Animal Models of Exfoliation Syndrome, Now and Future

Simon W.M. John, PhD,†‡ Jeffrey M. Harder, PhD,† John H. Fingert, MD, PhD,§ and Michael G. Anderson, PhD§‖¶*

Abstract: At present, no animal models fully embody exfoliation syndrome or exfoliation glaucoma. Both genetic and environmental factors appear critical for disease manifestation, and both must be considered when generating animal models. Because mice provide a powerful mammalian platform for modeling complex disease, this paper focuses on mouse models of exfoliation syndrome and exfoliation glaucoma.

Key Words: exfoliation syndrome, animal models, knockout mice, transgenic mice, alleles

(*J Glaucoma* 2014;23:S68–S72)

Exfoliation syndrome (XFS) is the most common identifiable cause of open-angle glaucoma. Despite its prevalence, little is understood about its molecular etiology or about the factors determining susceptibility and progression to exfoliation glaucoma (XFG). It is clear that XFS is a complex, age-related disease affected by both genetic and environmental factors. Nevertheless, both the genetic and environmental factors need better definition.[1–4] A strong association between single-nucleotide polymorphisms (SNPs) in the lysyl oxidase–like 1 (*LOXL1*) gene and XFS was identified in the Swedish and Icelandic populations using a genome-wide association study.[5] This association was replicated in other populations.[6–22] However, risk alleles of *LOXL1* are very common in unaffected controls, and the mechanisms of action of these alleles are not clear.[7,23] Risk alleles at contactin-associated protein-like 2 (*CNTNAP2*) loci also have significant associations between XFS and XFG in some but not all populations.[24–26] Mutations in *CNTNAP2* cause neurologic disease, and it is not clear if allelic, genetic background or other differences modulate the phenotypic consequences of mutation in this gene. A host of environmental factors have been suggested to influence XFS, but findings are often inconsistent across studies.[3,4]

THE CHALLENGE OF MODELING XFS/XFG

To understand the molecular mechanisms, underlying XFS and progression to XFG tractable animal models are needed. Extremely few publications have studied animal models of XFS, and to our knowledge only 2 animal models have been reported.[27,28] There are no reports of models with all of the features of XFS with XFG. The lack of animal models has been suggested to reflect the typical occurrence of XFS at old ages, with the belief that model species do not live long enough to develop the condition. Although possible, we do not feel that the shorter lifespan of model species in absolute years prevents development of XFS. Disease susceptibility in model species increases with age relative to lifespan in a similar manner to that in people.[29] For example, within a 2 years life span, mice often develop complex, age-related diseases that occur later in human life. In contrast, differences in environment may be critical and profoundly impact whether or not model species develop XFS. The degree and nature of exposure to light (UV), low temperature, viruses, and caffeine have all been suggested to impact development of XFS.[4,30–33] Most laboratory animals are housed in cages with limited UV exposure, constant controlled temperature, limited or no exposure to pathogens, and lack of caffeine and other lifestyle factors. Genetic differences across species may also be important. In humans a high-risk genotype at the *LOXL1* locus is typically necessary for manifestation of XFS, as high-risk alleles are present in almost all patients with XFS.[23] Thus, the challenge of modeling XFS/XFG in animals is to develop models reflecting these complex genetic and environmental risk factors.

CURRENT MODELS

Porcine Model

The first animal model was reported in pigs.[27] The authors fed pigs a high sucrose, high salt diet to induce cataracts. They reasoned that cataracts are common in XFS and that mature cataracts shed exfoliative material. After a few months on the diet the pigs developed cataracts and had an exfoliation-like material that contained crystallins. We are not aware of any follow-up studies. The relevance of this model to the human disease is not yet clear, although it may caution against high salt and sugar intakes.

