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Transcriptome Study of rd1Mouse Brain and Association with Parkinson's Disease

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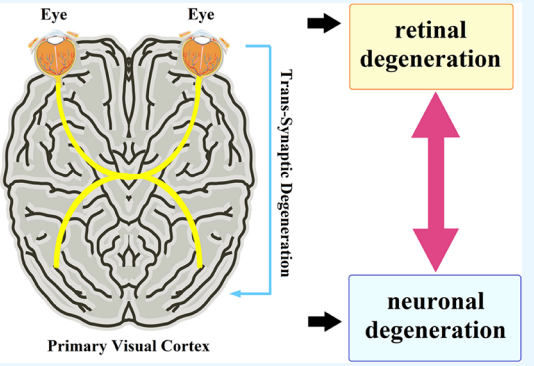
ABSTRACT: Degeneration of the retina is intrinsically associated with the pathogenesis and progression of neurodegenerative diseases. However, the cellular and molecular mechanisms underlying the association between neurodegeneration and retinal degeneration are still under exploration due to the complexity of the connectivity network of the nervous system. In this study, RNA-seq data from the brains of model retinitis pigmentosa (RP) mice and previously studied Parkinson's disease (PD) mice were analyzed to explore the commonalities between retinal degenerative and neurodegenerative diseases. Differentially expressed genes in RP were compared with neurodegenerative disease-related genes and intersecting genes were identified, including Cnr1 and Septin14. These genes were verified by quantitative real-time reverse transcription PCR and Western blotting experiments. The key proteins CNR1 and SEPTIN14 were found to be potential cotherapeutic targets for retinal degeneration and neurodegenerative disease. In conclusion, understanding the commonalities between retinal degenerative diseases and neurodegenerative processes in the brain will not only facilitate the interpretation of the underlying pathomechanisms but also contribute to early diagnosis and the development of new therapeutic strategies.



1. INTRODUCTION

Neurodegenerative diseases are a heterogeneous group of diseases characterized by progressive structural and functional degeneration due to degeneration and apoptosis of neurons in the central nervous system (CNS) or the myelin sheaths of neurons in the peripheral nervous system.[1] Neurodegenerative diseases share many common features, including the progressive degeneration of neuronal populations and the formation of abnormal protein aggregates. Although these common features increase the conservatism of pathogenic mechanisms, the clinical and pathological features of neurodegenerative diseases demonstrate significant differences. Neurodegenerative diseases have become one of the most harmful groups of disorders to human health worldwide due to the continual aging of the population. The pathogenesis of neurodegenerative diseases is extremely complex and not completely understood. The lack of understanding of the molecular mechanisms underlying neurodegenerative diseases has led to the absence of effective diagnostic and therapeutic approaches to date. Many studies have demonstrated associations between neurodegenerative diseases and both the gut[2,3] and the retina.[4,5] In particular, retinal changes may be excellent alternative biomarkers for neurodegenerative diseases such as Parkinson's disease (PD) and can be used to evaluate new therapies, both experimental and clinical.

PD is one of the most common neurodegenerative diseases.[6] Numerous studies have confirmed the presence of symptoms of visual dysfunction in patients with PD.[7−9] Moreover, visual dysfunction has been reported as a potential biomarker with high specificity in the early stage of neurodegenerative diseases.[10,11] In recent years, retinal degeneration in patients with PD has received widespread research attention and has become one of the research hotspots in the field of neurodegenerative diseases.[12] It has been confirmed that retinal degeneration is present in the early stage of PD.[13] Further, retinal degeneration is strongly associated with the course and severity of PD. Retinal degeneration is an important cause of nonmotor symptomatic visual dysfunction in PD.[14] It has been demonstrated that neurodegenerative lesions can propagate bidirectionally along neuronal projections in the visual pathway.[15] These studies have revealed a close interrelationship between retinal and brain degenerative diseases. However, the molecular mechanisms underlying the association between retinal and brain degenerative diseases are still unclear. A comprehensive understanding of the connectivity between retinal and brain degenerative diseases is important for the

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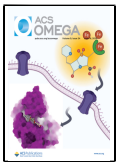

