Genetics

Survey of Common Eye Diseases in Laboratory Mouse Strains

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PURPOSE. As in human populations, in which founder mutations have been identified in groups of families, a number of founder mutations have been observed across strains in mice. In this report, we provide a phenotype and genotype survey of three common eye diseases in the collection of JAX mice strains at The Jackson Laboratory (JAX). These eye diseases are retinal degeneration 1 (*Pde6b^{rd1}*), retinal degeneration 8 (*Crb1^{rd8}*), and cone photoreceptor function loss 3 (*Gnat2^{cpfl3}*).

METHODS. Ocular lesions for *rd1* and *rd8* were evaluated by fundus examination and fundus photography, and the abnormal retinal function observed in mice homozygous for *cpfl3* was assessed by ERG. Genotyping protocols for *rd1*, *rd8*, and *cpfl3* mutations were performed by PCR with appropriate primers.

RESULTS. We have actively screened retired breeders for surface dysmorphologies, and for intraocular defects by indirect ophthalmoscopy, slit-lamp biomicroscopy, and ERG to discover new spontaneous mutations in strains from the Genetic Resource Science (GRS) production colony. Through this process, we have found that of the strains screened, 99 strains carried the *rd1* mutation, 85 strains carried the *rd8* mutation, and 20 strains carried the *cpfl3* mutation.

CONCLUSIONS. Of the 1000 of strains screened during this study, 204 carried one of three founder mutations in *Pde6b*, *Crb1*, or *Gnat2*. Since these three retinal mutations occur commonly in various mouse strains, genotyping for these mutations, and/or avoiding mouse strains or stocks carrying these mutant alleles when studying new retinal disorders is recommended. The robust PCR genotyping protocols to test for these common alleles are described herein.

Keywords: retinal degeneration, fundus photography, spontaneous mutation, animal model, eye diseases

D iscovery of human retinal degenerations generally is identified by self-reports of visual problems with subsequent eye examinations. However, determining the etiology and the disease progression, elucidating the mechanisms underlying the disease, and exploring methods for delaying or ameliorating retinal degenerations can be impeded by ethical considerations of exploratory experimentation on humans. Also, human eye tissue (including biopsies) seldom is available for study because it is difficult to obtain tissue from the eye without risk of further damage to the patient's vision, and if samples are available, they usually are at the end stages of the disease process.

Animal models have advanced our understanding of eye diseases significantly as they allow for sampling of tissue throughout development and disease progression, for invasive studies, and for rapid genetic analysis. Use of mouse models, in particular, has increased through the past decades due to their ability to be modified genetically. In addition to manipulation by genetic engineering, a large number of spontaneous and chemically-induced mutations have been identified by screening mice using indirect ophthalmoscopy and ERG at The Jackson Laboratory (JAX; Bar Harbor, ME), where many mouse models of retinal degeneration and diseases have been discovered.[1,2] These mouse models have increased significantly

our understanding of the function of specific genes and how they lead to disease when disrupted. Studies of mouse models of human retinal degeneration have contributed to our understanding of the pathophysiology, as well as the etiology, of these diseases, and allowed for development of gene-based therapy to delay the progression of disease and increase visual function.[3,4]

Historically, the first retinal degeneration model was described by Clyde E. Keeler more than 80 years ago. It subsequently was identified as a disruption in phosphodiesterase 6 beta (*Pde6b^{rd1}*, formerly *rd1*, *rd*, identical to Keeler rodless retina, *r*).[5–7] The second retinal degeneration model was discovered 50 years later, and named retinal degeneration-slow (*Rds* or *Rd2*).[8] The third retinal degeneration was discovered in 1993 and named retinal degeneration 3, *rd3*.[9] Subsequently, during the last 20 years, many additional mouse models of retinal degeneration have been discovered as the tools used commonly in human clinical eye examinations have been adapted for use with the smaller mouse eyes.[10,11]

