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Review Article

Genetically modified animal models of hereditary diseases for testing of gene-directed therapy

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Abstract

Disease-causing genes have been identified for many severe muscular and neurological genetic disorders. Advances in the gene therapy field offer promising solutions for drug development to treat these life-threatening conditions. Depending on how the mutation affects the function of the gene product, different gene therapy approaches may be beneficial. Gene replacement therapy is appropriate for diseases caused by mutations that result in the deficiency of the functional protein. Gene suppression strategy is suggested for disorders caused by the toxic product of the mutant gene. Splicing modulators, genome editing, and base editing techniques can be applied to disorders with different types of underlying mutations. Testing potential drugs in animal models of human diseases is an indispensable step of development. Given the specific gene therapy approach, appropriate animal models can be generated using a variety of technologies ranging from transgenesis to precise genome editing. In this review, we discuss technologies used to generate small and large animal models of the most common muscular and neurological genetic disorders. We specifically focus on animal models that were used to test gene therapies based on adeno-associated vectors and antisense nucleotides.

Keywords

animal models of human diseases, gene therapy, antisense oligonucleotides (ASO), hereditary diseases, adeno-associated virus (AAV), CRISPR/Cas9, transgenesis.

Introduction

Gene therapy is an innovative treatment option for devastating hereditary diseases that cannot be cured. The first gene therapy drugs reached patients in the past decade and demonstrated good efficiency in clinical trials (He et al. 2021; Hill and Meisler 2021). Neurological and neuromuscular diseases rank first among diseases with genetic causes in terms of the size of the gene therapy market and its growth rate. Among the already approved therapeutics are adeno-associated virus (AAV)-based drugs for spinal muscular atrophy (Zolgensma) and Leber congenital amaurosis (Luxturna). Antisense oligonucleotide (ASO) therapies released onto the market include Spinraza for spinal muscular atrophy and Eteplirsen for Duchenne muscular dystrophy.

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Prior to advancing gene-targeted therapies to trials in humans, a large number of processes are performed: the choice of a therapeutic strategy, the development of a drug candidate, primary screening *in vitro* and *in vivo*, and preclinical studies of efficacy and safety. The therapeutic strategy for gene therapy is selected based on the type of the disease-causing mutation (Fig. 1). Gene replacement therapy is appropriate for diseases caused by loss-of-function (LoF) mutations leading to deficiency in the functional gene product. LoF mutations are typically recessive, while haploinsufficiency (i.e., a condition where a single functioning copy of a gene is not sufficient to preserve normal function) is caused by a dominant mutation in dosage-sensitive genes. Gene replacement therapy provides a functional copy of a gene to compensate for the deficiency due to the LoF mutation. Another pathogenic mechanism of disease-causing mutations is the synthesis of a toxic gene product with enhanced, altered, or new functional activity. Such mutations are dominant and classified as gain-of-function (GoF) or dominant-negative. For these types of mutations, gene suppression therapy is proposed. The therapeutic approach involves downregulation of the mutant gene in an allele-selective or nonselective manner and can be combined with gene replacement therapy. Universal gene therapy strategies that can be applied to LoF mutations and dominant disorders include splicing modulators and mutation correction using genome editing and single base editing techniques.

At the development stage, gene therapy approaches can be tested in various *in vitro* and *in vivo* models. Primary drug screen can be conducted in cell culture-based models of the disease. The advantage of *in vitro* models is the simplicity of analysis and high repeatability among series of experiments. Drug efficacy can be demonstrated at the mRNA and protein levels as well as in functional tests (Danilov et al. 2020). However, to evaluate drug characteristics, such as safety, dosage, and biodistribution, animal models are indispensable.

Animal models of human diseases are routinely created in small laboratory animals, such as mice and rats. Large animal models (rabbits, dogs, pigs, primates, etc.) are used more rarely, but also available. Various technologies are employed to introduce disease-causing mutations to the genome of animals including transgenesis, single-gene knockouts and knock-ins, conditional gene modifications, and chromosomal rearrangements (Justice et al. 2011; Gurumurthy and Lloyd 2019).

The most common approach for disease modeling is transgenesis (Gurumurthy and Lloyd 2019). Transgenesis involves the introduction of the mutant or normal gene copy (transgene) into a model organism. Inserting a mutated gene variant leads to disease phenotype development and genetic model generation. In a classical variant, linear DNA is injected into a fertilized oocyte. The limitations of this approach are low integration efficiency following DNA transfer into the one-cell-stage zygote, non-directed integration prone to gene silencing, and relatively short DNA fragments allowed for injection. To overcome these restrictions, transgenesis methods were modified. The use of retroviral vectors facilitated

