· 综　　述 ·

CRISPR/Cas9 基因编辑技术在构建眼科疾病动物模型中的应用

马啸辰　综述　刘红玲　审校
哈尔滨医科大学第一附属医院眼科医院，哈尔滨 150001
通信作者：刘红玲，Email：hydliuhl@163.com

【摘要】 成簇规律间隔短回文重复序列及其相关蛋白 9（CRISPR/Cas9）系统是一种利用 RNA 指导核酸内切酶进行基因编辑的技术，具有操作简便、靶向精准、周期短、基因敲除效率高等特点，被广泛用于多个物种的基因编辑及疾病基因治疗中。目前，采用该技术已构建了多种眼科疾病动物模型，如角膜营养不良模型（*UBIAD1*、*TGF-β* R124C 基因突变）、青光眼模型（*MYOC* Y435H、*OPTN* E50K 和 *PMEL* 基因突变）、白内障模型（*GJA8*、*KPNA4*、*c-Maf*、*AQP5* 和 *PIKFYVE* 基因突变）、Leber 先天性黑矇动物模型（*KCNJ13* 和 *LCA5* 基因突变）、视网膜母细胞瘤动物模型（*RB1/RBL* 基因突变）和视网膜色素变性动物模型（*HKDC1*、*C8ORF37*、*CERKL*、*PRCD*、*ASRGL1*、*LRAT* 和 *PDE6B* 基因突变）等。还有研究者采用该基因编辑技术进一步证实了 *MFRP*、*CPAMD8*、*Pax6* 和 *FREM* 基因在动物眼部发育过程中的作用。本文就 CRISPR/Cas9 基因编辑技术在构建眼科疾病动物模型中的应用进行综述。

【关键词】 CRISPR-Cas 系统；基因编辑；眼病；动物模型

基金项目： 国家自然科学基金项目（81301325）；中国初级卫生保健基金会眼科新技术孵化基金项目（FB2022008629）

DOI：10.3760/cma.j.cn115989-20190930-00425

Application of CRISPR/Cas9 gene editing technology in the construction of animal models of ophthalmic diseases

Ma Xiaochen, Liu Hongling
Department of Ophthalmology, First Affiliate Hospital of Harbin Medical University, Harbin 150001, China
Corresponding author: Liu Hongling, Email: hydliuhl@163.com

[**Abstract**] The clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated endonuclease 9 (Cas9) technology is a gene editing technology that uses RNA to guide endonucleases. This technology is rapidly used in gene editing and disease gene therapy in multiple species because of its easy operation, precise targeting, short cycle, and high gene knockout efficiency. At present, the corneal dystrophy model (*UBIAD1*, *TGF-β* R124C gene mutations), glaucoma model (*MYOC* Y435H, *OPTN* E50K and *PMEL* gene mutations), cataract model (*GJA8*, *KPNA4*, *C-MAF*, *AQP5* and *PIKFYVE* gene mutations), Leber congenital amaurosis animal model (*KCNJ13* and *LCA5* gene mutations), retinblastoma animal model (*RB1/RBL* gene mutations) and retinitis pigmentosa models (*HKDC1*, *C8ORF37*, *CERKL*, *PRCD*, *ASRGL1*, *LRAT* and *PDE6B* gene mutations) have been constructed by using this technology. The role of *MFRP*, *CPAMD8*, *Pax6*, and *FREM* genes in animal eye development has been further confirmed via this technology. The application of CRISPR/Cas9 gene editing technology in the construction of animal models of ophthalmic diseases was reviewed in this article.