Interestingly, through screening many retired breeders of different mouse strains at JAX, mutations in *Pde6b*, *Crb1*, and *Gnat2* have been found to recur in high frequency. Because the strains examined all bore the same respective mutations within these particular genes, this suggested that they were founder
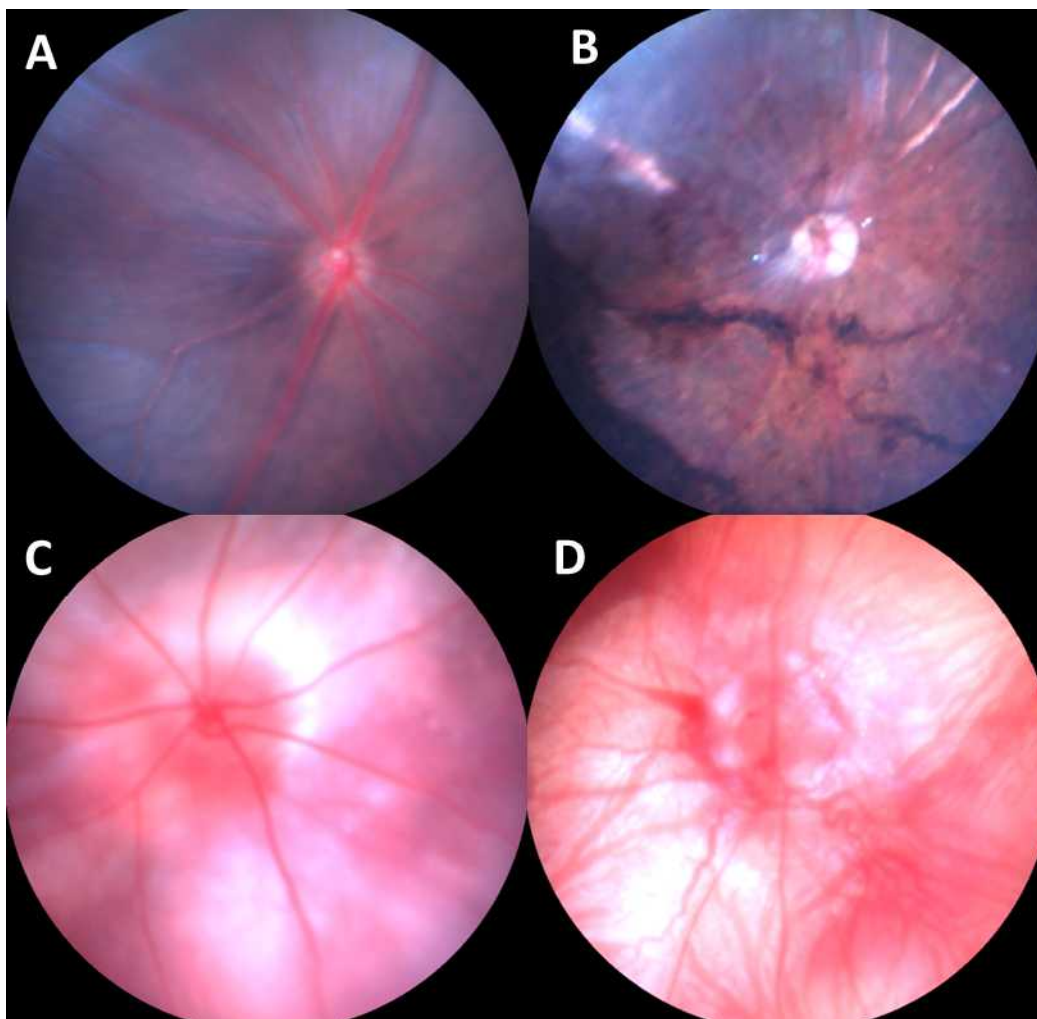
Figure 1. Fundus photographs of normal pigmented (**A**) and albino (**C**) mice as well as fundus photographs of mice bearing the $Pdeb6^{rd1}$ mutation in pigmented (**B**) and albino (**D**) background.

mutations that had been propagated across multiple strains through the years of model development and inbreeding.[12–14]

As significant retinal pathology occurs in the presence of $Pde6b^{rd1}$ and $Crb1^{rd8}$, and functional deficits occurs in the presence of $Gnat2^{cpfl3}$, interpretation of disease phenotypes resulting from different gene/mutations and experimental studies where behavioral performance is an end point, respectively, may be confounded. Knowledge of the genotype for the common disease alleles in these experimental models will aid in the proper selection of mouse strains for use in research studies and interpretation of data generated from them.

MATERIALS AND METHODS

Mice

The mice screened in this study were bred and maintained in standardized conditions at the Production and Research Animal Facilities at JAX. They were maintained on a NIH31 6% fat chow diet and provided acidified water, in a pathogen-free vivarium environment with a 14-hr light/10-hr dark cycle. One pair of retired breeders from the JAX Mouse Repository was screened for each strain. All experiments were approved by the Institutional Animal Care and Use Committee, and conducted

in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Clinical Retinal Evaluation

Mouse eyes, dilated with 1% atropine (Alcon Laboratories, Inc., Fort Worth, TX), were evaluated by indirect ophthalmoscopy with a 90 diopter lens. If a mouse strain showed signs of early and rapid retinal degeneration, such as attenuation of retinal vessels or areas of depigmentation (Fig. 1B), the mouse strain was genotyped for the $rd1$ mutation. If a mouse strain in the C57BL/6 genetic background or related background showed a retinal spotting phenotype (Fig. 2A), the mouse strain was typed for the $rd8$ mutation. Fundus photographs were taken with the Micron III retinal imaging microscope from Phoenix Research Laboratories (Phoenix, AZ).

