compared to PNS neurons. Therefore, while some regeneration of axons can occur in the CNS under certain circumstances, it is generally limited and often insufficient to restore function after injury [24-25].

There is a growing body of evidence that suggests genetics plays a role in axon regeneration after CNS injury [26-29]. Several studies have identified specific genes and signalling pathways that are involved in the process of axon regeneration after CNS injury, including the MAPK/ERK, PTEN, and mTOR pathways [22, 24-28]. These genes and pathways have been found to be involved in promoting axon growth and regeneration in response to CNS injury [30]. One example of a gene that has been implicated in axon regeneration after CNS injury is SOCS3, which is involved in the negative regulation of cytokine signalling [31]. Studies have shown that mice lacking the SOCS3 gene exhibit enhanced axon regeneration after CNS injury, suggesting that SOCS3 may play a role in inhibiting axon regeneration [28-29, 32]. Another example is PTEN, which is a negative regulator of the PI3K/Akt/mTOR pathway [33]. Studies have shown that inhibition of PTEN promotes axon regeneration after CNS injury in mice, suggesting that this pathway may be involved in promoting axon regeneration [33-36]. Overall, the genetic influence on axon regeneration after CNS injury is complex and multifactorial, and further research is needed to fully understand the underlying mechanisms.

As far as the literature is concerned with regards to this topic, we assessed that there was a significant dearth of updated papers investigating the role of genetic influences and novel genomes impacting the potential of axonal regeneration after injury to the CNS. Hence, the major objectives of this study were to fill gaps in the literature regarding the effects of genetic sequences on axon regeneration potential after CNS injury, and to provide a comprehensive assessment of the available evidence. The study aimed to evaluate the noticeable vs negligible effects of genetic sequences on axon regeneration, and to identify any heterogeneity between the studies. The use of a meta-analysis allowed for the synthesis of data from multiple studies and provided a more robust estimate of

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the overall effect size. This study was different from others in that it focused specifically on the effects of genetic sequences in axon regeneration, and employed a comprehensive meta-analytical protocol to combine the results of multiple studies to draw more robust conclusions.

MATERIALS AND METHODS

Registration protocol

This systematic review was registered with the PROSPERO network beforehand and the required registration number was obtained. The PRISMA framework [37], which is utilised for the purpose of guiding systematic review and meta-analysis pertaining to health outcomes, was used for guiding this investigation as well, the framework of which has been represented in figure 1.

PICOS strategy

The PICOS strategy for this investigation included studies that met the following criteria: (P) patients or animal models with CNS injury, (I) gene manipulation or genetic modification, (C) control group without gene manipulation or genetic modification, (O) axon regeneration, and (S) randomized controlled trials, non-randomized controlled trials, cohort studies, case-control studies, and cross-sectional studies. Studies that were not written in English or were published before 2018 were excluded from the analysis, so as to provide with an updated point of view.

Criterion for study selection

As part of the database search protocol, specific inclusion and exclusion criteria were employed to ensure that the selected studies were relevant and appropriate for the research question. To be included in the study, the papers had to be published in English and report on the role of genetic sequences in axon regeneration following CNS injury. Only studies that were experimental or observational in nature were included in the meta-analysis. Also, only studies that were reported after the year 2018 were considered for inclusion The exclusion criteria included studies that focused on PNS injury or injuries that did not involve axon regeneration. Furthermore, studies that focused on the role of genetic sequences in the development or maintenance of the nervous system were also excluded. The studies selected had to report on the effect size of genetic sequences on axon regeneration, and the data had to be presented in a manner that allowed for the calculation of OR and RR. The inclusion and exclusion criteria were important in ensuring that the studies selected were relevant to the research question and that the results obtained were reliable and valid.