[**Key words**] CRISPR-Cas systems; Gene editing; Eye diseases; Disease models, animal

Fund program: National Natural Science Foundation of China (81301325); Ophthalmology New Technology Incubation Fund Project of Primary Health Care Foundation of China (FB2022008629)

DOI：10.3760/cma.j.cn115989-20190930-00425

基因编辑是近年来眼科实验研究的热点领域。眼球解剖结构独特，角膜透明，借助裂隙灯显微镜、光相干断层扫描等仪器，方便对眼部疾病进行观察[1]；另外，眼部缺乏淋巴管结构，眼球处于相对免疫赦免状态，对新抗原的免疫应答反应较弱，更适合外源性基因编辑治疗[2]。Ⅱ型 CRISPR/Cas 系统由前核糖体 RNA、Cas9 和反式激活 CRISPR RNA（crRNA）组成，其中，Cas9 经 crRNA 引导后与互补 DNA 靶序列结合，进而产生位点特异性 DNA 双链断裂，理论上可以在任何基因位点上实现

中华医学会杂志社 Chinese Medical Association Publishing House 版权所有 违者必究

DNA 切割[3]。CRISPR/Cas9 基因编辑技术不仅在目的基因设计合成筛选上更为简便快捷，而且可以在单个细胞内同时靶向编辑多个基因位点，成倍地提高了基因编辑的效率，但 Cas 蛋白的不稳定性和脱靶效应在一定程度上限制了其应用[4]。基因编辑动物属于原发性疾病模型，其表型持续时间长，并可稳定遗传。对于已明确致病基因的疾病，构建基因编辑动物模型可真实地模拟疾病发生和演变过程。应用胚胎细胞克隆技术，还可批量制备基因型一致的动物模型，减少个体差异对实验结果的影响[5]。本文就 CRISPR/Cas9 基因编辑技术在构建眼科疾病动物模型中的应用进展进行综述。

1　角膜疾病

Schnyder 角膜营养不良是一种罕见的常染色体显性遗传病，病因可能为染色体 1P36 位点的 UBIA 异戊二烯基转移酶结构域（UbiA prenyltransferase domain containing 1，*UBIAD1*）基因突变，角膜出现渐进性混浊，胆固醇和磷脂沉积，最终导致视力下降[6]。Dong 等[7] 采用 CRISPR/Cas9 技术构建 *Ubiad1*（*N100S*）点突变小鼠模型，小鼠角膜可见点状沉积物；角膜上皮细胞、基质细胞和内皮细胞中，线粒体形态结构异常，甘油磷脂代谢发生障碍。转化生长因子 β（transforming growth factor-β，*TGF-β*）基因突变可导致角膜营养不良，其中 *TGF-β*（R124C）突变与格子状角膜营养不良密切相关[8]。Kitamoto 等[9] 采用 CRISPR/Cas9 基因编辑技术构建 *TGF-β*（R124C）基因突变小鼠，40 周龄时，约 80% 纯合 *TGF-β*（R124C）基因突变小鼠和 9.1%杂合小鼠出现角膜混浊，角膜基质层内有嗜酸性沉积，角膜出现大量 TGF-β 突变蛋白。以上研究有助于加深对角膜病发病机制的理解。

2　青光眼

Myocilin（*MYOC*）基因与青光眼密切相关[10]。Lynch 等[11] 采用 CRISPR 基因编辑技术构建 *Myoc* 基因突变大鼠模型，大鼠小梁网组织中可检测到 MYOC 突变蛋白表达，但大鼠眼压并未升高。*Optineurin*（*OPTN*）E50K 基因突变可导致正常眼压性青光眼，其特点是视网膜神经节细胞（retinal ganglion cell，RGC）进行性变性，最终可致盲[12]。Liu 等[13] 和 Hou 等[14] 采用 CRISPR/Cas9 技术构建 *Optn* E50K 突变青光眼小鼠模型，蛋白组学分析显示自噬和线粒体功能相关的通路发生障碍；16 月龄时，小鼠 RGC 轴突运输减慢；3 月龄小鼠 RGC 轴突线粒体形态结构明显异常，且随年龄增长而加重；小鼠成年后出现视力损害和视网膜变性特征，但眼压正常。色素性青光眼以虹膜释放的色素沉积在前房为特点。黑素小体特异性蛋白（premelanosome protein，PMEL）参与黑色素合成、储存和运输，*PMEL* 基因突变可导致色素性青光眼的发生[15]。Lahola-Chomiak 等[15] 采用 CRISPR/Cas9 技术构建 *pmel* 基因突变斑马鱼模型，斑马鱼眼部出现大量色素沉积。以上研究进一步扩充并验证了青光眼的致病基因数据库，为后续治疗研究提供了动物模型保障。