Simplified Screening for Functional ERG Alterations

Attenuation of retinal vessels and disturbances in the retinal pigment epithelium are detected easily by indirect ophthalmoscopy. However, even if the retina has a normal appearance, retinal function still may be impaired. Therefore, all strains with a normal fundus appearance by indirect
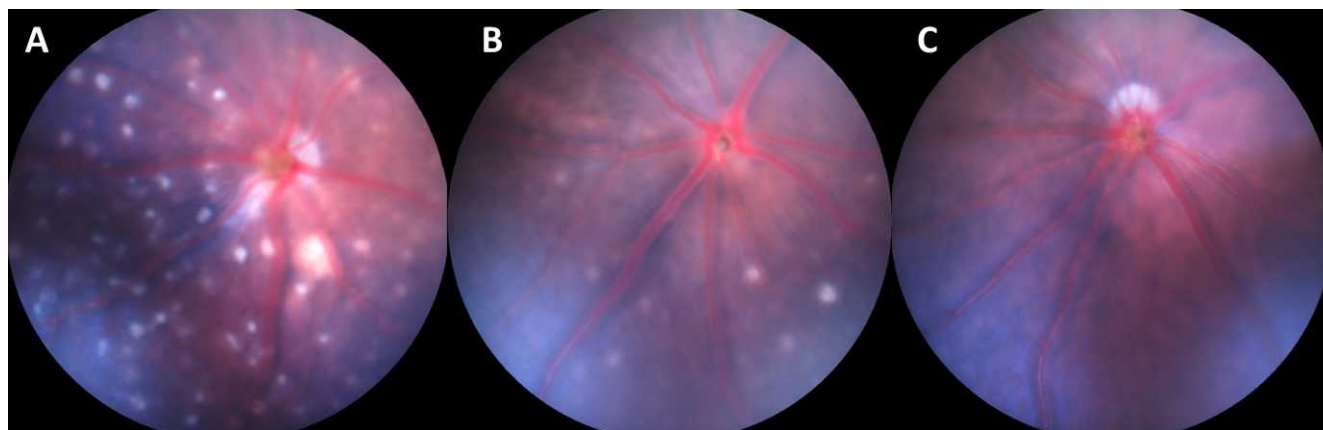
Figure 2. Fundus photographs of mice homozygous for the *Crb1rd8* mutations with the typical pattern of white retinal spots (**A**), with a few white retinal spots (**B**), and with no retinal white spots (**C**).

ophthalmoscopy were screened for functional deficits by ERG. Because the standard ERG test is time consuming, requiring approximately 30 to 60 minutes per mouse (not including the time necessary for dark adaption), we have developed a simple screening ERG test that takes less than 10 minutes per mouse to detect for functional deficits, such as retinal cone photoreceptor function loss (achromatopsia)[14,15] and no b-wave (*nob*) mutations.[16,17]

Briefly, one eye per mouse was dilated with 1% cyclopentolate hydrochloride ophthalmic solution (Bausch & Lomb, Inc., Tampa, FL). A group of 5 to 10 mice could be dilated at the same time. Mice were weighed to determine the proper dosage of anesthesia (0.1 mL/20 g body weight of a mixture of 0.8 mL ketamine [Ketamine KCL injection USP, 100 mg/mL; Butler Schein Animal Health, Dublin, OH], 0.8 mL xylazine [Xylazine sterile solution, 20 mg/mL; Butler Schein Animal Health], and 3.4 mL 0.9% sodium chloride). Before administering the anesthetic via intraperitoneal injection, the eye to be tested was treated with cyclomydril (0.2% cyclopentolate hydrochloride, 1% phenylephrine hydrochloride ophthalmic solution, sterile; Alcon Laboratories, Inc.) to moisten the surface of the eye. The sedated mouse was placed on a 37°C heating pad to maintain the temperature of the animal during testing. A needle probe was inserted just under the skin at the base of the tail for grounding, a gold loop electrode was placed between the gum and cheek, and the active gold loop electrode was placed gently on the cornea slightly below the middle of the eye. A drop of 2.5% Gonioscopic Prism Solution (Wilson Ophthalmic, Mustang, OK) was placed on the cornea and electrode to assure a good contact and to prevent drying of the eye surface. In the simplified test, 10 flashes of the highest light intensity (light intensity = 1.9 log cd/m²) were averaged. If a mouse had a photopic b-wave amplitude at or above 100 μV and implicit times at approximately 50 ms, the mouse was considered normal (Fig. 3A), whereas a mouse with a b-wave amplitude at or below 50 μV and/or implicit times longer than 50 ms was considered abnormal (Fig. 3B). An abnormal ERG response was confirmed by the longer, standard ERG test with appropriate dark/light adaptations.[18]

Genotyping Protocols

Genomic DNA for genotyping mice was prepared from tail tips by the rapid, hot sodium hydroxide and Tris (HotSHOT) procedure,[19] and 1 μL of the DNA supernatant was used in a 10 μL PCR reaction. Amplicons were visualized with ethidium bromide after electrophoretic separation on a 4% agarose gel.
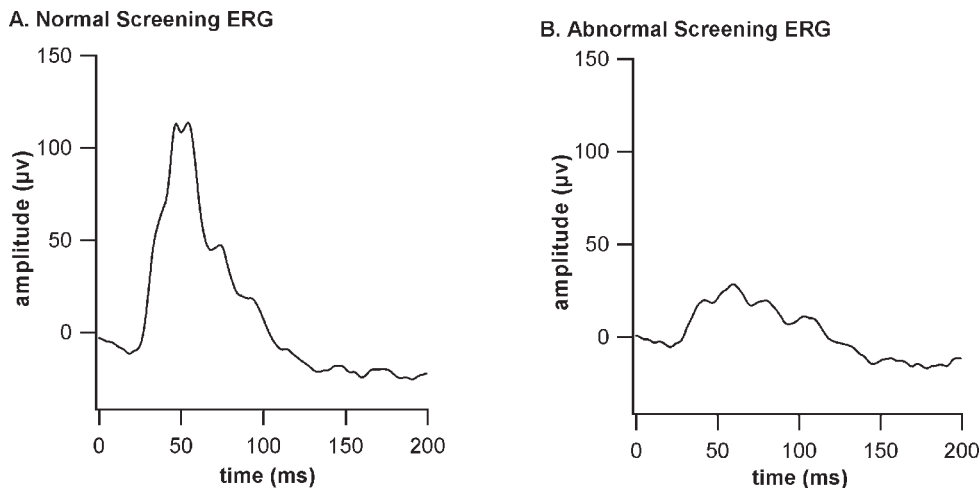

Figure 3. Representative ERG responses to a bright flash obtained from a mouse with normal retinal function (**A**) and a mouse with abnormal retinal function (**B**).

