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Gene therapy shines light on congenital stationary night blindness for future cures

Yi Zhang[1†], Siqi Lin[1†], Lingqi Yu[1†], Xiang Lin[1,2], Shuai Qu[1,2], Qingyang Ye[3], Mengting Yu[4], Wenfeng Chen[1,2*] and Wenjie Wu[4*]

Abstract

Congenital Stationary Night Blindness (CSNB) is a non-progressive hereditary eye disease that primarily affects the retinal signal processing, resulting in significantly reduced vision under low-light conditions. CSNB encompasses various subtypes, each with distinct genetic patterns and pathogenic genes. Over the past few decades, gene therapy for retinal genetic disorders has made substantial progress; however, effective clinical therapies for CSNB are yet to be discovered. With the continuous advancement of gene-therapy tools, there is potential for these methods to become effective treatments for CSNB. Nonetheless, challenges remain in the treatment of CSNB, including issues related to delivery vectors, therapeutic efficacy, and possible side effects. This article reviews the clinical diagnosis, pathogenesis, and associated mutated genes of CSNB, discusses existing animal models, and explores the application of gene therapy technologies in retinal genetic disorders, as well as the current state of research on gene therapy for CSNB.

Introduction

Light from the natural environment enters the eye and is refracted through the lens and vitreous body before being transmitted to the retina, which serves as the first station for visual signal formation. The retina is primarily composed of five main types of neuronal cells: photoreceptors, bipolar cells (BCs), retinal ganglion cells (RGCs), horizontal cells (HCs), and amacrine cells (ACs) (Fig. 1). In the process of visual signal transmission, photoreceptor cells capture light signals and convert them into electrical signals, which are then relayed to the brain's visual center through bipolar and ganglion cells (Fig. 1). HCs and ACs are intermingled within two consecutive synaptic layers, providing lateral inhibition. This mechanism shapes signal transmission from photoreceptors to BCs and from BCs to RGCs, respectively [1]. The transmission of retinal signals mainly occurs through synaptic neurotransmitter release; for example, photoreceptors and BCs activate their postsynaptic partners by releasing glutamate [2]. In reality, the transmission of visual signals in the retina involves various regulatory mechanisms, making it significantly more complex than described. Overall, the retina plays a crucial role in vision formation, with its structured layers closely interconnected. However, in some cases, mutations in genes associated within different retinal structures can disrupt this 'harmony', leading to visual impairments.

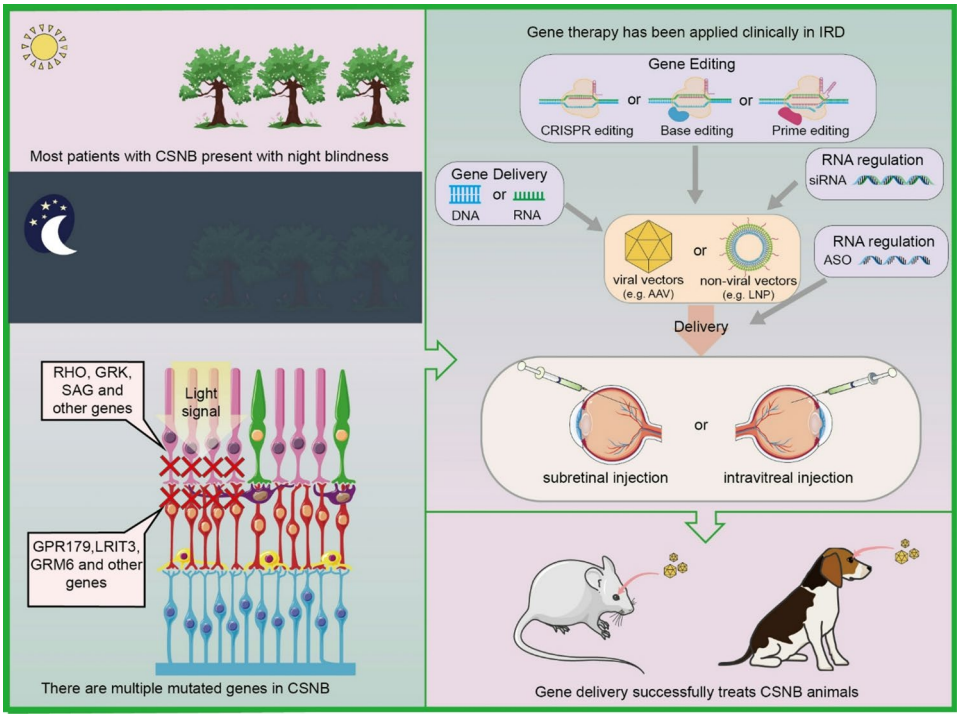
CSNB is a non-syndromic inherited retinal disease (IRD) characterized primarily by night blindness, with some patients also exhibiting myopia, strabismus, nystagmus, and fundus abnormalities [3]. As a genetically heterogeneous disorder, CSNB can result from mutations

†Yi Zhang, Siqi Lin and Lingqi Yu contributed equally to this work.

*Correspondence:
Wenfeng Chen
chenwenfeng@fzu.edu.cn
Wenjie Wu
wenjie_wu@foxmail.com

Full list of author information is available at the end of the article

Graphical abstract



Keywords Congenital stationary night blindness, Gene therapy, Retina, Inherited retinal disease, Full-field electroretinography, Animal models, Gene editing

in several genes, such as *RDH5*, *GRM6*, *NYX*, and *LRIT3*. These mutations primarily affect photoreceptor and BCs, leading to dysfunction in these cell types [3]. Due to the clinical and genetic heterogeneity of CSNB, an accurate diagnosis requires not only a fundoscopic examination but also full-field electroretinography (ffERG) to determine the CSNB subtype and guide subsequent treatment.

Gene therapy has recently emerged as a promising technique for treating genetic disorders, showing significant progress over the past few decades. It mainly involves gene replacement approaches or gene editing to correct defective genes. In the case of retinal genetic disorders, since the FDA's approval of the first gene therapy drug, Luxturna, multiple gene therapies for IRDs have entered clinical trials [4], most of which are based on gene replacement strategies. Currently, there are no widely adopted treatment options for CSNB, and research is still primarily at the preclinical stage, with studies using animal models being reported. In this review, we summarize the current research on the pathological mechanisms of CSNB, the development and application of related animal models, and the treatment outcomes observed in these models. Additionally, we explore the potential of emerging gene editing technologies for CSNB treatment and discuss the challenges that need to be addressed for future gene therapy approaches.

CSNB and its clinical diagnosis

CSNB refers to a group of clinically and genetically heterogeneous IRDs causing synaptic dysfunction that is non-progressive. They are characterised by impaired night vision or delayed dark adaptation, typically presenting in infancy. Associated symptoms may include myopia or hyperopia, strabismus, nystagmus, reduced visual acuity, or an abnormal findings in the fundus. Fundus appearances [5–8] in CSNB patients may be normal or abnormal, depending on the subtype [3]. Due to the characteristics of CSNB, in addition to fundus examination, ffERG is essential for the diagnosis and classification of CSNB, particularly in patients with normal fundus findings. Furthermore, the review of the patient's medical history and molecular genetic testing are also important for the classification of CSNB. Full-field ERG is a non-invasive retinal testing method that assesses retinal function through electrical responses to flashes and recordings from corneal electrodes (Fig. 2A). It primarily evaluates retinal function under dark adaptation (DA) and light adaptation (LA). According to the standards set by the

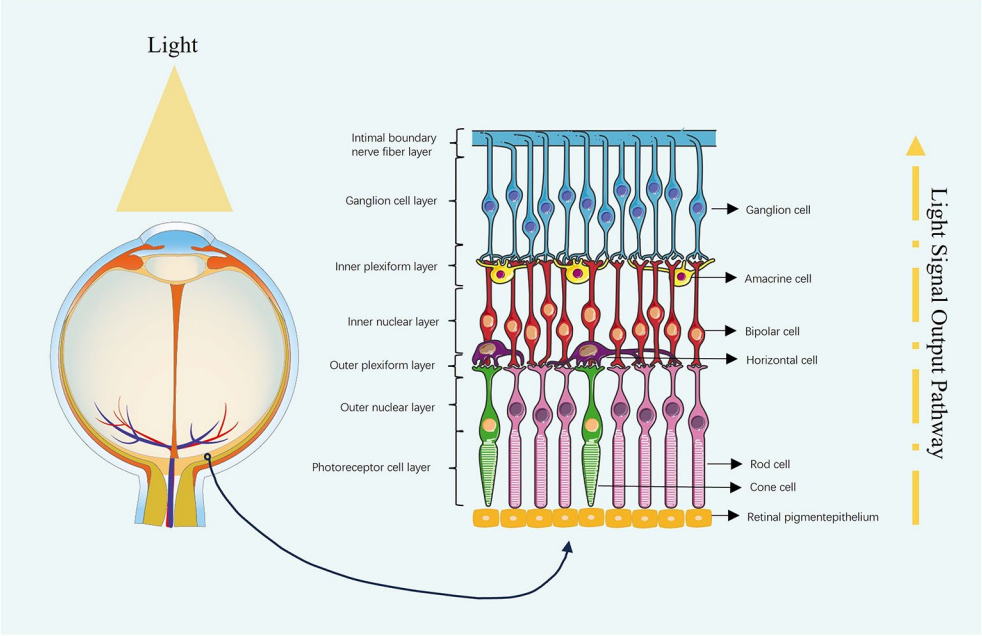


Fig. 1 Diagram of the visual pathway in retina. When light passes through the cornea and vitreous body of the eye and is focused onto the retina, the photoreceptor cells convert the light signal into an electrical signal. This signal is then transmitted step by step via BCs and other structures, eventually being relayed to the brain through ganglion cells, where it generates visual perception.

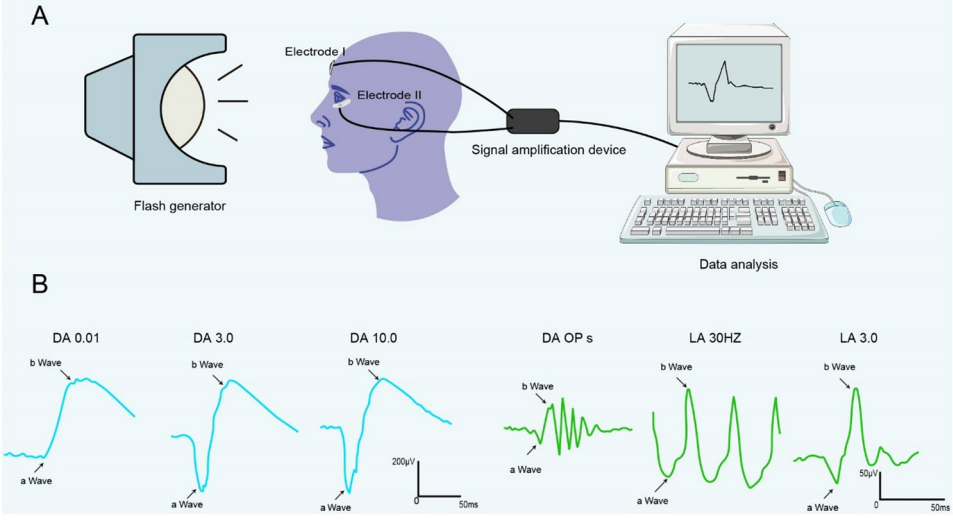


Fig. 2 Full-field electroretinography (ffERG) testing. (**A**). ERG testing diagram: Two electrodes are placed on the patient's forehead and lower eyelid. When the patient is exposed to stimuli from a flash stimulator, the eye generates corresponding electrical currents. These currents are amplified by a signal amplifier and displayed as waveforms in the program. (**B**). ERG of a normal individual: According to the standards set by the ISCEV, the CSNB waveform of a normal individual is shown as depicted

International Society for Clinical Electrophysiology of Vision (ISCEV) [9], DA assessments involve flash intensities of 0.01 (DA 0.01), 3.0 (DA 3.0), and 10.0 (DA 10.0) cd•s/m^2, while LA assessments involve a flash intensity of 3.0 cd•s/m^2 superimposed on a 30 cd•s/m^2 background, along with single signals LA 3.0 and 30 Hz flashing (LA 30 Hz) (Fig. 2B).

After 20 min of dark adaptation, the signal in the DA 0.01 ERG is predominantly represented by the positive b-wave generated by BCs, while the a-wave produced by rod cells is weaker (Fig. 2B). The DA 3.0 and DA 10.0 ERGs indicate mixed rod-cone responses, both displaying a pronounced negative a-wave primarily generated within photoreceptor cells, followed by a positive b-wave produced by ON-type BCs (Fig. 2B). Additionally, the ERG waveforms for DA 3.0 and DA 10.0 contain a higher-frequency, lower-amplitude waveform known as the oscillatory potential (OP), which typically reflects the

activity of ACs and some vascular functions in the inner retina (Fig. 2B). In LA, the LA 3.0 and LA 30.0 Hz ERGs show distinct a-waves and b-waves, with the a-wave mainly generated by OFF-type BCs and the b-wave co-dominated by ON-type and OFF-type BCs, regulated by long, medium, and short-wavelength cone cells (Fig. 2B). The LA 30.0 Hz ERG originates from ON-type and OFF-type BCs but is primarily influenced by long-wavelength and medium-wavelength cone cells [9, 10].