Lyst Mutant Mouse Model

The other model is an inherited mouse model, which shares several features with human XFS. In addition to the accumulation of fibrillar material in the eye, patients with XFS have characteristic saw-tooth morphology of the iris pigment epithelium.[34,35] This results in iris transillumination defects characterized by a specific concentric, circular transillumination pattern.[36] Similar to human patients, *Lyst* mutant mice have microscopically detectable deposits of fibrillin 1-positive material in the eye.[28] They also replicate the saw-tooth morphology of the iris pigment epithelium and have the same pattern of transillumination defects as human patients (Fig. 1). In human XFS, increased susceptibility to oxidative stress has been

Received for publication August 11, 2014; accepted August 11, 2014.
From *The Howard Hughes Medical Institute; †The Jackson Laboratory, Bar Harbor, ME; ‡Department of Ophthalmology, Tufts University School of Medicine, Boston, MA; Departments of §Ophthalmology and Visual Sciences; ‖Molecular Physiology and Biophysics, University of Iowa; and ¶Center for the Prevention and Treatment of Visual Loss, Iowa City VA Health Care System, Iowa City, IA.
Disclosure: The authors declare no conflict of interest.
Reprints: Simon W.M. John, PhD, The Jackson Laboratory, 600 Main St., Bar Harbor 04609, Maine (e-mail: simon.john@jax.org).

DOI: 10.1097/IJG.0000000000000121

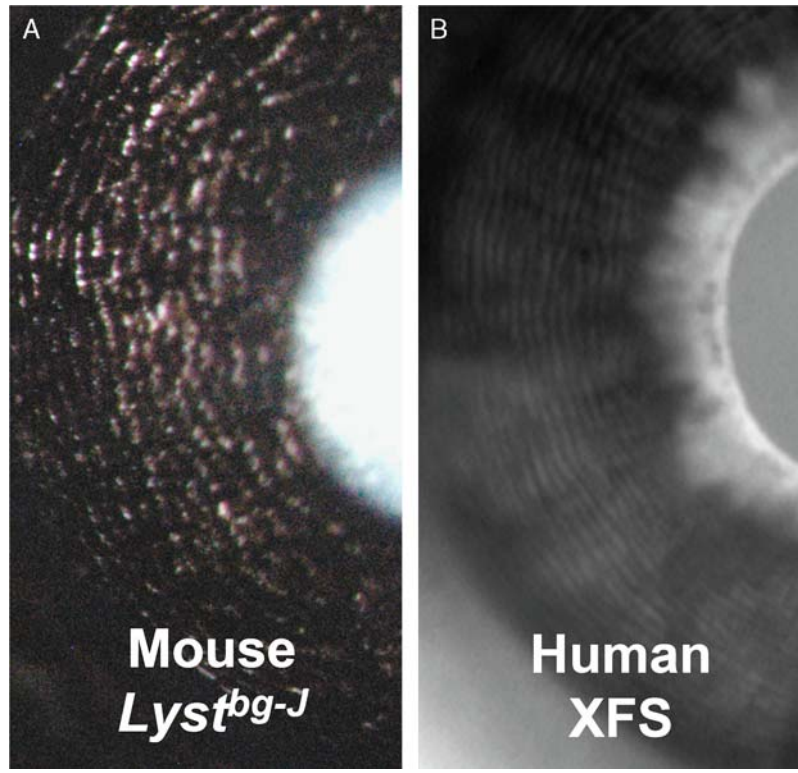

FIGURE 1. Characteristic iris transillumination defects of XFS/XFG. (A) Mouse homozygous for the *Lyst*$^{bg-J}$ mutation imaged with standard slit-lamp illumination. Note concentric circles of transillumination defects (red). (B) Human XFS patient imaged with infrared videography showing concentric circles of transillumination defects (white). The 2 images have been scaled differently to promote comparison.

suggested to contribute to the pathology[37,38] and, as in other glaucomas, levels of transforming growth factor-β(TGF-β) superfamily members are elevated.[39–42] The *Lyst* mutant iris disease involves oxidative damage[43] and TGF-β levels in *Lyst* mutant eyes remain to be tested. Thus, the mice have some XFS-like phenotypes but lack clinically obvious XFS deposits or glaucoma. Although the degree of relevance of this mouse to human XFS is not yet clear, it is currently the most similar available model. Further evaluation of this model will be important. Similarly, evaluation of the *LYST* gene and functionally related genes in human patients is worthwhile.