3　白内障

GJA8 基因编码晶状体缝隙连接蛋白，该基因突变可引起先天性白内障[16]。Yuan 等[17] 将 Cas9/sgRNA mRNA 注射到兔受精卵中构建 *Gja8* 基因突变白内障家兔模型，结果表明约 78.9% *Gja8* 基因突变家兔具有小眼球和晶状体混浊表型。Mei 等[18] 采用 CRISPR/Cas9 技术构建 *pikfyve* 基因突变斑马鱼模型，斑马鱼晶状体中出现大量液泡样改变，晚期内体与该液泡共定位，晶状体纤维细胞明显肿胀，细胞核形态不规则。Karyopherin alpha 4（KPNA4）参与经典核转运过程，维持细胞内稳态[19]。Ping 等[19] 采用 CRISPR/Cas9 系统构建 *kpna4* 基因突变斑马鱼模型，在胚胎期可观察到晶状体混浊，晶状体上皮细胞层变薄，形态不规则。水通道蛋白 5（aquaporin 5，AQP5）是膜水通道的重要组成成分[20]。Tang 等[21] 采用 CRISPR/Cas9 技术构建 *Aqp5* 突变小鼠模型，6 月龄时晶状体出现轻度混浊，9 月龄时发生严重混浊。白内障的发病基因具有多样性，但针对特定基因位点构建基因编辑动物模型有助于筛选特定的发病基因，为后续的白内障基因回补治疗奠定基础。

4　眼底疾病

4.1　Leber 先天性黑矇

KCNJ13 基因表达于视网膜色素上皮（retinal pigment epithelium，RPE）细胞顶侧，其突变可导致 Leber 先天性黑矇[22]。Zhong 等[23] 采用 CRISPR/Cas9 技术构建 *Kcnj13* 突变小鼠，17 周龄小鼠感光细胞几乎消失，视网膜结构严重受损。1%～2%的 Leber 先天性黑矇是由编码纤毛蛋白的 *LCA5* 基因突变引起的[24]。Qu 等[25] 采用 CRISPR/Cas9 技术敲除斑马鱼 *lca5* 基因，视网膜电图（electroretinography，ERG）检查示斑马鱼视觉异常；视杆和视锥细胞光感受器进行性变性，外节膜盘排列紊乱。

4.2　视网膜母细胞瘤

RB1 等位基因失活可导致视网膜母细胞瘤（retinoblastoma，RB）发生。Naert 等[26] 采用 CRISPR/Cas9 技术改造非洲爪蟾 RB 基因，*RB1* 或 *RBL1* 单一基因突变的非洲爪蟾并未出现 RB，而 *RB1*/*RBL1* 等位基因突变蝌蚪迅速发展为 RB 表型；视网膜内出现大量嗜碱性肿块。

4.3　视网膜色素变性

Hexokinase Domain-Containing Protein 1（HKDC1）表达于视网膜光感受器内[27]。Zhang 等[28] 采用 CRISPR/Cas9 技术构建 *Hkdc1* 敲除小鼠模型，小鼠出现暗视 ERG 反应降低、外核层变薄、视紫红质蛋白异常定位和光感受器细胞凋亡增多等视网膜色素变性（retininitis pigmentosa，RP）病理特征。Chromosome 8 Open Reading Frame 37（*C8ORF37*）是常染色体隐性遗传 RP 的致病基因[29]。Sharif 等[29] 采用 CRISPR/Cas9 技术构建 *C8orf37* 基因突变小鼠模型，小鼠出现进行性视杆和视锥细胞退化，光感受器外节段膜盘大量解体。Parain 等[30] 采用 CRISPR/Cas9 技术对视紫红质基因进行改造，建立非洲爪蟾和热带爪蟾 RP 模型，爪蟾表现为视锥细胞形态异常并伴光感受器外节段萎缩