Clinical features of CSNB

CSNB presents with various clinical phenotypes, which can be categorized based on the fundus appearance into normal fundus CSNB and abnormal fundus CSNB (Fig. 3A).

Abnormal fundus CSNB

Abnormal fundus CSNB mainly consists of two types: Oguchi disease (OD) and Fundus albipunctatus (FA). These two types differ in symptoms and ERG findings, yet both are inherited in an autosomal recessive manner.

Oguchi disease

Patients with Oguchi disease exhibit congenital night blindness while maintaining normal color vision, visual acuity, and visual fields. These patients display abnormal fundus characteristics known as Mizuo-Nakamura, which feature golden-yellow lesions that disappear after prolonged DA [11, 12]. In ERG testing, following brief dark adaptation, the b-wave is significantly reduced or absent under DA 0.01 conditions, and both the a-wave and b-wave show reductions under DA 10.0, indicating dysfunction in rod cells. However, after extended dark adaptation, the a-wave and b-wave can return to near-normal levels with single flash stimulation, only to revert to abnormal levels in subsequent tests [13, 14]. Under LA, the ERG is typically normal in Oguchi patients [15] (Fig. 4A).

Fundus albipunctatus disease

Fundus albipunctatus is another autosomal recessive disorder characterized by night blindness and delayed dark adaptation. Their fundus shows significant abnormalities, characterized by white spots in the mid-peripheral and posterior regions, except for the macula; these spots may improve or worsen over time [16]. ERG examinations of FA patients reveal that under DA 0.01 conditions, the b-wave is severely reduced or even absent. Additionally, in the DA 10.0 ERG, both the b-wave and a-wave show reductions, indicating primary rod dysfunction. However, with prolonged dark adaptation, most patients' ERG results tend to normalize [17] (Fig. 4B).

Normal fundus CSNB

Normal fundus CSNB is mainly categorized into two types: Schubert-Bornschein type and Riggs type.

Schubert-Bornschein type CSNB

The Schubert-Bornschein type CSNB was discovered and reported by Schubert and Bornschein. Patients with this type exhibit a normal fundus, while their dark-adapted ERG shows a normal a-wave with a disappearance of the b-wave [18]. This is the most common form of CSNB, with inheritance patterns including autosomal recessive and X-linked inheritance [19]. The Schubert-Bornschein type can be further divided into incomplete

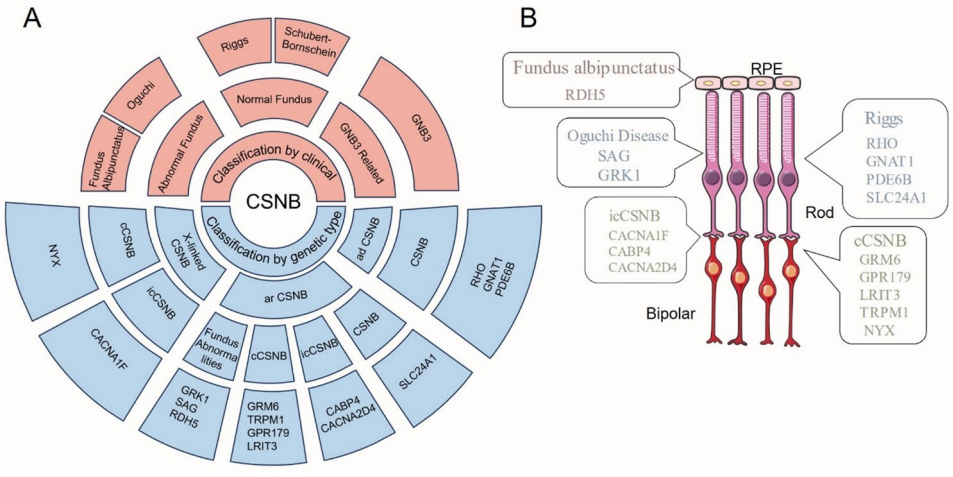


Fig. 3 Clinical and molecular characteristics of CSNB. (**A**). Upper: Based on clinical characteristics, CSNB can be categorized into three main types: normal fundus type, abnormal fundus type, and GNB3 type; Lower: Although CSNB is a rare disease, it exhibits multiple inheritance patterns, including X-linked CSNB, autosomal recessive (arCSNB) and autosomal dominant (adCSNB). (**B**). CSNB related genes and their distribution: Mutations associated with CSNB are primarily located in rod cells and BCs, which explains why the visual impairment in CSNB patients mainly occurs in the signal transmission between photoreceptor cells and BCs

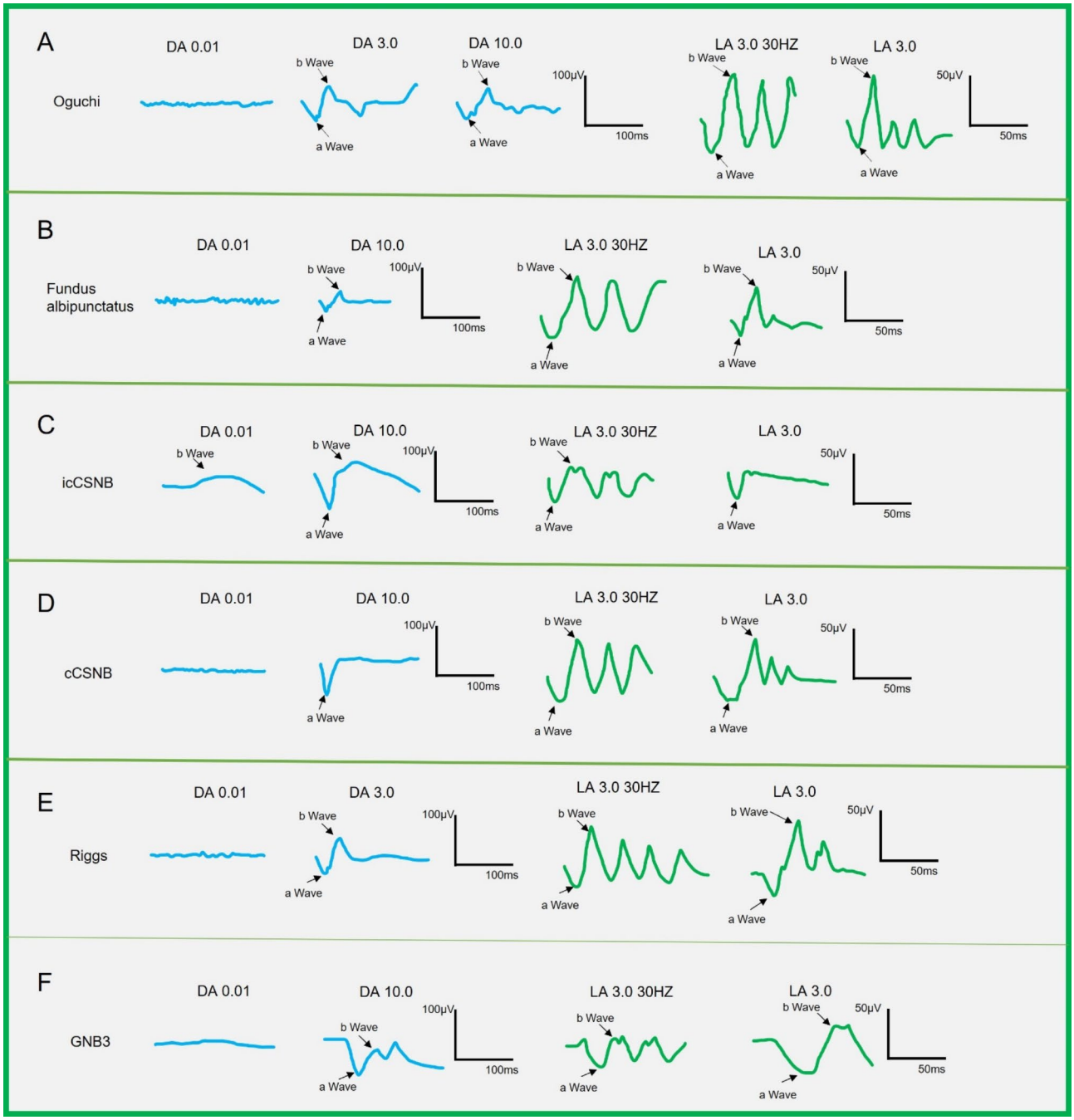


Fig. 4 Electrophysiological findings of different CSNB patients. (**A**). ERG of OD patients: OD patients exhibit abnormal fundus. The ERG shows a complete disappearance of the b-wave under dark-adapted (DA) 0.01 conditions. Subsequent tests reveal a reduction in both a- and b-waves, while the light-adapted (LA) response is generally normal [14]. (**B**). ERG of FA patients: FA patients have white spots in the fundus. The ERG is characterized by a severely reduced b-wave under DA 0.01 conditions, and both a- and b-waves are reduced under DA 10.0 conditions [17]. (**C**). ERG of icCSNB patients: icCSNB patients have a normal fundus. Under DA 0.01 conditions, the b-wave is significantly reduced but still detectable. Under DA 10.0 conditions, the b-wave shows further reduction while the a-wave remains normal. Under LA 3.0 30 Hz conditions, both a- and b-waves are reduced and delayed. Similarly, under LA 3.0, both a- and b-waves show reductions [34]. (**D**). ERG of cCSNB patients: cCSNB patients have a normal fundus. The b-wave is completely absent under DA 0.01 conditions, while under DA 10.0, the b-wave is significantly reduced but the a-wave remains normal. Under LA 3.0 30 Hz conditions, the waveform broadens, and under LA 3.0, the a-wave is normal but the trough broadens and the b-wave becomes steeper [34]. (**E**). ERG of Riggs patients: Riggs patients have a normal fundus. Under DA 0.01 conditions, the b-wave is severely reduced or absent, and both a- and b-waves are reduced under DA 10.0 conditions. Their light-adapted response is mostly normal [28]. (**F**). ERG of GNB3 related CSNB: GNB3 patients are rare and show varying phenotypes. In the cases mentioned, the ERG mainly shows a reduction in the b-wave under DA 0.01 or DA 10.0 conditions. Under LA 3.0 30 Hz, the ERG may show weakening or delays, while under LA 3.0, the a-wave is normal but may be delayed, and the b-wave is weakened and delayed [33]

CSNB (icCSNB) and complete CSNB (cCSNB) [20–22], with these two forms representing distinct diseases [23].

Incomplete CSNB Patients with icCSNB exhibit functional impairments in both ON-type and OFF-type BCs. In ERG testing under DA 0.01 conditions, the b-wave is reduced but does not disappear, while the a-wave remains normal. Under DA 10.0 conditions, only a decrease in the b-wave is observed, indicating that rod phototransduction is functioning normally in icCSNB patients [22]. In LA, the LA 30 Hz ERG response is significantly weakened and delayed, yet a clear biphasic response can still be seen. The LA 3.0 ERG also shows reductions, with similar amplitudes for the a-wave and b-wave [19] (Fig. 4C). Prolonged stimulation leads to dysfunction in both ON-type and OFF-type BCs [24]. Compared to cCSNB, icCSNB presents unique characteristics; for example, mutations in *CACNA1F* can result in icCSNB patients having less night vision impairment [24, 25], contrasting with the heightened sensitivity to light stimuli seen in most icCSNB patients [25]. Additionally, icCSNB patients may experience myopia, hyperopia, nystagmus, strabismus, reduced vision, and color vision deficiencies [25].

Complete CSNB Patients with cCSNB exhibit dysfunction in ON-type BCs. In dark-adapted ERG testing, under DA 0.01 conditions, the b-wave completely disappears, while under DA 10.0 conditions, the b-wave is significantly reduced and the a-wave remains normal, indicating that rod phototransduction is functioning properly [22]. In light-adapted ERG testing, under LA 3.0 and 30 Hz, the amplitude is normal, but the trough widens; at LA 3.0, a normal a-wave is present, yet the trough still widens and the b-wave becomes steeper [22, 26] (Fig. 4D). Prolonged stimulation confirms that patients have ON-type BCs dysfunction, while OFF-type BCs remain unaffected [24]. In addition to night blindness and reduced vision, cCSNB patients may also experience myopia, nystagmus, and strabismus [25].

Riggs type CSNB

Riggs type CSNB represents a form of CSNB characterized by dysfunction in rod photoreceptors [27]. Unlike Schubert-Bornschein type, Riggs type shows a significant reduction in ERG response under dark adaptation; specifically, the b-wave is severely reduced or absent under DA 0.01 conditions, and both the b-wave and a-wave decrease in DA 10.0 ERG. These findings indicate primary rod cell dysfunction during dark adaptation, while the ERG results in light conditions are comparable to normal [28] (Fig. 4E). Riggs type CSNB is known to include both autosomal dominant [29] and autosomal recessive inheritance patterns [30]. Patients with Riggs type CSNB typically experience milder night blindness, with a minority exhibiting myopia [28, 31].

