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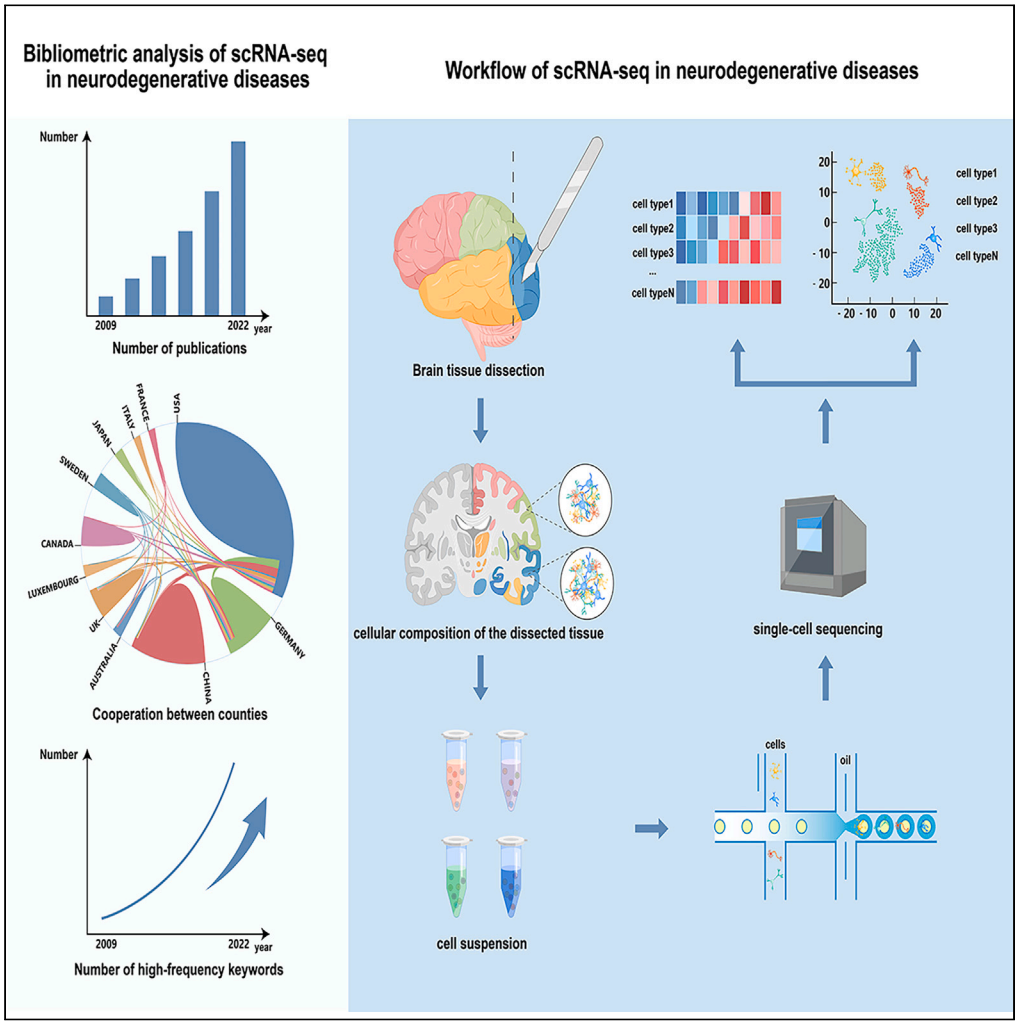

Wei Wang, Tianhua Li, Zheng Wang, ..., Chaodong Wang, Xinli Hu, Shibao Lu

cdongwang01@126.com (C.W.)
huxinli123@qq.com (X.H.)
shibaolu@xwh.ccmu.edu.cn (S.L.)

Highlights

scRNA-seq in neurodegenerative diseases is experiencing rapid growth

We summarized scRNA-seq research on neurodegenerative diseases

scRNA-seq offers deep insights into nervous system complexity

Many challenges arise when using scRNA-seq in neurodegenerative diseases

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Bibliometric analysis of research on neurodegenerative diseases and single-cell RNA sequencing: Opportunities and challenges

Wei Wang,[1,2] Tianhua Li,[3] Zheng Wang,[1,2] Yaxin Yin,[1,2] Sitao Zhang,[1,2] Chaodong Wang,[4,*] Xinli Hu,[1,2,5,*] and Shibao Lu[1,2,*]

SUMMARY

Neurodegeneration, characterized by the progressive deterioration in neuronal structure or function, presents an elusive mechanism. The use of single-cell RNA sequencing (scRNA-seq) technology in the clinic is becoming increasingly prevalent in recent decades. This technology offers unparalleled cell-level insights into neurodegenerative diseases, establishing itself as a potent tool for elucidating these diseases underlying mechanisms. Here, we made a deep investigation for scRNA-seq research in neurodegenerative diseases using bibliometric analysis from 2009 to 2022. We observed a robust upward trajectory in the number of publications on this subject. The United States stood out as the principal contributor to this expanding field. Specifically, the University of California System exhibited notable research prowess in this field. Alzheimer disease and Parkinson disease were the diseases most frequently investigated. Key research hotspots include the creation of a molecular brain atlas and identification of vulnerable neuronal subpopulations and potential therapeutic targets at the transcriptomic level.

INTRODUCTION

Neurodegenerative diseases primarily manifest as structurally and functionally progressive degeneration of neurons in the central nervous system.[1] There are many specific types of neurodegenerative diseases, among which Alzheimer disease (AD), Parkinson disease (PD), multiple sclerosis (MS), Huntington disease, and motor neuron disease are common.[2] Neurodegenerative diseases are more common in elderly individuals. In recent years, population aging has become a worldwide problem, and the number of elderly people is increasing, so the number of patients with neurodegenerative diseases is also increasing. Approximately 50 million people worldwide are affected by neurodegenerative diseases.[3] They are difficult to treat because of the complex nature of the nervous system.[4] The cell types that make up the neural circuits within the central nervous system are highly diverse, and the exact extent of their diversity is not well understood and has been the subject of ongoing academic debate.[5] For example, do cortical interneurons comprise a few, dozens, or >100 distinct cell types and which types of cells play a role in neurodegenerative diseases and how much they contribute to neurodegenerative diseases.[6] Because of the cellular diversity mentioned earlier, the interactions between these different types of interconnected cells are also very complex, which greatly increases the difficulty of studying and treating neurodegenerative diseases.[7] Accordingly, to further our understanding of the effects of cell types in neurodegenerative diseases, we should enhance and enrich the knowledge of neurodegenerative diseases at the cellular level. Single-cell RNA sequencing (scRNA-seq) technology can provide us with partial answers in such conditions.