TABLE 1. JAX Mice With Retinal Degeneration 1 Mutation ($Pde6b^{rd1}$)

TABLE 1. Continued

#	Strain Name	Stock Number
1	STOCK Th^{tm1Srt}/J	008779
2	STOCK Tg(Stra8-cre)1Reb/J	008208
3	STOCK Tg(Neurog1-cre)1Jejo/J	012859
4	STOCK Tg(Nefh-cre)12Kul/J	009102
5	STOCK Tg(Myh6-Prkca)1Jmk/J	010579
6	STOCK Tg(Myh6-Ppp3ca)37Eno/J	009075
7	STOCK Tg(mI56i-FLPe)39Fsh/J	010815
8	STOCK Tg(KRT5-rtTA)T2D6Sgkd/J	017519
9	STOCK Tg(I12b-cre/ERT2,-ALPP)37Fsh/J	014600
10	STOCK Tg(CAG-Venus)1Hadj/J	011107
11	STOCK $Smn1^{tm1Msd}$ Tg(H2-K1-tsA58)6Kio Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb/J	006553
12	STOCK $Sirt6^{tm1.1Cxd}$/J	017334
13	STOCK $Kcna2^{tm1Tem}$/J	010744
14	STOCK Fxn^{tm1Mkn} Tg(FXN)YG22Pook/J	010963
15	STOCK $En1^{tm7(cre/ESR1)Alj}$/J	007917
16	STOCK $En1^{tm2Alj}$/J	007912
17	STOCK $En1^{tm2(cre)Wrst}$/J	007916
18	STOCK $Blm^{tm4Ches}$/J	008670
19	STOCK Axl^{tm1Grl}/J	011121
20	$Smn1^{tm3(SMN2/Smn1)Mrpb}$Tg(SMN2*delta7)4299Ahmb	008869
21	SJL/J-Chr Y^{B10}/TeuJ	016840
22	SJL.129S2(C)-$Cxcr2^{tm1Mwm}$/RmraJ	013043
23	RSV/LeJ	000268
24	HPG/BmJ	000804
25	FVB-Tg(Ttr-Igf1)1Sykr/J	012662
26	FVB-Tg(tetO-Ppargc1b)7Dpk/J	012385
27	FVB-Tg(tetO-Ppargc1a)1Dpk/J	012387
28	FVB-Tg(tetO-MET)23Rwng/J	008695
29	FVB-Tg(tetO-Cib1)1Jmol/J	013780
30	FVB-Tg(tetO-Cacnb2)1Jmol/J	013778
31	FVB-Tg(Pbsn-IGF1*)5305Ng/J	008874
32	FVB-Tg(Pbsn-Ar*E231G)7353Ng/J	008878
33	FVB-Tg(NPEPPS)1Skar/J	013732
34	FVB-Tg(Myh6-TRPC3*)6.6Jmol/J	016570
35	FVB-Tg(Myh6-SOD2,Tyr)3Pne/J	009438
36	FVB-Tg(Myh6-cre)2182Mds/J	011037
37	FVB-Tg(Myh6/tetO-Itpr2)3.11Jmol/J	014153
38	FVB-Tg(Myh6/tetO-Itpr1)22.3Jmol/J	014155
39	FVB-Tg(Myh6/tetO-Gata6)2Jmol/J	016571
40	FVB-Tg(MECP2)1Hzo/J	008679
41	FVB-Tg(Lactb)74.2Lus/J	008784
42	FVB-Tg(Gstm5-EGFP)1Ilis/J	010947
43	FVB-Tg(GFAP-luc,GAPDH-rluc)172.9Mes/J	009638
44	FVB-Tg(GFAP-CRYAB)141.6Mes/J	010676
45	FVB-Tg(GAS7)63.2Lus/J	010515
46	FVB-Tg(Cdh5-tTA)D5Lbjn/J	013585
47	FVB-Tg(CAG-luc,-GFP)L2G85Chco/J	008450
48	FVB-Tg(C3-1-TAg)cJeg/JegJ	013591
49	FVB-Tg(ACTA1-PABPN1*A17)1Drub/DrubJ	016193
50	FVB;129S6-$Snca^{tm1Nbm}$ Tg(SNCA)1Nbm/J	010710