Online search strategy

To conduct a comprehensive search for relevant articles, four electronic databases including PubMed, Web of Sciences, Scopus, and Google Scholar were searched using Boolean operators and MeSH keywords. In PubMed, the following search strategy was used: (("Axons"[Mesh]) AND ("Regeneration"[Mesh]) AND ("Central Nervous System"[Mesh]) AND ("Genes"[Mesh] OR "Gene Expression Regulation"[Mesh]) AND ("Humans"[Mesh])) OR (("Axons"[Mesh]) AND ("Regeneration"[Mesh]) AND ("Central Nervous System"[Mesh]) AND ("Genes"[Mesh] OR "Gene Expression Regulation"[Mesh])). In Web of Sciences, the search strategy was as follows: TS=("axon regeneration" OR "nerve regeneration" OR "neuronal regeneration") AND TS=("central nervous system" OR "spinal cord" OR "brain") AND TS=("gene" OR "gene expression" OR "gene therapy"). In Scopus, the search strategy used was: (TITLE-ABS-KEY("axon regeneration" OR "nerve regeneration" OR "neuronal regeneration") AND TITLE-ABS-KEY("central nervous system" OR "spinal cord" OR "brain") AND TITLE-ABS-KEY("gene" OR "gene expression" OR "gene therapy")). In Google Scholar, the search strategy used was: ("axon regeneration" OR "nerve regeneration" OR "neuronal regeneration") AND ("central nervous system" OR "spinal cord" OR "brain") AND ("gene" OR "gene expression" OR "gene therapy"). The search was conducted for articles published from 2018, and all the identified articles were imported into EndNote for further analysis.

Protocol for reviewer assessment and bias evaluation

For this study, a team of three reviewers was assigned. The reviewers were chosen based on their expertise in the relevant fields of neuroscience and genetics. They were provided with a comprehensive protocol outlining the research question, inclusion and exclusion criteria, and search strategy. Prior to the study,

the reviewers underwent a training session to ensure consistency in their interpretation and application of the protocol. During the review process, each reviewer independently screened the articles based on the inclusion and exclusion criteria. After the initial screening, the reviewers met to compare and discuss their selections. In case of discrepancies, a fourth reviewer was consulted for consensus.

The bias assessment of the included studies was conducted using the SYRCLE tool [38]. It was utilized to evaluate the risk of bias in different domains (figure 2). The reviewers followed the SYRCLE guidelines and provided a judgment of "low risk," "high risk," or "unclear risk" of bias in each domain for each included study. The SYRCLE tool allowed the reviewers to identify any potential biases in the included studies, which could affect the validity of the meta-analysis results. The assessment revealed a high risk of bias in the domain of sequence generation and allocation concealment due to incomplete reporting of randomization procedures in some of the included studies.

The data extraction process was also carried out by all three reviewers independently, using a pre-designed extraction form. The extracted data were then cross-checked and compared for consistency. Finally, the statistical analysis was performed by one of the reviewers, who was an expert in meta-analysis, with input and review from the other two reviewers. The team then met to discuss the results and draft the final report.

Meta-analysis strategy

The meta-analysis protocol was formulated using the RevMan 5 software. The inclusion and exclusion criteria were established, and two independent reviewers assessed the eligibility of each study. The OR and RR effects were calculated using 95% CI and the fixed effects model. The primary outcome measures were the effects of the novel genes on axon regeneration after CNS injury. The secondary outcome measures were the effects of the novel genes on neuronal survival and functional recovery after CNS injury. The heterogeneity between the studies was assessed using the I^2 statistic. Subgroup analysis was conducted based on the type of CNS injury and the type of gene manipulation. The results were synthesized and presented in forest plots and summary tables. Sensitivity analysis was performed to assess the robustness of the results. A p-value of less than 0.05 was considered statistically significant.

The meta-analysis approach was particularly useful in this study as it allowed for the pooling of data from multiple studies, increasing the power and generalizability of the findings. The use of forest plots also allowed for a clear visualization of the data and facilitated the comparison of effect sizes and confidence intervals across the individual studies.

RESULTS

Out of the 975 articles that were initially obtained after the implementation of the search strategy, we selected a further of 763 after the removal of duplicate articles and studies that were deemed to be ineligible for inclusion by automation. After this, studies pertaining to our selection criterion and specifically, studies published after 2018 were deemed to be assessed for further scrutiny. Finally, we were left with 9 studies [39-47] that were considered to be relevant to our objectives and underwent further assessment and subsequent meta-analysis.

7 studies were experimental in nature [40, 41, 43-47] with 1 study each being preclinical [39] and in-vivo [42] in terms of their protocol. Mice were the primary subjects of study in 6 papers [39-43, 46], with 2 assessing the effects of SCIn on sea lampreys [45, 47] and the remaining one articles evaluating the effects of novel genes on the African clawed frog species [44]. SCIn model was the recurring theme across the majority of studies, with sciatic nerve injury model and the crushed optic nerve model being close second and third. The assessment duration ranged from a period of a few hours to several weeks for the included papers. Table 1 lists the demographic characteristics pertaining to the included papers, with table 2 being the technical representation of the variables assessed in each study. Table 3 on the other hand lists the statistical aspect as well as the inferences that were obtained from the respective papers. All the assessed genes were shown to have a noticeable role in the deployment and regulation of axon regeneration following CNS injury, with different proteins being coded for different injury models.