中华医学会杂志社 Chinese Medical Association Publishing House 版权所有 违者必究

和坏死。Domènech 等[31]采用 CRISPR/Cas9 技术敲除小鼠神经酰胺激酶样(ceramide kinase-like, *CERKL*)基因,小鼠视锥细胞数量减少、光感受器外节段形态异常和 RPE 细胞吞噬功能下降。进行性杆锥变性(progressive rodecone degeneration, PRCD)蛋白定位于光感受器外节段膜盘内[32]。Sechrest 等[32]采用 CRISPR/Cas9 技术构建 *Prcd* 突变小鼠,小鼠光感受器外节段膜盘迂曲变形,视网膜中视紫红质表达水平显著下降。Zhou 等[33]采用 CRISPR/Cas9 技术构建异天冬酰胺肽酶 1(asparaginase like 1, *Asrgl1*)基因突变小鼠模型,9 个月时小鼠视网膜外核层厚度开始减少;光感受器内、外节段变薄,视杆细胞和视锥细胞进行性变性。Koster 等[34]采用 CRISPR/Cas9 技术构建卵磷脂视黄醇酰基转移酶(lecithin retinol acyltransferase, *LRAT*)基因突变大鼠模型,大鼠视网膜整体厚度变薄,ERG 振荡电位降低。Yeo 等[35]采用 CRISPR 技术构建 *Pde6b* 突变大鼠模型,3 周时大鼠眼底可见色素沉着,视网膜光感受器出现变性,视网膜外核层厚度变薄。以上研究通过基因编辑技术模拟 RP 的发病过程,为 RP 治疗靶点的选择提供了依据。

5　眼部发育障碍

膜卷曲相关蛋白(membrane frizzled-related protein, *MFRP*)基因突变可导致眼球萎缩、上睑下垂、RPE 细胞缺失和视盘玻璃疣等[36]。Collery 等[36]采用 CRISPR/Cas9 系统在斑马鱼 *Mfrp* 基因中构建多个移码突变,斑马鱼视敏度降低、眼轴缩短和视网膜下巨噬细胞聚积。眼前段发育不全(anterior segment dysgenesis, ASD)常表现为角膜混浊、角膜 Schwalbe 线向前移位、虹膜发育不全、虹膜粘连和白内障[37]。Bonet-Fernández 等[38]采用 CRISPR/Cas9 技术构建 *cpamd8* 基因突变斑马鱼模型,该突变体斑马鱼出现严重 ASD 体征,其眼部病变包括虹膜角膜发育不全、眼球萎缩、前房浅和角膜基质胶原排列紊乱。哺乳动物 *Pax6* 基因定位于无眼基因座,其突变常导致眼部发育异常。Nakanishi 等[39]采用 CRISPR/Cas9 技术改造水蚤的 *Pax6* 基因,部分存活幼体眼球形态异常,约 8.2%第 2 代水蚤出现眼部异常。Yasue 等[40]采用 CRISPR/Cas9 技术构建 *Pax6* 基因缺陷小鼠,胚胎期小鼠出现不同程度眼部发育异常。*FREM2* 基因突变可导致胚胎皮肤水疱、隐眼、并指症和泌尿生殖系统畸形等[41]。Zhang 等[42]采用 CRISPR/Cas9 技术构建 *Frem* 基因突变小鼠模型,小鼠出现双侧或单侧隐眼表型,伴眼球发育不良,还可并发角膜新生血管和虹膜粘连。