Future Models

The housing environment may critically interact with genetics to determine if model species develop XFS. Thus, environment should be considered and manipulated when working to produce new models of XFS. Among other factors, the degree and nature of exposure to light (UV), low temperature, presence of specific viruses/microbes, dietary composition, and caffeine intake may impact the development of XFS.[4,30–33,44] Using animal models, the importance of specific environmental factors could be clearly determined. It is now possible to abrogate or greatly decrease the amount of gene product produced by mutating genes in various species and a variety of species may contribute to improved understanding of XFS. Because of their small size, high fecundity, relatively low cost, and the most powerful array of available genetic resources and tools for dissecting complex diseases and humanizing their genomes, mice will remain a very important model species.[45,46] Mice are likely to add substantially to our mechanistic understanding of XFS (Fig. 2).

MICE WITH HUMAN ALLELES OF LOXL1 AND CNTNAP2

As the *LOXL1* genotype is critically important in the development of XFS, an important step in producing a new mouse model of XFS is to make mice with variant forms of the *Loxl1* gene. Mice with a completely nonfunctional allele of *Loxl1* have an elastic tissue disease[47] but do not develop XFS.[48,49] Although it is worth aging and assessing these mice in different environments, it remains possible that a null allele will not cause XFS. As the specific DNA change(s) that render susceptibility to XFS are not clearly defined and may be intronic, making mice that are transgenic for a human bacterial artificial chromosome (BAC) containing a high-risk human allele of *LOXL1* is a high priority. If multiple high-risk alleles are defined from different populations, mice can be made with alleles conferring different degrees of risk with initial bias toward those with the strongest effect. Further, it will be important to evaluate multiple transgenic mouse lines with different expression levels.

LOXL1 genotype alone is unlikely to be sufficient to induce XFS, as many individuals with a high-risk allele do not have the disease. Thus, it will be important both to

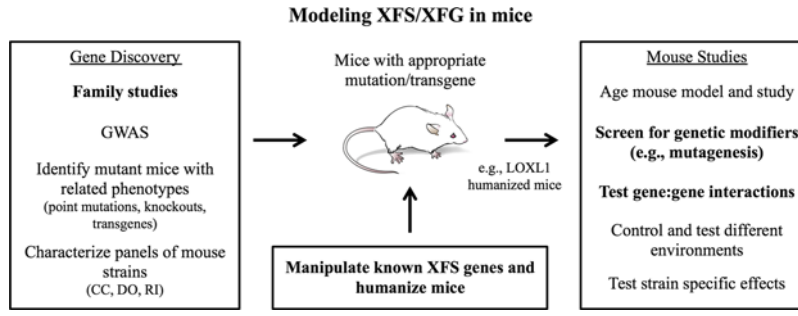

FIGURE 2. Strategies to model XFS/XFG in mice. *Gene discovery*: a main priority is to identify genes associated with XFS. Large families provide a rare but important resource for identifying causal genes—they may provide the most effective mode of gene discovery. Genome-wide association studies (GWAS) remain important, but gene discovery can be difficult because of complexity and very large sample sizes are needed for further progress. The characterization of mice with mutations or transgenes affecting specific genes may also identify new XFS genes. Another avenue for gene discovery is characterization of a series of mouse strains that are specifically tailored for identifying genes that underlie complex diseases like XFS, such as collaborative cross (CC), diversity outcross (DO), and recombinant inbred (RI) mice. Mice can be screened for XFS based on the presence of exfoliation, concentric circular iris defects, or high IOP and glaucoma. *Mouse models*: Once human genes are identified, mouse models can be made by targeted mutagenesis or by making transgenic mice. A top priority is to create a humanized mouse model containing a high-risk allele of *LOXL1*. BAC transgenic mice with a human *LOXL1* allele and its regulatory elements will likely best recapitulate *LOXL1*-associated disease risk. *Mouse studies*: Humanized *LOXL1* mice and other mouse models need to be studied in the context of aging, genetic modifiers, environmental conditions, and gene-environment interactions. Using chemical mutagenesis to induce random point mutations in humanized *LOXL1* mice is an unbiased way to uncover genetic modifiers of *LOXL1*. Generating combined mutants with *LOXL1* variants and *Lyst*, humanized *CNTNAP2*, or newly discovered XFS genes is also an effective strategy to understand genetic interactions. Priority areas that may provide the quickest route to useful mouse models are indicated in bold.