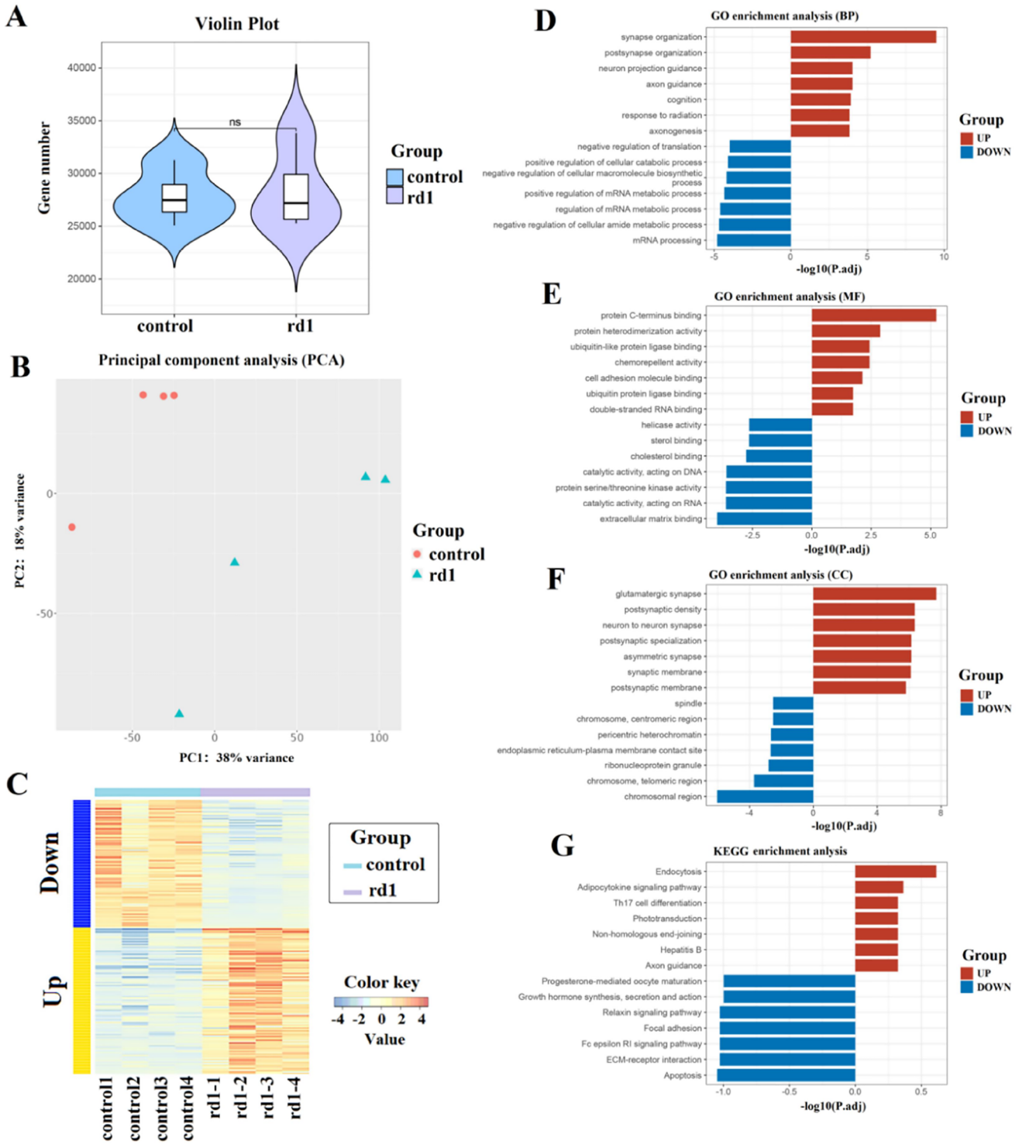

Figure 1. (A) Mean value of the number of genes obtained from the libraries of the normal control and rd1 mouse groups was around 20,000. Moreover, the difference between the rd1 and normal groups was not statistically significant ($P < 0.05$). (B) rd1 Group could be distinguished well from the normal group by PCA. (C) There were significant DEGs between the rd1 and normal control groups with Log_2|Fold change|>1 and P-value < 0.05. (D) GO pathway of the biological process involving upregulated and downregulated DEGs. (E) GO pathway of molecular function involves upregulated and downregulated DEGs. (F) GO pathway of cellular composition (CC) involves upregulated and downregulated DEGs. (G) KEGG pathway involving upregulated and downregulated DEGs.

early diagnosis of both diseases and the development of new therapeutic strategies.

There is an urgent need for basic biological studies that comprehensively explore the connection between retinal and brain degenerative diseases. The cellular and molecular mechanisms underlying the association between neurodegeneration and retinal degeneration are still under exploration due to the complexity of the connectivity network of the nervous system.[16] In this study, RNA-seq data from the brains of model RP mice and previously studied PD mice were analyzed to

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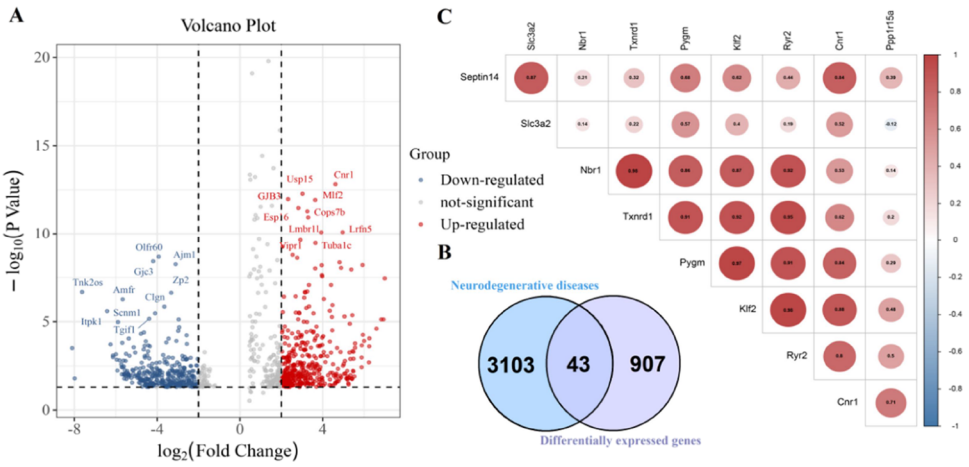

Figure 2. (A) Volcano plots of the DEGs between the rd1 and normal control groups. (B) There are 43 genes with intersection between the DEGs of rd1 and the neurodegenerative diseases. A detailed list of intersecting genes can be found in Table S1. (C) Heatmaps of the expression of these intersecting genes showed a positive correlation between their expression.

explore the commonalities between retinal degenerative and neurodegenerative diseases.[17] Intersecting genes were identified between differentially expressed genes (DEGs) in RP and PD and neurodegenerative disease-related genes, including Cnr1 and Septin14. The results of this research will not only improve our understanding of the pathomechanisms underlying RP and PD but also contribute to the early diagnosis of these diseases and the development of new therapeutic strategies.