51	FVB-N-Tg(tetO-Fasl)BDepa/J	014547
52	FVB/N-Tg(Myh6-Gnaq)40Gwd/J	012460
53	FVB/N-Tg(Myh6*tetO-Capn1)L2Gwd/J	012459
54	FVB/N-Tg(LRRK2*R1441G)135Cjli/J	009604
55	FVB/N-Tg(LRRK2*G2019S)1Cjli/J	009609
56	FVB/N-Tg(LRRK2)1Cjli/J	009610
57	FVB/N-Tg(HTT*97Q)IXwy/J	008197
58	FVB/N-Tg(Hoxc13)61B1Awg/J	011032
59	FVB/N-Tg(GFAP-HTT*160Q)31Xjl/J	012630
60	FVB/N-Tg(Dazl-EGFP)10Rarp/J	009354
61	FVB/N-Tg(CAG-EGFP,-ALPP)2.6Ggc/J	008200
62	FVB/N-Tg(Acta2-RAC1*G12V)33Pjgc/J	010634
63	FVB/N-$Midn^{Tg(Tyr)2261EOve}$/J	017438
64	FVB/NJ-Tg(Slc6a3-PARK2*Q311X)AXwy/J	009090
65	FVB/NJ-Tg(Hspa1a-luc,-EGFP)2Chco/J	012370
66	FVB/N-$Ckap5^{TgTn(sb-cHS4,Tyr)2320F-1Ove}$/J	017437
67	FVB.C-$Prdm16^{csp1}$/J	013100
68	FVB.Cg-Tg(tetO-cre)1Jaw/J	008244
69	FVB.Cg-$Smn1^{tm6(SMN2)Mrpb}$/J	009381
70	FVB.Cg-$Smn1^{tm1Msd}$ Tg(SMN2)89Ahmb Tg(SMN2*A111G)591Ahmb/J	009134
71	FVB.Cg-$Gt(ROSA)26Sor^{tm1(CAG-lacZ,-EGFP)Glb}$/J	012429
72	FVB.B-Wld^{S}/UmonJ	008820
73	FVB.A-$Irf6^{clft1}$/BeiJ	012655
74	FVB.129X1(B6)-$Trfr2^{tm1Slu}$/J	005215
75	FVB.129S6-$Fmn1^{tm2Made}$/J	008665
76	FVB.129S4-$Ptpn1^{tm1Bbk}$/Mmjax	032242
77	FVB.129S4(B6)-$Scn4a^{tm1.1Ljb}$/J	011033
78	FVB.129-$Fmr1^{tm1Rbd}$/J	008909
79	FVB.129(Cg)-$Slc9a3^{tm1Ges}$/J	012563
80	FVB.129(B6)-$Smn1^{tm5(Smn1/SMN2)Mrpb}$/J	008604
81	FVB.129(B6)-$Smn1^{tm4(SMN2)Mrpb}$/J	008713
82	FVB.129(B6)-$Igf1^{tm1Dlr}$/J	012663
83	FVB(Cg)-Tg(Ghrhr-cre)3242Lsk/J	011034
84	C3h-f	009101
85	C3H/HeOuJ-$Maoa^{Tg(H2-K1-Ifnb1)8Seif}$/J	014132
86	C3;B6-Tg(AAVS1)A1Xob/J	009601
87	C3.129S7(B6)-$Ifng^{tm1Ts}$/J	008228
88	C3.129S4-$Mtnr1b^{tm1Drw}$/J	010488
89	C3.129S4(B6)-$Mtnr1a^{tm1Rep}$/J	009681
90	BXSB.129P2(Cg)-$Tcra^{tm1Mjo}$/TheoJ	007848
91	B6;SJL-Tg(Thy1-COP3/EYFP)8Gfng/J	012348
92	B6;SJL-Tg(tetO-Erbb2*)8-4Jek/J	010577
93	B6;SJL-Tg(MMTV-rtTA)4-1Jek/J	010576
94	B6;SJL-Tg(LRRK2)66Mjff/J	013725
95	B6;SJL-Tg(Gh1-rtTA)4-3Jek/J	010574
96	B6;129S2-$Nfatc3^{tm1Glm}$/J	010589
97	B6.Cg-Tg(Tek-cre)1Ywa/J	008863
98	B6.Cg-Tg(Nlrp1b)1Die/DieJ	006840
99	B6.129S2-$Sele^{tm1Hyn}$ $Selp^{tm1Hyn}$/J	008437