GNB3 gene-related CSNB

GNB3-related CSNB presents a phenotype distinct from the previously mentioned types [32, 33], with currently reported cases being relatively few and phenotypically varied. Under dark-adapted conditions, such as DA 0.01 or DA 10.0, the b-wave is reduced while the a-wave remains normal, indicating that rod phototransduction is functioning properly. In LA, the ERG response may show weakening or delays at LA 3.0 and 30 Hz, with a normal a-wave that may also be delayed, and a weakened and delayed b-wave. Prolonged stimulation reveals abnormal responses in ON-type BCs (Fig. 4F), while OFF-type BCs responses remain normal. Patients with GNB3-related CSNB may exhibit night blindness, myopia, and nystagmus, though not all patients display these symptoms.

CSNB molecular pathology

CSNB is a type of hereditary retinal disease, with known inheritance patterns including X-linked, autosomal recessive, and autosomal dominant (Fig. 3A). Numerous genes associated with CSNB and their mutations have been identified, which are summarized in Table 1. The mutations in CSNB can be broadly categorized into two groups: one primarily causes dysfunction in rod phototransduction within photoreceptors, as seen in mutations related to Riggs, FA, and OD; the other affects the signaling pathway between photoreceptors and BCs, leading to mutations associated with cCSNB and icCSNB (Fig. 3B).

Defects in rod phototransduction cascade-related genes

Mutations associated with Riggs, FA, and OD types lead to dysfunction in rod photoreceptors, particularly affecting genes that encode critical proteins in the rod phototransduction cascade. This cascade is a vital system for converting light into electrical signals in the retina, primarily operating through G-protein pathways. In a dark environment, the proteins involved in phototransduction remain in an inactive state. During this time, cyclic guanosine 3',5'-monophosphate (cGMP)-regulated cation channels remain open, leading to depolarization of the photoreceptors. As a result, glutamate, functioning as a neurotransmitter, is released into the synaptic cleft. When exposed to light, rhodopsin captures photons, activating G-protein signaling, which triggers the hydrolysis of cGMP, closing the ion channels, resulting in hyperpolarization of the photoreceptors and a decrease in glutamate release (Fig. 5).

In Riggs-type CSNB, mutations have been identified in genes such as *RHO*, *SLC24A1*, *PDE6B*, and *GNAT1*. Mutations in the *RHO* gene (e.g., Gly90Asp, Thr94Ile, Ala292Glu, Ala295Val) are commonly associated with

Table 1 Gene mutations related to the CSNB

Disease	Defective gene	mRNA length	Chromosome location	Mutation type	Number of CSNB-related pathogenic mutations	CDS length	Inheritance	NCBI number
Fundus Albipunctatus	*RDH5*	1274 bp	12q13.2	Including single base mutations, frameshift mutations, deletions and insertions	36/273*	954 bp	autosomal recessive	NM_002905.5
	RPE65	2,605 bp	1p31.3	Single base mutations (splice donor mutations)	1/210	1599 bp	autosomal recessive	NM_000329.3
	RLBP1	1,638 bp	15q26.1	Single base mutation (missense mutation)	4/36	951 bp	autosomal dominant/recessive	NM_000326.5
Riggs	*GNAT1*	3,599 bp	3p21.31	Single base mutation (missense mutation, nonsense mutation)	5/18	1050 bp	autosomal dominant	NM_144499.3
	PDE6B	3,294 bp	4p16.3	Single base mutation (missense mutation), deletion	6/110	2562 bp	autosomal dominant	NM_000283.4
	SLC24A1	5,762 bp	15q22.31	Single base mutation (missense mutation), deletion	7/34	3297 bp	autosomal recessive	NM_004727.3
	RHO	2,768 bp	3q22.1	Single base mutation (missense mutation)	7/34	1044 bp	autosomal dominant	NM_000539.3
cCSNB	*NYX*	2,414 bp 2,629 bp	Xp11.4	Single base mutations (missense mutations, nonsense mutations), deletions, insertions, frameshift mutations, duplications	9/30	1428 bp	X-linked	NM_001378477.3 NM_022567.2
	GRM6	6,025 bp	5q35.3	Single base mutations (missense mutations, splice acceptor mutations)	5/20	2631 bp	autosomal recessive	NM_000843.4
	TRPM1	5,787 bp	15q13.3	Single base mutations (missense mutations, splice acceptor mutations) duplications, frameshift mutations, deletions	20/59	4875 bp	autosomal recessive	NM_001252024.2
	GPR179	9,165 bp	17q12	Single base mutations (missense mutations, splice acceptor mutations), deletions, duplications	11/34	7101 bp	autosomal recessive	NM_001004334.4
	LRIT3	3,661 bp	4q25	Single base mutation (missense mutation), frameshift mutation	5/7	2037 bp	autosomal recessive	NM_198506.5
icCSNB	*CABP4*	1,429 bp 3,991 bp	11q13.2	Single base mutations (missense mutations, 5 prime UTR mutations, 3 prime UTR mutations, synonymous mutations, intron mutations), deletions, insertions, duplications	4/23	1410 bp	autosomal recessive	NM_206997.1 NM_145200.5
	CACNA2D4	5,343 bp	12p13.33	insert	0/9**	825 bp	autosomal recessive	NM_172364.4
	CACNA1F	6,006 bp	Xp11.23	Single base mutations (missense mutations, splice acceptor mutations), deletions, duplications	28/95	5898 bp	X-linked	NM_001256789.3
Oguchi Disease	*SAG*	1,596 bp	2q37.1	Single base mutation (missense mutation), deletion	12/26	1215 bp	autosomal recessive	NM_000541.5
	GRK1	4,233 bp	13q34	Single base mutation (missense mutation), deletion	4/4	1689 bp	autosomal recessive	NM_002929.3
CSNB 1 H	*GNB3*	1,657 bp	12p13.31	Single base mutation (unintentional mutation), deletion	2/2	1020 bp	autosomal dominant	NM_002075.4

Note: The data in the table are obtained through NIM (https://www.ncbi.nlm.nih.gov/) query statistics

*: Currently, 49 types of FA caused by *RDH* have been confirmed, and only 36 types have been registered on the NIM website. **: The mutation of *Cacna2d4* underlies a novel channelopathy leading to cone–rod dysfunction in the visual system of mice, which may become a new candidate gene for human icCSNB [74]

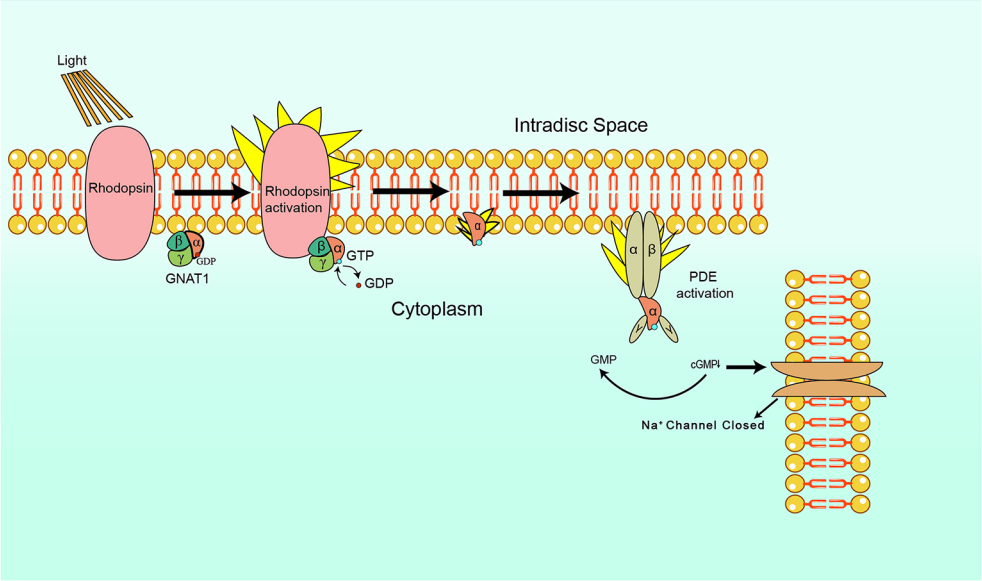


Fig. 5 Rod phototransduction cascade. Upon absorbing photons, rhodopsin becomes activated and interacts with the G protein transducin (GNAT1), leading to the activation of the α subunit of transducin. The activated α subunit then binds to the γ subunit of phosphodiesterase-6 (PDE6), subsequently activating the αβ subunits of PDE6. The activated phosphodiesterase reduces the intracellular levels of cGMP, causing the closure of cGMP-regulated cation channels [35]

autosomal dominant inheritance in affected families [36], while *SLC24A1* mutations are primarily associated with autosomal recessive inheritance, such as p.F538CfsX23 and p.(Glu801*) mutations [37, 38]. The p.Gly38Asp mutation in the *GNAT1* gene was identified [39], and additional missense mutations have been found with autosomal dominant inheritance [29, 31]. Moreover, a homozygous missense mutation in *GNAT1* was identified with autosomal recessive inheritance [30]. The *PDE6B* gene encodes the β subunit of phosphodiesterase, with the p.His258Asn mutation confirmed in a family [40], while another mutation in *PDE6B* has only been reported in a family with autosomal dominant inheritance [41]. The specific mechanisms of these gene mutations remain unclear, but "constitutive activation" may explain the desensitization and reduced light response observed in mutations of *RHO*, *GNAT1*, and *PDE6b*. The *RHO* gene encodes rhodopsin, a G-protein coupled receptor (GPCR), while the *PDE6b* gene encodes phosphodiesterase-6, and the *GNAT1* gene encodes the transducin protein. GPCRs typically exist in equilibrium between inactive (R) and active (R*) conformations. Upon photon absorption by photoreceptor cells, rhodopsin transitions to its activated state (R*), which exhibits high affinity for transducin. Upon binding, it initiates GDP-GTP exchange in transducin, activating its α subunit GNAT1. The activated α subunit subsequently activates phosphodiesterase-6, leading to a reduction in intracellular cGMP levels. This decrease causes the closure of cGMP-gated channels, promoting the release of glutamate to transmit light signals to downstream cells [19, 42] Meanwhile, the *SLC24A1* gene encodes a transporter protein that is believed to play a key role in intracellular Ca^{2+} homeostasis, though its precise mechanism requires further investigation [37].

In the rod phototransduction cascade, mutations in genes such as *RDH5*, *SAG*, and *GRK1* not only lead to rod dysfunction but also result in abnormal fundus appearances. In FA-type CSNB, the *RDH5* gene, which encodes for 11-cis-retinol dehydrogenase 5, has been associated with 49 identified mutations [43]. This gene plays a crucial role in the conversion of 11-cis-retinol to 11-cis-retinal in the retinoid cycle within retinal pigment epithelium (RPE) cells. Patients with *RDH5* mutations may exhibit "bleached" white fundus spots, which disappear after extended DA as rhodopsin levels normalize [19]. In OD -type CSNB, mutations primarily involve two genes: *SAG*, which encodes arrestin-1, and *GRK1*, which encodes rhodopsin kinase. *SAG* and *GRK1* are essential for shutting down the phototransduction cascade [44, 45]. When these genes are mutated, it would prolong the activated state of rhodopsin under light stimulation. Similarly, after extended dark adaptation, both the fundus appearance and ERG results in patients may return to normal.

Genetic defects associated with Schubert-Bornschein type CSNB

The transmission of signals from photoreceptor cells to BCs primarily depends on neurotransmitters release at the synapse. Photoreceptors consist of cone cells and rod cells. The terminal end of cone cells, known as the cone

pedicle, contains numerous synaptic vesicles filled with glutamate and features a specialized structure called the synaptic ribbon. This structure primarily forms a tight triad, composed of ON-type BC terminals synapsing within the invaginated portion of the cone cell, flanked by synapses from HCs. In contrast, OFF-type BCs form contact points at the base of the cone pedicle (Fig. 6A) [46, 47]. Most cone BCs are connected to multiple cone pedicles, while only a small proportion link to a single cone pedicle. The synaptic terminal of rod cells, known as the rod spherule, is structurally similar to the cone pedicle, featuring synaptic vesicles and a synaptic ribbon at the terminal (Fig. 6B) [48]. The cone pedicle terminal contains multiple synaptic ribbons, allowing it to transmit information to several BCs simultaneously. In contrast, the rod spherule has only a single synaptic ribbon. The mechanism of signal transmission between photoreceptor cells and BCs varies depending on lighting conditions. When exposed to light, the release of glutamate from photoreceptor cells decreases. Under these conditions, ON-type BCs depolarize, while OFF-type BCs hyperpolarize, subsequently transmitting signals to RGCs (Fig. 6C) [48, 49]. Conversely, in a dark environment, photoreceptors continuously release glutamate. Under these conditions, OFF-type BCs depolarize, while ON-type BCs hyperpolarize (Fig. 6C).

