HHS Public Access

Author manuscript

J Glaucoma. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

J Glaucoma. 2018 July ; 27(Suppl 1): S78–S82. doi:10.1097/IJG.0000000000000911.

Update on Animal Models of Exfoliation Syndrome

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Abstract

Animal models are powerful tools for studying diseases that affect the eye, such as exfoliation syndrome. Two types of animal models have been used to investigate the pathophysiology of exfoliation syndrome and glaucoma. One class of animal models is engineered to have key features of a disease by alteration of their genome (genotype-driven animal models). *LOXL1* is the first gene known to increase the risk for developing exfoliation syndrome in humans. Two transgenic mouse models with altered *Loxl1* genes have been generated to study exfoliation syndrome. One strain of mice, *Loxl1* deficient mice, also known as *Loxl1* knock-out mice, have had the *Loxl1* gene removed from the genomes of these mice. Another strain of mice has been engineered that produces excess amounts of the protein produced by the *Loxl1* gene, or Loxl1 over-expression. A second class of animal models includes naturally occurring strains of mice that exhibit key clinical features of a disease. Studies of these phenotype-driven animal models may identify genes that cause disease and may also provide a valuable resource for investigating pathogenesis. One strain of mice, B6-*Lyst*$^{bg\text{-}J}$, has several key features of human exfoliation syndrome, including ocular production of exfoliation-like material, and stereotypical iris abnormalities. Studies of this range of mice and other public mouse genetic resources have provided some important insights into the biology of exfoliation syndrome and may be useful for future studies to test the efficacy of drug therapies.

Keywords

Animal model; mice; exfoliation syndrome; glaucoma; *LOXL1*; *LYST*

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Competing interest: None

Animal models fuel medical research by facilitating studies that complement patient-based studies or those not possible with human tissue. Limited availability of human ocular tissue, disease heterogeneity among human patients, and slowly progressive diseases have challenged the use of humans and human tissues in exfoliation syndrome (XFS) research. The tissues affected by XFS, the lens, zonules, and iridocorneal angle structures (i.e. the trabecular meshwork), are readily obtained for research from animal studies. Consequently, animal models are powerful tools for investigating pathogenesis, natural history of disease, and testing new therapeutic interventions.[1,2]

Mice are an especially useful organism for studying human disease for many reasons. There are many anatomical similarities between mouse and human eyes. Most of the clinical techniques used to detect exfoliative glaucoma (XFG) in human eyes have also been successfully modified for use with mice, including slit lamp examination, gonioscopy, tonometry, and optical coherence tomography (Figure 1). Additionally, the eyes of inbred mice have much more uniform features than human eyes, which may be another strength for developing disease models with predictable features. The broad range of powerful tools that have been developed for genetic manipulation of the mouse genome is another major advantage of mouse studies of human disease. Finally, mice are widely available at relatively low cost.

However, developing mouse models that recapitulate disease features is not always straightforward. Though mice and humans have similar anatomy, physiology, and genomes, they are not identical and species-specific differences can create difficult hurdles. For example, although mutations in *MYOC* were the first known association with primary open angle glaucoma in humans,[3] it took over a decade before a mouse model with *Myoc* manipulations was identified that actually developed elevated intraocular pressure and glaucoma.[4] In this instance, a subtle difference in amino acid sequence led to key differences in how the mutant mouse and mutant human isoforms of the protein were trafficked in cells. While discovering this species-specific difference did eventually provide new insights into the basic biology of MYOC-glaucoma, it also delayed development of a mouse model of glaucoma. Similarly, the development of robust mouse models of XFG is trailing the discovery of genes associated with XFS/XFG in humans.

Here, we review the characterization of mouse models accomplished to date and bring attention to some of the remaining obstacles to development of a mouse model with the key features of exfoliative material deposition, elevated intraocular pressure, and glaucoma. As we explain below, mice have thus far been used in at least two different ways to study XFS. In genotype-driven approaches, animal models were engineered to harbor mutations in genes associated with XFS in human patients (i.e. *Loxl1* and *Cacna1a* mutations). In phenotype-driven approaches, clinical screening of many different strains of mice led to the discovery of naturally-occurring mutations that produce features of XFS in mice (i.e. *Lyst* mutations). Currently available genotype- and phenotype-driven mouse models of XFS all have limitations and the search continues for more robust animal models of XFS/XFG.