Brain and spinal cord cells are biological organizations with high complexity, and their functions are defined molecularly by complex programs for gene expression.[5] Therefore, the subtle differences among these cells must be fully analyzed to help us understand their specific molecular characteristics, which requires much work to measure thousands of genes. It is precisely because the differences between different cells are so subtle that they cannot be measured by traditional sequencing methods, which can only help us to understand the average diversity of cells but fail to disclose and obtain the specific heterogeneity information.[8] In summary, it is difficult to clarify cell-to-cell changes due to the complexity of neurodegenerative disease with averaged datasets obtained from traditional sequencing methods. In this case, the researchers could not know the specific roles of different cells in neurodegenerative diseases and could not identify the key cells. The inception of scRNA-seq technology, pioneered by Norman Iscove and James Eberwine in the early 1990s, brought forth an innovative tool to solve these problems. Their seminal experiments involved the reverse transcription and exponential amplification of RNA from single cells using polymerase chain reaction (PCR), as well as linear amplification through *in vitro* transcription (IVT).[9,10] These methods allowed for an amplified

[1]Department of Orthopedics, Xuanwu Hospital, Capital Medical University, Beijing 100053, China
[2]National Clinical Research Center for Geriatric Diseases, Xuanwu Hospital, Capital Medical University, Beijing 100053, China
[3]Department of Neurosurgery, Xuanwu Hospital, Capital Medical University, Beijing 100053, China
[4]Department of Neurology, National Clinical Research Center for Geriatric Diseases, Xuanwu Hospital of Capital Medical University, Beijing, China
[5]Lead contact
*Correspondence: cdongwang01@126.com (C.W.), huxinli123@qq.com (X.H.), shibaolu@xwh.ccmu.edu.cn (S.L.)
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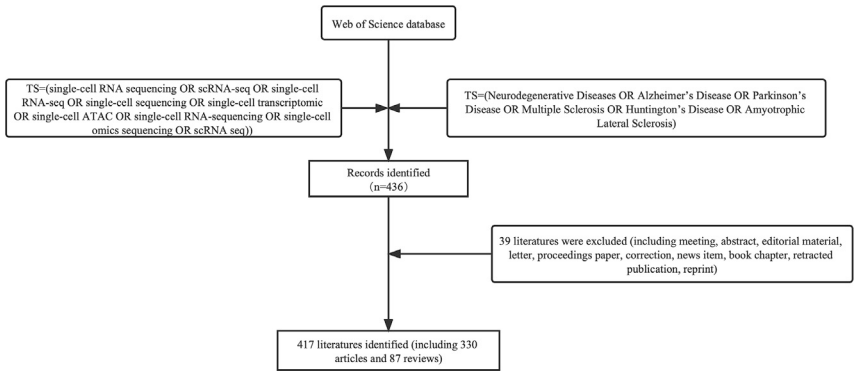

Figure 1. Flow chart of data collection

representation of individual cellular transcriptomes that could be used as templates for PCR or southern blots against genes of interest. Subsequent advancements such as the introduction of microarray technology and next-generation sequencing (NGS) have dramatically transformed the field. These innovations enabled researchers to perform transcriptome-wide analyses, with NGS emerging as the preferred method due to its comprehensive and high-throughput capabilities. In recent years, the field of scRNA-seq has undergone a significant revolution, bolstered by numerous technical modifications to the protocol. These enhancements have made the technology not only easier and faster but also more reliable. Moreover, they have equipped researchers with the ability to profile an ever-increasing number of cells, thereby opening new opportunities for in-depth cellular studies.[11] In 2009, Tang et al. proposed a method to study the mRNA transcriptome using single-cell high-throughput sequencing in a single mammalian cell, which is a new approach and indicates that scRNA-seq technologies will be increasingly widely used in scientific research.[12] Recently, scRNA-seq technologies have been increasingly applied in research on neurodegenerative disorders.[13–15] However, it is still challenging to learn about the current landscape of scRNA-seq in neurodegenerative disorders in a rapid way. After investigation, we found no published works on bibliometric analyses of scRNA-seq in neurodegenerative diseases. Therefore, this work was developed based on CiteSpace and VOSviewer to systematically analyze applied research topics, hotspots, and new views of scRNA-seq in neurodegenerative diseases. The purpose of this work is to provide valuable ways to better understand the research status and future research trends in scRNA-seq in neurodegenerative diseases.

RESULTS

Overview

We obtained 436 papers from the WOS, including the WOSCC, BIOSIS Previews, KCI Korean Journal, MEDLINE, Russian Science Citation Index, and SciELO Citation Index, and finally included 330 original articles and 87 review articles. Figure 1 illustrates how to search the above articles.

The contributions of countries and institutions to global publications

Since 2009, the mRNA transcriptome has been implemented by high-throughput sequencing in a single mammalian cell. The volume of articles on scRNA-seq in neurodegenerative diseases is given in Figure 2A, which demonstrates that it fluctuates slightly overall. The number of studies was the highest in 2021 (n = 127) and the lowest in 2011 (n = 1). One to three publications were published per year before 2012, whereas the increase in the number of publications was obviously noticeable in the last 10 years, especially from 2019 (n = 35) to 2021 (n = 127).

The United States exhibited the highest contribution to research on scRNA-seq in neurodegenerative diseases (n = 234), followed by China (n = 70) and Germany (n = 66), which is shown in Table 1 and Figure 2B. Meanwhile, the average number of citations in the Netherlands was the highest (121.95 times), followed by England (60.89 times), Sweden (59.33 times), Switzerland (59.31 times), Scotland (47.62 times), and Canada (39.46 times).

In Figure 3A, we observed that only three countries, the United States, Canada, and Germany, were studying scRNA-seq in neurodegenerative diseases in 2009. In the following years, few countries joined the groups studying scRNA-seq in neurodegenerative diseases. However, with the spread of the technology, an increasing number of countries have joined the research of scRNA-seq in neurodegenerative diseases. In addition, in terms of international cooperation, the United States cooperates with other countries with the highest frequency of all countries, and China cooperates less, although it contributed the second most publications (Figure 3B). The top 10 prolific organizations were the University of California System (UCS) (58), Harvard University (Harvard) (46), Massachusetts Institute of Technology (MIT) (33), Broad Institute (BI) (29), Harvard Medical School (HMS) (27), University of California San Francisco (UCSF) (20), Helmholtz Association of German Research Centers (HGF) (19), University of London (UL) (19), University of Texas System (UTS) (17), and Karolinska Institute (KI) (20) (Table 2). In addition, we noticed that MIT, BI, and HMS possessed the most citations, which were all from the United States. Therefore, we can infer that the United

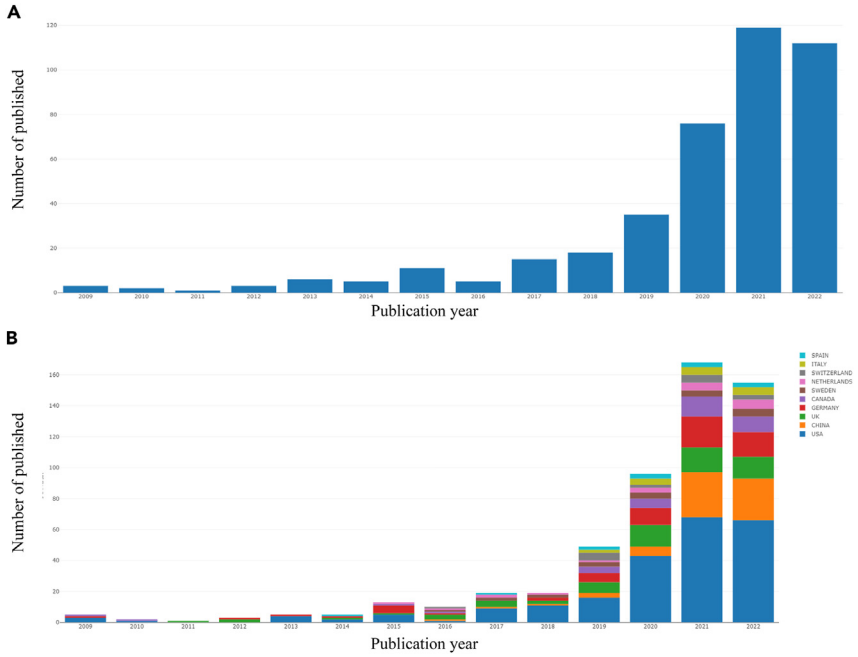

Figure 2. Trends of scRNA-seq in neurodegenerative diseases publications with the years

(A) The number of annual publications of scRNA-seq in neurodegenerative diseases from 2009 to 2022. (B) The growth trends of the top 10 countries in field of scRNA-seq in neurodegenerative diseases from 2009 to 2022.