Genotyping for $Pde6b^{rd1}$

The genotyping for $Pde6b^{rd1}$ was done by three-primer PCR.[20,21] A pair of primers, G1(5′-CCTGC ATGTGAACCCAG TATTCTATC) and G2 (5′-CTACAGCCCCTCTCCAAGGTTTAT AG) generated a product (240 base pair) from normal control DNA, while primer G2 paired with primer XMV (5′-AAGCTAGCTGCAGTAACGCCATTT), which primes within the virus sequence, generated a larger product (560 base pair) from the rd1 mutant allele. These primers were unique and were able to detect the mutant allele.

Genotyping for $Crb1^{rd8}$

Allele-specific PCR was used on genomic DNA to confirm the presence of the $Crb1^{rd8}$ mutation[22] using the primers: Crb1-mF1: 5′-GTGAAGAAGACAGCTACAGTTCTGATC; Crb1-mF2: 5′-GCCCCTGTTTGCATGGAGGAAACTTGGAAGACAGCTACAGTT CTTCTG; and Crb1-mR: 5′-GCCCCATTTGCACACTGAT. We made some sequence changes in each primer to improve the genotyping results.

TABLE 2. JAX Mice With the Retinal Degeneration 8 (*Crb1^{rd8}*) Mutation

#	Strain Name	Stock Number	Retinal Spots
1	STOCK *Wap^{tm2(rtTA)Kuw}*/J	016116	N
2	STOCK *Trpa1^{tm2Kykw}* Tg(CAG-cre/Esr1*)5Amc/J	008813	Y
3	STOCK Tg(Tph2-icre/ERT2)6Gloss/J	016584	N
4	STOCK *Sst^{tm2.1(cre)Zjh}*/J	013044	N
5	STOCK *Nphp1^{tm1Jgg}*/J	013169	Y
6	STOCK *Mov10l1^{tm1.1Eno}*/J	014179	N
7	STOCK *Gt(ROSA)26Sor^{tm6(Gli1)Amc}*/J	013123	N
8	STOCK *Gad2^{tm1(cre/ERT2)Zjh}*/J	010702	N
9	STOCK Et(icre/ERT2)14602Rdav/J	012692	N
10	STOCK *Epn3^{tm1.1Pdc}*/J	014108	N
11	STOCK *Dclk2^{tm1Jgg}*/J	013172	N
12	STOCK *Cck^{tm1.1(cre)Zjh}*/J	012706	N
13	CXB9/HiAJ	001630	N
14	CXB5/ByJ	000355	N
15	CXB3/ByJ	000353	N
16	CXB2/ByJ	000352	N
17	CXB12/HiAJ	001633	N
18	C57BL/6-*Zbtb7b^{tm1.2Litt}*/J	008775	N
19	C57BL/6-Tg(tetO-EDN1,-lacZ)9Mhus/J	013729	N
20	C57BL/6-Tg(LacZpl)60Vij/J	002754	Y
21	C57BL/6-Tg(Defa2-Myd88)1Lvh/J	016133	N
22	C57BL/6N-Tg(Slc6a3-icre/ERT2)2Gloss/J	016583	Y
23	C57BL/6N-Tg(Slc32a1-icre/ERT2)3Gloss/J	016582	N
24	C57BL/6N-*Pawr^{tm1Rang}*/J	015823	N
25	C57BL/6N-*Agtr1a^{tm1Uky}*/J	016211	N
26	B6N;129-*Egr1^{tm1Jmi}*/J	012924	N
27	B6N.Cg-Tg(UGT1A1*28)1Rhtu/J	014170	N
28	B6N.129-*Ptpn5^{tm1Pjlo}*/J	016556	N
29	B6N.129-*Ptch1^{tm1Habn}*/J	012457	Y
30	B6N.129-*Ptch1^{tm1Habn}*/J	011052	N
31	B6N.129-*Ptch1^{tm1Habn}*/J	012457	N
32	B6N.129P2-*Adora1^{tm1Bbf}*/J	014161	N
33	B6129S-Del(7Slx1b-Sept1)4Aam/J	013128	N
34	B6;DBA-Tg(Cited1-TagRFP)26Amc/J	015853	N
35	B6;D2-Tg(tetO-SNCA)1Cai/J	012450	N
36	B6;CBA-Tg(Thy1-spH)21Vnmu/J	014651	N
37	B6;C3-Tg(PDGFB-LRRK2*R1441C)574Djmo/J	016576	N
38	B6;129X1-*Snca^{tm1Rosl}*/J	003692	N
39	B6;129X1-*Dusp6^{tm1Jmol}*/J	009069	Y
40	B6;129X1-*Dkkl1^{tm1.1Mldp}*/J	012711	N
41	B6;129S-*Syn2^{tm1Sud}*/J	002477	Y
42	B6;129S-*Sele^{tm2Hyn}* *Sell^{tm4Hyn}*/J	003806	Y
43	B6;129S-*Prkd1^{tm1Eno}*/J	014181	N
44	B6;129S-*Nr1h2^{tm1Djm}*/J	014635	N
45	B6;129S4-*Ighm^{tm1Che}*/J	003751	Y
46	B6;129S4-*Col1a1^{tm1(tetO-Pou5f1,-Klf4,-Sox2,-Myc)Hoch}*/J	011001	Y
47	B6;129S2-*Itgb3^{tm1Hyn}*/J	004669	N
48	B6;129S1-*Dnm3^{tm1.1Pdc}*/J	013543	N
49	B6;129-*Nrxn3^{tm4.1Sud}*/J	016194	N
50	B6;129-*Mavs^{tm1Zjc}*/J	008634	N
51	B6;129-*Iis1^{tm3(CAG-Bgeo,-tdTomato/TEVP,-Dlg4,-GFP)Nat}*/J	012587	N
52	B6;129-*Iis1^{tm2(CAG-Bgeo,-Ndp,-EGFP)Nat}*/J	011077	N
53	B6;129-*Igf1r^{tm2Arge}*/J	012251	N
54	B6;129-*Hprt^{tm1Detl}*/J	003183	Y
55	B6;129-*Crhr1^{tm1Klee}*/J	004454	Y
56	B6;129-*Cd3e^{tm1Lov}*/J	004177	Y
57	B6.SJL-Tg(Vil-cre)997Gum/J	004586	Y
58	B6.FVB-Tg(CMA1-cre)6Thhe/J	014643	N
59	B6.Cg-Tg(UBC-TVA)1Clc/J	015808	N
60	B6.Cg-Tg(tetO-Ifng)184Pop/J	009344	Y
61	B6.Cg-Tg(Itgax-TGFBR2)1Flv/J	008378	N
62	B6.Cg-Tg(Col1a1-cre/ERT2)1Crm/J	016241	N
63	B6.Cg-Tg(CMV-CASP3)17Edge/J	016908	N
64	B6.Cg-*Selplg^{tm1Fur}*/J	004201	Y