GENOTYPE-DRIVEN APPROACH (Engineered mouse models)

Genome-wide association studies (GWAS) have successfully identified genes associated with XFS. Variants in two genes, lysyl oxidase like 1 (*LOXL1*)[5] and calcium voltage-gated channel subunit alpha 1A (*CACNA1A*),[6] are highly associated with XFS/XFG, suggesting that they have an important role in the pathogenesis of this form of glaucoma. Consequently, there is interest in studying how abnormalities in the *LOXL1* and *CACNA1A* genes might influence the development of glaucoma in mice. *LOXL1* was the first human gene to be associated with XFS. Consequently, initial attempts to generate mouse models of XFS focused on using a genotype-driven approach to generate and study mice with manipulations to the analogous gene in the mouse genome, the *Loxl1* gene.

Loxl1 knockout mice (*Loxl1* deficient mice)

The function of the human *LOXL1* gene in health and in disease was first investigated by studying mice that have no functional *Loxl1* genes in their genomes. These mice (*Loxl1*^{tm1Tili}; hereafter referred to as *Loxl1* knockout mice) were engineered by disrupting *Loxl1* gene structures that are necessary for its activity. Such *Loxl1* knockout mice are viable; the *Loxl1* gene is not required for survival. However, these mice have abnormalities in elastin synthesis and develop vascular, pulmonary, and uterine malformations. Predominant clinical features of these mice are uterine prolapse, aortic aneurysm, enlarged airspaces in the lungs, and redundancy and laxity of skin.[7]

Janey Wiggs and coworkers examined the eyes of homozygous *Loxl1* knockout mice for signs of XFS and observed numerous ocular abnormalities. Immunohistochemical analysis of ocular tissues from these mice showed reduced elastin in iris and ciliary body. Moreover, monomeric elastin, a building block for mature elastin fibers, was found to accumulate in the anterior segment tissues of *Loxl1* deficient mice, further suggesting that the decreased amount of elastin observed in *Loxl1* mice was due to diminished elastin production.[8] Other ocular abnormalities detected included: reduced integrity of the blood aqueous barrier, a unique form of cataract, and subcapsular vesicles in the anterior cortical lens.[8]

Although molecular abnormalities in elastin synthesis associated with the loss of function of the *Loxl1* gene were detected, no clinical signs typical of XFS and XFG were observed in *Loxl1* knockout mice. No exfoliation material was detected in the anterior segment via examination with slit lamp microscopy (*in vivo* analysis) or light microscopy (histological analysis).[8] The intraocular pressures (IOPs) of *Loxl1* knockout mice were also examined as they aged. *Loxl1* knockout mice had the same IOPs as control mice. No signs of optic nerve damage consistent with glaucoma were observed by histological analysis.[8] In sum, although variants in the human *LOXL1* gene are highly associated with XFS,[5] loss of function mutation of the murine *Loxl1* gene does not recapitulate key features of the disease in mice.

While the *Loxl1* knockout mice do not develop the classic signs of XFS (deposition of exfoliation material in the anterior segment, elevated IOP, or glaucomatous optic nerve damage), they do exhibit some non-ocular features of XFS. A recent association has been made between pelvic organ prolapse and human XFS.[9] *Loxl1* knockout mice have a pelvic floor abnormality, uterine prolapse,[7] which suggests that these transgenic mice may be

useful for studying the etiology of this non-ocular feature of XFS. In depth study of the human *LOXL1* locus has raised the possibility that *LOXL1-AS1*, a long non-coding RNA transcribed from the opposite strand, has a role in XFS.[10] An ortholog of *LOXL1-AS1* has not been identified in the mouse genome (Figure 2) and is one potential explanation for the discrepancy in ocular phenotypes between human and mouse.