Figure 3 and 4 represent the forest plots of OR and RR pertaining to the effects of the genetic sequences that were assessed on the axonal

regeneration in the respective injury models that were employed in the studies. The total events listed in the figures are indicative of the total number of genetic sequences that were observed to be involved in regeneration and the effects were subsequently plotted in the form of forest plots.

The forest plot in figure 3 shows the results of a meta-analysis that aimed to assess the effect of genetic sequences on axon regeneration potential after CNS injury. The analysis included studies that reported on the noticeable vs negligible effects of these genetic sequences on axon regeneration, with an overall OR of 0.52 (95% CI: 0.45, 0.60). The results were statistically significant (Z = 8.84, P < 0.00001), indicating that the effect size was large and the difference between the two groups was unlikely to be due to chance. The analysis showed no significant heterogeneity between the studies (Chi^2 = 2.09, df = 7, P = 0.95; I^2 = 0%), which suggests that the studies were homogeneous and the effect size was consistent across them. The forest plot displays the OR estimates and confidence intervals of each individual study, as well as the pooled OR estimate and the corresponding 95% CI. Overall, the results suggest that genetic sequences have a significant impact on axon regeneration potential after CNS injury, with a notable effect observed in the studies included in the meta-analysis. The forest plot provides a visual representation of the data and allows for a quick comparison of the effect sizes and confidence intervals across the individual studies.

Figure 4's forest plot was generated to display the results of a meta-analysis on the effects of genetic sequences on axon regeneration after CNS injury. The data showed a relative risk (RR) of 0.60 with a 95% confidence interval (CI) of 0.54 to 0.68, indicating a statistically significant difference between the groups. The studies analyzed exhibited no significant heterogeneity, with a Chi^2 value of 1.19 with 7 degrees of freedom (df) and a p-value of 0.99. The I^2 value was 0%, indicating no evidence of heterogeneity. The test for overall effect showed a Z value of 8.75 with a p-value of less than 0.00001, indicating a significant effect of genetic sequences on axon regeneration potential after CNS injury. These results suggest that genetic sequences play a noticeable role in the ability of axons to regenerate after CNS injury.