States still dominates scRNA-seq technology in neurodegenerative diseases. Furthermore, the co-occurrence relations are dispersed in Figure 4. In summary, the roles of most of the above institutions in cooperation were insufficient.

Journal

We listed the top 10 prolific journals with articles focusing on scRNA-seq in neurodegenerative diseases. Based on data analysis, we found that the most prolific journal was *Frontiers In Immunology* and *Nature Communications,* which had 20 and 18 documents, respectively. The impact factors (IFs) of *Nature* and *Frontiers In Cellular Neuroscience* were the highest and lowest, respectively. From the perspectives of the number of articles published and the IF, *Frontiers In Immunology* and *Nature Communications* might exhibit the greatest influencing effects (Table 3).

Authors

The primary authors were determined based on the number of documents, the total citations, and the H-index. There are so many primary authors, and we listed the first 10 in Table 4. Amit, Ido and Chun, Jerold and Prinz, and Marco were ranked in the first 3 articles. Amit and Ido were the first with 6 articles and 1889 citations, whereas Colonna and Marco from Washington University had the most citations (2,048) with 5

Table 1. The top 10 countries/regions contributing to publications about scRNA-seq in neurodegenerative diseases

Rank	Countries	Article counts	Percentage (%)	H-index	Total number of citations	Average number of citations
1	USA	234	56.12	42	9120	38.97
2	PEOPLES R CHINA	70	16.79	12	398	5.69
3	GERMANY	66	15.83	23	2528	38.30
4	ENGLAND	56	13.43	23	3410	60.89
5	CANADA	37	8.87	14	1460	39.46
6	SWEDEN	21	5.04	11	1246	59.33
7	NETHERLANDS	20	4.80	10	2439	121.95
8	ITALY	16	3.84	9	252	15.75
9	SWITZERLAND	16	3.84	9	949	59.31
10	SCOTLAND	13	3.12	8	619	47.62

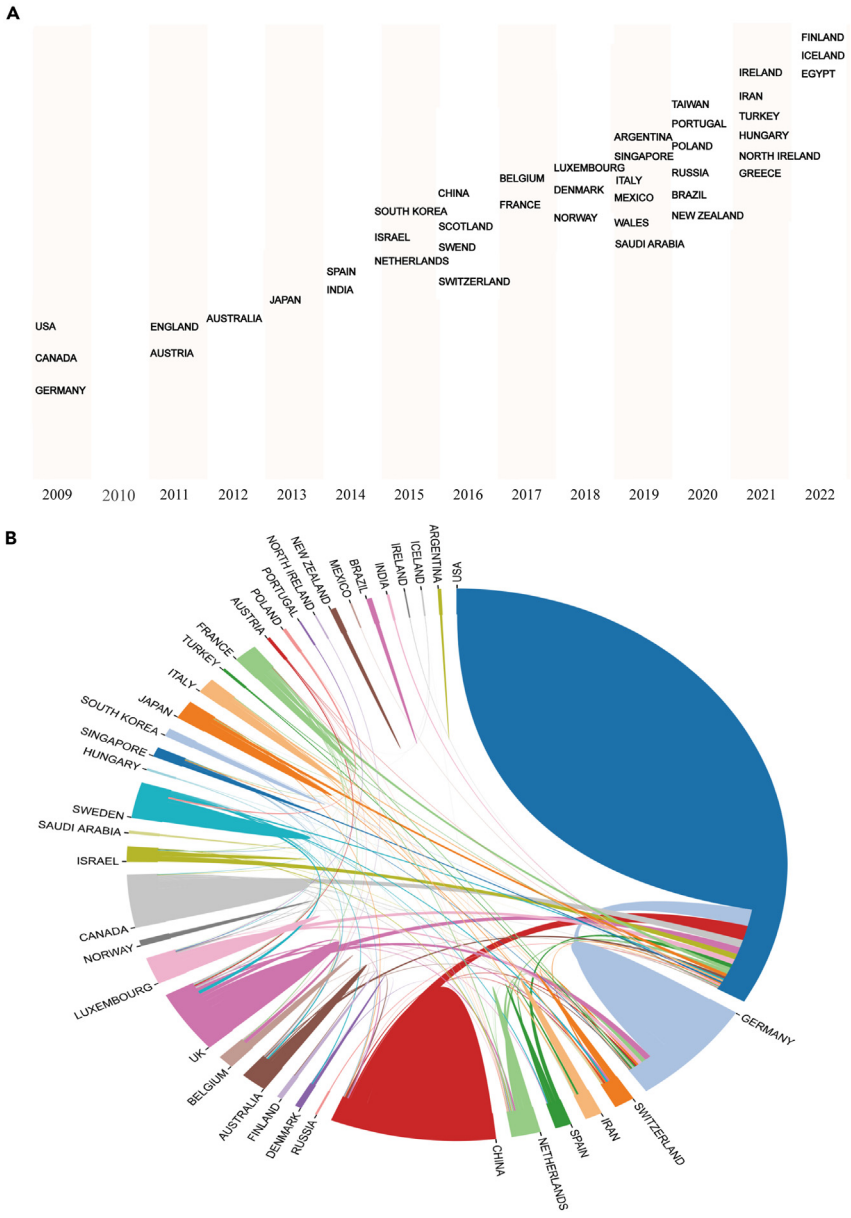

Figure 3. Contributions of various countries to the research of scRNA-seq in neurodegenerative diseases

(A) Countries involved in the application of scRNA-seq in the research of neurodegenerative diseases each year.

(B) Cooperative relations among countries.

articles. Prinz, Marco and Bennett, David A and Colonna, and Marco had five H-index scores. Figure 5 shows the co-authorship relations of authors. The clustering information suggested that they could be grouped into three modules. Wang Y, Wang X, and Zhang B from China were included in Module 1, which enrolls many researchers who are famous in scRNA-seq in neurodegenerative diseases. Module 2 includes Bennett D and Preinz M. In the top 10 productive authors, they occupy the two positions in the number of articles about scRNA-seq in neurodegenerative diseases. Module 3 contains Antel J and Bennett J, who fail to contact frequently with authors in other modules but contribute outstandingly to scRNA-seq in neurodegenerative diseases.

Top 10 citations of included records

We analyzed the citations of the included records to understand how many times an article was cited, and the results are displayed in Table 5, with the first 10 listed. There were 274–1794 citations in total. The article titled "*A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease*" exhibited the highest citation frequency (1,794 times), which was published in 2017 with the primary author of

Table 2. The top 10 institutions contributing to publications about scRNA-seq in neurodegenerative diseases

Rank	Institutions	Article counts	Percentage (%)	H-index	Total number of citations	Average number of citations
1	University of California System	58	13.91	19	1707	29.43
2	Harvard University	46	11.03	19	3182	69.17
3	Massachusetts Institute of Technology Mit	33	7.91	16	3033	91.91
4	Broad Institute	29	6.95	14	2829	97.55
5	Harvard Medical School	27	6.48	12	2361	87.44
6	University of California San Francisco	20	4.80	11	715	35.75
7	Helmholtz Association	19	4.56	10	632	33.26
8	University of London	19	4.56	13	647	34.05
9	University of Texas System	17	4.08	6	184	10.82
10	Karolinska Institutet	16	3.84	11	1233	77.06

Keren-Shaul. It was followed by Hammond TR's "*Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes*" published in 2019. The third one referred to the "*Single-cell transcriptomic analysis of Alzheimer's disease*", and the "*Altered human oligodendrocyte heterogeneity in multiple sclerosis*" ranked last, with 274 citations. In Figure 6, we summarized the references with the strongest citation bursts from the first 1 to 19. The article developed by Zeisel et al. tanked the first with an 8.1 strength index during the period from 2016 to 2019, followed by that of Macosko et al. (strength index: 5.38) and that of Darmanis et al. (strength index: 4.54). The data summarized here demonstrate that the citation burst was extremely obvious in 2017 and continued to 2020.