TABLE 2. Continued

#	Strain Name	Stock Number	Retinal Spots
65	B6.Cg-*Pkd2l1*$^{tm1.1Yuni}$/J	016853	N
66	B6.Cg-*Gt(ROSA)26Sor*$^{tm1(rtTA*M2)Jae}$ *Col1A1*$^{tm6(tetO-MSI2)Jae}$/J	014588	N
67	B6.129S4-*Mtor*$^{tm1.2Koz}$/J	011009	N
68	B6.129S4(FVB)-*Insr*^{tm1Kbn}/J	006955	Y
69	B6.129S4(Cg)-*Cdk5*$^{tm1.1Lbt}$/J	014156	N
70	B6.129S2-*Pak4*^{tm1Amin}/J	015829	N
71	B6.129S2-*Mapk9*^{tm1Flv}/J	004321	Y
72	B6.129S2-*Il10rb*^{tm1Agt}/J	005027	N
73	B6.129S2-*H2-DMa*^{tm1Doi}/J	004513	Y
74	B6.129S1-Nf1^{tm1Cbr}/J	007923	Y
75	B6.129S1-*Mapk10*^{tm1Flv}/J	004322	Y
76	B6.129S1(Cg)-*Dnm2*$^{tm1.1Pdc}$/J	013542	N
77	B6.129S(Cg)-*Id2*$^{tm1.1(cre/ERT2)Blh}$/ZhuJ	016222	N
78	B6.129S(C)-*Batf3*^{tm1Kmm}/J	013755	N
79	B6.129P-*Cx3cr1*^{tm1Litt}/J	005582	N
80	B6.129P2-*Icos*^{tm1Mak}/J	004859	N
81	B6.129P2-*Axin2*^{tm1Wbm}/J	009120	N
82	B6.129-*Bace1*^{tm1Pcw}/J	004714	Y
83	B6(Cg)-*Il10*$^{tm1.1Karp}$/J	014530	N
84	B6(Cg)-*Crb*$^{tm1(cre)Zjh}$/J	012704	N
85	B6(Cg)-*Cck*$^{tm2.1(cre/ERT2)Zjh}$/J	012710	Y

Genotyping for *Gnat2*^{cpfl3}

The *Gnat2*^{cpfl3} mutation resulted in a new *MseI* site that enabled us to genotype the *cpfl3* mutation with a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) assay described previously.[14]

RESULTS

JAX, which has the world's largest collection of mouse mutant stocks and genetically diverse inbred strains (available in the public domain at http://jaxmice.jax.org/index.html), has been an ideal place to discover and characterize models of heritable retinal disorders. By screening approximately 1000 strains of JAX mice from the JAX Mouse Repository, three highly prevalent retinal disorders have been detected.

Detection of the *Pde6b*^{rd1} Mutation

Mice homozygous for the *Pde6b*^{rd1} mutation have a severe, early onset retinal degeneration associated with a murine viral insertion and a second nonsense mutation in exon 7 of the *Pde6b* gene, which encodes the beta subunit of cGMP-phosphodiesterase. Mice with the *Pde6b*^{rd1} mutation can be detected easily by indirect ophthalmoscopy. Retinal vessels are attenuated (Figs. 1B, 1D) and areas of retinal depigmentation are observed (Fig. 1B). In this survey, once the *rd1*-like retinal degeneration phenotype (Figs. 1B, 1D) was detected, *Pde6b*^{rd1} allele specific genotyping was done to confirm the *rd1* mutation. By screening approximately 1000 mouse strains (the entire JAX mouse collection currently contains over 6000 strains) from JAX, 99 strains were confirmed to carry the *Pde6b*^{rd1} mutation (Table 1).

Presence of the *Crb1*^{rd8} Mutation

Mice homozygous for *Crb1*^{rd8} mutation exhibit large white retinal spots covering the inferior nasal quadrant of the retina (Fig. 2A) and a slow progressive retinal degeneration. It is caused by a single nucleotide deletion in the *Crb1* gene. Because the degree of the spotting phenotype is highly variable (Figs. 2A–C) in mice homozygous for the *rd8* mutation, it is difficult to determine whether a strain carries the *rd8* mutation by clinical fundus examination alone. During the period between 2003 and 2011, 17 strains (Table 2) were found to carry the *Crb1*^{rd8} mutation by fundus examination and confirmed by *rd8* genotyping. In the past year, the *Crb1*^{rd8} genotyping protocol was added to our Eye Mutant Resource screening program. By screening 302 strains, 66 strains were found to be homozygous for the *Crb1*^{rd8} mutation by genotyping (Table 2). However, only 6 of them had the retinal white spots phenotype (Table 2).