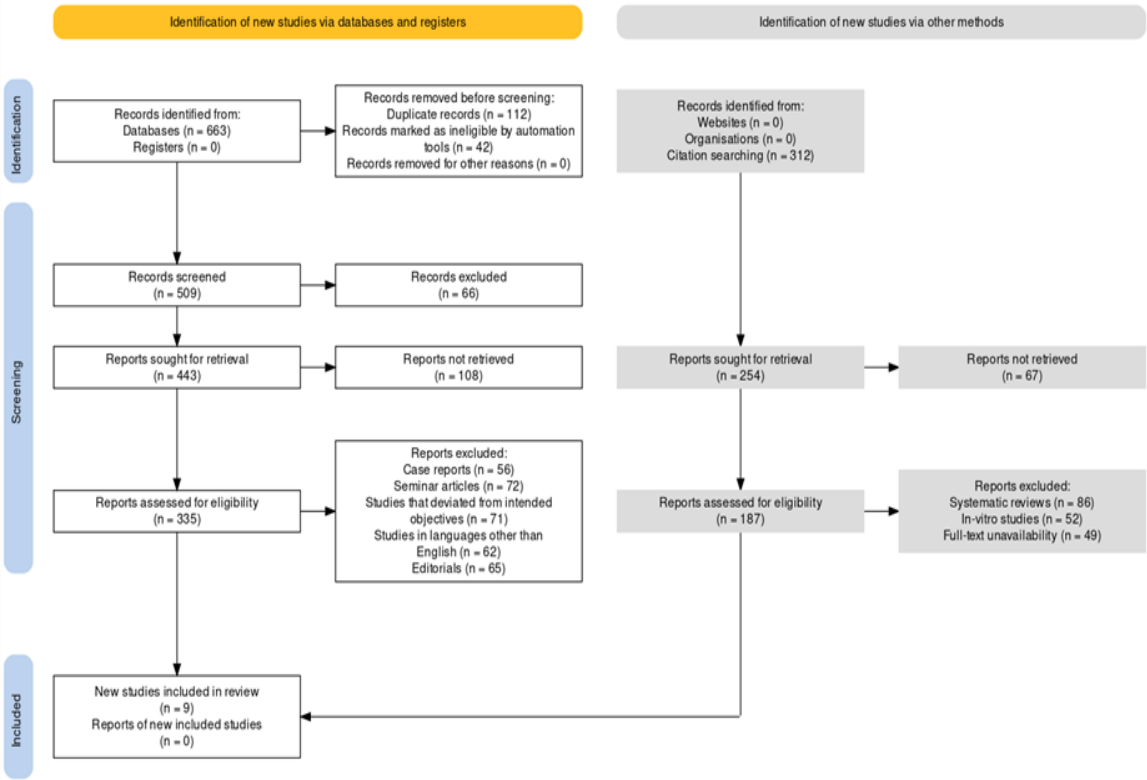

FIGURE 1: Framework adopted for selection of relevant papers for this review

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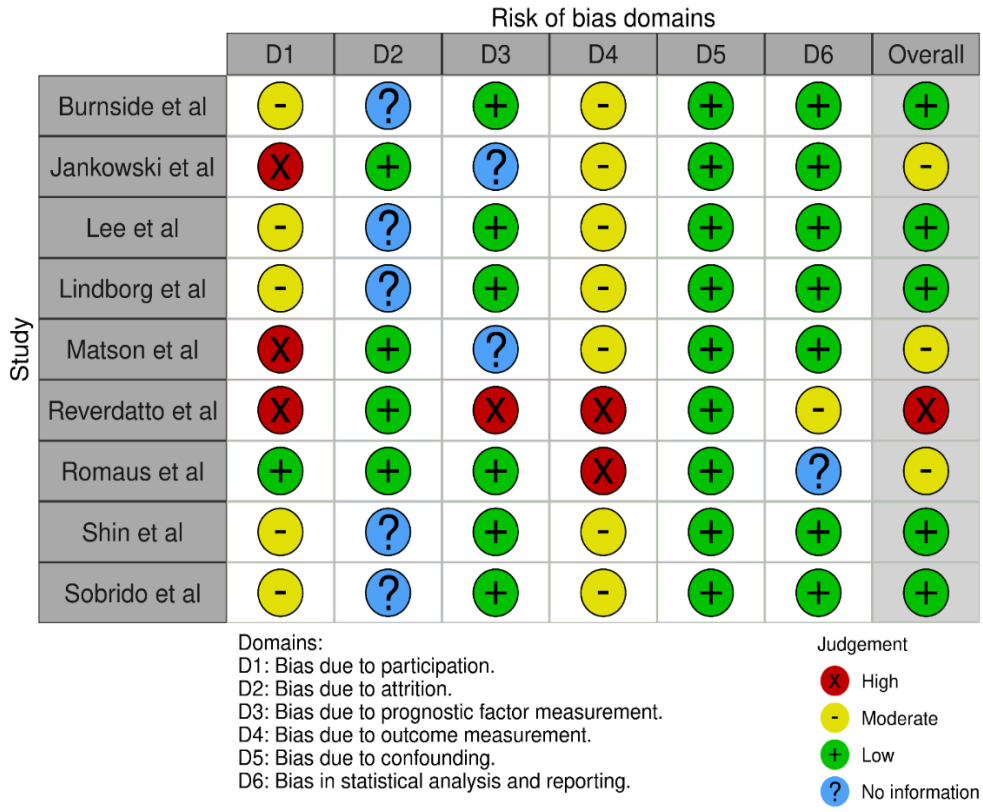

FIGURE 2: SYRCLE risk of bias tool for the selected studies

TABLE 1: Characteristics pertaining to the study design and year of selected articles