Keywords

If the keywords of an article appear frequently, it means this article may focus on a current hot topic. Keywords with a high frequency represent hot topics. With the frequency of occurrence as the criterion, we sorted the recurring keywords and then put out the top 30 words with the highest

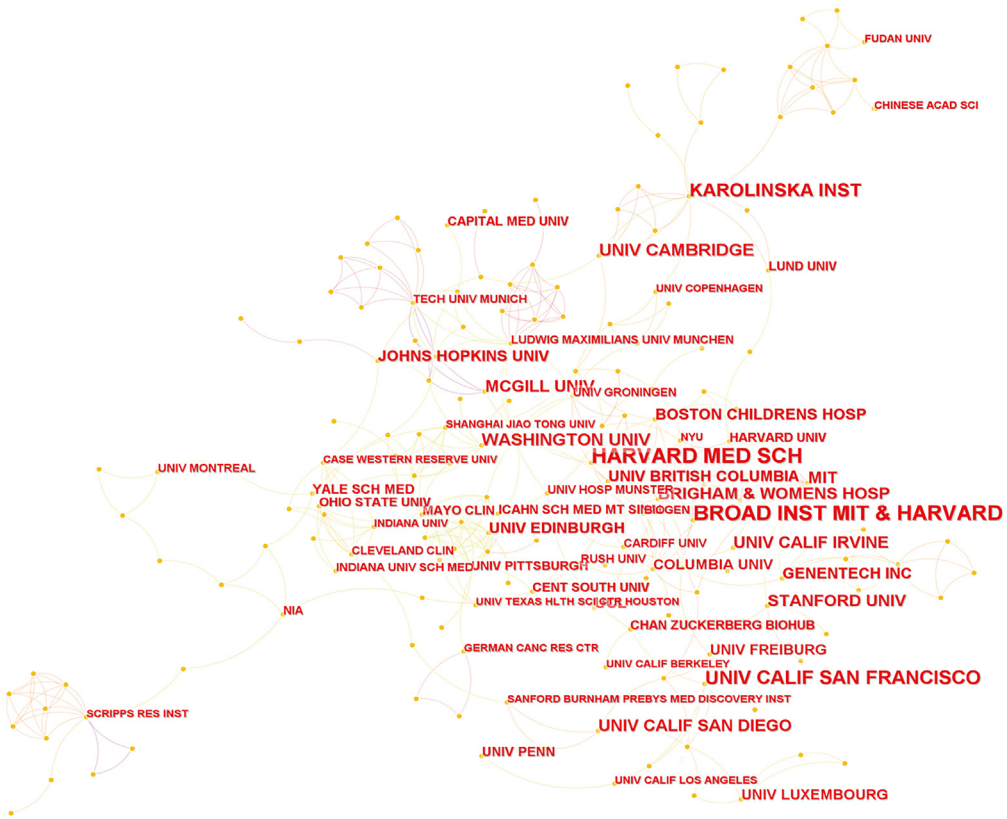

Figure 4. Cooperative relations among institutions

Table 3. The top 10 most active journals that published articles about scRNA-seq in neurodegenerative diseases

Rank	Journal title	Article counts	Percentage (%)	H-index	Total number of citations	Average number of citations	IF	JCR
1	Frontiers In Immunology	20	4.80	8	364	18.20	8.786	Q1
2	Nature Communications	18	4.32	13	765	42.50	17.694	Q1
3	Cell Reports	13	3.12	7	561	43.15	9.995	Q1
4	Cells	10	2.40	4	41	4.10	7.666	Q2
5	Frontiers In Cellular Neuroscience	10	2.40	6	83	8.30	6.147	Q1
6	Nature Neuroscience	10	2.40	7	453	45.30	28.771	Q1
7	Proceedings of The National Academy of Sciences of The United States of America	10	2.40	6	121	12.10	12.779	Q1
8	Journal of Neuroinflammation	8	1.92	3	60	7.50	9.587	Q1
9	Nature	8	1.92	8	1469	183.63	69.504	Q1
10	Acta Neuropathologica Communications	6	1.44	4	108	18.00	7.578	Q1

frequency (Table 6), which was carried out based on some similar keywords combined with the most representative topics in scRNA-seq in neurodegenerative diseases as much as possible. The high frequency of keywords related to scRNA-seq indicates that scRNA-seq is a hot topic at present, including "AD" "PD", "MS", and "aging," which also involves the field of cells, such as microglia, astrocytes, and oligodendrocytes.

According to the changes in the time when keywords appear, we can understand the specific development situation of the field of keywords in different periods, which helps us to estimate the recent development trend and direction. A high-frequency topic keyword figure from 2009 to 2022 is shown in Figure 7. At present, the focus of study gradually shifts from disease study to therapeutic research. It is recommended to pay attention to the latest hot spots, such as growth factor, extracellular vesicle, and gene regulator network. In addition, the number of keywords is increasing, suggesting that the field has strong vitality.

DISCUSSION

This work performed a bibliometric analysis of literature extracted from public databases to discover and identify hot topics and future developments in scRNA-seq for neurodegenerative diseases. After specific analysis, some interesting views are obtained, which are worth our

Table 4. The top 10 most productive authors contributed to publications about scRNA-seq in neurodegenerative diseases

Rank	Author	Institution	Article counts	H-index	Total number of citations	Average number of citations
1	Amit, Ido	Weizmann Inst Sci, Dept Immunol, Rehovot, Israel	6	4	1889	314.83
2	Chun, Jerold	Sanford Burnham Prebys Med Discovery Inst, Translat Neurosci Initiat, La Jolla, CA USA	6	3	66	11.00
3	Prinz, Marco	Univ Freiburg, Inst Neuropathol, Fac Med, Freiburg, Germany	6	5	230	38.33
4	Regev, Aviv	Broad Inst MIT & Harvard, Cambridge, MA USA	6	4	795	132.50
5	Skupin, Alexander	Univ Luxembourg, Luxembourg Ctr Syst Biomed, Esch Belval, Luxembourg	6	3	147	24.50
6	Zhang, Bin	Icahn Sch Med Mt Sinai, Dept Genet & Genom Sci, New York, NY USA	5	3	152	30.40
7	Bennett, David A.	Rush Univ, Rush Alzheimers Dis Ctr, Med Ctr, Chicago, IL USA	5	5	858	171.60
8	Colonna, Marco	Washington Univ, Sch Med, Dept Neurol, St Louis, MO USA	5	5	2048	409.60
9	Friedman, Brad A.	Genentech Inc, Dept Bioinformat & Computat Biol, San Francisco, CA USA	5	3	180	36.00
10	Parmar, Malin	Lund Univ, Lund Stem Cell Ctr, Dept Expt Med Sci, S-22184 Lund, Sweden	5	4	204	40.80