Loxl1 transgenic mice (*Loxl1* overexpression)

Another approach to investigate the role of *LOXL1* in human XFS is to study the effects of over activity of the *Loxl1* gene and overproduction of LOXL1 protein in mice. Ernst Tamm and coworkers explored *Loxl1* function by engineering mice to have lens-specific overexpression of murine *Loxl1* under the control of the chicken βB1-crystallin promoter. These *Loxl1* transgenic mice exhibited increased LOXL1 protein levels in aqueous humor; however, exfoliation material was not observed in their eyes, nor did the mice develop elevated IOP or glaucoma. Studies of these βB1-crystallin-*Loxl1* transgenic mice suggest that increased lenticular LOXL1 production may not be sufficient to cause XFS.[11]

Additional resources—In addition to the *Loxl1* knockout and transgenic mice discussed above, there are other murine resources for studying *Loxl1* that warrant mention. First, the KOMP project has generated *Loxl1* deletion strains (*Loxl1*$^{tm1(KOMP)Vlcg}$ and *Loxl1*$^{tm1.1(KOMP)Vlcg}$) that are available as a cryopreserved resource (https://www.komp.org/index.php). The mice are on a C57BL/6NTac genetic background and, thus, presumably also harbor the *rd8* mutation.[12] Ocular phenotype data collected by a JAX pipeline is publically available via the International Mouse Phenotyping Consortium (http://www.mousephenotype.org) and indicates that *Loxl1*$^{tm1.1(KOMP)Vlcg}$ homozygotes had largely normal anterior chambers as determined via slit-lamp exams. Although no exfoliative material was detected in these mice, it is important to note this negative result may be due to inadequate aging; inadequate sample size (number of mice examined); and/or absence of specific examination for exfoliative material. With these caveats, it seems unlikely that this strain will have overt signs of XFS. Second, another mouse strain with a constitutive knockout of *Loxl1* (*Loxl1* - Model 9550 – KO; C57BL/6-*Loxl1*$^{tm1.2\ Mrl}$) is commercially as a cryopreserved resource from Taconic (https://www.taconic.com). To our knowledge, ocular phenotypes of these mice have not been examined.

PHENOTYPE-DRIVEN APPROACH (Naturally-occurring mouse models)

Lyst mutant mice

The B6-*Lyst*$^{bg-J}$ strain of inbred mouse (hereafter referred to as *Lyst* mice) arose when a homozygous mutation in the *Lyst* gene (*beige-J*) spontaneously occurred in the standard C57BL/6J inbred strain.[13] C57BL/6J mice have a black coat color; however, the *beige-J* mutation causes the *Lyst* mouse to have a lighter, beige coat color (Figure 3). The *Lyst* mouse was identified as a potential model of XFS through a slit lamp survey of several strains of mice conducted by Michael Anderson. Examination of *Lyst* mice revealed several ocular features of human XFS.[13]

The central feature of XFS is the fibrillary, proteinaceous material that is deposited in extracellular spaces throughout the body but is most easily detected on ocular structures (i.e. on the anterior lens capsule, on the pupillary margin of the iris, and in the trabecular meshwork) with routine clinical eye examination techniques. No obvious exfoliation material was observed with slit lamp examination of *Lyst* mice. However, a trace amount of exfoliation-like material was detectable with further investigation using transmission electron microscopy. This exfoliation-like material was discovered within the iris stroma, on the posterior surface of the iris, and spilling into the aqueous humor.[13] Further characterization of the exfoliation-like material is challenged by the limited amount produced in very small mouse eyes.

Iris abnormalities are also important features of the *Lyst* mouse (Figure 1B). A concentric pattern of iris transillumination defects was identified in *Lyst* mice that closely mimics a subtle iris irregularity seen in human XFS patients.[13,14] While the mouse transillumination defect is readily visible using white light, the analogous abnormality in humans is typically only visible using more sensitive infrared videography. The source of the concentric iris transillumination defects in *Lyst* mice and XFS patients is an abnormality in the iris pigment epithelium. A breakdown in the adhesion between iris pigment epithelium cells that form the posterior surface of the iris may cause a shift from a flat configuration to a saw-tooth configuration that is evident on histopathological examination.[13,14] This unusual saw-tooth configuration of the iris pigment epithelium and the resultant concentric transillumination defects may be due to the radial strain produced during constriction and dilation of the iris, though this hypothesis is largely untested. Regardless of its origin, the saw-tooth morphology of the iris pigment epithelium would likely increase both pigment and exfoliation material liberation during incidental lenticular contact, thus also explaining why XFS often includes pigment accumulations in the iridocorneal angle.