combine the human *LOXL1* transgene with mutations in other genes (such as *Lyst*) and to assess the effects of different environments. Decreased antioxidant capacity and increased propensity to inflammation may be important in XFS and XFG.[38,50,51] Mutations that alter these systems can be introduced into the transgenic mice by breeding. Altered TGF-β signaling has been implicated in XFS, but transgenic or knockout mice affecting this pathway have not been demonstrated to develop XFS. Combining human *LOXL1* transgenes with mutations affecting TGF-β pathways may prove valuable, especially for the development of high IOP and glaucoma.[52–55] Although less clearly important, mutations in *CNTNAP2* are also implicated in XFS and it is worth assessing mice with *CNTNAP2* mutations and humanizing mice with human high-risk alleles.

OTHER XFS GENE AND MODELS

Various efforts to identify XFS genes are underway, including genome-wide association studies and genetic studies in human families. Because of the complexity of the disease these studies are not easy, but once more genes are identified they can be assessed in mice using the approaches discussed above. Genetic studies in families with many affected individuals may have the highest likelihood of success and are very important. Mutagenesis screens in mice are another powerful approach for providing animal models and discovering disease mechanism.[46,56–59] With adequate aging and attention to environment, such screens may provide key new models of XFS and XFG. One important strategy for discovering XFS genes and pathways would be to perform a mutagenesis screen using mice that are transgenic for human high-risk alleles of *LOXL1*. Modifier screens to identify genetic differences that alter phenotypes caused by the *LOXL1* transgene (or other human alleles) between mouse strains may prove valuable.[60–63]

REFERENCES

1. Schlötzer-Schrehardt U. Genetics and genomics of pseudoexfoliation syndrome/glaucoma. *Middle East Afr J Ophthalmol*. 2011;18:30–36.
2. Sein J, Galor A, Sheth A, et al. Exfoliation syndrome: new genetic and pathophysiologic insights. *Curr Opin Ophthalmol*. 2013;24:167–174.
3. Damji KF, Bains HS, Stefansson E, et al. Is pseudoexfoliation syndrome inherited? A review of genetic and nongenetic factors and a new observation. *Ophthalmic Genet*. 1998;19:175–185.
4. Stein JD, Pasquale LR, Talwar N, et al. Geographic and climatic factors associated with exfoliation syndrome. *Arch Ophthalmol*. 2011;129:1053–1060.
5. Thorleifsson G, Magnusson KP, Sulem P, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. *Science*. 2007;317:1397–1400.
6. Aragon-Martin JA, Ritch R, Liebmann J, et al. Evaluation of LOXL1 gene polymorphisms in exfoliation syndrome and exfoliation glaucoma. *Mol Vis*. 2008;14:533–541.
7. Challa P, Schmidt S, Liu Y, et al. Analysis of LOXL1 polymorphisms in a United States population with pseudoexfoliation glaucoma. *Mol Vis*. 2008;14:146–149.
8. Chen L, Jia L, Wang N, et al. Evaluation of LOXL1 polymorphisms in exfoliation syndrome in a Chinese population. *Mol Vis*. 2009;15:2349–2357.
9. Fan BJ, Pasquale L, Grosskreutz CL, et al. DNA sequence variants in the LOXL1 gene are associated with pseudoexfoliation glaucoma in a US clinic-based population with broad ethnic diversity. *BMC Med Genet*. 2008;9:5.
10. Fingert JH, Alward WL, Kwon YH, et al. LOXL1 mutations are associated with exfoliation syndrome in patients from the midwestern United States. *Am J Ophthalmol*. 2007;144:974–975.
11. Fuse N, Miyazawa A, Nakazawa T, et al. Evaluation of LOXL1 polymorphisms in eyes with exfoliation glaucoma in Japanese. *Mol Vis*. 2008;14:1338–1343.
12. Hayashi H, Gotoh N, Ueda Y, et al. Lysyl oxidase-like 1 polymorphisms and exfoliation syndrome in the Japanese population. *Am J Ophthalmol*. 2008;145:582–585.
13. Hewitt AW, Sharma S, Burdon KP, et al. Ancestral LOXL1 variants are associated with pseudoexfoliation in Caucasian