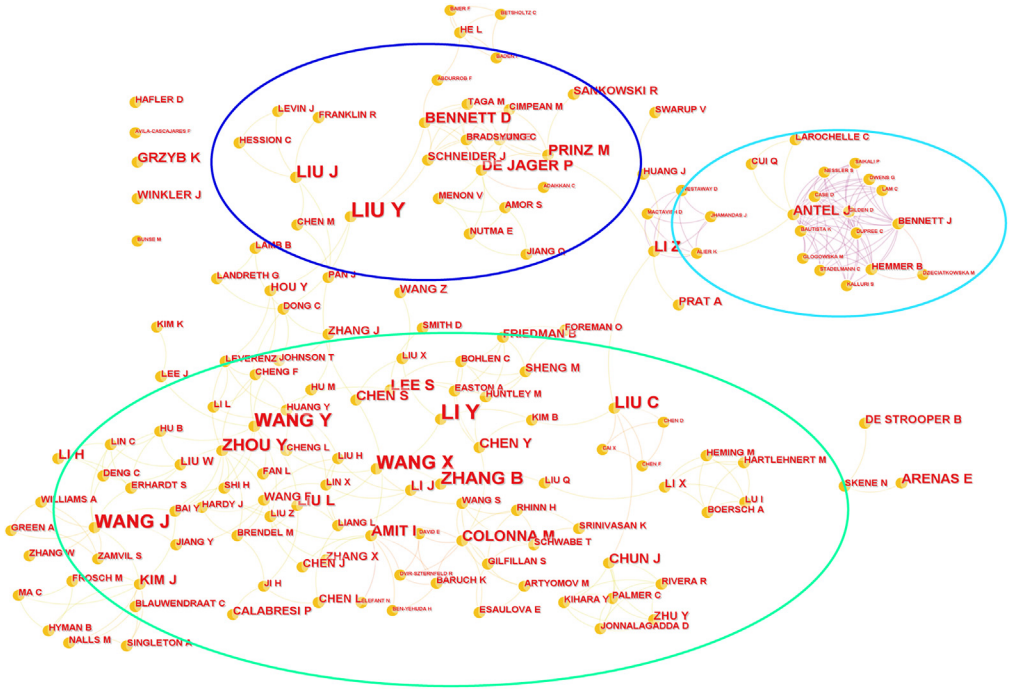

Figure 5. The network map of productive authors

thinking. The number of academic articles published each year can be an important indicator of the trend of scRNA-seq for neurodegenerative diseases. We retrieved 436 articles published during the establishment of WOSCC to the end of October 2022, but fewer were published before 2018. This fact indicates that the application of scRNA-seq in neurodegenerative diseases is still in the preliminary stage of research. The overall increase in the number of studies in 2018 suggests that the use of scRNA-seq in neurodegenerative diseases is becoming more common internationally. Therefore, it can be predicted that the research literature on this field will increase year by year in the following years. Furthermore, we analyzed the sources of authors of these articles published in WOS and found that the United States was dominant in scRNA-seq applied in neurodegenerative diseases. Although most articles are published by authors from the United States, the contributions of Chinese authors are large. The authors' institutions and the countries were matched according to geographical locations. Institutions from the United States (UCS, Harvard, MIT, and BI) have dominated research on scRNA-seq applied in neurodegenerative diseases. From the perspective of authors, the top five authors each published the six articles, and Colonna Marco showed the greatest influence and possessed the most citations. The top 10 most productive authors are basically from the United States, indicating that American researchers have shown strong academic influence and great contribution in scRNA-seq applied in neurodegenerative diseases. By analyzing the network of coauthors, we found that the cooperative relationships among these authors present a dispersive distribution, indicating that most of the cooperated authors were from the same units. This may be one reason why there is no obvious centrality to the most prolific authors. Based on the results and discussion here, we recommend encouraging the authors to cooperate with others from different units or countries. On the other hand, the most involved and cited journals were *Frontiers In Immunology* and *Nature*, respectively. The IF of all the top 10 journals was >5.000, whereas that of *Nature* was 69.504. This shows a preference for high-impact papers.

Future perspectives of scRNA-seq in neurodegenerative diseases

The hotspots of single-cell sequencing in neurodegenerative diseases mainly manifest in (1) continuous optimization of scRNA-seq technology itself; (2) the use of scRNA-seq in studies related to the mechanisms of neurodegenerative diseases; and (3) providing information about possible new therapeutic targets and biomarkers for neurodegenerative diseases through scRNA-seq.

Single-cell sequencing

scRNA-seq is an innovation that began with the pioneering work of Norman Iscove and James Eberwine in the early 1990s. The initial experiments conducted by Iscove and Eberwine centered on reverse transcription and exponential amplification of RNA from single cells using PCR or linear amplification with IVT.[9,10] The amplified cellular transcriptomes were subsequently utilized as templates for PCR or southern blots against specific genes of interest. The emergence of microarray technology and next-generation sequencing (NGS) propelled the field to new heights, allowing for transcriptome-wide analyses. Today, these comprehensive analyses are becoming increasingly common.[16] In recent years, the field has witnessed an exciting revolution thanks to numerous technical modifications in scRNA-seq. These advancements have

Table 5. The top 10 high-cited papers about scRNA-seq in neurodegenerative diseases

Rank	Article title	Journal	Authors	Publication year	Total citation	Average annual frequency of citations	IF	JCR
1	A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease	Cell	Keren-Shaul, H; Spinrad, A; (…); Amit, I	2017	1794	299.00	66.850	Q1
2	Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes	Immunity	Hammond, TR; Dufort, C; (…); Stevens, B	2019	679	169.75	43.474	Q1
3	Single-cell transcriptomic analysis of Alzheimer's disease	Nature	Mathys, H; Davila-Velderrain, J; (…); Tsai, LH	2019	675	168.75	69.504	Q1
4	The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease	Nature Reviews Genetics	Stewart, JB and Chinnery, PF	2015	442	55.25	59.581	Q1
5	Intrathecal Pathogenic Anti-Aquaporin-4 Antibodies in Early Neuromyelitis Optica	Annals Of Neurology	Bennett, JL; Lam, C; (…); Hemmer, B	2009	400	28.57	11.274	Q1
6	Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells	Cell	La Manno, G; Gyllborg, D; (…); Linnarsson, S	2016	384	54.86	66.850	Q1
7	Single-Cell Genomics Unveils Critical Regulators of Th17 Cell Pathogenicity	Cell	Gaublomme, JT; Yosef, N; (…); Regev, A	2015	345	43.13	66.850	Q1
8	Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution	Cell Reports	Mathys, H; Adaikkan, C; (…); Tsai, LH	2017	308	51.33	9.995	Q1
9	The genetic architecture of Parkinson's disease	Lancet Neurology	Blauwendraat, C; Nalls, MA and Singleton, AB	2020	289	96.33	59.935	Q1
10	Altered human oligodendrocyte heterogeneity in multiple sclerosis	Nature	Jakel, S; Agirre, E; (…); Castelo-Branco, G	2019	274	68.50	69.504	Q1

made the technology faster, more reliable, and capable of profiling an ever-increasing number of cells. These improvements have greatly expanded the potential of scRNA-seq, opening new avenues for exploring the complexity of the cellular landscape. Because of this, scRNA-seq has become increasingly popular. RNA transcriptome sequencing was first applied in mammalian cells in 2009 by Tang et al.[12] Since then, there has been a significant increase in the number of publications in this area during 2010–2019, especially after 2014. To date, the application and development time of scRNA-seq technology in mammals has lasted for nearly 10 years, and some achievements have been made, so there are some landmark research studies. First, after the study of Tang et al. first applied RNA transcriptome sequencing to mammalian cells in 2009, Navin et al. published an article focusing on the first genomic sequencing of single human cells.[17] Subsequently, several relevant articles were published successively in the following 3 years; the most representative of the article is "*Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor*" by Xu et al.[18] and "*Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain*" by Evrony et al.[19] In addition, the analysis revealed an almost exponential increase in the number of publications in recent years, mainly due to the ability of scRNA-seq to support neurological research.[20,21]

Over the last decade, scRNA-seq has made impressive strides, greatly enhancing our understanding of cellular interplay in health and disease. However, the maturity of scRNA-seq methods does not signal the end of improvements. Indeed, there remain challenge to address and promising applications to explore.[22] One such challenge lies in the process of capturing individual cells, which tends to compromise cellular integrity and functionality. This process results in an immediate loss of spatial resolution and fails to depict spatial relationships among cells within the original tissue context. Another hurdle comes with the generation of high-dimensional data by sequencing, often accompanied by significant noise.[23] Therefore, devising strategies to mitigate this noise is indispensable to enhance the accuracy of high-throughput scRNA-Seq data analysis. Besides these technical hurdles, the financial burden of scRNA-Seq analysis, especially when compared with alternative analytical techniques, poses a significant barrier to its wider adoption as a standard tool.[24] Currently, the primary application of scRNA-Seq focuses on the gene level,