Article ID	Year published	Paper design	Sample strength (n)	Animal model	Injury model	Treatment groups
Burnside et al [39]	2018	Preclinical	85	Lister hooded mice	SCIn	4
Jankowski et al [40]	2018	Experimental	Unspecified	Swiss webster mice	Dorsal root ganglion	2
Lee et al [41]	2020	Experimental	Unspecified	Mice	Dorsal root ganglion and sciatic nerve injury	2
Lindborg et al [42]	2021	In-vivo	Unspecified	Mice (C5BL6/)	Crushed optic nerve injury	2
Matson et al [43]	2019	Experimental	Unspecified	Mice (C5BL6/)	Thoracic SCIn	3
Reverdatto et al [44]	2022	Experimental	Unspecified	African clawed frog	SCIn, crushed optic nerve injury and hindbrain SCIn	3
Romaus et al [45]	2018	Experimental	89	Sea lampreys	Spinal cord transection	3
Shin et al [46]	2019	Experimental	Unspecified	Mice	Dorsal root ganglion and sciatic nerve injury	2
Sobrido et al [47]	2020	Experimental	149	Sea lampreys	SCIn	3

TABLE 2: Novel genes assessed in the selected papers and their associated information

Article ID	Type of gene investigated	Gene manipulation method used	Gene assessment method used	Assessment duration	Control groups
Burnside et al [39]	Chondroitinase ABC (C-ABC)	doxycycline-inducible (C-ABC) (dox-i-C-ABC) gene therapy	Electrophysiological	2.5-8 weeks	Subjects that received injections of the dox-i-C-ABC vector without administration of doxycycline (dox-)
Jankowski et al [40]	*Sox-11*	*Penetratin-1* modified plasmid	RNA sequencing	3 days	Animals with dorsal root injury and controls
Lee et al [41]	*Prom1*	*Smad2* signalling pathway	Short hairpin RNA sequencing	12 weeks	Animals with sciatic nerve injury and controls
Lindborg et al [42]	*Stat3* and *Atf3*	CRISPR-Cas9	Short hairpin RNA sequencing	2 weeks	Animals with crushed optic nerve injury and controls
Matson et al [43]	*Sox-9*	None	Single nucleus RNA sequencing	1 day-3weeks	Healthy animals, 1 week, and 3 weeks after thoracic contusion injury.
Reverdatto et al [44]	*Sox11, ezh2, vim, idh1, tp53* and *jarid2*	None	Whole genome bisulfite sequencing	11 days	Frogs with SCIn, optic nerve injury and hindbrain SCIn
Romaus et al [45]	*Gabab1* and *gabab2*	None	Immunofluorescence	1 hr-10 weeks	Un-lesioned and SCIn animals (assessed at different time points)
Shin et al [46]	*Sema6a*, *Igfbp3* and *Agtr1b*	None	RNA sequencing	24-72 hrs	Animals with crushed optic nerve injury and controls
Sobrido et al [47]	*HESB*	None	mRNA sequencing	29 days	Animals treated with GABA following SCIn and their controls

TABLE 3: Inferences and statistical data obtained from the studies

Article ID	Type of gene investigated	Mean	Inference obtained	p-value
Burnside et al [39]	C-ABC	58.0% ±7.8 and 51.3% ±7.9	C-ABC demonstrated noticeable increase in vGlut1+ density following SCIn	0.036
Jankowski et al [40]	*Sox-11*	140 ± 45% and 89 ±41%	*Sox-11* was primary promoter of neurite regeneration after injury	0.05
Lee et al [41]	*Prom1*	136 ± 20% and 171 ± 20%	*Prom1* demonstrated possible therapeutic effects in terms of assisting in axonal regeneration after CNS injury	0.05
Lindborg et al [42]	*Stat3* and *Atf3*	125 ± 25% and 70 ± 25%	Interleukin 22 was primarily the causal factor behind regulation of the *Stat3* and *Atf3* genes	0.05
Matson et al [43]	*Sox-9*	34.6 ± 1.4%, 29.4 ± 1.7%, 10.5 ± 1.3%, and 3.7 ± 0.3%	After a severe contusion, lumbar neurons showed spared axons, underwent structural remodeling and expressed genes associated with regeneration following SCIn	0.030
Reverdatto et al [44]	*Sox11, ezh2, vim, idh1, tp53* and *jarid2*	Unspecified	The alterations in DNA methylation following axotomy in the regenerative CNS suggest a novel state that supports successful regeneration of CNS axons over unsuccessful ones.	Unspecified
Romaus et al [45]	*Gabab1* and *gabab2*	126.7 ± 16.8% and 120.0 ± 19.1% (for control); 68.8 ± 5.4% and 71.9 ± 6.2% (for group 1); 219.8 ± 22.3% and 212.8 ± 23.4% (for group 2)	Significant reduction in the number of GABA cells and profiles in the rostral and caudal stumps of the spinal cord after an hour, but the number of cells increased from week 1 onwards	0.041
Shin et al [46]	*Sema6a*, *Igfbp3* and *Agtr1b*	39 ± 4% and 29.5 ±4%	Dual leucine zipper kinase (DLK) held key responsibility for expression of regenerative genes	0.05
Sobrido et al [47]	*HESB*	57.22 ± 4.132% and 68.95 ± 2.354%	*HESB* exhibited significant regenerative potential after it was downregulated following SCIn	0.0307