综上所述,CRISPR 技术构建动物模型对于后续的基因编辑治疗具有重要意义,有助于扩充并验证疾病的致病基因数据库。目前,Mouse Knockout 联合会等正在广泛使用该技术生成基因突变小鼠,以期探索疾病的发病机制[43]。但我们仍应注意到,动物疾病模型并不能完全模拟疾病的发生过程,可能会由于基因缺失而引发一系列代偿反应。完全性基因敲除往往会导致胚胎死亡,条件性基因敲除因可在某一特定的时间阶段或组织器官内调控靶基因的表达而越来越受到重视。此外,基因与其相应表型并非具有必然的对应关系,遗传背景及其他因素使基因编辑的结果更加复杂[44]。尽管如此,借助基因编辑技术构建疾病动物模型仍是一种非常有前景的探索疾病起源的方法,值得深入研究。

利益冲突　所有作者均声明不存在利益冲突

参考文献

[1] Spaide RF, Fujimoto JG, Waheed NK, et al. Optical coherence tomography angiography[J]. Prog Retin Eye Res, 2018, 64: 1-55. DOI: 10.1016/j.preteyeres.2017.11.003.

[2] Pleyer U, Pohlmann D. Anatomy and immunology of the eye[J]. Z Rheumatol, 2017, 76(8): 656-663. DOI: 10.1007/s00393-017-0344-y.

[3] Cho S, Shin J, Cho BK. Applications of CRISPR/Cas system to bacterial metabolic engineering[J/OL]. Int J Mol Sci, 2018, 19(4): 1089[2022-03-10]. https://pubmed.ncbi.nlm.nih.gov/29621180/. DOI: 10.3390/ijms19041089.

[4] Suzuki K, Tsunekawa Y, Hernandez-Benitez R, et al. *In vivo* genome editing via CRISPR/Cas9 mediated homology-independent targeted integration[J]. Nature, 2016, 540(7631): 144-149. DOI: 10.1038/nature20565.

[5] 吴世靖,睢瑞芳. CRISPR/Cas9 技术在遗传性眼病基因治疗中的应用[J]. 中华实验眼科杂志, 2018, 36(11): 892-896. DOI: 10.3760/cma.j.issn.2095-0160.2018.11.016.
Wu SJ, Sui RF. Applications of CRISPR/Cas9 genome editing technology in gene therapy for hereditary eye diseases[J]. Chin J Exp Ophthalmol, 2018, 36(11): 892-896. DOI: 10.3760/cma.j.issn.2095-0160.2018.11.016.

[6] Xie J, Li L. Functional study of SCCD pathogenic gene *UBIAD1* (review)[J/OL]. Mol Med Rep, 2021, 24(4): 706[2022-03-12]. https://pubmed.ncbi.nlm.nih.gov/34368857/. DOI: 10.3892/mmr.2021.12345.

[7] Dong F, Jin X, Boettler MA, et al. A mouse model of schnyder corneal dystrophy with the N100S point mutation[J/OL]. Sci Rep, 2018, 8(1): 10219[2022-03-15]. https://pubmed.ncbi.nlm.nih.gov/29977031/. DOI: 10.1038/s41598-018-28545-0.

[8] Han KE, Choi SI, Kim TI, et al. Pathogenesis and treatments of TGFBI corneal dystrophies[J]. Prog Retin Eye Res, 2016, 50: 67-88. DOI: 10.1016/j.preteyeres.2015.11.002.

[9] Kitamoto K, Taketani Y, Fujii W, et al. Generation of mouse model of TGFBI-R124C corneal dystrophy using CRISPR/Cas9-mediated homology-directed repair[J/OL]. Sci Rep, 2020, 10(1): 2000[2022-03-16]. https://pubmed.ncbi.nlm.nih.gov/32029872/. DOI: 10.1038/s41598-020-58876-w.

[10] Kwon YH, Fingert JH, Kuehn MH, et al. Primary open-angle glaucoma[J]. N Engl J Med, 2009, 360(11): 1113-1124. DOI: 10.1056/NEJMra0804630.

[11] Lynch JM, Li B, Katoli P, et al. Binding of a glaucoma-associated myocilin variant to the αB-crystallin chaperone impedes protein clearance in trabecular meshwork cells[J]. J Biol Chem, 2018, 293(52): 20137-20156. DOI: 10.1074/jbc.RA118.004325.