TABLE 3. JAX Mice With the Cone Photoreceptor Function Loss 3 (*Gnat2*^{cpfl3}) Mutation

#	Strain Name	Stock Number
1	STOCK Zp2^{tm1Dean}/J	004129
2	STOCK Tg(Ttr-RFP)1Hadj/J	011108
3	STOCK Tg(Trp53A135V)L3Ber/J	003262
4	STOCK Tg(tetO-Ipf1,EGFP)956.6Macd/J	005699
5	STOCK Tg(tetO-HIST1H2BJ/GFP)47Efu/J	005104
6	STOCK Tg(Stra8-cre)1Reb/J	008208
7	STOCK Tg(Neurog3-cre)C1Able/J	005667
8	STOCK Tg(Neurog1-cre)1Jejo/J	012859
9	STOCK Tg(Fos-lacZ)34Efu/J	004623
10	STOCK Tg(CAG-mRFP1)1F1Hadj/J	005645
11	STOCK Tg(CAG-KikGR)33Hadj/J	013753
12	STOCK Tg(CAG-EGFP)B5Nagy/J	003115
13	STOCK Tg(CAG-ECFP)CK6Nagy/J	003773
14	STOCK Sgk3^{fz-ica}/McirJ	006135
15	STOCK Bcl2^{tm1Irt}/J	008882
16	SENCARC/PtJ	002748
17	SENCARB/PtJ	002747
18	SENCARA/PtJ	002746
19	PN/nBSwUmabJ	005052
20	CD10/JlsJ	006180

Frequency of the *Gnat2^{cpfl3}* Mutation

Homozygous *Gnat2^{cpfl3}* mice exhibit reduced cone-mediated ERG responses as early as 3 weeks of age with normal rod-mediated ERG responses. The *cpfl3* mutation originally was discovered in ALS/LtJ mice by routine ERG screening of retired breeders. Sequence analysis revealed a missense mutation due to a single base pair substitution in exon 6 of the *Gnat2* gene. Subsequently, seven additional strains also were confirmed to carry the *Gnat2^{cpfl3}* mutation (Table 3).[14] In our report, using the simple screening ERG method described above, once a strain showed an abnormal ERG response (Fig. 3B), it was genotyped using the *Gnat2^{cpfl3}* genotyping protocol. By genotyping the strains with abnormal ERG responses, 13 additional strains were confirmed to carry the *Gnat2^{cpfl3}* mutation (Table 3).

Discussion

Major advantages of using the mouse as a model system for ocular diseases include the physiologic and anatomic similarities of the mouse and human eye, the availability of clinical tools to characterize the models, and the well-developed resources available for data mining, for biochemical and physiologic studies, and for molecular manipulation of their genomes. For example, the ability to target and alter specific gene(s) is an important tool to produce mouse models with mutations in genes and tissues of choice. Inducing mutations by transgenesis or homologous recombination is termed "reverse genetics," as opposed to "forward genetics" approaches whereby spontaneous/induced mutations are discovered as a result of overt phenotypes and the underlying gene/mutation subsequently is identified. Through "forward" and "reverse" genetic approaches, mouse models in >100 genes that underlie human retinal diseases have been studied.[23]

Examination of the mouse fundus by indirect ophthalmoscopy is a very powerful, noninvasive tool for evaluating the appearance of the mouse retina. It also is an effective approach for high throughput screening for mouse models of human retinal degeneration and diseases. Many examples of mouse models of retinal degeneration have been discovered by fundus examination.[1,2] However, given the high frequency of the *Pde6b^{rd1}* and *Crb1^{rd8}* mutations, it is important to avoid mouse strains or stocks carrying these mutant alleles, or to exclude these mutant alleles before studying new retinal models. Both of these founder mutations can be identified easily by genotyping. While mice with the *Pde6b^{rd1}* mutation can be typed by fundus examination (Fig. 1) other mutations, such as *Pde6a^{nmf363}*, *Pde6a^{282}*, and B6.129-*tulp1^{-/-}*, are known to have similar disease characteristics, onset, and progression.[24,25]

The spontaneous *Crb1^{rd8}* mutation is likely to have occurred during the mid 1950s, when C57BL/6J mice were transferred to the National Institutes of Health (NIH; Bethesda, MD).[13] During the intervening 60 years, this mutation has been widely disseminated, as indicated by the number of strains affected. Ocular models derived from the C57BL/6N strain background, which carries the *Crb1^{rd8}* mutation, should be genotyped even if a retinal spotting phenotype is not observed (Fig. 2, Table 2). Unfortunately, many embryonic stem cell lines that are used to generate genetically engineered models also carry the *Crb1^{rd8}* mutation as they were B6N derived.[13] Despite the lack of retinal spots, *Crb1^{rd8}* mice all show a fragmented outer limiting membrane that may confound the interpretation of the disease characteristics of new retinal models being characterized.[22]

The simple screening ERG used in our study is another powerful, noninvasive and high throughput method to evaluate mouse retinal function, as mice with a normal fundus appearance still may be impaired functionally.[14]

The results from our survey provide information regarding the frequency of three commonly found mutations among different mouse strains. Strains that were positive for these three commonly found mutations will be listed in the JAX mice database (available in the public domain at http://jaxmice.jax.org/index.html). The genotyping protocols described should assist in identifying these founder mutations in new models of ocular diseases and for selection against these mutations to exclude their contributions to ocular phenotypes when they are found.

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