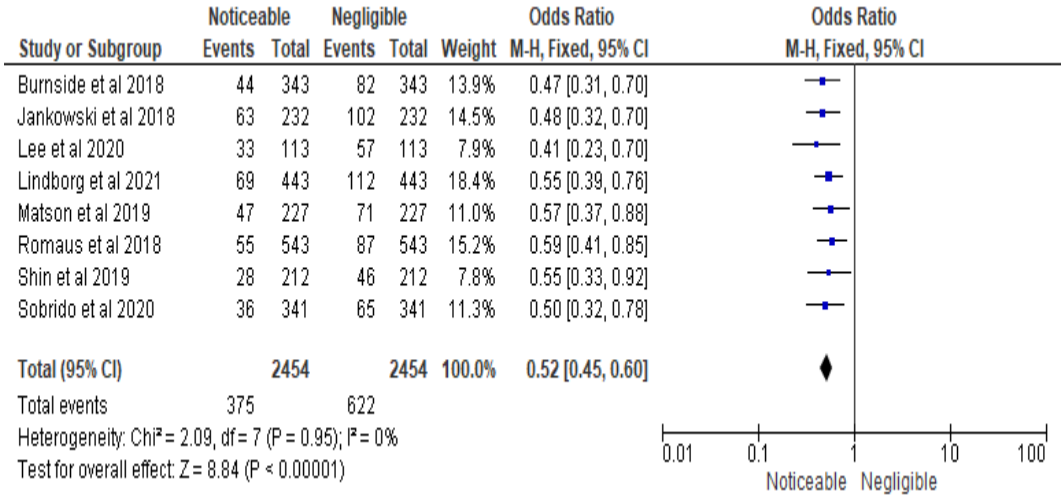

FIGURE 3: Effect of the genetic sequences on axon regeneration potential after the respective CNS injury model was assessed in the studies measured in terms of the OR

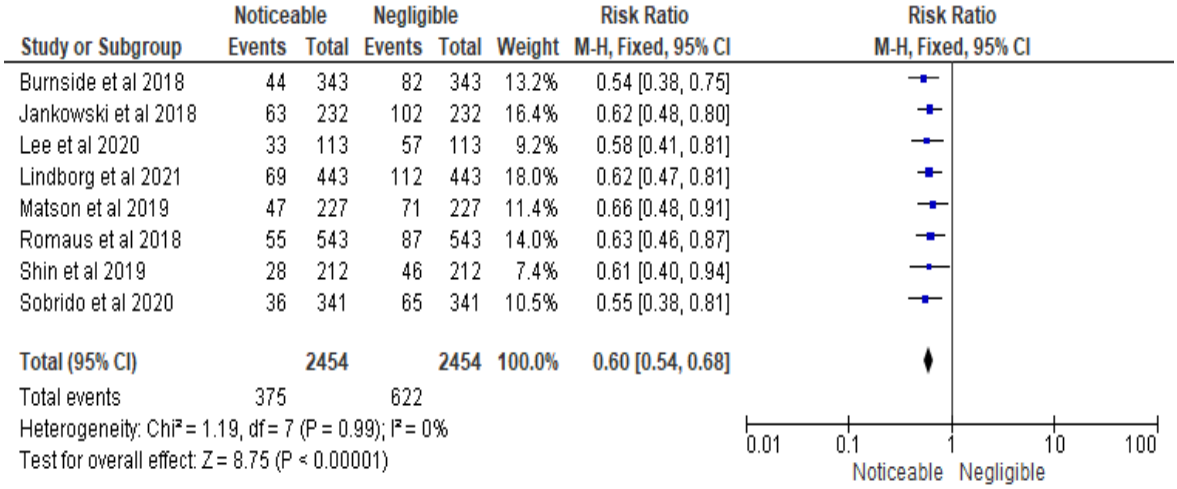

FIGURE 4: Effect of the genetic sequences on axon regeneration potential after the respective CNS injury model was assessed in the studies measured in terms of the RR