The release of glutamate is influenced by intracellular Ca^{2+} concentration, which is primarily regulated by the voltage-gated Ca^{2+} channel (Cav1.4) [3]. Mutations in genes such as *CACNA1F*, *CABP4*, and *CACNA2D4* affect components of the Cav1.4. *CACNA1F* encodes the α1 subunit, while *CABP4* encodes calcium-binding protein 4, and *CACNA2D4* encodes the α-2/δ subunit 4. These subunits are crucial for calcium channel function at photoreceptor terminals, and their mutations often lead to icCSNB [50–52]. The *CACNA1F* gene has been found to harbor missense mutations, deletions, duplications, and splice site mutations. In addition, intronic and synonymous mutations in *CACNA1F* have been discovered to cause splicing defects [53]. Mutations in the *CABP4* and *CACNA2D4* genes affect proper targeting of synaptic membrane channels, regulation of calcium currents, and calcium channel activity [54–58]. Furthermore, mutations in *CACNA1F*, *CABP4*, and *CACNA2D4* may also result in a reduction in their own gene expression, leading to loss of function, which can decrease calcium channel activity, thereby disrupting glutamate release [3].

Bipolar cells can be classified into ON-type and OFF-type, which express different glutamate receptors and thus respond differently to light stimuli. ON-type BCs express metabotropic glutamate receptor 6 (GRM6/mGluR6) [59–61], showing a depolarizing response to light [62–64], while OFF-type BCs express ionotropic

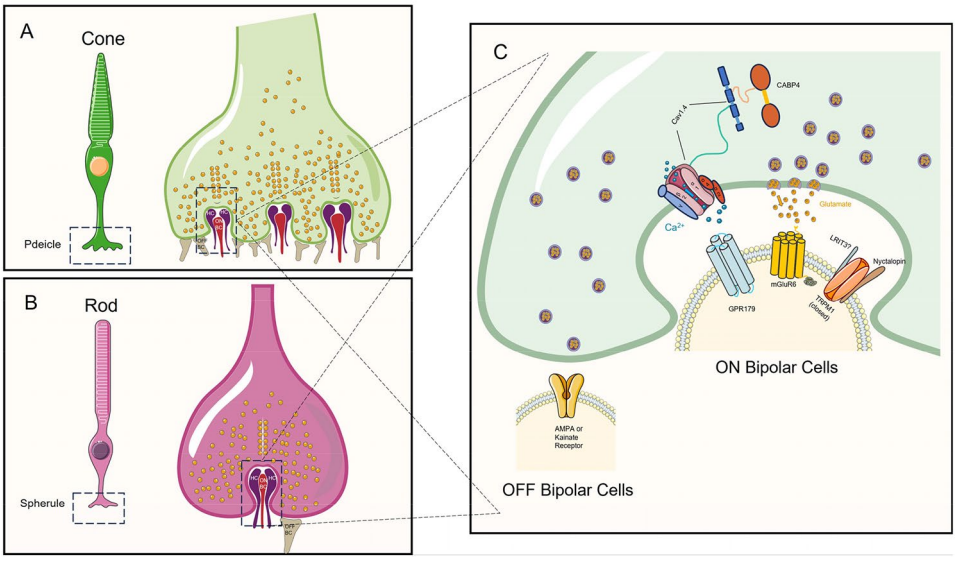


Fig. 6 Signal transmission between photoreceptors and BCs. (**A**). A cone pedicle, located at the synaptic terminal of cone cells, features synaptic ribbons situated on the invaginated dendrites of HCs and ON bipolar cells (ON BC). This synaptic arrangement is referred to as the "triad" structure. Multiple synaptic ribbons are present at the cone cell terminal. The dendrites of OFF bipolar cells (OFF BC) form contacts at the base of the cone pedicle. (**B**). A rod spherule, located at the synaptic terminal of rod cells, contains a single synaptic ribbon at the terminal, positioned on the invaginated dendrites of HC and ON BC. The dendrites of OFF BC form contacts at the base of the rod spherule. (**C**). Transmission of light signals between photoreceptors and BCs. In well-lit conditions, the release of glutamate into the synaptic cleft decreases. This leads to the depolarization of ON BCs, while the reduction in glutamate signals received by the AMPA or kainate receptors of OFF BCs causes their hyperpolarization. In contrast, under dark conditions, glutamate release increases. The mGluR6 receptors on ON BC receive the glutamate, causing hyperpolarization of ON BC, while OFF BC depolarize. The specific mechanism of Lrit3 remains unclear

glutamate receptors and undergo hyperpolarization when the light exposure [49, 65–67]. This difference in receptor expression helps explain the distinct phenotypes seen in patients with icCSNB.

In cCSNB, ON-type BCs are primarily affected due to mutations in genes like *GRM6*, *GPR179*, *LRIT3*, *TRPM1*, and *NYX*. The *GRM6* gene encodes metabotropic glutamate receptor 6 (mGluR6), which, upon receiving the glutamate signal [59, 60], activates the α-subunit of G protein [68], thus controlling the closure of the TRPM1 channel. Under light conditions, reduced glutamate release from photoreceptors leads to the opening of the TRPM1 channel, causing depolarization of ON-type BCs [62]. In this process, the *NYX* and *LRIT3* genes influence the localization of the TRPM1 channel at the dendritic tips of BCs [69, 70], while the *TRPM1* gene encodes transient receptor potential cation channel subfamily M member 1, which contributes to the formation of the TRPM1 ion channel [71]. *GPR179*, which encodes G protein-coupled receptor 179, regulates mGluR6 activity and can directly interact with TRPM1 [72]. Mutations in these genes include missense mutations, splice site mutations, large deletions, small deletions, and duplications [19], which result in the absence of b-wave in the ERG of cCSNB patients.

Defects related to the GNB3 gene

The GNB3 gene encodes the β3 subunit, which is a component of the G-protein heterotrimer. GNB3 is expressed in both cone photoreceptors and ON-type BCs. Biallelic mutations in GNB3 result in dysfunction of the G-protein complexes, specifically Gαt2β3γt2 in cone photoreceptors and Gαoβ3γ13 in cone and ON-type BCs. Consequently, patients with CSNB caused by GNB3 mutations exhibit a dual molecular phenotype and reduced cone photoreceptor sensitivity [32, 73].

Animal models related to CSNB

Animal models are invaluable tools for studying and developing treatments for human diseases. In the case of CSNB, many pathogenic mechanisms and genetic defects have been uncovered through research on animal models. Furthermore, prior to clinical studies, animal models serve as the best candidates for exploring CSNB treatments. Specific animal models of CSNB are either naturally occurring mutations [75] or are artificially constructed. One of the most effective methods involves using genome-editing techniques to directly create animal models with specific mutated genes [76]. Animal models can closely mimic the genetic mutations found in human patients, but there are still genetic structural differences compared to humans. Gene-humanized animal models can help reduce these differences to some extent. Unlike directly mutating the animal genome, gene-humanized models replace the corresponding animal gene with a specific human gene in situ through genome-editing techniques, such as homology-directed repair (HDR) [77, 78], which not only simulates the mutation but also better reflects the human genomic structure. These models thus provide more valuable data for preclinical studies.

In this review, we have summarized several CSNB animal models in Table 2, including mice, horses, and dogs, along with their disease phenotypes. The advantage of animal models lies in their ability to provide a direct understanding and analysis of pathological features, which can then be extrapolated to humans. Additionally, behavioral tests can be conducted on animal models to assess disease symptoms or the effectiveness of treatments. Despite their advantages, animal models also have limitations. Species-specific differences in tissue structures (e.g., the eye) and genetics must be considered when extrapolating results to humans. Nevertheless, these models remain irreplaceable in understanding and developing treatments for CSNB.

Principles and methods of gene therapy

Gene therapy, as an emerging medical technology, aims to correct abnormal gene expression in the body through methods such as gene editing or replacement, thereby restoring normal protein function and cellular signaling. The current methods used in gene therapy primarily include: gene replacement therapy, which employs viral vectors (such as adeno-associated virus, AAV) to introduce healthy genes into retinal cells to replace defective ones; gene editing, which utilizes gene editing technologies like CRISPR/Cas9 to directly repair mutated genes; and gene regulation, which involves modulating the expression of mutated genes through RNA interference or other means.

Gene replacement therapy

Gene replacement therapy involves delivering healthy genes to specific cells using viral or non-viral vectors to replace defective ones. This method is well-suited for treating single-gene mutations. One major advantage of gene replacement therapy is that it can restore normal gene expression without permanently altering the original gene, thereby minimizing the risk of unintended consequences. The first ophthalmology gene therapy drug approved by the FDA, Luxturna, successfully rescued patients with Leber congenital amaurosis (LCA) caused by *RPE65* gene mutations through the delivery of the normal *RPE65* gene via AAV2 [4]. Apart from the approved Luxturna, gene delivery treatments for retinal diseases such as retinitis pigmentosa (RP), color blindness, and Usher syndrome are still in clinical trials. Although gene replacement therapy is straightforward and effective, its

Table 2 Animal models of CSNB

Species	defective gene	source	mutation type	disease phenotype	ref
Cat	*Rdh5*	naturally occurring	Missense mutations in *Rdh5* c.542G > T;p.Gly181Val	Thinning of central retinal area.	[79]
Dog	*Lrit3*	naturally occurring	A base deletion that occurs in *Lrit3* c.762_763delG	The b-wave disappears in dark-adapted ERG images Bright-adapted ERG b-wave reduction.	[80]
Dog	*Sag*	naturally occurring	Exon 16 c.1216T > C p.*405Rext*25	Data not shown, but late onset of initial visual loss in dim light (night blindness), which gradually progress in some cases to total blindness. Thin retinal blood vessels, pallid optic nerve head, irregular pattern of tapetal reflectivity.	[81]
Fly	*mtt*	RNAi interference	Decreasing the expression of *mtt* gene through RNAi interference	*Drosophila* eye structures are disrupted, especially in adult flies.	[82]
Horse	*Grm6*	naturally occurring	Missense mutations in *Grm6* c.533 C > T, p.178Thr > Met	Dark-adapted ERG b-wave disappears, Ming-adapted ERG a-wave amplitude is normal but the trough becomes wider.	[83]
Horse	*Trpm1*	naturally occurring	Downregulation of Trpm1, 1378 bp long terminal repeat (LTR) insertion in intron 1 of *Trpm1*	Dark and light adapted: b-wave missing.	[84]
Mouse	*Gpr179*	KO	A gene cassette containing lac Z and neomycin is inserted into intron 1 of *Gpr179*	In dark-adapted ERG images, b-wave disappears, light-adapted ERG a and b-waves are delayed, b-wave amplitude is significantly reduced, and RGS7, RGS11 and GNB5 are missing at the dendritic tips of ON-type bipolar cells.	[85]
Mouse	*Lrit3*	KO	Knocking out the remaining part of exon 3 (21 bp) of exon 4 and inserting c.611_2046delinsGCCCATAG resulted in premature termination. p.Phe204Trpfs*3	In dark-adapted ERG images, b-wave disappears, light-adapted ERG a and b-waves are delayed, b-wave amplitude is significantly reduced, and the INL layer becomes thinner.	[86]
Mouse	*Cacna1f*	naturally occurring	A single base change and a 10 bp insertion occurred between exons 1, 3 and 14 of the *Cacna1f* gene. The insertion resulted in a frameshift mutation in exon 14.	The b-wave of dark-adapted ERG images is severely reduced, the OPL layer becomes thinner, and retinal M and S proteins decrease with age. In addition, there is miss localization of retinal proteins.	[87]
Mouse	*Grm6*	naturally occurring	Missense mutation in *Grm6* gene p.Met66Leu	Dark adaptation ERG b-wave weakening The b-wave of ERG is severely weakened during bright adaptation.	[88]
Mouse	*Rho*	naturally occurring	Exon 1 c.269G > A and c.270 A > C p. Gly90Asp	Loss of rod sensitivity. Depending on the amount of endogenous rhodopsin and p. Gly90Asp allele retinal degeneration.	[89]
Mouse	*Gnat1*	genetically modified	Exon 2 probably c.113G > A p.Gly38Asp	Reduced rod photoreceptor responses.	[90]
Mouse	*Gnat1*	genetically modified	Exon 6 not clear p.Gln200Leu	Not clear if in Gnat1þ/ background. But in high levels of transgene reduced rod photoreceptor responses.	[91]
Mouse	*Gnat1*	genetically modified	In exon 4–5 neomycin selection cassette inserted	Gnat1/ no rod b-wave, no a wave (from rods) but normal cone b-wave Gnat1þ/ morphology normal; Gnat1/ mild age dependent retinal degeneration.	[92]
Mouse	*Pde6b*	genetically modified	Exon 4 c.772 C > A p.His258Asn	Selective loss of b-wave or a and b-wave reduction. ERG findings dependent on genetic background.	[35]
Mouse	*Rdh5*	KO	In exon 1, 2 and 3 neomycin selection cassette inserted	Delayed dark adaptation. Delayed 11-cis-retinal regeneration, delayed dark adaption kinetics no other ERG abnormalities in dark adapted KO mice in fully dark-adapted mice.	[93]
Mouse	*Sag*	KO	In 5'promoter elements and in the first two exons neomycin selection cassette inserted	Prolonged Photoresponses Increased susceptibility to light damage, fundus abnormalities not investigated.	[94]
Mouse	*Grk1*	KO	In exon 1 neomycin selection cassette inserted	Prolonged Photoresponses Increased susceptibility to light damage, fundus abnormalities not investigated.	[94]
Mouse	*Cacna1f*	Cacna1fDEx7 Total KO	In exon 7 self-excising Cre-loxneo cassette inserted p.Gly305*	No CACNA1F in the OPL, thinner than normal OPL, develop ectopic neurites from DBCs and horizontal cells, absence of optokinetic responses Severe icCSNB or cone-rod dystrophy.	[95]