2. RESULTS AND DISCUSSION

2.1. Transcriptome Study of rd1 Mouse Brain Identifies Neurodegenerative Disease-Associated Genes. The rd1 mouse was the first identified model mouse for retinitis pigmentosa (RP), a degenerative disease of the human retina.[18] RP is one of the most common causes of vision loss due to progressive degeneration of retinal neurons.[19,20] To date, most of the research on RP has focused on the retina. There are no detailed studies of the brain in RP. Retinal neurodegenerative diseases are caused by irreversible damage to retinal neuronal cells. There is no effective treatment for these diseases. Patients with retinal neurodegenerative diseases not only suffer from visual impairment but also exhibit degenerative brain lesions. Due to the limitations of human studies, it is necessary to use reliable animal models to elucidate the molecular mechanisms of brain lesions in retinal degenerative diseases in order to further understand the pathogenesis of these diseases and identify effective therapeutic options.

Four libraries were constructed for the normal control and rd1 mouse groups, respectively. The mean number of genes obtained from the libraries of both groups was around 20,000. There was no statistically significant difference between the rd1 group and the normal group ($P < 0.05$) (Figures 1A and S1). The rd1 group could be distinguished from the normal group by principal component analysis (PCA) (Figure 1B). Significant DEGs were observed between the rd1 and normal control groups ($Log_2|Fold\ change|>1$, $P < 0.05$). The upregulated and downregulated DEGs in the rd1 group relative to the normal control group were analyzed separately (Figure 1C). The RNA-seq results of the brain tissue samples from the rd1 group and the normal control group revealed that the DEGs were involved in neurodegenerative disease-related pathways.

The GO pathway of biological process (BP) involving upregulated DEGs is mainly synapse organization, postsynapse organization, neuron projection guidance, axon guidance, cognition, response to radiation, and axonogenesis (Figure 1D). The GO pathway of BP involving downregulated DEGs is mainly negative regulation of translation, positive regulation of cellular catabolic process, negative regulation of cellular macromolecule biosynthetic process, positive regulation of mRNA metabolic process, regulation of mRNA metabolic process, negative regulation of cellular amide metabolic process, and mRNA processing. The GO pathway of molecular function (MF) involving upregulated DEGs is mainly protein C-terminus binding, protein heterodimerization activity, ubiquitin-like protein ligase binding, chemorepellent activity, cell adhesion molecule binding, ubiquitin protein ligase binding, and double-stranded RNA binding (Figure 1E). The GO pathway of MF involving downregulated DEGs is mainly helicase activity, sterol binding, cholesterol binding, catalytic activity, acting on DNA, catalytic activity, acting on RNA, and extracellular matrix binding. The GO pathway of cellular composition (CC) involving upregulated DEGs is mainly glutamatergic synapse, postsynaptic density, neuron-to-neuron synapse, postsynaptic specialization, asymmetric synapse, synaptic membrane, and postsynaptic membrane (Figure 1F). The GO pathway of CC involving downregulated DEGs includes spindle, chromosome, centromeric region, pericentric heterochromatin, endoplasmic reticulum-plasma membrane contact site, ribonucleoprotein granule, chromosome, telomeric region, and chromosomal region. The KEGG pathway involving upregulated DEGs includes endocytosis, adipocytokine signaling pathway, Th17 cell differentiation, phototransduction, nonhomologous end-joining, Hepatitis B, and axon guidance (Figure 1G). The KEGG pathway involving downregulated DEGs includes progesterone-mediated oocyte maturation, growth hormone synthesis, secretion and action, relaxin signaling pathway, focal adhesion, Fc epsilon RI signaling pathway, ECM−receptor interaction, and apoptosis.

The volcano plots of the DEGs between the rd1 and normal control groups are shown in Figure 2A. A detailed list of other DEGs is presented in Table S1. The DEGs were compared to the genes related to neurodegenerative diseases in the NCBI database. Figure 2B demonstrates that there were 43 intersecting genes between the DEGs of rd1 and the genes of neuro-