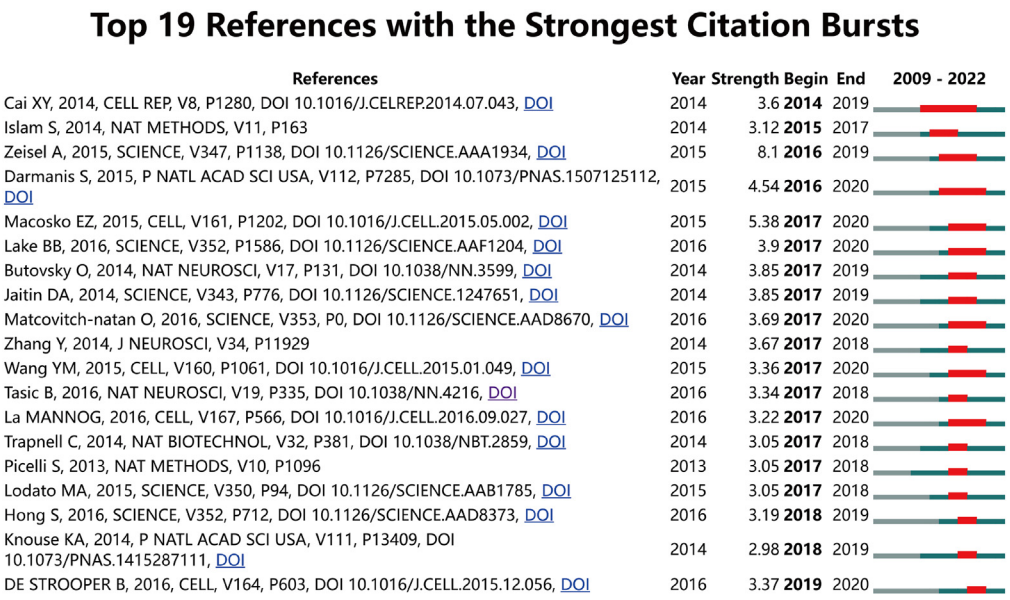

Figure 6. The top 19 references with the strongest citation bursts were presented

facilitating the exploration of specific regulatory gene activity mechanisms. Yet, there is an urgent need for more in-depth systematic studies in areas such as disease diagnostics, personalized clinical treatment, reproductive development, and drug action mechanisms. These studies demand the integration of innovative technologies at the metabolic and protein expression levels, such as spatial transcriptomics, single-cell proteomics, spatial metabolomics, and mass spectrometry flow technology.[25] The combination of these technologies provides a comprehensive and accurate representation of life science phenomena and drug action mechanisms. Spatial transcriptomics allows for the capturing of individual cells' original spatial positions. Combined with scRNA-Seq, it localizes cells with unique transcriptional signatures, creating high-resolution maps of cell subpopulations.[26] Similarly, single-cell proteomics permits both quantitative and qualitative protein analysis among individual cells, assisting in constructing protein molecular maps.[27] Spatial metabolomics enables the precise identification and characterization of functional metabolites in tissues. Coupled with scRNA-Seq, it supports high-resolution localization of diverse cell types and states within tissues.[28] Mass spectrometry flow technology facilitates large-scale cell counting of specific proteins, enabling detailed cell sorting and precise analysis of cellular pathways and cell-cycle stages.[25] The progress in scRNA-Seq and its integration with other multi-omics technologies play a crucial role in elucidating individual cell growth and differentiation, gene regulatory interplay, and cellular heterogeneity within complex tissues. This knowledge can further illuminate disease pathogenesis and assist in the development of novel therapeutic agents.

Neurodegenerative disease

As a well-known neurodegenerative disease, PD is prevalent in people aged >60 years, and more than 1% of them suffer from this disease.[29] scRNA-seq has been used in the field of neuroscience for several years, but its application to study the pathogenesis of PD is still in its infancy, especially in research on postmortem brain tissue.[30] In recent studies, by establishing animal and human models of PD *in vitro*, relevant experimental studies have helped us to understand the types, characteristics, and diverse expression of glial cells in brain neurons of animals and humans, on which we have determined the roles of these cells in the development of PD. These scRNA-seq data were supported by experiments and can be used as the basis for the development of targeted functional research tools. Taking it as a starting point, PD-specific transcriptome characteristics can be well correlated with the spatial and physiological environment in which it lives. In a study, cell samples were collected from six neocortical regions, and a scRNA-seq assay found 16 neuronal subtypes and demonstrated the distinct spatial organization of different interneuron cell populations and excitatory neurons.[20] Smajić et al. assessed the cell-type-specific risk for PD based on sc-RNA and discovered a neuronal cell cluster characterized by CADPS2 gene overexpression and low tyrosine hydroxyl levels. This cluster was formed by dysfunctional dopaminergic neurons, which were exclusively present in idiopathic PD midbrains.[31] Through the application of high-resolution, single-cell transcriptomic analysis, Lang and colleagues studied iPSC-derived dopamine neurons that carry the PD risk variant GBA-N370S.[32] They discovered a progressive shift in gene expression, which culminated in endoplasmic reticulum stress. Using pseudotime analysis of the genes exhibiting differential expression along this trajectory, they pinpointed histone deacetylase 4 (HDAC4), a transcriptional repressor, as an upstream regulator modulating disease progression. In the PD iPSC-derived dopamine neurons, HDAC4 was found to be incorrectly localized to the nucleus, which resulted in early repression of genes along the disease trajectory, subsequently causing deficits in protein homeostasis. Administering HDAC4-modulating compounds to iPSC-derived dopamine neurons resulted in the upregulation of genes earlier in the differential expression trajectory, rectifying PD-associated cellular phenotypes. Their research underscores how single-cell transcriptomics can be harnessed to delve into cellular heterogeneity, unmasking disease processes and pinpointing potential therapeutic targets.[32] In conclusion, it is believed that the continuous

Table 6. The top 30 high-frequency keywords about scRNA-seq in neurodegenerative diseases

Key words	Frequency
Single-cell RNA sequencing	122
Alzheimer disease	78
Microglia	50
Parkinson disease	30
Multiple sclerosis	30
Neuroinflammation	21
Neurodegeneration	38
Aging	17
Transcriptomics	13
Heterogeneity	9
Astrocyte	9
Biomarker	9
S disease	8
B cell	8
Inflammation	6
Mosaicism	6
Aneuploidy	5
Oligodendrocyte	5
Epigenetics	5
Somatic mutation	5
GWAS	5
Bioinformatics	4
Brain	4
Neurons	4
TREM2	4
Dopaminergic neurons	4
Hippocampus	4
Proteomics	4
Huntington disease	4
GM-CSF	3

improvement and application of experiments and analytical tools using scRNA-seq technology can greatly improve the development of some new capabilities, including the development of gene-based biomarkers, and further make corresponding contributions to the diagnosis, prognosis, and targeted therapy of PD.