Lyst mice were also assessed for evidence of glaucoma. Despite pigment accumulation in the iridocorneal angle (Figure 1C), IOP in *Lyst* mice is no different than IOP in wild-type control mice. *Lyst* mice do not develop ocular hypertension. The optic nerves and retinal ganglion cells of *Lyst* mice were also examined for evidence of glaucomatous damage using a variety of quantitative assessments including manually counting optic nerve axons. However, no evidence of optic nerve damage was detected in *Lyst* mice.[13]

In sum, *Lyst* mice exhibit several features of XFS including deposition of exfoliation material, iris pigment epithelium cell shape abnormalities, and iris transillumination defects that mirror human XFS. However, *Lyst* mice don’t develop elevated IOP or glaucomatous optic nerve damage.[13] Consequently, *Lyst* mice may be a better model of XFS than of XFG.

The *beige-J* mutation in the *Lyst* gene has only been studied on the C57BL/6J genetic background. It is possible that the C57BL/6J strain is not permissive for development of XFG. Consequently, it is a plausible that the *beige-J* mutation might cause ocular hypertension and glaucoma, if it were placed in the genome of a different inbred mouse strain with a permissive genetic background. Further studies of the *beige-J* mutation are clearly warranted.

FUTURE DIRECTIONS

Many recent advances in human genetic studies of XFG have the potential to be extended with mouse resources and additional studies. A second gene, the calcium voltage-gated channel subunit alpha1A (*CACNA1A*) has recently been associated with XFS in a large, multicenter genome-wide association study.[6] The CACNA1A protein has long been appreciated to have important roles in neuronal function and the neuroscience community has already generated multiple mouse strains with targeted mutations. The encoding gene is also large and, when mutated, often associated with a dominant behavioral phenotype (ataxia), resulting in isolation of several strains with spontaneous mutations. Among these numerous strains, eleven different strains are currently listed as available via cryo-recovery at The Jackson Laboratory. Careful examination of these mice may identify signs of XFS and provide evidence that these mice are good models of human disease.

A second future direction pertains to close examination of mouse models with mutations in genes associated with microfibrils or other types of extracellular fibrillar material components,[15,16] some of which might have exfoliative phenotypes. Finally, a worthwhile direction, albeit challenging, is to consider non-coding genetic elements physically linked to GWAS-identified loci, but functionally distinct from genes such as *LOXL1* or *CACNA1A*.[10] In all of these endeavors, collaborations between clinicians and scientists studying XFG are likely to be key in increasing the power of the research community to discover more XFG genes, to develop better animal models, and to test future therapies for this important disease.

Acknowledgments

This research presented in this manuscript was supported in part by funds from the Glaucoma Foundation, the Research to Prevent Blindness, and Marlene and Leonard Hadley. The authors thank Hannah Mercer for help providing photographs used in the figures. MGA is supported in part by NIH grants R01 EY017673 and Department of Veterans Affairs grant I01 RX001481. The contents do not represent the the views of the U.S. Department of Veterans Affairs or the United States Government.

Funding: Glaucoma Foundation, Research to Prevent Blindness, NIH RO1EY017673, The Department of Veterans Affairs I01 RX001481, and Leonard and Marlene Hadley.

References

1. Using genetic mouse models to gain insight into glaucoma: Past results and future possibilities. Exp Eye Res. 2015; 141:42–56. [PubMed: 26116903]
2. Zeiss CJ. Translational models of ocular disease. Veterinary ophthalmology. 2013; 16(Suppl 1 (s1)): 15–33. [PubMed: 23750503]
3. Stone EM, Fingert JH, Alward WLM, et al. Identification of a Gene That Causes Primary Open Angle Glaucoma. Science. 1997; 275(5300):668–670. [PubMed: 9005853]
4. Zode GS, Kuehn MH, Nishimura DY, et al. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. J Clin Invest. 2011; 121(9):3542–3553. [PubMed: 21821918]
5. Thorleifsson G, Magnusson KP, Sulem P, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science. 2007; 317(5843):1397–1400. [PubMed: 17690259]
6. Aung T, Ozaki M, Mizoguchi T, et al. A common variant mapping to CACNA1A is associated with susceptibility to exfoliation syndrome. Nat Genet. Feb.2015