Australians but with markedly lower penetrance than in Nordic people. *Hum Mol Genet*. 2008;17:710–716.
14. Lee KY, Ho SL, Thalamuthu A, et al. Association of LOXL1 polymorphisms with pseudoexfoliation in the Chinese. *Mol Vis*. 2009;15:1120–1126.
15. Mabuchi F, Sakurada Y, Kashiwagi K, et al. Lysyl oxidase-like 1 gene polymorphisms in Japanese patients with primary open angle glaucoma and exfoliation syndrome. *Mol Vis*. 2008;14:1303–1308.
16. Mori K, Imai K, Matsuda A, et al. LOXL1 genetic polymorphisms are associated with exfoliation glaucoma in the Japanese population. *Mol Vis*. 2008;14:1037–1040.
17. Mossbock G, Renner W, Faschinger C, et al. Lysyl oxidase-like protein 1 (LOXL1) gene polymorphisms and exfoliation glaucoma in a Central European population. *Mol Vis*. 2008;14:857–861.
18. Ozaki M, Lee KY, Vithana EN, et al. Association of LOXL1 gene polymorphisms with pseudoexfoliation in the Japanese. *Invest Ophthalmol Vis Sci*. 2008;49:3976–3980.
19. Pasutto F, Krumbiegel M, Mardin CY, et al. Association of LOXL1 common sequence variants in German and Italian patients with pseudoexfoliation syndrome and pseudoexfoliation glaucoma. *Invest Ophthalmol Vis Sci*. 2008;49:1459–1463.
20. Ramprasad VL, George R, Soumittra N, et al. Association of non-synonymous single nucleotide polymorphisms in the LOXL1 gene with pseudoexfoliation syndrome in India. *Mol Vis*. 2008;14:318–322.
21. Tanito M, Minami M, Akahori M, et al. LOXL1 variants in elderly Japanese patients with exfoliation syndrome/glaucoma, primary open-angle glaucoma, normal tension glaucoma, and cataract. *Mol Vis*. 2008;14:1898–1905.
22. Yang X, Zabriskie NA, Hau VS, et al. Genetic association of LOXL1 gene variants and exfoliation glaucoma in a Utah cohort. *Cell Cycle*. 2008;7:521–524.
23. Chen H, Chen LJ, Zhang M, et al. Ethnicity-based subgroup meta-analysis of the association of LOXL1 polymorphisms with glaucoma. *Mol Vis*. 2010;16:167–177.
24. Krumbiegel M, Pasutto F, Schlötzer-Schrehardt U, et al. Genome-wide association study with DNA pooling identifies variants at CNTNAP2 associated with pseudoexfoliation syndrome. *Eur J Hum Genet*. 2011;19:186–193.
25. Malukiewicz G, Lesiewska-Junk H, Linkowska K, et al. Analysis of CNTNAP2 polymorphisms in Polish population with pseudoexfoliation syndrome. *Acta Ophthalmol*. 2012; 90:e660–e661.
26. Shimizu A, Takano Y, Shi D, et al. Evaluation of CNTNAP2 gene polymorphisms for exfoliation syndrome in Japanese. *Mol Vis*. 2012;18:1395–1401.
27. Veromann S, Sunter A, Juronen E, et al. Eye lens crystallins: a component of intraocular pseudoexfoliative material. *Ophthalmic Res*. 2004;36:51–54.
28. Trantow CM, Mao M, Petersen GE, et al. Lyst mutation in mice recapitulates iris defects of human exfoliation syndrome. *Invest Ophthalmol Vis Sci*. 2009;50:1205–1214.
29. Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell*. 2005;120:449–460.
30. Pasquale LR, Wiggs JL, Willett WC, et al. The Relationship between caffeine and coffee consumption and exfoliation glaucoma or glaucoma suspect: a prospective study in two cohorts. *Invest Ophthalmol Vis Sci*. 2012;53:6427–6433.
31. Ringvold A. Exfoliation syndrome immunological aspects. *Acta Ophthalmol Suppl*. 1988;184:35–43.
32. Detorakis ET, Kozobolis VP, Pallikaris IG, et al. Detection of herpes simplex virus in pseudoexfoliation syndrome and exfoliation glaucoma. *Acta Ophthalmol Scand*. 2002;80: 612–616.
33. Resnikoff S, Filliard G, Dell'Aquila B. Climatic droplet keratopathy, exfoliation syndrome, and cataract. *Br J Ophthalmol*. 1991;75:734–736.
34. Asano N, Schlötzer-Schrehardt U, Naumann GO. A histopathologic study of iris changes in pseudoexfoliation syndrome. *Ophthalmology*. 1995;102:1279–1290.