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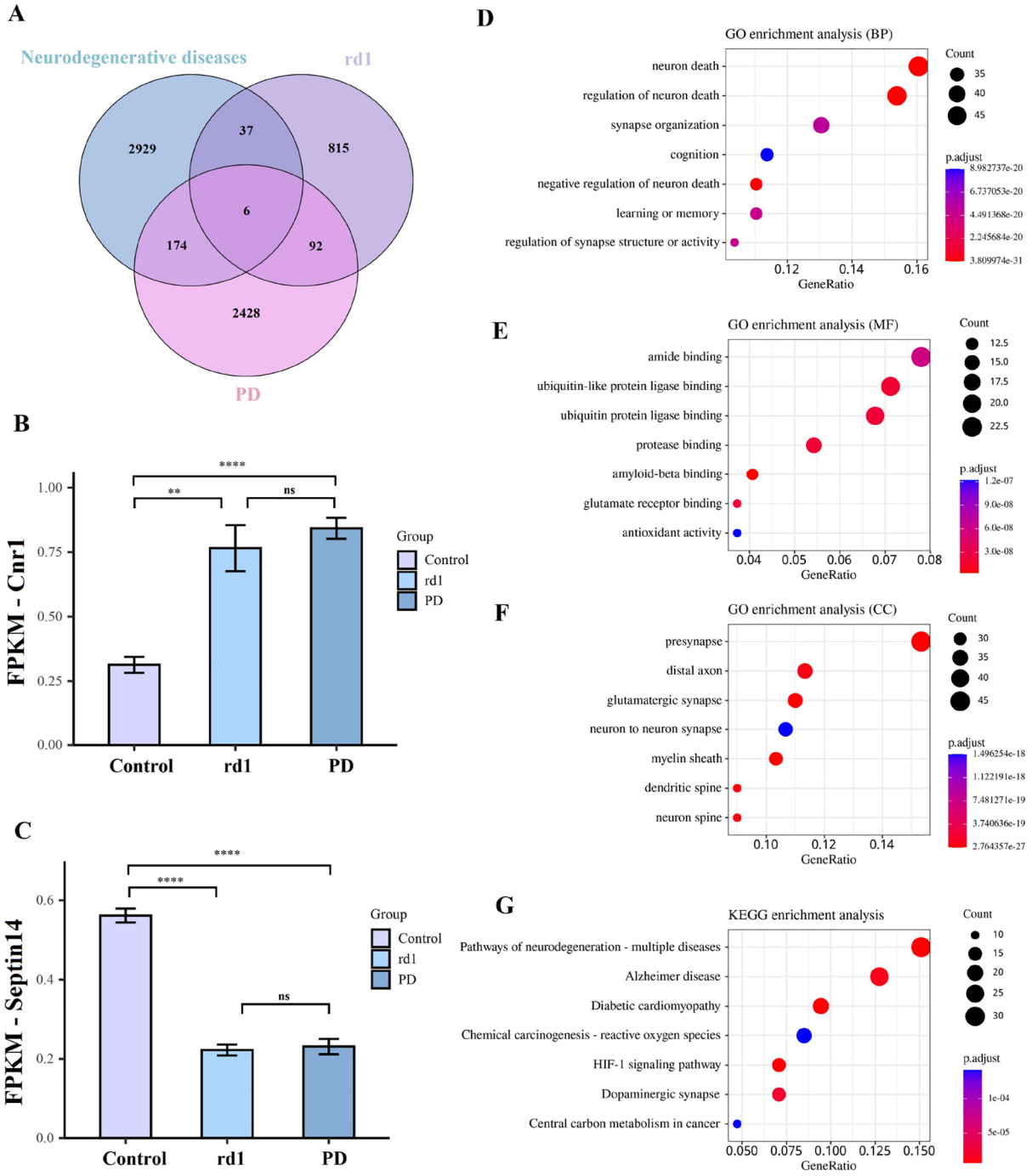

Figure 3. (A) Data of the rd1 mice were further compared with the previous data of PD. The two sets of data were further intersected with neurodegenerative disease-related genes. As a result, six genes were found to be intersected in the three data sets. (B) It was found that Cnr1 was upregulated in the rd1 group and also upregulated in the PD group. (C) Septin14 was downregulated in the rd1 group and also downregulated in the PD group. (D) GO pathway of BP involving those intersecting DEGs is mainly neuron death, regulation of neuron death, synapse organization, negative regulation of neuron death, learning or memory, and regulation of synapse structure or activity. (E) GO pathway of MF involving intersecting DEGs is mainly amide binding, ubiquitin-like protein ligase binding, and antioxidant activity. (F) GO pathway of CC involving intersecting DEGs is mainly presynapse, distal axon, glutamatergic synapse, neuron-to-neuron synapse, myelin sheath, dendritic spine, and neuron spine. (G) These intersecting genes are mainly involved in the pathways of neurodegeneration and Alzheimer's disease. The most important KEGG pathway is "pathways of neurodegeneration-multiple diseases."

degenerative diseases. A detailed list of intersecting genes can be found in Table S1. Heatmaps of the expression of these intersecting genes showed positive correlations between their expressions (Figure 2C).

An analysis of how retinal degenerative diseases contribute to neurodegeneration in the brain is needed. Studies have shown that neurodegenerative lesions can propagate bidirectionally along neuronal projections in the visual pathway.[15] The damage to retinal ganglion cells leads to degeneration of the visual cortex, also known as paracrine trans-synaptic degeneration. Conversely, degeneration of the visual cortex in the brain leads to retinal degeneration, also known as retrograde trans-synaptic

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degeneration. The ability to efficiently communicate with a high degree of complexity between retinal neurons is attributed to the organization of various retinal cell types. Communication between neurons occurs within the two plexiform layers, where the dendrites and axons of neighboring neurons make synaptic connections, achieving an extensive network of both vertical and lateral contacts. RP is a group of severe blinding retinal degenerative diseases characterized by photoreceptor apoptosis secondary to retinal tissue remodeling. The cell bodies of retinal neurons are located in the outer nuclear layer and the inner nuclear layer. Retinal neuron apoptosis in RP further affects brain lesions through synaptic connections via retinal ganglion cells.