[12] VanderWall KB, Huang KC, Pan Y, et al. Retinal ganglion cells with a glaucoma *OPTN* (E50K) mutation exhibit neurodegenerative phenotypes when derived from three-dimensional retinal organoids[J]. Stem Cell Reports, 2020, 15(1): 52-66. DOI: 10.1016/j.stemcr.2020.05.009.

[13] Liu X, Wang Q, Shao Z, et al. Proteomic analysis of aged and *OPTN* E50K retina in the development of normal tension glaucoma[J]. Hum Mol Genet, 2021, 30(11): 1030-1044. DOI: 10.1093/hmg/ddab099.

[14] Hou M, Shao Z, Zhang S, et al. Age-related visual impairments and retinal ganglion cells axonal degeneration in a mouse model harboring *OPTN* (E50K) mutation[J/OL]. Cell Death Dis, 2022, 13(4): 362[2022-03-16]. https://pubmed.ncbi.nlm.nih.gov/35436991/. DOI: 10.1038/s41419-022-04836-3.

[15] Lahola-Chomiak AA, Footz T, Nguyen-Phuoc K, et al. Non-synonymous variants in premelanosome protein (PMEL) cause ocular pigment dispersion and pigmentary glaucoma[J]. Hum Mol Genet, 2019, 28(8): 1298-1311. DOI: 10.1093/hmg/ddy429.

[16] Ding N, Chen Z, Song X, et al. Novel mutation of *GJA8* in autosomal

中华医学会杂志社 Chinese Medical Association Publishing House 版权所有 违者必究

dominant congenital cataracts[J/OL]. Ann Transl Med, 2020, 8(18): 1127[2022-03-26]. https://pubmed.ncbi.nlm.nih.gov/33240976/. DOI: 10.21037/atm-20-4663.

[17] Yuan L, Yao H, Xu Y, et al. CRISPR/Cas9-mediated mutation of αA-crystallin gene induces congenital cataracts in rabbits[J]. Invest Ophthalmol Vis Sci, 2017, 58(6): BIO34-BIO41. DOI: 10.1167/iovs.16-21287.

[18] Mei S, Wu Y, Wang Y, et al. Disruption of PIKFYVE causes congenital cataract in human and zebrafish[J/OL]. Elife, 2022, 11: e71256[2022-03-18]. https://pubmed.ncbi.nlm.nih.gov/35023829/. DOI: 10.7554/eLife.71256.

[19] Ping X, Cheng Y, Bao J, et al. KPNA4 is involved in cataract formation via the nuclear import of p53[J/OL]. Gene, 2021, 786: 145621[2022-03-18]. https://pubmed.ncbi.nlm.nih.gov/33798680/. DOI: 10.1016/j.gene.2021.145621.

[20] Gletten RB, Cantrell LS, Bhattacharya S, et al. Lens aquaporin-5 inserts into bovine fiber cell plasma membranes via unconventional protein secretion[J/OL]. Invest Ophthalmol Vis Sci, 2022, 63(8): 5[2022-03-18]. https://pubmed.ncbi.nlm.nih.gov/35816045/. DOI: 10.1167/iovs.63.8.5.

[21] Tang S, Di G, Hu S, et al. AQP5 regulates vimentin expression via miR-124-3p.1 to protect lens transparency[J/OL]. Exp Eye Res, 2021, 205: 108485[2022-03-19]. https://pubmed.ncbi.nlm.nih.gov/33582182/. DOI: 10.1016/j.exer.2021.108485.

[22] Kanzaki Y, Fujita H, Sato K, et al. *KCNJ13* gene deletion impairs cell alignment and phagocytosis in retinal pigment epithelium derived from human-induced pluripotent stem cells[J/OL]. Invest Ophthalmol Vis Sci, 2020, 61(5): 38[2022-03-22]. https://pubmed.ncbi.nlm.nih.gov/32437550/. DOI: 10.1167/iovs.61.5.38.