35. Eagle RC Jr, Font RL, Fine BS. The basement membrane exfoliation syndrome. *Arch Ophthalmol*. 1979;97:510–515.
36. Fingert JH, Burden JH, Wang K, et al. Circumferential iris transillumination defects in exfoliation syndrome. *J Glaucoma*. 2013;22:555–558.
37. Schlötzer-Schrehardt U, Naumann GO. Ocular and systemic pseudoexfoliation syndrome. *Am J Ophthalmol*. 2006;141: 921–937.
38. Erdurmus M, Yagci R, Atis O, et al. Antioxidant status and oxidative stress in primary open angle glaucoma and pseudoexfoliative glaucoma. *Curr Eye Res*. 2011;36:713–718.
39. Schlötzer-Schrehardt U, Zenkel M, Küchle M, et al. Role of transforming growth factor-beta1 and its latent form binding protein in pseudoexfoliation syndrome. *Exp Eye Res*. 2001;73:765–780.
40. Picht G, Welge-Luessen U, Grehn F, et al. Transforming growth factor beta 2 levels in the aqueous humor in different types of glaucoma and the relation to filtering bleb development. *Graefes Arch Clin Exp Ophthalmol*. 2001;239: 199–207.
41. Kottler UB, Jünemann AG, Aigner T, et al. Comparative effects of TGF-beta 1 and TGF-beta 2 on extracellular matrix production, proliferation, migration, and collagen contraction of human Tenon's capsule fibroblasts in pseudoexfoliation and primary open-angle glaucoma. *Exp Eye Res*. 2005;80:121–134.
42. Inatani M, Tanihara H, Katsuta H, et al. Transforming growth factor-beta 2 levels in aqueous humor of glaucomatous eyes. *Graefes Arch Clin Exp Ophthalmol*. 2001;239:109–113.
43. Trantow CM, Hedberg-Buenz A, Iwashita S, et al. Elevated oxidative membrane damage associated with genetic modifiers of Lyst-mutant phenotypes. *PLoS Genet*. 2010;6:e1001008.
44. Yilmaz A, Ayaz L, Tamer L. Selenium and pseudoexfoliation syndrome. *Am J Ophthalmol*. 2011;151:272–276 e271.
45. Nguyen D, Xu T. The expanding role of mouse genetics for understanding human biology and disease. *Dis Model Mech*. 2008;1:56–66.
46. John SW, Anderson MG, Smith RS. Mouse genetics: a tool to help unlock the mechanisms of glaucoma. *J Glaucoma*. 1999;8:400–412.
47. Liu X, Zhao Y, Gao J, et al. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nat Genet*. 2004;36:178–182.
48. Wiggs JL, Pasquale LR, Pawlyk B, et al. LOXL1 null mouse phenotype suggests that loss of lysyl oxidase like 1 function contributes to exfoliation glaucoma. *59th Annual Meeting of The American Society of Human Genetics*. Honolulu, HI. October 20–24, 2009.
49. 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;55:856–864.
50. Zenkel M, Kruse FE, Naumann GO, et al. Impaired cytoprotective mechanisms in eyes with pseudoexfoliation syndrome/glaucoma. *Invest Ophthalmol Vis Sci*. 2007;48: 5558–5566.
51. Schlötzer-Schrehardt U. Oxidative stress and pseudoexfoliation glaucoma. *Klin Monbl Augenheilkd*. 2010;227:108–113.
52. Fleenor DL, Shepard AR, Hellberg PE, et al. TGFbeta2-induced changes in human trabecular meshwork: implications for intraocular pressure. *Invest Ophthalmol Vis Sci*. 2006; 47:226–234.
53. Bachmann B, Birke M, Kook D, et al. Ultrastructural and biochemical evaluation of the porcine anterior chamber perfusion model. *Invest Ophthalmol Vis Sci*. 2006;47: 2011–2020.
54. Shepard AR, Millar JC, Pang IH, et al. Adenoviral gene transfer of active human transforming growth factor-{beta}2 elevates intraocular pressure and reduces outflow facility in rodent eyes. *Invest Ophthalmol Vis Sci*. 2010;51:2067–2076.
55. Robertson JV, Golesic E, Gauldie J, et al. Ocular gene transfer of active TGF-beta induces changes in anterior segment morphology and elevated IOP in rats. *Invest Ophthalmol Vis Sci*. 2010;51:308–318.