Table 2 (continued)

Species	defective gene	source	mutation type	disease phenotype	ref
Mouse	*Cacna1f*	Spontaneous (nob2) in AXB-6/PgnJ strain, incomplete KO	In exon 2 transposon insertion leading to 2 transcripts: 90%:* in exon 2, 10%:	light adapted: b-wave, cone responses are reduced No CACNA1F in the OPL, thinner than normal OPL, develop ectopic neurites from DBCs and horizontal cells, but normal optokinetic responses.	[96]
Mouse	*Cacna1f*	KO	In exon 14–17 neomycin cassette inserted	Dark adapted: a-wave normal, b-wave absent, light adapted: b-wave absent cone function reduced by behavioural tests; heterozygous mice reduction of b-wave and cone function in between WT and hemizygous mice consistent with X-inactivation, No CACNA1F in the OPL, and the IPL, develop ectopic neuritis from DBCs and horizontal cells; heterozygous mice patchy pattern probably due to mosaic defects.	[97]
Mouse	*Cacna1f*	Knock-in mimicking gain of function human mutation Exon 17 c.2234T > C p.Ile745Th	Exon 17 c.2267T > C p.Ile756Thr	Dark adapted: reduced b waves, light adapted: b-wave, cone responses are reduced Disperse staining of CACNA1F, extending into ONL. Most synapses immature but some mature, thinner OPL, ONL, cones were shorter and few cones sprout, develop ectopic neurites from DBCs, reduction in expression of Cacna1f, β_2 and $\alpha_2\delta$-4.	[98]
Mouse	*Cabp4*	Cabp4DEx1-2 KO	In exon 1 and part of exon 2 neomycin cassette inserted, protein is not detectable	Dark adapted: a-wave reduced, b-wave missing, light adapted, a- slightly reduced, b-wave severely reduced Thinner OPL, reduction in the number of synaptic ribbons and photoreceptor terminals and deflation of rod spherules and cone pedicles. Formation of ectopic synapses between rods and rod bipolar or horizontal cells in the outer nuclear layer.	[99]
Mouse	*Cacna2d4*	naturally occurring	In exon 25 c.2451dup p.Gly818Argfs*15 reduced RNA	a-wave reduced, b-wave missing, cone ERG absent Thinner OPL, loss of ribbon synpases, degeneration of rods.	[74]
Mouse	*Nyx*	naturally occurring	Exon 4 c.567_651del p.Ile189Metfs171*	Dark and light adapted: b-wave missing heterozygous mice reduction of b-wave consistent with X-inactivation.	[100]
Mouse	*Grm6*	KO	In Exon 8, neomycin selection cassette inserted	Dark and light adapted: b-wave missing.	[101]
Mouse	*Grm6*	naturally occurring	Mutation in intron 2 leads to splice site with larger transcript: c.486þ648C > T, r.486_487þ486ins582_6446, p.Ile163Glyfs*103	Dark and light adapted: b-wave missing.	[102]
Mouse	*Grm6*	N-ethyl-N-nitrosourea (ENU) induced (nob4)	Exon 3 c.553T > C, p.Ser185Pro	Dark and light adapted: b-wave missing.	[102]
Mouse	*Trpm1*	KO	In exons 4–6 neomycin selection cassette inserted	Dark and light adapted: b-wave missing.	[62]
Mouse	*Trpm1*	ENU induced	Exon 23 c.3202G > A, p.Ala1068Thr	Dark and light adapted: b-wave missing Heterozygous mice show reduction of b-wave.	[100]
Mouse	*Gpr179*	naturally occurring	Transposon insertion (~6.5 kb) in intron 1	Dark adapted: b-wave missing, light adapted: severely reduced till missing.	[103]
Mouse	*Grm6*	naturally occurring	A G > A transition occurred at the last position of *Grm6* exon 8, resulting in incorrect splicing and extending exon 8 by 28 base pairs.	The b-wave is missing in both dark-adapted and light-adapted ERGs, and mGluR6 protein is missing at the tips of DBC dendrites.	[104]
Mouse	*Rho*	KI	A DNA fragment of approximately 1.3 kb containing the G90D-Rho cDNA was inserted before the start codon of the arrestin-1 gene in mice.	Mutant G90D-rhodopsin produces dark continuous noise.	[105]

Table 2 (continued)

Species	defective gene	source	mutation type	disease phenotype	ref
Rat	*Cacna1f*	naturally occurring	Exon 23 c.2941 C > T p.Arg981*	a-wave reduced, b-wave missing, cone responses reduce Rod bipolar and horizontal cells reduced, but neither rod bipolar nor horizontal cells dendrites were observed to extend beyond the OPL in the rat, behavioral differences.	[106]
Zebrafish	*Cacnafa*	ENU induced	Exon 5 c.626T > A p.Leu209* c.3430 C > T p.Gln1144*	Reduced a-wave, delayed and reduced b-wave Thinner OPL.	[107, 108]
Zebrafish	*Nyx*	KO	Morpholino against the translation site	b-wave missing.	[109]
Zebrafish	*Grm6b*	KO	Morpholino against the translation site	b-wave missing.	[110]
Zebrafish	*Gpr179*	KO	Morpholino against the translation site	b-wave missing.	[103]

efficacy is limited by the packaging capacity of the vectors, and continuous administration is required during treatment. Additionally, the effectiveness of the therapy can be influenced by the expression of the gene [111].

Gene editing therapy

Gene editing therapy offers the potential for a lifelong cure by directly correcting mutated genes. Early gene editing primarily relied on zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). However, due to the complexity of their processes and the large number of components required, their use in hereditary retinal diseases has been limited [112]. In recent years, the CRISPR gene editing system has become a popular tool in the field. Like ZFNs and TALENs, the CRISPR system performs gene editing by inducing double-strand breaks (DSBs). However, CRISPR is guided by single guide RNA (sgRNA) rather than relying on nuclease proteins, making it possible to edit different target sites simply by altering the sgRNA sequence [113, 114].

Among the CRISPR protein family, the most commonly used is the Type II CRISPR/Cas9 protein [115]. Under the guidance of sgRNA, CRISPR/Cas9 induces DSB in DNA, which are primarily repaired through two pathways: HDR and non-homologous end joining (NHEJ). The HDR pathway, using provided DNA as a template, allows precise insertion of any desired edit, whereas NHEJ-guided repair, which requires no template, results in insertions and deletions (Fig. 7A). However, since HDR mainly occurs in dividing cells, and most retinal cells are in the post-mitotic phase, the editing efficiency of HDR in retinal cells is significantly reduced [116]. To address this, an early method called microhomology-mediated end joining (MMEJ) was developed to facilitate gene editing in post-mitotic cells. By taking advantage of NHEJ's activity at any stage of the cell cycle, microhomology arms of about 5–25 bp are added to both ends of the DSB and the homology arms, allowing gene insertion [117]. Building on this approach, Suzuki et al. designed a method called homology-independent targeted integration (HITI). This technique uses Cas9-targeting sites designed at both ends of the template gene segment to integrate the segment directly into the target site without the need for homology arms. Compared to MMEJ, HITI offers higher editing efficiency and more precise results (Fig. 7A). However, a downside of HITI is that the integrated genome still retains some "scars" from the cutting process, which may introduce uncontrollable risks [118]. In more recent research, a repair pathway called single homology arm donor-mediated targeted integration (SATI) was demonstrated. In SATI, one side of the donor contains both a Cas9-targeting site and a homology arm. Through a combination of HITI and an HDR mechanism that has not yet been fully elucidated, donor DNA is precisely integrated into the target genome, with all integrations being perfectly seamless [119].

With continued research on the CRISPR system, various engineered variants have been developed, such as base editors (BEs) and prime editors (PE). BEs can induce single-base mutations without generating DSBs. The initial base editors enabled base conversion from C•G to T•A (CBE) and A•T to G•C (ABE) through deamination reactions after binding a DNA deaminase to the DNA strand [120, 121] (Fig. 7B). Base editors are more suitable for correcting genetic diseases caused by single-base mutations, theoretically capable of correcting 30% of human pathogenic mutations using CBE or ABE [122]. As research on base editing continues, more highly active and low off-target BEs are being developed. Currently, base editors can achieve conversions from A to C, A to Y, and C to G [123]. Recently, gCBE and gTBE, which do not rely on deaminases, were developed. These new editors efficiently convert C to G without deaminases (gCBE) and, for the first time, enable T to C or T to G conversions (gTBE) [124], further expanding the application of

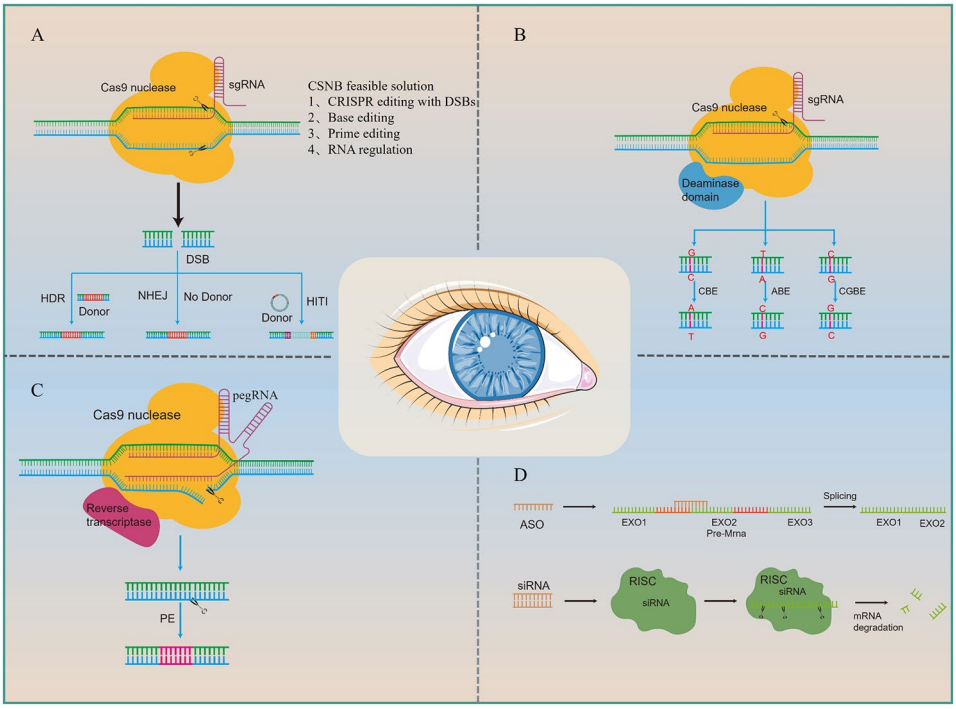


Fig. 7 Possible gene therapy strategies for CSNB. (**A**). CRISPR-mediated gene editing: Taking Cas9 as an example, gene editing occurs by creating a double-strand break at the target site, followed by editing through the DNA repair mechanisms, including HDR (Homology-Directed Repair), NHEJ (Non-Homologous End Joining), or HITI (Homology-Independent Targeted Integration). (**B**). Single-base editing: Classic single-base editing does not require cutting the DNA strand; instead, it achieves single-base conversion through deamination reactions. Currently, various types of base editors have been developed, as illustrated in the image, including CBE (Cytosine Base Editor), ABE (Adenine Base Editor), and CGBE (Cytosine-Guanine Base Editor). (**C**). Prime editing: A novel editing approach that introduces specific sequences from the RT template of pegRNA through reverse transcription after cutting one strand of DNA, theoretically allowing the introduction of any mutation at any position. (**D**). RNA regulation: The process of correcting erroneous RNA expression by regulating RNA splicing or processing through ASO (Antisense Oligonucleotides) or siRNA (small interfering RNA)

base editors. PE is the latest CRISPR/Cas9-based editing method, theoretically capable of introducing all types of point mutations [122]. The PE editor consists of a Cas9 nuclease and a prime editing guide RNA (pegRNA). The pegRNA serves both as a guide and as a reverse transcriptase template, allowing the introduction of desired edits through reverse transcription (Fig. 7C). Like BEs, prime editing does not generate DSBs and poses no risk of bystander editing [125]. Since the first PE1, the PE editing system has undergone several iterations. The latest generation, PE6a-g, is smaller and more efficient than its predecessors, making it more suitable for in vivo delivery and further expanding its range of applications [126].