7. Liu X, Zhao Y, Gao J, et al. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat Genet. 2004; 36(2):178–182. [PubMed: 14745449]
8. Wiggs JL, Pawlyk B, Connolly E, et al. Disruption of the blood-aqueous barrier and lens abnormalities in mice lacking lysyl oxidase-like 1 (LOXL1). Invest Ophthalmol Vis Sci. 2014; 55(2):856–864. [PubMed: 24425853]
9. Wirostko BM, Curtin K, Ritch R, et al. Risk for Exfoliation Syndrome in Women With Pelvic Organ Prolapse: A Utah Project on Exfoliation Syndrome (UPEXS) Study. JAMA ophthalmology. 2016; 134(11):1255–1262. [PubMed: 27632406]
10. Hauser MA, Aboobakar IF, Liu Y, et al. Genetic variants and cellular stressors associated with exfoliation syndrome modulate promoter activity of a lncRNA within the LOXL1 locus. Hum Mol Genet. 2015; 24(22):6552–6563. [PubMed: 26307087]
11. High Amounts of Lysyl Oxidase-like Protein-1 in the Aqueous Humor of Transgenic Mice Do not Cause Ultrastructural Changes of Zonular Fibers and Ciliary Body. Invest Ophthalmol Vis Sci. 2014; 55:5661. [PubMed: 25201925]
12. Mattapallil MJ, Wawrousek EF, Chan C-C, et al. The Rd8 mutation of the Crb1 gene is present in vendor lines of C57BL/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes. Invest Ophthalmol Vis Sci. 2012; 53(6):2921–2927. [PubMed: 22447858]
13. Trantow CM, Mao M, Petersen GE, et al. Lyst mutation in mice recapitulates iris defects of human exfoliation syndrome. Invest Ophthalmol Vis Sci. 2009; 50(3):1205–1214. [PubMed: 19029039]
14. Fingert JH, Burden JH, Wang K, Kwon YH, Alward WLM, Anderson MG. Circumferential iris transillumination defects in exfoliation syndrome. J Glaucoma. 2013; 22(7):555–558. [PubMed: 22525123]
15. Kuchtey J, Kuchtey RW. The microfibril hypothesis of glaucoma: implications for treatment of elevated intraocular pressure. Journal of ocular pharmacology and therapeutics. 2014; 30(2–3): 170–180. [PubMed: 24521159]
16. Zenkel M, Schlötzer-Schrehardt U. Expression and regulation of LOXL1 and elastin-related genes in eyes with exfoliation syndrome. J Glaucoma. 2014; 23(8 Suppl 1):S48–S50. [PubMed: 25275906]