[23] Zhong H, Chen Y, Li Y, et al. CRISPR-engineered mosaicism rapidly reveals that loss of Kcnj13 function in mice mimics human disease phenotypes[J/OL]. Sci Rep, 2015, 5: 8366[2022-03-25]. https://pubmed.ncbi.nlm.nih.gov/25666713/. DOI: 10.1038/srep08366.

[24] Corton M, Avila-Fernandez A, Vallespín E, et al. Involvement of LCA5 in Leber congenital amaurosis and retinitis pigmentosa in the Spanish population[J]. Ophthalmology, 2014, 121(1): 399-407. DOI: 10.1016/j.ophtha.2013.08.028.

[25] Qu Z, Yimer TA, Xie S, et al. Knocking out *lca5* in zebrafish causes cone-rod dystrophy due to impaired outer segment protein trafficking[J]. Biochim Biophys Acta Mol Basis Dis, 2019, 1865(10): 2694-2705. DOI: 10.1016/j.bbadis.2019.07.009.

[26] Naert T, Colpaert R, Van Nieuwenhuysen T, et al. CRISPR/Cas9 mediated knockout of rb1 and rbl1 leads to rapid and penetrant retinoblastoma development in Xenopus tropicalis[J/OL]. Sci Rep, 2016, 6: 35264[2022-03-25]. https://pubmed.ncbi.nlm.nih.gov/27739525/. DOI: 10.1038/srep35264.

[27] Wang F, Wang Y, Zhang B, et al. A missense mutation in *HK1* leads to autosomal dominant retinitis pigmentosa[J]. Invest Ophthalmol Vis Sci, 2014, 55(11): 7159-7164. DOI: 10.1167/iovs.14-15520.

[28] Zhang L, Sun Z, Zhao P, et al. Whole-exome sequencing revealed *HKDC1* as a candidate gene associated with autosomal-recessive retinitis pigmentosa[J]. Hum Mol Genet, 2018, 27(23): 4157-4168. DOI: 10.1093/hmg/ddy281.

[29] Sharif AS, Yu D, Loertscher S, et al. C8ORF37 is required for photoreceptor outer segment disc morphogenesis by maintaining outer segment membrane protein homeostasis[J]. J Neurosci, 2018, 38(13): 3160-3176. DOI: 10.1523/JNEUROSCI.2964-17.2018.

[30] Parain K, Lourdel S, Donval A, et al. CRISPR/Cas9-mediated models of retinitis pigmentosa reveal differential proliferative response of müller cells between Xenopus laevis and Xenopus tropicalis[J/OL]. Cells, 2022, 11(5): 807[2022-08-25]. https://pubmed.ncbi.nlm.nih.gov/35269429/. DOI: 10.3390/cells11050807.

[31] Domènech EB, Andrés R, López-Iniesta MJ, et al. A new cerkl mouse model generated by CRISPR-Cas9 shows progressive retinal degeneration and altered morphological and electrophysiological phenotype[J/OL]. Invest Ophthalmol Vis Sci, 2020, 61(8): 14[2022-03-25]. https://pubmed.ncbi.nlm.nih.gov/32658961/. DOI: 10.1167/iovs.61.8.14.

[32] Sechrest ER, Murphy J, Senapati S, et al. Loss of PRCD alters number and packaging density of rhodopsin in rod photoreceptor disc membranes[J/OL]. Sci Rep, 2020, 10(1): 17885[2022-03-25]. https://pubmed.ncbi.nlm.nih.gov/33087780/. DOI: 10.1038/s41598-020-74628-2.

[33] Zhou Y, Tian W, Jiang X, et al. Deletion of Asrgl1 leads to photoreceptor degeneration in mice[J/OL]. Front Cell Dev Biol, 2021, 9: 783547[2022-03-26]. https://pubmed.ncbi.nlm.nih.gov/35118070/. DOI: 10.3389/fcell.2021.783547.