Currently, there are no reported cases of gene editing therapy for CSNB, but gene editing has already been applied to the treatment of other IRDs. For example, an intronic mutation (IVS26) in the CEP290 gene is known to cause aberrant splicing of the protein, leading to LCA10. Maeder et al. utilized the principles of CRISPR editing and employed Staphylococcus aureus Cas9 (SaCas9) with a dual-cut strategy to remove the harmful mutation, thereby restoring the normal splicing of the CEP290 transcript [127]. Building on this work, Editas Medicine developed a gene therapy agent called EDIT-101, which was used in a Phase 1/2 clinical trial for LCA10 (NCT03872479). EDIT-101 employs a single AAV vector to deliver SaCas9 driven by the GRK1 promoter, along with two sgRNAs. This approach targets the IVS26 point mutation in intron 26 of the CEP290 gene, introducing DSBs at both ends of the mutation to excise the mutated sequence and restore normal protein splicing. The results demonstrated that EDIT-101 exhibited good safety and significantly improved vision in some patients [128]. In addition to EDIT-101, two other therapies have also entered the clinical stage: ACDN-01 (NCT06467344), targeting retinal degeneration, and ZVS203e (NCT05805007), aimed at treating RP. While there are currently no clinical reports on base editing or prime editing, these approaches have demonstrated their safety and efficacy in animal studies [129].

Gene regulation by antisense oligonucleotides (ASOs) and RNA interference (RNAi)

ASOs are short oligonucleotides, typically ranging from 15 to 30 nucleotides in length. These artificially designed ASOs regulate RNA transcripts by binding to them through base pairing. ASOs primarily achieve RNA-level regulation through two mechanisms: first, by targeting

and binding to RNA transcripts, leading to their degradation and a subsequent reduction in gene expression; second, ASOs can bind to precursor mRNA to obstruct the normal function of splicing factors, thereby causing exon skipping (Fig. 7D) [130]. Consequently, ASOs can not only silence the transcription of dominant pathogenic genes but also correct aberrant splicing caused by gene mutations (Fig. 7D). Early-designed ASOs were highly susceptible to degradation in vivo, but the third generation of phosphorodiamidate morpholino oligomers (PMOs) demonstrates exceptional stability and targeting affinity [131], enabling their use in clinical trials. Although recent studies on wild-type mouse retinas have shown that ASOs modified with 2'O-methyl-phosphorothioate (2-OMe/PS) and 2'O-methoxyethyl-phosphate (2-MOE/PS) exhibit comparable efficacy and safety. In contrast, octa-guanidine-dendrimer-conjugated in vivo PMO-oligonucleotides (ivPMO) caused toxicity [132]. The c.2991 + 1655 A > G mutation (p.Cys998X) in the CEP290 gene causes abnormal mRNA splicing and the production of a nonfunctional CEP290 protein, leading to LCA10. ProQR developed an ASO therapy called Sepofarsen, initiating clinical trials for its use in treating LCA10. In an open-label Phase 1b/2 clinical trial (NCT03140969), 11 participants (6 adults and 5 children) experienced improved vision following treatment. However, varying degrees of adverse reactions were observed in subsequent follow-ups [133]. Similarly, in a subsequent Phase 2/3 study (ILLUMINATE) evaluating the treatment of 36 patients (NCT03913143), the results failed to meet expectations [129]. Similarly, ProQR's other ASO therapy, Ultevursen, encountered adverse reactions during clinical trials for the treatment of retinitis pigmentosa (RP) caused by exon 13 mutations in the USH2A gene (Sirius) (NCT05158296). These findings highlight the need for further improvements in the safety of ASOs therapies in the future.

siRNA is a double-stranded RNA approximately 19–22 nucleotides long, which can be introduced into cells exogenously. It binds to various proteins to form RNA-induced silencing complexes (RISC) that cleave and degrade RNA, thereby silencing mutated RNA (Fig. 7D). This mechanism is known as RNAi [134]. Age-related macular degeneration (AMD) is the third leading cause of blindness globally. In addition to established environmental risk factors and genetic predisposition, miRNA regulation is considered a pathogenic mechanism. As an acquired retinal degeneration, vision loss in AMD is typically caused by abnormalities in the RPE and the formation of retinal drusen, progressing to geographic atrophy and/or choroidal neovascularization [135]. Two clinical drugs, Cand5 (NCT00306904) and siRNA-027 (NCT00363714), have been developed for the treatment of AMD. While these treatments led to some degree of vision recovery in patients, most exhibited adverse reactions following the therapy [135].

Genetic mutations in inherited diseases can generally be categorized into three types: loss-of-function mutations, gain-of-function mutations, and dominant negative mutations. When conducting gene therapy, it is essential to select the appropriate treatment strategy based on the specific mutation involved. For CSNB, most mutations in patients are loss-of-function mutations [5, 136, 137]. We believe that gene replacement therapy is suitable for most CSNB patients. However, in cases with harmful mutations (e.g., the c.698 C > T, p.(Pro233Leu) mutation in the VSX2 gene [138]), where the deleterious gene continues to be expressed, gene replacement may not be the most appropriate strategy. As for gene editing, this method corrects the mutated gene at the DNA level, and since DNA is the "starting point" for functional expression, we believe gene editing could be applicable to all types of mutations. ASO and RNAi, which regulate at the RNA level, have more limited applications. For example, ASO is particularly suitable for cases of CSNB caused by RNA splicing errors.

Delivery strategy selection

Effective gene therapies for retinal diseases require efficient and safe delivery to the targeted tissues. Currently, the main routes of administration for retinal diseases are intravitreal injection (IVT) and subretinal injection (SRI). IVT is a relatively safe and straightforward ocular injection technique (Fig. 8A). The drug injected into the vitreous body can diffuse throughout the vitreous fluid and reach the retina. However, in practice, the diffusion of the drug from the vitreous can be affected by various factors, such as the dilution of the vitreous fluid [139], interference from immune response mechanisms [140, 141], barriers posed by the internal limiting membrane [142], and diffusion distance [143]. These factors limit the diffusion efficiency of the drug within the vitreous. SRI is currently a more commonly used route of administration in clinical settings. By injecting the drug directly into the subretinal space, a smaller volume of drug can achieve better results compared to intravitreal injection (Fig. 8B). The immune privilege of the subretinal space results in a smaller immune response [111]. However, the disadvantages of SRI should not be overlooked. Since the subretinal space cannot be directly visualized, this method often requires the use of precise instruments to minimize operational risks. Additionally, because SRI is a mechanical procedure, it may potentially lead to retinal detachment [144]. Even when operators are professionally trained, caution is essential during the procedure.

To effectively target specific sites, drugs require suitable carriers for encapsulation and delivery. Retinal drug carriers are typically classified into viral and non-viral

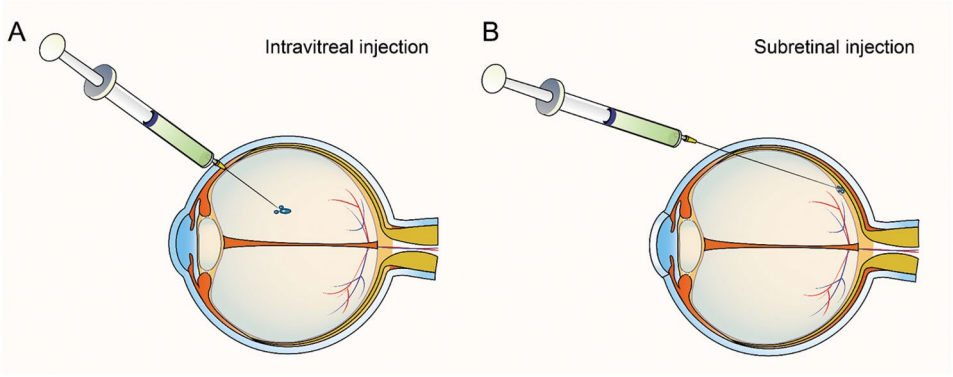


Fig. 8 Different ocular drug delivery methods

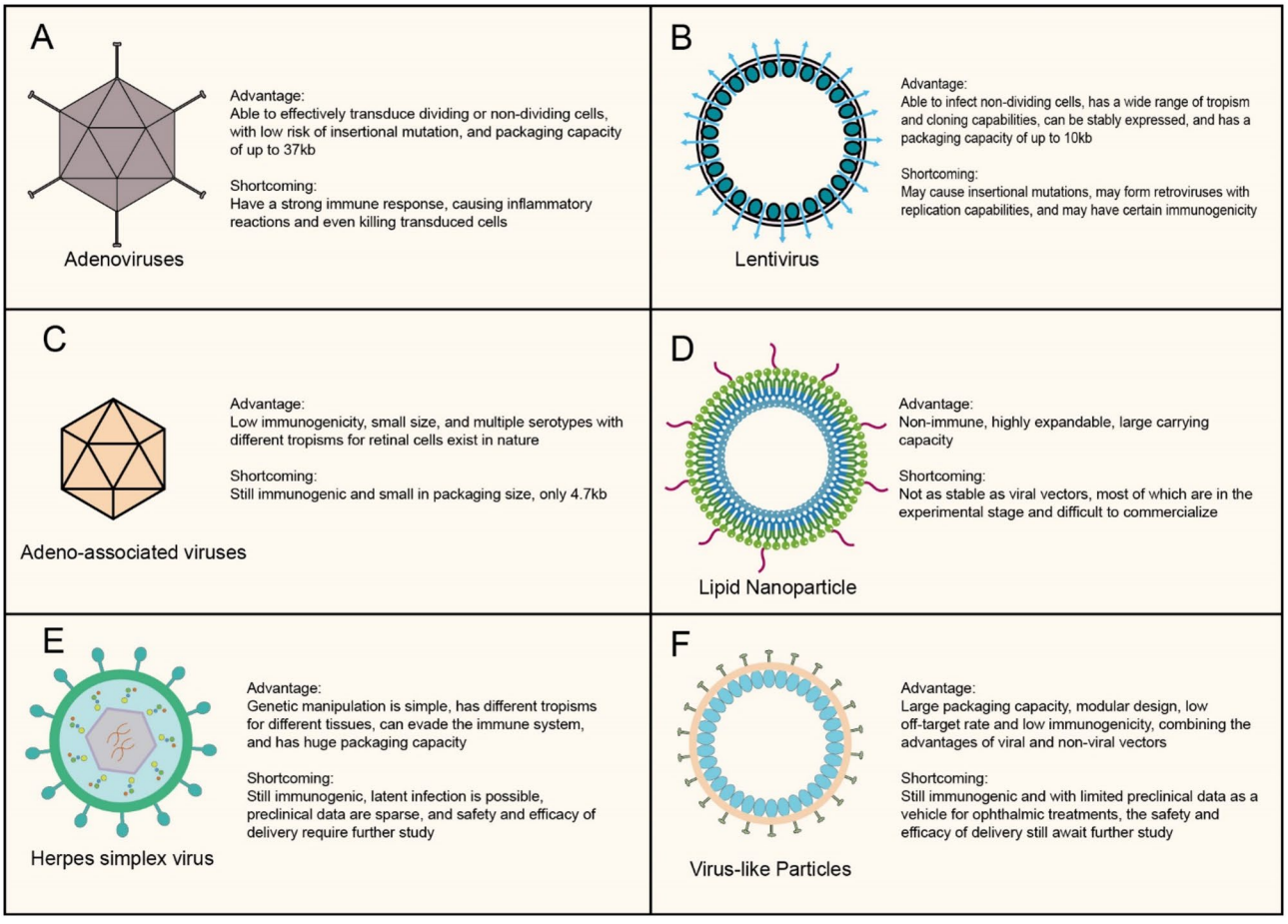


Fig. 9 Comparison of different delivery carriers in ophthalmic diseases. The primary vectors for gene therapy in the eye are either viral or non-viral. Viral vectors include lentivirus, adenovirus, AAV, and herpes simplex virus, with AAV being widely used in clinical applications. Among non-viral vectors, nanoparticles such as lipid nanoparticles (LNPs) and virus-like particles (VLPs) are prominent. However, since these technologies have emerged more recently, most remain in the preclinical stage

vectors (Fig. 9). Viral vectors mainly include lentiviruses [145], adenoviruses [146], and AAVs [147]. Among these, AAVs exhibit varying tropisms for different retinal structures [148] and do not induce disease. Their small size (approximately 4.7 kb) enables them to overcome most barriers within the eye, making AAVs the preferred viral vectors for ocular gene therapy. The key to effective AAV delivery lies in the selection of serotype. The AAV serotype determines its targeting ability and delivery efficiency. Currently, different wild-type AAV serotypes have been identified, with AAV-1, 2, 5, 8, and 9 confirmed to transduce RPE and photoreceptor cells upon subretinal

injection [149]. AAV-1, 2, 4, and 5 have been shown to achieve high levels of retinal gene delivery [150].