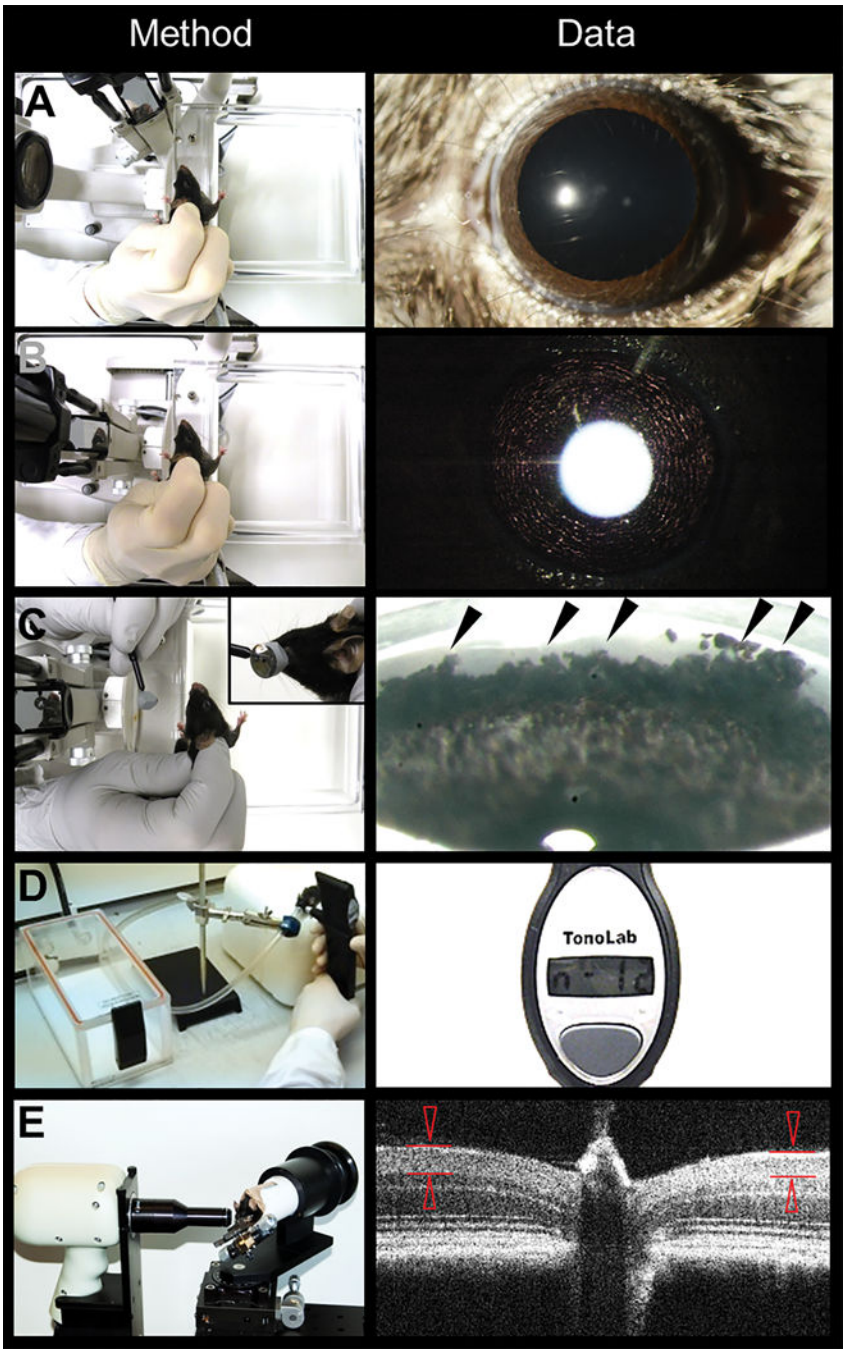

Figure 1. Ocular examination of mice for signs of exfoliation syndrome and glaucoma

Most examination techniques for human eyes can be employed to study mouse eyes (setup, *left column*; resultant data, *right column*). **(A)** Slit-lamp examination of a dilated eye with broad-beam illumination is a primary tool that can be used to screen for exfoliation syndrome in mice. Mice with overt deposits of exfoliative material in the anterior chamber have not yet been discovered; the expectation is that exfoliative material would most likely be present in a “bulls-eye” pattern on the lens. Pigment accumulation in the inferior angle would likely also be observable. Wild-type C57BL/6J eye pictured. **(B)** Concentric iris

transillumination can be detected in affected eyes by shining a beam of light through an undilated pupil, as seen in the eyes of B6-*Lyst*$^{bg\text{-}J}$ mice shown here. **(C)** Gonioscopy is used to visualize the iridocorneal angle and can be used to screen for the presence of exfoliative material and liberated pigment (*arrowheads*), as seen in the eyes of B6-*Lyst*$^{bg\text{-}J}$ mice shown here. **(D)** Rebound tonometry is used to assess intraocular pressure of mice; compared to strain- and age-matched controls, mice with secondary glaucoma would be expected to have an elevated intraocular pressure **(E)** Retinal optical coherence tomography can identify thinning of the retinal ganglion cell complex (optically dense retinal layer between *red lines* with accompanying *arrowheads*) in glaucomatous eyes. Wild-type retina shown.