In addition, dopamine (DA) neurons in the retina may be involved in this process. Dopamine is a chemical neurotransmitter in the retina that facilitates synaptic effects and visual information, thereby regulating the electrical activity and retinal motion. After retinal degeneration, DA cells are stimulated to release functional dopamine. Several dopaminergic cells can spontaneously activate and alter their dopamine levels in the dark. In turn, DA affects photoreceptor cells, bipolar cells, amacrine cells, and horizontal cells, thereby remodeling photomechanical movement and survival, modulating cellular communication for visual stimuli, and protecting the retina nerve fibers layer.[21] Furthermore, inhibition of DA signaling has been shown to inhibit cGMP accumulation in rd1 retinal organ cultures.[22] DA inhibition can act via nitric oxide to reduce the level of cGMP synthesis in the CNS and retina in a variety of species. The mechanism may involve activation of the protein kinase G signaling pathway or cross-talk with DA signaling via the cyclic AMP pathway. Together, the results presented above suggest that there is a link between retinal degeneration and neurodegeneration. Therefore, the association between retinal degeneration and PD was further investigated in this study.

2.2. Correlation Analysis of Transcriptomic Data from rd1 Mouse Brain with PD Data. Both α-synuclein (α-syn) aggregates in the retina and retinal microvascular changes may contribute to the pathology of PD. Previous studies have revealed a relationship between α-syn aggregates in the retina and impaired vision in PD.[23] It has been demonstrated that the main pathological features of the loss of DA neuron degeneration and deposition of α-syn are present in the retina of PD patients.[24] Further, the aggregation of α-syn in the inner retinal layers and the loss of full retinal thickness have been observed in PD. It has been proposed that retinal degeneration in PD is associated with the deposition of α-syn, degeneration of dopamine neurons, trans-synaptic degeneration, and alterations in the vascular system.[25] It has been demonstrated that the deposition of phosphorylated α-syn in the retina occurs in parallel with Lewy body lesions in the brain tissue in the early stages of PD.[26] As PD progresses, α-syn is deposited in the retina which causes progressive apoptosis of ganglion cells and results in retinal degeneration.[12] In addition, dynamic changes in retinal microvascular morphology accompanied by pathological accumulation of α-syn deposits have been observed. The above findings suggest that retinal microvascular pathology is an important pathophysiological marker in PD.[27] Therefore, the retina may be closely related to the onset and progression of PD, as it is a potential predisposing factor outside the CNS.

In this study, data from rd1 mice were compared with those from PD mice. The DEGs in the rd1 and PD groups were identified by comparison of the diseased mice with the normal controls. The brain tissue samples for the rd1 and PD groups were obtained from the hippocampal regions of mice of the same age. The gene expression was normalized by the fragments per kilobase per million transcripts (FPKM) value. The intersecting DEGs of the groups were identified. Then, the two sets of data were further intersected with neurodegenerative disease-related genes. As a result, six intersecting genes were found among the three data sets (Figure 3A). The FPKM values of these intersecting genes were analyzed in detail. The results revealed that Cnr1 was upregulated in the rd1 and PD groups (Figure 3B). The difference between the rd1 and normal control groups was statistically significant ($P < 0.05$). Septin14 was downregulated in the rd1 group and also in the PD group (Figure 3C). The results of the associated protein−protein interaction (PPI) networks analyses are shown in Figure S2.

Then, the intersecting DEGs between the rd1 group and neurodegenerative diseases were analyzed. All intersecting genes were found to be strongly associated with CNS diseases. For example, PPP1R15A is expressed in a variety of structures, including the CNS.[28,29] It is involved in the CNS myelin regeneration in an inflammatory environment. Nbr1 is associated with mitochondrial dysfunction.[30] Becn1 is known to play a role in a variety of cellular processes, including tumorigenesis, neurodegeneration, and apoptosis.[31] Klf2 plays a role in adipogenesis, embryonic erythropoiesis, epithelial integrity, inflammation, and T-cell viability.[32] Moreover, Klf2 is involved in inflammatory responses. The Klf2-Vav1-Rac1 axis promotes axonal regeneration after a peripheral nerve injury. In addition, Klf2 may serve as a novel molecular therapeutic target for clinical nerve injury repair.[33]