AD is a common form of dementia and can be extremely devastating. Data show that more than 24 million people worldwide have been found to have AD. They can study the changes and regulation of cell type specificity at the single-cell level to understand the molecular mechanisms that contribute to the pathological and physiological processes of dementia. In recent years, there have been several studies using scRNA-seq to study the brains of older adults and AD, and these studies have identified several brain cell groups associated with AD.[13,33] Mathys and colleagues recently published a paper describing the application of scRNA-seq to study transcriptional changes in brain cells in elderly patients with AD.[13] By analyzing the differences in gene expression in different cell types, they found that the homeostatic signature of excitatory and inhibitory neurons was severely inhibited, making them the most affected cells. In the early stage of AD, the molecular changes in neurons are small, but with the deterioration of the disease, there will be a large pathological change. They discovered that excitatory neurons and oligodendrocytes in AD exhibited upregulated myelination-related genes such as LINGO1. Furthermore, activation of microglia and astrocytes in AD was suggested by the upregulation of genes such as CD81 and GFAP, respectively.[13] In addition, Grubman et al. characterized the transcriptional changes and cellular heterogeneity in the entorhinal cortex in the brains of patients with AD using scRNA-seq. They identified that the risk gene APOE of AD is specifically repressed in corresponding oligodendrocyte progenitor cells and astrocyte subpopulations; meanwhile, it is upregulated in an AD-specific microglial subpopulation.[34] This investigation disclosed an elevation in stress response gene expressions such as mitochondrial genes, heat shock genes, and molecular chaperone genes across diverse cell types. In AD samples, neuronal genes such as GABA receptor (GABRA2, GABRB1), glutamate receptor (GRIA2, GRID2), and neurexin (NRXN1, NRXN3) demonstrated a pronounced downregulation. This observation is consistent with the

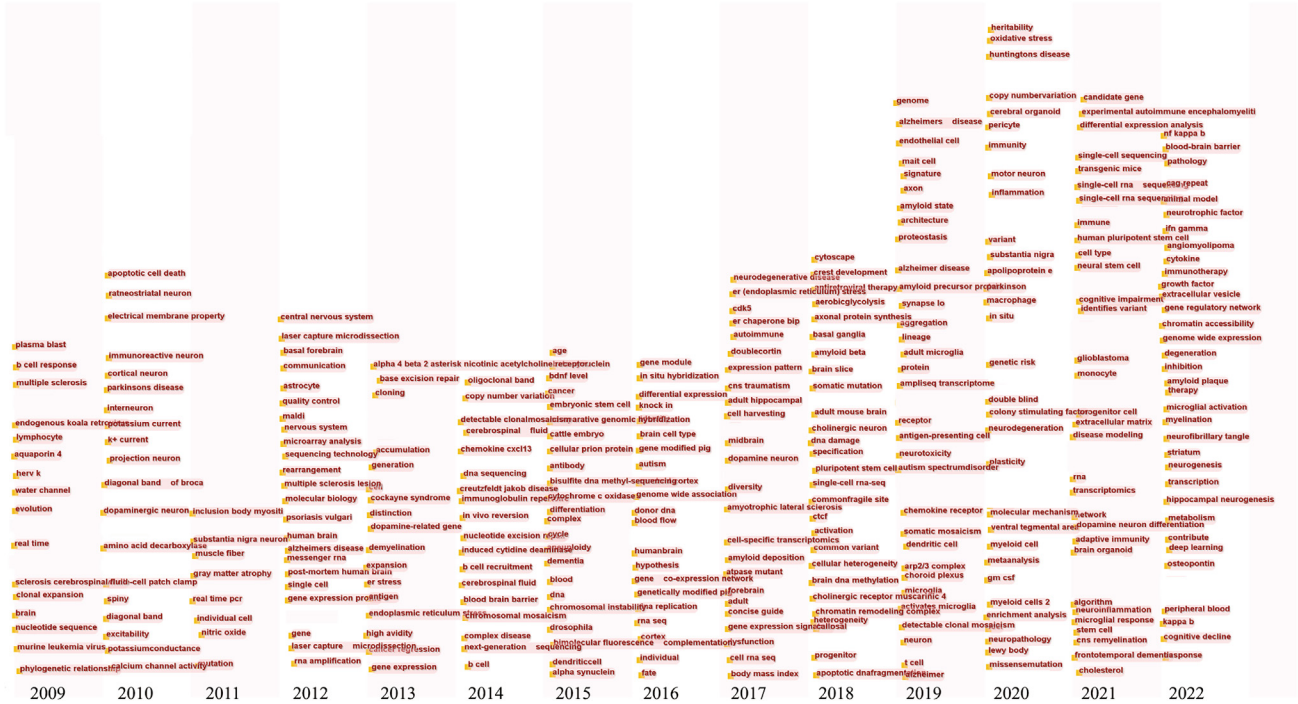

Figure 7. A high-frequency topic keywords figure from 2009 to 2022

synaptic loss characteristic of AD. Furthermore, genes associated with glial cell evolution and differentiation, including BIN1 and CNTN2, displayed upregulation in astrocytes and oligodendrocytes, which might represent a compensatory mechanism to myelin loss. AD's endothelial cells also showed heightened gene expressions related to cytokine secretion and immune responses, encompassing HLA-E, MEF2C, and NFKBIA.[34] Utilizing these data, researchers developed gene regulatory networks to explore the interplay between transcription factors and downstream target genes pertinent to AD. A significant observation was the escalated expression of the TFEB gene in AD astrocytes relative to control astrocytes. TFEB, a transcription factor, acts as a master regulator of lysosomal function and operates upstream of numerous AD-associated genes, as identified in genome-wide association studies (GWASs). These genes, including POLN, STK32B, EDIL3, AKAP12, HECW1, WDR5, LEMD2, BIN1, and CLDN11, also exhibited increased expression in AD astrocytes.[34] Lastly, an independent study delved into the impact of different TREM2 gene variants on single-nucleus gene expression in postmortem brains of AD patients. TREM2, a microglial receptor, bears mutations that impair the microglia's capacity to encircle AD-associated plaques, thereby intensifying neuronal dysfunction. The R62H and R47H TREM2 variants, relative to the common TREM2 variant, were associated with an escalated risk of AD.[35] Therefore, scRNA-seq can be applied further to assess the effects and relative contributions of underlying genetic factors to the described cellular and transcriptomic changes in AD.

MS is an inflammatory demyelinating disease that occurs in the CNS. Two million people in the global population have some degree of MS.[36] Single-cell sequencing is also increasingly being used in multiple sclerosis. Masuda and colleagues used scRNA-seq to describe the microglia of MS patients in detail from the molecular perspective. Enriching the microglia of healthy people can indicate the homeostatic phenotype. On the other hand, in the microglial cell analysis results of MS patients, they found that this homeostatic phenotype was divided into several phenotypes with decreased expression.[37] In addition, a specific study employed scRNA-seq to interrogate the white matter derived from postmortem samples of MS patients.[38] The investigative focus was directed toward oligodendrocytes, which revealed seven transcriptionally distinct oligodendrocyte subtypes. The research findings indicated a decreased prevalence of oligodendrocyte progenitor cells (OPCs) within MS samples, accompanied by the diminished presence of certain oligodendrocyte subtypes, alongside an enriched manifestation of others. Furthermore, a noticeable enhancement of myelin-associated gene expression, encompassing MBP, CNP, and MAG, was observed in oligodendrocytes from MS samples in comparison to control oligodendrocytes.[38] In summary, although a handful of scRNA-Seq studies have provided some insights into MS, additional investigations are required to enhance our comprehension of the disease's etiology at the distinct cellular level.

New therapeutic targets and biomarkers

The application of single-cell RNA sequencing (scRNA-seq) technology has significantly expanded our understanding of the regenerative potential of the brain. This innovative approach was notably used in a study that investigated the subventricular zone of adult mice. The research team managed to isolate 130 cells and successfully characterized a pool of dormant neural stem cells (NSCs) within this region.[39] These NSCs were found to express unique combinations of lineage-specific genes, shedding new light on the intricate workings of brain regeneration. In a further pioneering step, the study delved into the behavior of these NSCs in the face of brain ischemia.[39] It was observed

that the dormant stem cells transitioned to an activated state following injury. This transition was significantly influenced by interferon gamma (IFN) signaling, a vital component of the immune response to injury.[39] This was the first research effort to compare NSCs *in vivo* in a pathological context, opening new avenues for potential therapeutic interventions aimed at brain regeneration.