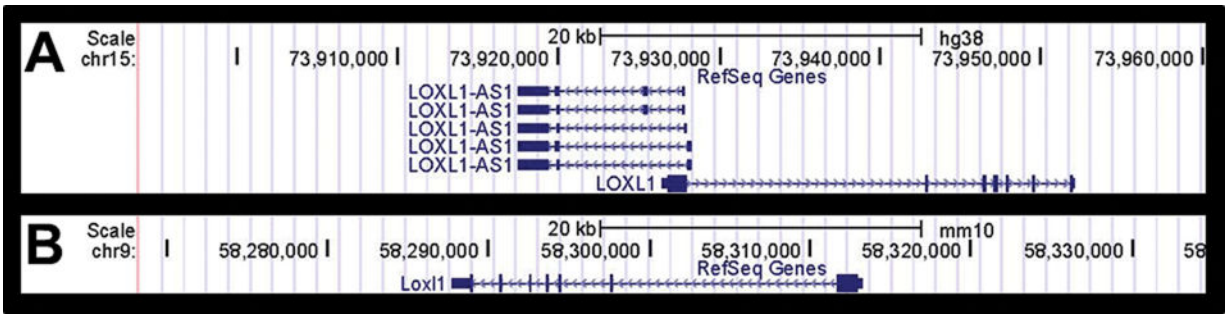

Figure 2. Human and mouse LOXL1 genomic locus

The LOXL1 locus displayed on the RefSeq genes track of the UCSC genome browser for **(A)** human genome build hg38 and **(B)** mouse genome build mm10 indicate that *LOXL1-AS1* is a human-specific gene. Note that the human and mouse genes are transcribed in opposite directions.

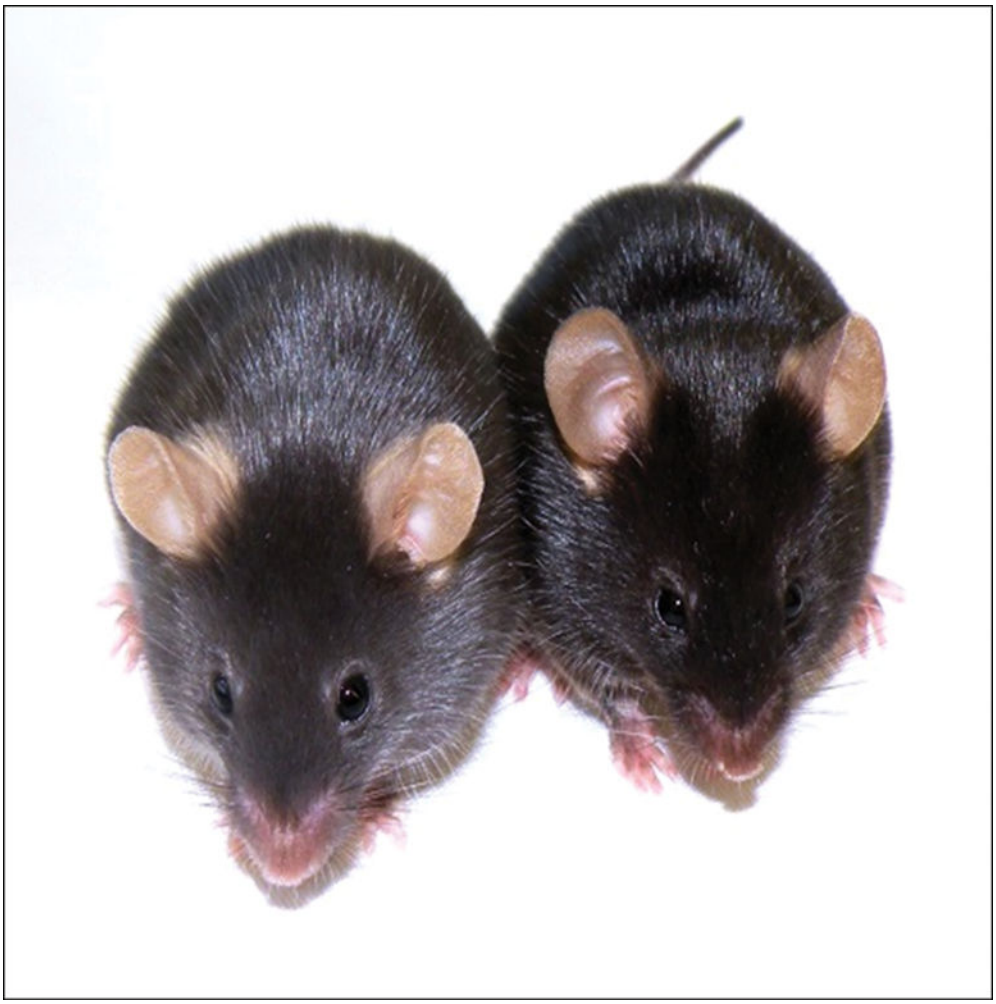

Figure 3. The B6-*Lyst*$^{bg\text{-}J}$ mouse has altered pigmentation

The most striking feature of the B6-*Lyst*$^{bg\text{-}J}$ mouse is its beige coat color, which differentiates it from its parental C57BL/6J strain, which has a black coat color.