The GO pathways of BP involving those intersecting DEGs are mainly neuron death, regulation of neuron death, synapse organization, cognition, negative regulation of neuron death, learning or memory, and regulation of synapse structure or activity (Figure 3D). The GO pathway of MF involving intersecting DEGs is mainly amide binding, ubiquitin-like protein ligase binding, ubiquitin protein ligase binding, protease binding, amyloid-beta binding, glutamate receptor binding, and antioxidant activity (Figure 3E). The GO pathway of CC involving intersecting DEGs is mainly presynapse, distal axon, glutamatergic synapse, neuron-to-neuron synapse, myelin sheath, dendritic spine, and neuron spine (Figure 3F). The KEGG pathway involving intersecting DEGs includes pathway of neurodegeneration-multiple diseases, Alzheimer's disease (AD), diabetic cardiomyopathy, chemical carcinogenesis-reactive oxygen species, HIF-1 signaling pathway, dopaminergic synapse, and central carbon metabolism in cancer (Figure 3G). The inflammatory hypothesis has been proposed to explain the pathogenesis of neurodegenerative diseases.[34] It has been demonstrated that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is expressed in activated microglia in the retinas of rd1 mice. Moreover, the invasion of the outer nuclear layer at the peak of photoreceptor apoptosis suggests that retinal microglia may generate extracellular reactive oxygen species (ROS) through the activation of NADPH oxidase, leading to apoptosis of optic rod cells. In addition, activated NADPH oxidase may also lead to an increase in intracellular ROS, which stimulates the production of several inflammatory factors and exacerbates neuronal damage such as CNS lesions.[35] The GO pathway results from both this study and the previous PD study demonstrated an association between oxidative stress and inflammation. To further validate the correlation between RP and PD, the intersecting genes were validated by RT-qPCR.

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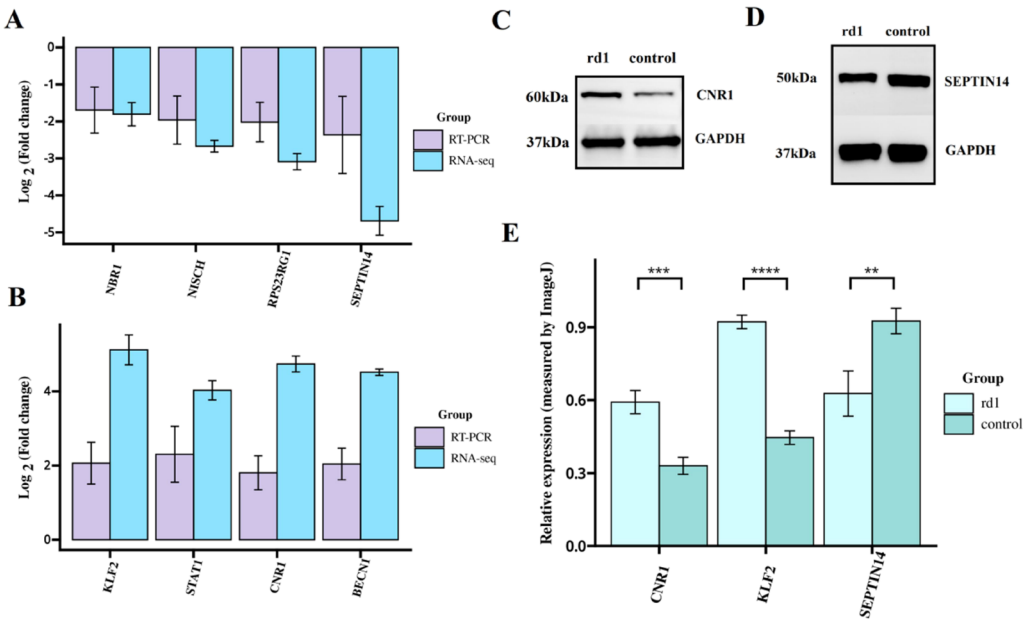

Figure 4. (A) It was further verified that Nbr1, Nisch, Rps23rg1, and Septin14 showed downregulation in both RNA-seq and RT-qPCR. (B) It was further verified that Klf2, Stat1, Cnr1, and Becn1 showed a trend of upregulation in both RNA-seq and RT-qPCR. (C) Upregulation trend of CNR1 in the rd1 group relative to the normal group was verified by the WB experiment. (D) Downregulation trend of SEPTIN14 in the rd1 group relative to the normal group was also verified by the WB experiment. (E) Area of the bands in each electropherogram was calculated by ImageJ. It was found that CNR1 and KLF2 were significantly upregulated in the rd1 group ($P < 0.05$). SEPTIN14 was significantly downregulated in the rd1 group ($P < 0.05$).

2.3. Validation of Intersecting Genes Using RT-qPCR and Western Blotting Experiments. Nbr1, Nisch, Rps23rg1, and Septin14 showed downregulation in both the RNA-seq and quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) analyses (Figure 4A). Further, Klf2, Stat1, Cnr1, and Becn1 showed upregulation in both the RNA-seq and RT-qPCR analyses (Figure 4B). The list of primers designed for RT-qPCR is listed in Table 1. The intersecting genes of the three groups are highlighted, including Cdk5r1, Cnr1, Nisch, Nlrp1a, Pygm, and Septin14. CNR1 and SEPTIN14 were verified by the Western Blotting (WB) experiment. The relevant antibodies are listed in Table 2. The upregulation of CNR1 in the rd1 group relative to the normal group was verified by the WB experiment (Figure 4C). The downregulation of SEPTIN14 in the rd1 group relative to the normal group was also verified by the WB experiment (Figure 4D). Additional raw electropherograms of the WB experiments are shown in Appendix Figure S3. The area of the bands in each electropherogram was calculated by ImageJ. It was found that CNR1 and KLF2 were significantly upregulated in the rd1 group ($P < 0.05$). SEPTIN14 was significantly downregulated in the rd1 group ($P < 0.05$) (Figure 4E).