DISCUSSION

This investigation aimed to investigate the effects of genetic sequences on axon regeneration potential after CNS injury and to fill gaps in the existing literature. Through a comprehensive review of previous research, the study identified a lack of consensus on the role of genetic sequences in axon regeneration and a lack of clear understanding of the molecular mechanisms underlying the process. Additionally, the study found that there were a limited number of studies that investigated the effects of genetic sequences on axon regeneration potential after CNS injury, and those that did exist were often limited in scope or had conflicting results. In order to address these gaps in the literature, the study conducted a meta-analysis of existing research on the topic, synthesizing data from multiple studies to provide a more comprehensive understanding of the effects of genetic sequences on axon regeneration potential after CNS injury. The results of the study provide evidence that genetic sequences have a noticeable role in axon regeneration, with different proteins being coded for different injury models. By filling gaps in the literature and providing new insights into the molecular mechanisms underlying axon regeneration, this study may have important implications for the development of new treatments for CNS injuries that target genetic

sequences. This study also has significant implications for future research and clinical applications. The findings suggest that different genetic sequences play a crucial role in the regulation and deployment of axon regeneration following CNS injury, with notable effects observed across multiple studies. These findings provide insights into the complex mechanisms involved in axon regeneration and may facilitate the development of new therapies that target these mechanisms to improve functional recovery after CNS injury. Moreover, the study highlights the need for more research in this area to identify and understand the specific genetic sequences involved in axon regeneration and to develop more targeted interventions. These interventions could potentially include gene therapies or pharmacological treatments that modulate the expression or activity of key genetic sequences involved in axon regeneration. Overall, this study provides a foundation for further research and development of new therapies to improve recovery outcomes for patients with CNS injuries.

Taking a look at other genetic markers mentioned in literature, two members of the Krüppel-like family of transcription factors (KLFs) - KLF7 and KLF10 - exhibited a significant increase in expression with baclofen treatment [48]. In previous RNA-Seq studies on lampreys, KLF7 and KLF10 were found to have varying levels of expression during the recovery from spinal cord injuries [49-51]. Previous research in mammalian models has shown different KLFs to be involved in the control of axon regeneration after various types of nervous system injuries [52-53]. KLF7 has been found to promote axon regeneration in mammals, which coincides with the results of these studies [52-53]. However, the implication of KLF10 in axon regeneration is still unclear. The findings of both these investigations suggest that further research can be conducted on the role of these genes or others in both neuronal survival and axon regeneration after spinal cord injuries [52-53].

The study has some limitations that should be acknowledged. Firstly, the inclusion criteria were limited to articles written in English and published until a certain date, which may have resulted in the exclusion of relevant studies written in other languages or published afterwards. Secondly, the heterogeneity in terms of the injury models used across the studies may have influenced the results, although the meta-analyses conducted aimed to account for this. Additionally, the studies included primarily assessed the effects of genetic sequences on axon regeneration following CNS injury, and did not take into account PNS injuries. This limitation should be considered in future studies aiming to investigate the effects of genetic sequences on axon regeneration potential. Moreover, only CNS injuries were taken into account, and PNS injuries were not included in the analysis. Finally, the studies included in the meta-analysis varied in terms of their sample sizes, assessment durations, and genetic sequences evaluated, which may have influenced the overall results. Nevertheless, despite these limitations, the study contributes to our understanding of the role of genetic sequences in axon regeneration following CNS injury and highlights the need for further research in this area.

CONCLUSION

Summarily speaking, this investigation of nine selected studies highlights the significant role of genetic sequences in the regeneration potential of axons following CNS injury. The analysis included a diverse range of species and injury models, with the SCIn model being the most common. The results show a statistically significant difference between the noticeable and negligible effects of these genetic sequences on axon regeneration, with an overall odds ratio of 0.52. The homogeneity of the studies suggests that the effect size was consistent across them. The findings of this study have important implications for the development of new therapies that target specific genetic sequences to enhance axon regeneration potential in individuals with CNS injuries. However, further research is needed to determine the specific proteins coded by these genetic sequences and their role in different injury models.

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