56. Vreugde S, Erven A, Kros CJ, et al. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. *Nat Genet*. 2002;30:257–258.
57. Nair KS, Hmani-Aifa M, Ali Z, et al. Alteration of the serine protease PRSS56 causes angle-closure glaucoma in mice and posterior microphthalmia in humans and mice. *Nat Genet*. 2011;43:579–584.
58. Lachke SA, Alkuraya FS, Kneeland SC, et al. Mutations in the RNA granule component TDRD7 cause cataract and glaucoma. *Science*. 2011;331:1571–1576.
59. Beckers J, Wurst W, de Angelis MH. Towards better mouse models: enhanced genotypes, systemic phenotyping and envirotype modelling. *Nat Rev Genet*. 2009;10:371–380.
60. Rubio-Aliaga I, Soewarto D, Wagner S, et al. A genetic screen for modifiers of the delta1-dependent notch signaling function in the mouse. *Genetics*. 2007;175:1451–1463.
61. Mohan S, Baylink DJ, Srivastava AK. A chemical mutagenesis screen to identify modifier genes that interact with growth hormone and TGF-beta signaling pathways. *Bone*. 2008;42:388–395.
62. Matera I, Watkins-Chow DE, Loftus SK, et al. A sensitized mutagenesis screen identifies Gli3 as a modifier of Sox10 neurocristopathy. *Hum Mol Genet*. 2008;17:2118–2131.
63. Liu X, Dobbie M, Tunningley R, et al. ENU mutagenesis screen to establish motor phenotypes in wild-type mice and modifiers of a pre-existing motor phenotype in tau mutant mice. *J Biomed Biotechnol*. 2011;2011:130947.