Table 1. Primers Used in RT-qPCR

genes	sequences
GAPDH	F:5′-CGTCCCGTAGACAAAATGGT-3′
	R:5′-TTGATGGCAACAATCTCCAC-3′
Cnr1	F:5′-AGCAAGGACCTGAGACATGC-3′
	R:5′-TGTTATTGGCGTGCTTGTGC-3′
Klf2	F:5′-GCGTACACACACAGGTGAGA-3′
	R:5′-CGCACAAGTGGCACTGAAAA-3′
Becn1	F:5′-GCCTCTGAAACTGGACACGA-3′
	R:5′-TAGCCTCTTCCTCCTGGGTC-3′
Stat1	F:5′-GGACGCCCTCTAGCCTTTTT-3′
	R:5′-AGGGTATGGAGCAGAGCTGA-3′
Nisch	F:5′-TCCCAGTTTGGAGAACGAGC-3′
	R:5′-GGAAGTCCTCACCAGCCTTC-3′
Rps23rg1	F:5′-TAATGAGCTGCACCCTGACG-3′
	R:5′-CGGGATCAGAAGTGGCATGA-3′
Septin14	F:5′-ATGACGAAGCGAGTGCTCAA-3′
	R:5′-TCCCGGAGCTTCACAAAGTC-3′
Nbr1	F:5′-TGACACTAACCACGTCCTGC-3′
	R:5′-CAGCTCGCAACCTTTGCTTT-3′

The endocannabinoid system is a neurotransmitter system widely dispersed throughout the central and peripheral nervous systems. It is associated with a variety of brain functions, including memory, mood, and motor control. The cannabinoid receptor type 1 (CB1) is encoded by CNR1 and is primarily involved in brain function.[36] CNR1 is expressed in the central and peripheral nervous systems. CB1 acts as an inhibitor of excitatory and inhibitory neurotransmitters. An important CB1 regulator is cannabinoid receptor interacting protein 1 (CNRIP1).[37] This regulator acts in the early fetal period and is associated with neural and ocular induction in all vertebrates.[38] Pathology in the CNRIP1-CB1 network has also been associated with ocular defects, epilepsy, hippocampal damage, and striatal damage.[39] It has been reported that glial CB1 receptors are significantly altered in AD mice.[40] The expression of the CB1 receptor is increased in the reactive microglia in AD. CNR1 plays a neuroprotective role in neurodegenerative diseases.[41] Furthermore, the pathogenesis and treatment of both AD and RP are closely linked with inflammation.[42] Most importantly, this study found a correlation between CNR1 expression in RP and PD. These findings provide insight into the role of the endocannabinoid system in the pathophysiology of neurodegenerative diseases.

In addition, SEPTIN14 is expressed in the human brain and has been reported to be associated with a reduced risk of PD.[43] The homologue of SEPTIN14 is expressed not only in the testis

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Table 2. Antibodies for WB

antibody name	primary antibodies					secondary antibodies			
	origin	item no.	molecular weight (kDa)	antibody source	antibody dilution ratio	antibody name	origin	item no.	antibody dilution ratio
GAPDH	Servicebio	GB15004	37	rabbit	1:1000	HRP-goat antirabbit	Servicebio	GB23303	1:5000
CNR1	Servicebio	GB111214	60	rabbit	1:1000	HRP-goat antirabbit	Servicebio	GB23303	1:5000
KLF2	ABclonal	A16480	37	rabbit	1:1000	HRP-goat antirabbit	Servicebio	GB23303	1:5000
SEPTIN14	Servicebio	GB114333	50	rabbit	1:1000	HRP-goat antirabbit	Servicebio	GB23303	1:5000

but also in the brain, particularly in the cerebrum, cerebellum, and hippocampus.[44] SEPTIN14 is essential for neuronal migration during cortical formation via an interaction with SEPTIN4.[45] SEPTIN4 plays an important role in DA transmission. A lack of SEPTIN4 reduced DA release. The potential interaction between SEPTIN14 and SEPTIN4 is extremely important for PD.[45] The results of the present study revealed that SEPTIN14 was downregulated in both RP and PD, which is consistent with previous findings. Previous research suggests that the indirect effect of SEPTIN14 on DA release can be considered as a potential therapeutic target for PD. Taken together, the findings indicate that SEPTIN14 may serve as a common target gene for neurodegenerative diseases and thus is worthy of further exploration.

Based on the current findings, both CNR1 and SEPTIN14 are potential therapeutic targets for retinal degeneration and neurodegenerative diseases. There are a number of studies of therapeutic targets related to PD.[46−48] However, there are no existing pharmacologic agents or experimental interventions specifically targeting CNR1 or SEPTIN14. Future studies may be possible to investigate the impact of CNR1 and SEPTIN14 on efficacy and safety in clinical studies of PD. A thorough understanding of the molecular mechanisms underlying brain degeneration in RP will not only help to elucidate the early pathogenesis of RP and neurodegenerative diseases but also contribute to the potential exploitation of the retina as a therapeutic target for neurodegenerative diseases. To this end, the cotarget genes CNR1 and SEPTIN14 may serve as therapeutic targets.