The exploration of CNS disease mechanisms has taken an alternative route, focusing on the use of peripheral cells as stand-ins for pathology.[40] This innovative approach has been employed by numerous research groups who have delved into the transcriptional changes observable in patient-derived blood cells. The goal of these investigations is to uncover distinctive signatures of neurological diseases and identify potential disease mechanisms, a task made possible through the advanced capabilities of scRNA-seq.[40,41] This line of research is particularly significant, as the concept of immune dysfunction playing a critical role in driving disease pathology is increasingly acknowledged within the neurodegeneration field.[42,43] Hence, the transcriptional landscape of peripheral cells, such as monocytes, could provide not only valuable disease biomarkers but also valuable insights into the mechanistic pathways involved in these diseases. One of the key advantages of using patient-derived blood cells in such research is their accessibility. Unlike many investigations into human neurodegenerative diseases, these studies do not require postmortem analysis. This critical limitation has often hindered the progress of research in the field. Moreover, these cells are amenable to scRNA-seq analysis, further broadening their utility.[44,45] The concept of using a subtype of peripheral blood cells characterized through scRNA-seq as a "window" into the brain is a particularly intriguing one. Coupling scRNA-seq analysis performed on peripheral blood cells with RNA-seq data obtained directly from the CNS, such as postmortem tissue from patients, could offer valuable insights. This combined approach could potentially elucidate critical mechanisms contributing to human neurodegeneration, thereby opening new avenues for therapeutic intervention.

Conclusion

The increased number of publications on scRNA-seq in neurodegenerative diseases makes it highly significant to review the history, understand the major research achievements, and identify research hotspots and future directions in this field. Until now, there has been no bibliometric analysis of this topic. We aimed to fill this gap by conducting the first bibliometric analysis, drawing insights from the most influential papers, current hotspots, and potential research directions related to scRNA-seq in neurodegenerative diseases. Our study uncovered a rapidly growing interest in this area, demonstrated by the dramatic rise in related publications from 2009 to 2022. The majority of these publications hail from the United States, with the University of California System and Harvard University standing out as the most active institutions. The diseases frequently studied in this context include Alzheimer disease, Parkinson disease, and multiple sclerosis. scRNA-seq has been at the forefront of neurodegenerative diseases research, serving three crucial objectives. Firstly, it aids in creating a comprehensive molecular brain atlas, expanding our understanding of the brain's structure, and informing us about connectivity patterns. This knowledge enables the design of innovative tools for targeted precision interventions in specific brain subpopulations and lineages. Secondly, scRNA-seq offers detailed molecular profiling to identify vulnerable neuronal subpopulations and potential therapeutic targets. This has led to an exciting exploration of novel drug targets specific to particular cells of the CNS. Lastly, scRNA-seq is critical in defining cellular targets at the transcriptomic level for pharmacological manipulations. In summary, our study provides a systematic visualization of the research literature on scRNA-seq in neurodegenerative diseases and provides guidance and reference for understanding the current research status of scRNA-seq in neurodegenerative diseases and discovering new research directions.

Limitations of the study

Of course, this work is subject to several shortcomings. First, the papers used in this study was limited to English and selected from WOSCC, but only those that can be found in WOS were selected as analysis data, even though our research process has strict procedures and good structure. In addition, we only analyzed articles and reviews. There may exist a selection bias. Second, we searched the publications before October 31, 2022, but data in the WOSCC update every day, so some newly published articles were omitted. Therefore, the currently collected data failed to completely reflect the condition in 2022 in addition to providing a reference. Third, the bibliometric analysis made in this work pays more attention to the quantitative analysis of the characteristics of the literature and ignores the detailed background investigation and analysis to a certain extent. Therefore, the specific results of bibliometric analysis are too universal and not detailed. Fourth, most articles included belonged to experimental research to uncover the direction of the original research, so the information collected was restricted.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

W.W. conceived the study and wrote the article. T.L. helped perform the data and statistical analysis. Z.W., Y.Y., and S.Z. drew the graphical abstract and tables. C.W., X.H., and S.L. revised this manuscript critically for important intellectual content. All authors have read and agreed to the published version of the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Peer-reviewed academic publications	Web of Science	https://www.webofscience.com/wos/woscc/basic-search
Software and algorithms		
VOSviewer version 1.6.18	Center for Science and Technology Studies, Leiden University	https://www.vosviewer.com/
Citespace (5.3. R4)	College of Computing and Information Science and Technology, Drexel University	https://citespace.podia.com

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Xinli Hu (huxinli123@qq.com).

Material availability

This study did not generate new materials.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report the original code.
- Any additional information required to re-analyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Our study does not use typical experimental models in the life sciences.

METHOD DETAILS

Bibliometric analysis fundamentally involves two crucial stages: the assembly of an exhaustive database of relevant articles, and the ensuing utilization of established software tools for meticulous examination. This document offers an in-depth exploration of these procedural steps.

Database creation

We conducted a bibliometric analysis based on the science citation index-expanded database. We implemented the following strategy in the WOS: TS = ("single-cell RNA sequencing" OR "single-cell RNA-seq" OR "scRNA-seq" OR "single-cell sequencing" OR "single-cell transcriptomic" OR "single-cell ATAC" OR "single-cell omics sequencing" OR "single-cell RNA-sequencing" AND TS = (Neurodegenerative Diseases OR Parkinson's Disease OR Alzheimer's Disease OR Huntington's Disease OR Multiple Sclerosis OR Amyotrophic Lateral Sclerosis). The publication dates were January 1, 2009-October 31, 2022.

Inclusion and exclusion criteria

First, we searched all mentioned records regardless of their type, including articles, reviews, comments, letters, and brief introductions on scRNA-seq techniques in neurodegenerative diseases. We focused on scRNA-seq technology applied in neurodegenerative diseases after single-cell RNA sequencing was first applied in mammalian cells in 2009.[12] We excluded comments, letters, conference abstracts, and those not in English, by which the consistency and accuracy of the collected information can be ensured. We set two teams composed of several reviewers to screen the publications independently and select those many to be applicable based on their titles and abstracts. If necessary, the reviewers can read the full texts to further check the applicability of the screened publications. Different views from two teams can be subjected to discussion.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analysis using citespace and VOSviewer

We used citespace (5.3. R4) to analyze the development trends, future dynamics, and hotspots in the relevant scientific literature on a given topic. Citespace, a citation analysis software grounded in scientometrics, uses data and information visualization to represent the distribution,

patterns, and relationships of scientific knowledge.[46] With the capacity to build visual networks and calculate intermediary centrality, it offers a unique perspective on emerging trends and research frontiers by performing burst detection and keyword tagging.[47] In this study, we employed CiteSpace for analyzing co-cited authors and documents, extending its use to pinpoint burst citations and keywords. Burst detection is adopted to identify the keywords and references that have a sudden and obvious change in appearance frequency within a certain period and become popular.

VOSviewer is a distance-dependent tool, based on which bibliometric networks can be visualized.[48] It assigns many nodes, which are tightly associated with each other, into several clusters, and the nodes with tighter correlations are indicated by the more similar color.[49] In addition, it can be combined with the overlay visualization map, which can show the distribution of these nodes in 2D spaces by using their colors and distances.[50] In this work, we adopted VOSviewer to investigate the collaboration networks of the authors and their units and the co-occurrence network reflecting the correlations among the major works or studies of these authors.