As research progresses, wild-type AAV serotypes alone are insufficient to meet current study demands. Two primary approaches, rational design and directed evolution, are used to obtain desired AAV serotypes. Rational design involves modifying the AAV capsid based on existing knowledge to enhance transduction efficiency [151]. In contrast, directed evolution employs extensive screening to isolate suitable AAVs, such as through targeted selection from existing AAV libraries [152]. AAV7m8 is a special AAV serotype generated through directed evolution, capable of efficiently delivering genes to all retinal layers in non-primate animals [143]. Similarly, AAV8BP2, derived from AAV8 via directed evolution, exhibits efficient transduction of BCs, unlike the 7m8 serotype [153]. However, due to interspecies differences in retinal structure, neither AAV7m8 nor AAV8BP2 performs as expected in non-human primates (NHP) evaluations [154]. A recent study showed that a rationally designed AAV variant, AAVv128, efficiently transduces photoreceptors and RPE cells through both intravitreal and subretinal injection. Notably, AAVv128 demonstrates broad transduction across different species, including mice, rabbits, and NHPs [155]. Besides these serotypes, other AAVs targeting retinal cells have also been reported, and many AAVs are approved for use in clinical trials for IRDs (Table 3). When using AAVs, considerations regarding their packaging capacity and potential immune responses are essential. The original AAV packaging size is about 4.7 kb, which limits its ability to carry gene editing tools or longer cDNA sequences. Researchers have developed a dual AAV delivery system, dividing the cargo into two segments loaded into different AAVs. After delivery, large gene fragments can be reassembled through trans-splicing, homologous recombination, or a combination of both [156], increasing the AAV capacity to 9 kb. Furthermore, a triple AAV system has been developed, enhancing capacity to 14 kb [157]. While dual or triple AAVs increase capacity, the success rate decreases with the number of segments combined. Additionally, studies indicate that multi-AAV systems may produce short peptides that could raise safety concerns [157–159]. Apart from multiple AAV delivery, developing smaller editing tools can also alleviate the AAV cargo burden [160, 161]. Since AAVs and their delivered genes are introduced exogenously, they can provoke immune responses in patients, especially with intravitreal injections. In clinical settings, the magnitude of immune responses is a safety indicator for AAV retinal therapies. Research has shown that high doses of AAV can lead to more severe immune reactions [162]. Therefore, reducing injection dosages can help mitigate inflammation. Additionally, strategies such as chemical modifications [163, 164], shell induction [165], and directed evolution or rational design to construct less immunogenic AAV capsids may reduce immune responses, though these approaches may require further development before clinical application.

In addition to commonly used viral vectors, herpes simplex virus (HSV) vectors are also under development. HSV, an enveloped virus, carries over 150 kb of double-stranded DNA [166], with approximately half of its genome being replaceable. This endows HSV with a significantly higher payload capacity than AAV [167]. Among its eight subtypes [168], HSV-1 demonstrates neurotropism [169] and has been shown to enable sustained expression in the RGC and RPE layers [170]. Compared to other viral vectors, HSV offers lower immunogenicity, a much larger packaging capacity, and cell-specific lytic effects [166]. Currently, HSV-based clinical trials are primarily focused on cancer therapies. For retinal genetic diseases, HSV remains in the early stages of research, with further preclinical data needed to confirm the safety and reliability of HSV-mediated gene therapy for eye treatments.

In addition to its packaging limitations, AAV vectors have been associated with approximately 35% of therapy-related serious adverse events in ophthalmic clinical trials [171]. As a result, some researchers are seeking safer, non-viral alternatives for ocular drug delivery, such as lipid nanoparticles (LNPs) and virus-like particles (VLPs).

With the advent of COVID-19 vaccines [172], LNPs have garnered significant attention. As non-viral vectors, LNPs are composed of structural lipids, cationic or ionizable lipids, PEG-lipids, and cholesterol in varying proportions [173]. They enter cells through endocytosis [174] and release their encapsulated cargo. The safety of LNPs has been validated in clinical settings [174, 175], and their modular design [176, 177] allows for customization based on specific needs. In retinal diseases, studies have demonstrated that amine-modified polyethylene glycol (PEG) [178] and peptide conjugation on the LNP surface [179] can facilitate gene editing in the retina. Notably, research has shown that LNPs can effectively transduce human retinal and RPE cells [180]. Many LNPs for ocular gene therapy are currently under development, and LNPs may emerge as a valuable therapeutic tool for retinal diseases alongside AAVs in the future.

VLPs are widely used in vaccine development, and in recent years, they have also been explored as vectors for gene delivery. VLPs are protein nanoparticles that resemble viruses but lack viral genetic material, meaning they cannot replicate and are non-pathogenic [181]. Typically formed through the spontaneous assembly and budding of retroviral polyproteins, VLPs have a high packaging capacity and can be engineered for cell-specific targeting by modifying their envelope glycoproteins [182]. Studies

Table 3 AAVs used for IRD clinical applications

AAV serotypes	Disease	Targeted genes (drugs)	Promoter	Injection method	Transduction site	Phase	Clinical number
AAV2	CHM	RPE1	CAG	subretinal	Photoreceptors and RPE	Phase 2	NCT02407678
AAV2	AMD	CFI complement factor	CMV	subretinal	Photoreceptors and RPE	Phase 1/2	NCT03846193
AAV2	RP	MCO	CMV	Intravitreal	Inner nuclear layer (INL)	Phase 1/2a	NCT04919473
AAV2	RP	MCO-010	N/A	Intravitreal	Bipolar cells	Phase 2	NCT04945772
AAV2	RP	hMERTK	VMD2	subretinal	RPE	Phase 1	NCT01482195
AAV2	LCA	RPE65v2	CBA	subretinal	RPE	Phase 3	NCT00999609
AAV2	LCA	RPE65	CMV	subretinal	RPE	Phase 1	NCT00481546
AAV2	CHM	CHM	CAG	subretinal	RPE	Phase 2	NCT03507686
AAV2	RP	ChrimsonR-tdTomato	CAG	Intravitreal	RGCs	Phase 1/2a	NCT03326336
AAV2	LCA	RPE65	CMV	subretinal	RPE	Phase 1	NCT00821340
AAV2	CHM	RPE1	CAG	subretinal	Photoreceptors & RPE	Phase 2	NCT02077361
AAV2	CHM	RPE1	CAG	subretinal	Photoreceptors & RPE	Phase 2	NCT02553135
AAV2	LHON	ND4	CMV	Intravitreal	Macular RGCs	Phase 3	NCT03293524
AAV2	AMD	sFLT-1	CMV	subretinal	Anti-angiogenesis	Phase 1/2	NCT01494805
AAV2	CHM	REP1	CAG	subretinal	Photoreceptors & RPE	Phase 2	NCT02671539
AAV2	CHM	REP1	CAG	subretinal	Photoreceptors & RPE	Phase 1	NCT01461213
AAV2	RP	rhodopsin	GRM6	Intravitreal	RGCs	Phase 1/2	NCT02556736
AAV2	CHM	CHM	CAG	subretinal	Photoreceptors & RPE	Phase 1/2	NCT02341807
AAV2	LHON	ND4	CMV	Intravitreal	RGC	—	NCT03672968
AAV2	AMD	VEGF-neutralizing proteins FLT01	CAG	Intravitreal	Anti-angiogenesis	Phase 1	NCT01024998
AAV2	CHM	REP1	CAG	subretinal	Photoreceptors & RPE	Phase 3	NCT03496012
AAV2	LCA	RPE65	CB^{o}	subretinal	RPE	Phase 1/2	NCT00749957
AAV4	LCA	RPE65	hRPE65	subretinal	RPE	Phase 1/2	NCT01496040
AAV5	LCA	RPE65	NA65p	subretinal	RPE	Phase 1/2	NCT02781480
AAV5	LCA	CEP290	hGRK1	subretinal	Photoreceptors	Phase 2	NCT03872479
AAV5	RP	RPGR	RK	subretinal	Photoreceptors	Phase 3	NCT04671433
AAV5	RP	RPGR	RK	subretinal	Photoreceptors	Phase 3	NCT04794101
AAV5	RP	RPGR	RK	subretinal	Photoreceptors	Phase 3	NCT03252847
AAV5	RP	PDE6B	RK	subretinal	Photoreceptors	Phase 1/2	NCT03328130
AAV5	RP	RPGR	GRK	subretinal	Photoreceptors	Phase 1/2	NCT03252847
AAV8	RS	RS1	hRS	Intravitreal	—	Early Phase 1	NCT06289452
AAV8	STGD	ABCA4	—	subretinal	—	Phase 1/2	NCT06467344
AAV8	RP	PDE6A	RHO	subretinal	Photoreceptors	Phase 1/2a	NCT04611503
AAV8	LCA	hopt- LCA5	CAG	subretinal	Photoreceptors	Phase 1/2	NCT05616793
AAV8	BCD	CYP4v2	CAG	subretinal	RPE	Early Phase 1	NCT05399069
AAV8	RP	RPGR	RK	subretinal	Photoreceptors	Phase 1/2	NCT03116113
AAV8	RP	RLBP1	CPK850	subretinal	Photoreceptors	Phase 1	NCT03374657
AAV8	BCD	CYP4V2	CAG	subretinal	RPE	Phase 1	NCT04722107
AAV2tYF	RP	hRPGRco	GRK1	subretinal	Photoreceptors	Phase 1/2	NCT06275620
AAV2tYF	RP	RPGR	GRK1	subretinal	Photoreceptors	Phase 1/2	NCT06333249
AAV2tYF	RP	RPGR	GRK1	subretinal	Photoreceptors	Phase 1/2	NCT03316560
AAV2tYF	RS	RS1	CAG	Intravitreal	Bipolarcells and PR	Phase 1/2	NCT02416622
AAV2tYF	ACH	CNGA3	PR1.7	subretinal	ConePR	Phase 1/2	NCT02935517
AAV2tYF	*ACH*	CNGB3	PR1.7	subretinal	ConePR	Phase 1/2	NCT02599922
AAV2-7m8	AMD	Aflibercept	CMV	Intravitreal	Anti-angiogenesis	—	NCT04645212
AAV2-7m8	AMD	Aflibercept	CMV	Intravitreal	Anti-angiogenesis	Phase 1	NCT03748784
AAV2-7m8	AMD	Aflibercept	CMV	Intravitreal	Anti-angiogenesis	Phase 2	NCT04418427
AAV2-7m8	RP	ChrimsonR-tdTomato	CAG	Intravitreal	RGCs	Phase 1/2a	NCT03326336
4D-R100	RP	RPGR	—	Intravitreal	Photoreceptors	Phase 1/2	NCT04517149
4D-R100	CHM	CHM	—	Intravitreal	Photoreceptors & RPE	Phase 1	NCT04483440
OCU400	—	—	—	—	—	—	NCT06574997

Table 3 (continued)

AAV serotypes	Disease	Targeted genes (drugs)	Promoter	Injection method	Transduction site	Phase	Clinical number
FT-002	—	—	—	—	—	—	NCT05874310
AAV2.NN	RP	CNGA1	—	Intravitreal	Photoreceptors	Phase 1	NCT06291935

CHM, Choroideremia; **AMD**, Age related Macular Degeneration; **RP**, Retintis Pigmentosa; **LCA**, Leber Congenital Amaurosis; **LHON**, Leber's Hereditary Optic Neuropathy; **RS**, Retinoschisis; **STGD**, Stargardt; **BCD**, Bietti crystalline Corneoretinal Dystrophy; **ACH**, Achromatopsia; **RPE**, Retinal Pigment Epithelium; **—**: Since the specific data of some clinical studies are not disclosed on the website, they are replaced by symbols

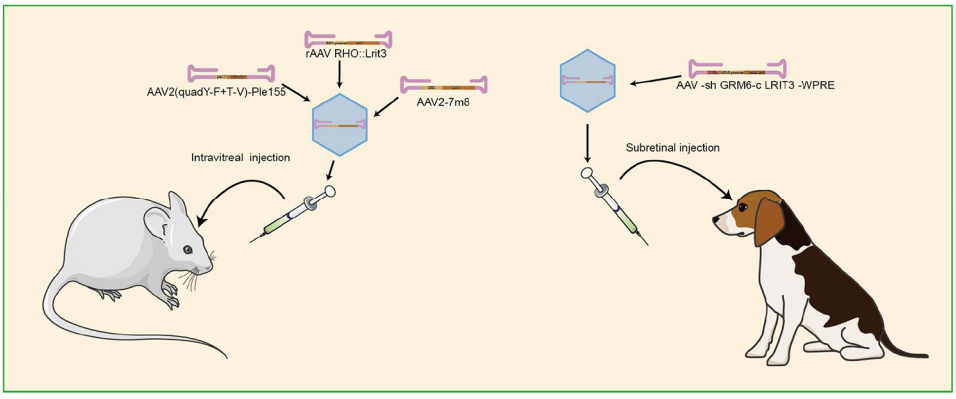


Fig. 10 Current results of gene therapy for CSNB. The currently published successful cases of gene therapy for CSNB primarily involve animal models, including *Nyx*^{nob} mice, *Lrit3*$^{-/-}$ mice, and Beagle dog models

also suggest that VLPs may significantly reduce off-target effects compared to viral vectors [183, 184]. With advantages such as high capacity, modular design, and lower immunogenicity than viral vectors, VLPs are emerging as promising tools for gene delivery. Currently, most VLP-based drug delivery studies are at the preclinical stage, with VLP-mediated gene therapy demonstrated in ocular animal models [183, 185]. However, further studies in larger animal models are needed to verify their efficacy and safety.