3. CONCLUSIONS

The results of this study confirm the existence of a close link between retinal degenerative diseases and neurodegenerative diseases of the brain. These findings provide insight into the mechanisms of retinal degeneration and neurodegenerative diseases and have implications for diagnosis and treatment. However, the limitations of this research must be acknowledged. First, this study had a small sample size and a limited number of data. Moreover, although this study demonstrated an association between RP and AD, this part of the hypothesis could not be confirmed due to the lack of samples from AD mice as verification. Future studies should address the above shortcomings and further investigate CNR1 and SEPTIN14 as novel molecular therapeutic targets for the treatment of neurodegenerative diseases.

4. METHODS

4.1. Animals and Transcriptome Library Building. Brain tissue samples from C57BL/6J background rd1 and wt control mice were used for all of the experiments. There were two groups of mice, including the rd1 mice group with retinitis pigmentosa (rd1, Pde6bem1 Cin purchased from Gem Pharmatech Co., Ltd., Nanjing, China) and the control mice group (C57BL/6J, purchased from Gem Pharmatech Co., Ltd.). The mice were euthanized by cervical subluxation after anesthesia. All animal procedures were performed in accordance with the ethical standards of the Ethics Committee of CUH, Southeast University. The animal ethics approval number for this experiment is 20230206002 and the approval date is February 6, 2023. The age of rd1 mice was consistent with those of PD mice and normal control mice, which were all 8 weeks old. The hippocampal regions of both rd1 mice and normal mice were first isolated. Then, RNA was extracted from crushed tissue blocks using an RNA Extraction Kit FT (Vazyme Biotech Co., Ltd., item no. RM203-01). The RNA was then reverse-transcribed into cDNA. Next, 5 ng of cDNA was treated with a One-step DNA Lib Prep Kit (ABclonal, RK20239) for Illumina sequencing. The library was successfully sequenced on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA, USA) sequencer with a paired-end pattern and inset size of 350 bp.

4.2. Analysis of RNA-seq Data and Statistical Methods. The fastq format raw data obtained after Illumina sequencing was screened to obtain high-quality clean data. Each library was quality-controlled by FastQC with the Q30 value in Table S1. Then, the clean data were compared with the mouse reference sequence using Hisat2 software. The number of counts obtained for each sample was measured using FeatureCounts in the subread Linux package. DEGs were analyzed using the DESeq2 R package (1.36.0) with Log_2|Fold change|>1 and P-value <0.05. Gene ontology (GO) enrichment and KEGG analysis of DEGs were performed using the Cluster Profiler R package. Comparisons between the two groups were made by t tests (paired two-sample mean analysis). Statistical significance was defined as $P < 0.05$.

4.3. Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction. RT-qPCR was performed using TB Green dye and specific primer pairs. The primer sequences are listed in Table 1.

Three replicates were performed for each sample. The cycle threshold (Ct value) was the number of cycles of fluorescent signaling in each reaction when the set threshold was reached. The samples were quantified using an internal reference method. The internal reference was glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of the mouse. The PCR amplification system consisted of the DNA amplification enzyme TB Green Premix Ex *Taq*II (Takara, RR420A, 2×), upstream and downstream primers (10 μm), a DNA template, and enzyme-free water to make up the volume to 20 μL. The ratios of the expression levels of DEGs between rd1 and normal control mice

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were calculated by the $2^{-\Delta\Delta Ct}$ method after normalization. The formula was as follows.

$$\Delta Ct_{disease/control} = Ct_{marker\ gene} - Ct_{internal\ reference} \quad (1)$$

$$\Delta\Delta Ct = \Delta Ct_{disease} - \Delta Ct_{control} \quad (2)$$

4.4. Western Blotting. Brain tissues from both the rd1 group and the normal control group were lysed and homogenized in RIPA lysis buffer (50 mM Tris−HCl, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.4) and 50× Cocktail protease inhibitor (Servicebio, G2006-250UL). Equal amounts of proteins were separated on SDS polyacrylamide gel and transferred to a PVDF membrane (0.45 μm, Servicebio, WGPVDF45). The proteins were standardized prior to SDS-PAGE separation and then transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk powder in TBST for 2 h at room temperature. The membranes were then incubated with the primary antibody in a blocking solution overnight at 4 °C. The primary and secondary antibodies used for Western blotting are shown in Table 2. The signals were developed with an Ultrasensitive ECL Chemiluminescence Kit (Servicebio, G2020-25 ML). The relative intensities of WB immunoreactive bands were quantified using the gel analysis tool provided by ImageJ software (1.8.0). At least three replicate Western blotting experiments were performed.

■ ASSOCIATED CONTENT

Data Availability Statement

The data used in this paper can be downloaded from the NCBI Web site: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1046153.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c09938.

Heatmap of differential expression between rd1 mouse group and normal control group; results of the associated protein−protein interaction networks analyses of the intersecting genes; raw electropherograms of the Western blotting experiments; and DEGs of Rd1 and PD (PDF)

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Y.Z.: data curation, methodology, writing-original draft. Y.Y.: data curation. T.Q.: data curation. Z.H.: writing review and editing. Q.G.: conceptualization, methodology. Z.L.: supervision.

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Notes

The authors declare no competing financial interest.

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