[34] Koster C, van den Hurk KT, Lewallen CF, et al. The Lrat$^{-/-}$ rat: CRISPR/Cas9 construction and phenotyping of a new animal model for retinitis pigmentosa[J/OL]. Int J Mol Sci, 2021, 22(13): 7234[2022-03-25]. https://pubmed.ncbi.nlm.nih.gov/34281288/. DOI: 10.3390/ijms22137234.

[35] Yeo JH, Jung BK, Lee H, et al. Development of a *Pde6b* gene knockout rat model for studies of degenerative retinal diseases[J]. Invest Ophthalmol Vis Sci, 2019, 60(5): 1519-1526. DOI: 10.1167/iovs.18-25556.

[36] Collery RF, Volberding PJ, Bostrom JR, et al. Loss of zebrafish Mfrp causes nanophthalmia, hyperopia, and accumulation of subretinal macrophages[J]. Invest Ophthalmol Vis Sci, 2016, 57(15): 6805-6814. DOI: 10.1167/iovs.16-19593.

[37] Prem Senthil M, Knight L, Taranath D, et al. Comparison of anterior segment abnormalities in individuals with *FOXC1* and *PITX2* variants[J]. Cornea, 2022, 41(8): 1009-1015. DOI: 10.1097/ICO.0000000000003020.

[38] Bonet-Fernández JM, Aroca-Aguilar JD, Corton M, et al. CPAMD8 loss-of-function underlies non-dominant congenital glaucoma with variable anterior segment dysgenesis and abnormal extracellular matrix[J]. Hum Genet, 2020, 139(10): 1209-1231. DOI: 10.1007/s00439-020-02164-0.

[39] Nakanishi T, Kato Y, Matsuura T, et al. CRISPR/Cas-mediated targeted mutagenesis in Daphnia magna[J/OL]. PLoS One, 2014, 9(5): e98363[2022-03-25]. https://pubmed.ncbi.nlm.nih.gov/24878568/. DOI: 10.1371/journal.pone.0098363.

[40] Yasue A, Kono H, Habuta M, et al. Relationship between somatic mosaicism of *Pax6* mutation and variable developmental eye abnormalities-an analysis of CRISPR genome-edited mouse embryos[J/OL]. Sci Rep, 2017, 7(1): 53[2022-03-26]. https://pubmed.ncbi.nlm.nih.gov/28246397/. DOI: 10.1038/s41598-017-00088-w.

[41] van Haelst MM, Maiburg M, Baujat G, et al. Molecular study of 33 families with Fraser syndrome new data and mutation review[J]. Am J Med Genet A, 2008, 146A(17): 2252-2257. DOI: 10.1002/ajmg.a.32440.

[42] Zhang X, Wang D, Dongye M, et al. Loss-of-function mutations in FREM2 disrupt eye morphogenesis[J]. Exp Eye Res, 2019, 181: 302-312. DOI: 10.1016/j.exer.2019.02.013.

[43] Moore BA, Flenniken AM, Clary D, et al. Genome-wide screening of mouse knockouts reveals novel genes required for normal integumentary and oculocutaneous structure and function[J/OL]. Sci Rep, 2019, 9(1): 11211[2022-03-26]. https://pubmed.ncbi.nlm.nih.gov/31371754/. DOI: 10.1038/s41598-019-47286-2.

[44] 范祥雨,徐建江. CRISPR/Cas9 基因组编辑技术在眼科疾病研究中的应用[J]. 中华实验眼科杂志, 2018, 36(7): 553-558. DOI: 10.3760/cma.j.issn.2095-0160.2018.07.015.
Fan XY, Xu JJ. Application of CRISPR/Cas9 genome editing technology in the research of eye diseases[J]. Chin J Exp Ophthalmol, 2018, 36(7): 553-558. DOI: 10.3760/cma.j.issn.2095-0160.2018.07.015.

（收稿日期:2022-03-30　修回日期:2022-08-29）

（本文编辑:刘艳　施晓萌）

中華醫學會雜誌社 Chinese Medical Association Publishing House 版权所有 违者必究