Overall, current ocular gene therapy focuses on developing safe and efficient delivery methods. Viral vectors, particularly AAV, are widely used in clinical applications due to their high transduction efficiency. However, as gene editing tools advance, they have also increased in size—such as PE tools—which further limits the already constrained packaging capacity of AAV. Additionally, AAV-based therapies are currently costly [186]. These factors have led some researchers to explore non-viral vectors as alternatives. Non-viral vectors like LNPs and VLPs offer greater capacity, lower immunogenicity, and excellent biocompatibility, enabling them to deliver genetic materials without the drawbacks of viral vectors, while also supporting large-scale commercial production [187]. Although nanocarriers partially address the limitations of viral vectors, their delivery efficiency in retinal cells—especially non-dividing retinal cells—still lags behind viral vectors [188]. Furthermore, additional preclinical data is required to validate their safety and efficacy.

A review of research on CSNB treatment

As a rare disease, there are currently no published clinical therapies for CSNB, but researchers have attempted various treatments for mammals (Fig. 10). One known pathogenic gene for CSNB is *NYX*; its deletion affects the localization of TRPM1 protein at the dendritic tips of ON BCs [69]. A mouse model, *Nyx*^{nob}, effectively mimics CSNB caused by *Nyx* mutations, exhibiting normal a-waves and absent b-waves in ERG similar to CSNB1 patients. Miranda and his team previously developed an AAV vector named AAV2(quadY-F + TV), which was engineered to enhance transduction efficiency in photoreceptor cells (PR) by targeted mutagenesis of tyrosine and threonine residues on the AAV2 capsid [189]. For treating *Nyx*^{nob} mice, they combined AAV2(quadY-F + TV) with a human mini-promoter, "Ple155" [190], and incorporated a YFP_Nyx fusion cDNA that matched the Nyxnob mice [191], resulting in AAV2(quadY-F + TV)-Ple155-YFP_Nyx. After intravitreal injection of AAV2(quadY-F + TV)-Ple155-YFP_Nyx, results showed that mice treated at postnatal day 2 (P2) had rescued b-waves, restoring TRPM1 protein localization at the dendritic tips of ON BCs. However, mice treated at postnatal day 30 (P30) did not exhibit rescue. Nonetheless, this study by Miranda L and his team marks the first successful attempt to rescue retinal function in a mouse model of CSNB [192].

Similar to the *Nyx*, the *Lrit3* also affects the transport and postsynaptic localization of TRPM1 protein in rod cells within the retina [193]. Abnormal expression of the

Lrit3 can lead to CSNB. Nazarul and colleagues, while demonstrating this mechanism, attempted to use AAV to treat *Lrit3*$^{-/-}$ mice. These mice are also classified as nob mice, exhibiting b-wave loss in dark-adapted ERG and a significant reduction in b-waves in light-adapted conditions [86]. The treatment strategy employed by Nazarul et al. was gene replacement therapy. In their experiments, they utilized rAAV RHO::*Lrit3*, which carries the human rhodopsin promoter (RHO) to drive the expression of the *Lrit3* in photoreceptors [194]. The results indicated that *Lrit3*$^{-/-}$ mice treated at postnatal day 5 (P5) showed restored transport of TRPM1 to the postsynaptic dendrites of red blood cells. In dark-adapted ERG, the average b-wave recovery in treated mice was as high as 50% compared to the control group, although no recovery was observed in LA. This lack of recovery may relate to the expression pattern of RHO in rod cells. Unlike the findings of Miranda et al., rAAV RHO::*Lrit3* also achieved rescue in adult mice (P35), albeit with less efficacy compared to treatment at P5 [193].

The *Grm6* encodes the mGluR6 receptor protein, which controls the closure of the TRPM1 channel in response to glutamate signaling in the synaptic cleft [195]. A *Grm6*$^{-/-}$ mouse model exhibits symptoms similar to those of patients with cCSNB, including a missing b-wave and a normal a-wave in ERG, along with a loss of mGluR6 expression [61]. Previous treatments for *Nyx* and *Lrit3* mice demonstrated that while gene replacement successfully rescued vision in young mice, challenges persisted in treating adult mice. Juliette and colleagues directly attempted to treat adult *Grm6*$^{-/-}$ mice. In their study, they injected AAV2-7m8 into the vitreous of mice at postnatal day 15 (P15). This experiment utilized AAV2-7m8 with two different promoters: the GRM6-200 bp/SV40 promoter [196] (GRM6-*Grm6*) and a fusion of the CAG promoter [143] (CAG-*Grm6*) to drive *Grm6* expression. The results indicated that although AAV2-7m8 treatment restored mGluR6 expression in the mice, the ERG phenotype did not recover. This lack of recovery may be attributed to retinal structural deformities caused by the absence of GRM6 during early retinal development, making it challenging to restore ERG function even after mGluR6 expression was reestablished [197]. Consequently, in the same year, Juliette and colleagues shifted their focus to rescuing adult *Lrit3*$^{-/-}$ mice. They injected AAV2-7m8 into P30 *Lrit3*$^{-/-}$ mice, where one group was equipped with an h GRK promoter to drive expression in both rod and cone photoreceptors [198], while another group utilized the 200 bp Grm6 promoter. The results demonstrated that the expression of LRIT3 and the localization of TRPM1 were both restored in the treated mice. In ERG tests, the b-wave recovery under DA reached up to 58% (driven by the hGRK promoter), and this effect was still detectable four months post-injection. These findings positively indicate the feasibility of gene replacement therapy for adult patients [199].

The results from previous teams suggest that gene replacement therapy can effectively rescue vision across different age groups of mice. However, large animals, with retinal structures more similar to humans, can provide valuable preclinical insights for treating CSNB. A wild-type CSNB beagle model has been identified, exhibiting a loss of b-wave during DA [200]. To develop an AAV variant that efficiently targets ON BCs, Takahashi and colleagues screened two AAV serotypes, AAV K9#4 and AAV K9#12, from wild canines, determining AAV K9#12 for subsequent experiments in NHPs. They tested different promoters in canines, including the lgGRM6 promoter (2.2 kb) and shGRM6 (0.7 kb) [201]. The lgGRM6 exhibited stronger expression than shGRM6; however, due to its longer length, they designed two combinatorial strategies: AAV-lgGRM6-cLRIT3 and AAV-shGRM6-cLRIT3-WPRE. The results showed that injecting AAV-shGRM6-cLRIT3-WPRE into adult beagles led to a stable recovery of the ERG b-wave, reaching up to 30% of wild-type levels. This recovery was detectable for over 1.2 years [202] and continued during follow-up assessments for up to 32 months [203].

Conclusion and perspectives

The most common feature of CSNB is that patients typically exhibit night blindness. With urbanization and the widespread use of electronic devices like smartphones, it has become difficult for individuals to notice night blindness, often leading to CSNB patients being overlooked in daily life. As a rare disease, CSNB exhibits high genetic heterogeneity, and its clinical features not only overlap among different types of CSNB but also with other IRDs, making it challenging to accurately identify specific CSNB types based solely on clinical observations. ffERG, a non-invasive clinical examination method, can provide precise diagnoses for CSNB patients by assessing their responses to various flash stimuli. Based on different ERG responses, CSNB patients can be further categorized into Schubert-Bornschein and Riggs types.

CSNB is primarily caused by genetic mutations, with associated genes mostly located in photoreceptor and BCs; several mutations, such as those in the *NYX*, *GRM6*, and *LRIT3* genes, have been confirmed. Research on CSNB treatment mainly focuses on genetics, with animal models widely used as effective research tools in retinal genetic disease studies. However, it has been found that although animal models can simulate mutant genes and their phenotypes well, there are significant differences in genes and retinal structures across species compared to humans. This interspecies variation makes it challenging to quickly translate research findings to clinical applications. Consequently, there is a shift towards designing

humanized animal models, which can reduce species gaps and expedite the clinical translation of experimental results.

Gene therapy, an emerging treatment approach for genetic diseases, has demonstrated effectiveness in preclinical and clinical studies of IRDs. While successful results have been achieved in animal models for CSNB gene therapy, clinical research still faces challenges: Firstly, identification of the pathogenic gene: The prerequisite for gene therapy is a clear understanding of the specific mutation at a particular site in the patient's genome, which requires the use of modern genetic diagnostic technologies for confirmation. Furthermore, gene therapy agents are often "customized," meaning that a specific gene therapy can only be used for a particular patient. In reality, the same disease can involve multiple different mutations in the same gene. Therefore, different agents must be developed for different patients, significantly increasing the cost of developing gene therapies. In practice, many patients neglect genetic diagnosis, which means they may not be eligible for gene therapy. Secondly, efficient and precise drug delivery strategy: Gene therapy relies on the targeting efficiency and dosage of the drug, particularly in areas like the retina, which has a dense and structured layout. Gene therapies typically cannot be directly injected (except for ASOs), so the key to effective treatment lies in finding a carrier (viral or non-viral) that can efficiently target the specific site. The carrier's shell and the promoters it carries often need to be specifically designed and selected to achieve optimal results. Thirdly, high treatment costs: The process from genetic diagnosis to the design of gene therapies and preclinical testing involves significant time and financial investment. For example, Luxturna, although it is effective in treating RPE patients, costs as much as $850,000 per injection, making it unaffordable for most patients. Therefore, if gene therapy is to become widely accessible, it is essential to develop cost-effective production methods. Lastly is safety: Based on existing clinical data, gene therapy not only presents immune reactions due to the drug but also other adverse events (such as those associated with ASO and RNAi drugs mentioned earlier). Therefore, in addition to focusing on the therapeutic efficacy of the drug, it is equally important to monitor and address potential adverse reactions. Overall, gene therapy as a novel treatment option shows great promise for hereditary retinal diseases. Despite the challenges in treating CSNB, researchers have made significant breakthroughs in the principles, technologies, and clinical applications of gene therapy. With continued research on CSNB-related genes and their associated molecular pathways, gene therapy may, in the future, enable CSNB patients to regain normal visual function and improve their quality of life.

Abbreviations

AAV	Adeno-associated virus
ABE	Adenine base editor
ACs	Amacrine cells
adCSNB	Autosomal dominant CSNB
arCSNB	Autosomal recessive CSNB
AMD	Age-related macular degeneration
ASO	Antisense oligonucleotides
BEs	Base editors
CBE	Cytosine base editor
cCSNB	Complete CSNB
CGBE	Cytosine-guanine base editor
cGMP	cyclic guanosine 3′,5′-monophosphate
CSNB	Congenital stationary night blindness
DA	Dark adaptation
DSBs	Double-strand breaks
FA	Fundus albipunctatus
ffERG	Full-field electroretinography
GPCRs	G-protein-coupled receptors
HC	Horizontal cells
HDR	Homology-directed repair
HITI	Homology-independent targeted integration
HSV	Herpes simplex virus
icCSNB	Incomplete CSNB
INL	Inner nuclear layer
IRD	Inherited retinal disease
ISCEV	International society for clinical electrophysiology of vision
IVT	Intravitreal injection
LA	Light adaptation
LCA	Leber congenital amaurosis
LNPs	Lipid nanoparticles
MMEJ	Microhomology-mediated end joining
NHEJ	Non-homologous end joining
NHP	Non-human primatesprimate
OD	Oguchi disease
OFF	BC OFF bipolar cells
ON	BC ON bipolar cells
OP	Oscillatory potential
PE	Prime editors
PEG	Polyethylene glycol
PMOs	Phosphorodiamidate morpholino oligomers
PR	Photoreceptor cells
pegRNA	Prime editing guide RNA
RGC	Retinal ganglion cell
RISC	RNA-induced silencing complexes
RNAi	RNA interference
RP	Retinitis pigmentosa
RPE	Retinal pigment epithelium
SaCas9	Staphylococcus aureus Cas9
SATI	Single homology arm donor-mediated targeted integration
	sgRNA Single guide RNA
siRNA	Small interfering RNA
SRI	Subretinal injection
TALENs	Transcription activator-like effector nucleases
VLPs	Virus-like particles
ZFNs	Zinc finger nucleases2-OMe/PS 2′O-methyl-phosphorothioate
2-MOE/PS	2′O-methoxyethyl-phosphate

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Selected artwork (Animals, Cell_membrane, Lab_apparatus, Neural_cells, Ophthalmology, Paraclinical_exams, Receptors_channels and Risk_Factorseyes) shown in the graphical abstract or Fig.s were partly used from or adapted from pictures provided by Servier Medical Art (Servier; https://smart.servier.com/), licensed under a Creative Commons Attribution 4.0 Unported License.

Authors' contributions

W.C. designed, supervised, and supported the whole project. Y.Z., S.L. and L.Y. wrote the manuscript. Y.Z. prepared the illustrations. W.C., X.L., S.Q., Q.Y. and M.Y. revised the manuscript. J.W. supervised the rewriting of the sections 'CSNB and its Clinical Diagnosis' and 'Principles and Methods of Gene Therapy.'

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Data availability
Due to its nature as a review article, all references are published articles. The data underlying this article are available in the Pubmed. Some data in the Table 1 are obtained through NIM (https://www.ncbi.nlm.nih.gov/) query statistics.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
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Author details
[1]Institute of Life Sciences, College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China
[2]Department of Biomedical Engineering, College of Biological Science and Engineering, Fuzhou University, Fuzhou 350108, China
[3]Hangzhou Bipolar Biotechnology Co., Ltd., Hangzhou 311199, China
[4]Department of Ophthalmology, Fuzhou University Affiliated Provincial Hospital, Fujian Provincial Hospital, Fuzhou 350028, China

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