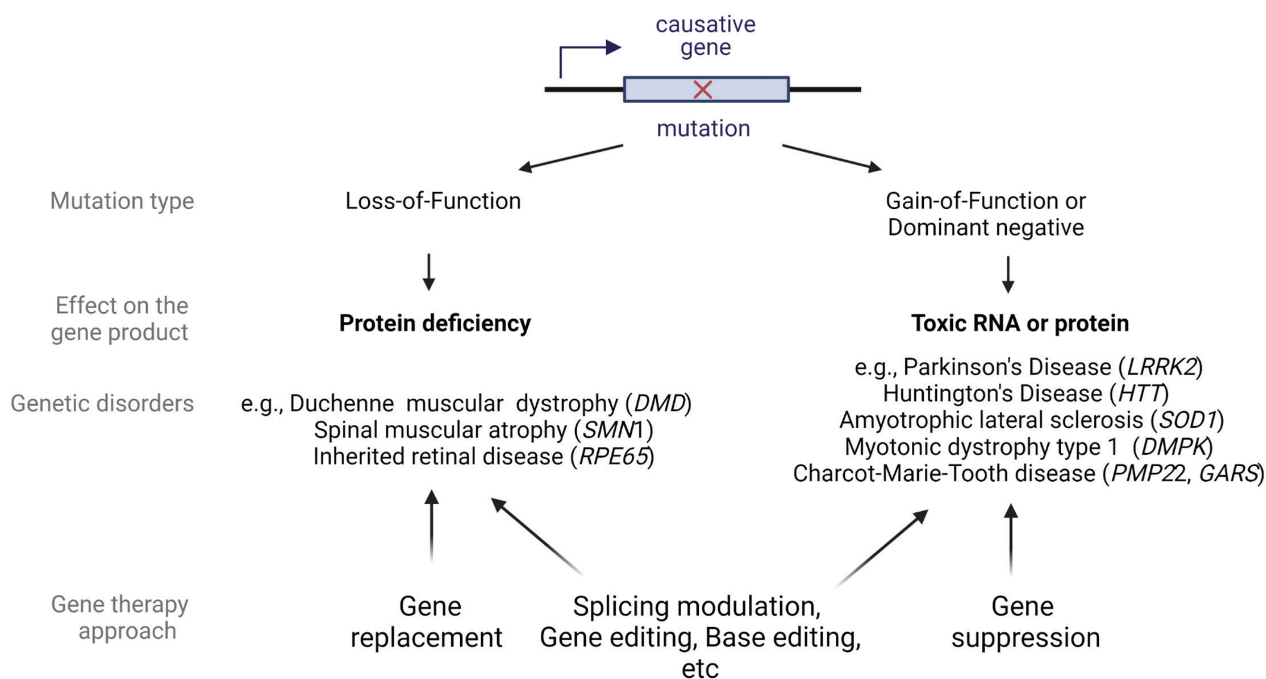

Figure 1. Type of the disease-causing mutations and gene therapy strategies for some hereditary muscular and neurological disorders.

integration into the genomes of animal models, and homologous recombination increased the likelihood of targeted integration. Transgene subcloning into yeast (YAC) or bacterial (BAC) artificial chromosomes allowed for the accommodation of larger DNA fragments for delivery. These larger transgenes may also contain native promoters and other regulatory sequences for gene expression and thus, more closely resemble endogenous genes. All of the above listed techniques can be used for transgene integration into genomic DNA of embryonic stem (ES) cells with subsequent clone selection. After ES transfer into the embryo at the morula or blastocyst stage, cells can differentiate into all cell types. In addition to coding and regulatory sequences, transgenes can include recombination sites and inducible expression cassettes for controlled expression. Using recombinases (Cre, Flp, or phiC31) and corresponding recombination sites (*loxP*, *FRT*, and *att*) allows for the restriction of transgene expression to the desired tissues or development stage. To create conditional knockout or knock-in models, first transgenic animals are generated with the target gene flanked by recombination sites or with a stop-cassette inserted into the target gene and flanked by recombination sites, respectively. Accompanying animal lines should contain corresponding recombinase with expression driven by a tissue-specific or inducible promoter. The crossing of these animals results in the desired genotype and allows one to model age-dependent conditions that are difficult to reproduce by single-step transgenesis. To simplify the production of such intricate animal models, many recombinase-expressing animal lines were developed and are available on demand (Gurumurthy and Lloyd 2019).

Breakthroughs in the development of precise genome editing techniques have further facilitated animal model generation for human diseases. These include TALEN (transcription activator-like effector nucleases), ZFN (zinc-finger nucleases), and CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein). Nucleases can be tailored to recognize specific nucleic sequences inside the genome for directed cleavage with subsequent repair by cellular enzymes. Depending on the outcome of the reparation, different mutations, including deletions, insertions, and inversions, can appear, allowing for the recapitulation of mutations found in patients. Due to the high effectiveness, editing complexes can be used both for direct injection into the zygote with subsequent offspring analysis and transfection of ES cells with selection and transfer of positive clones. Simultaneous delivery of the DNA repair template can lead to specific mutation introduction by homologous recombination or insertion into the cut site. Thus, directed cleavage of DNA increases the efficiency of gene-targeting compared to non-directed transgenesis.

Choosing from the variety of methods, it is critical to use the most suitable techniques to generate animal models, taking into account further application in human disease studies. Highly desirable disease models reproduce not only a patient's mutation but also the genetic context including regulatory sequences, gene copy number, and positioning within the genome. Animal models used for testing genome-targeted therapies should also have single-nucleotide polymorphisms around the mutation, making the sequence of the target site fully identical to the sequence of the patient. In this review, we provide examples of murine and large animal models generated for neurological and neuromuscular human diseases with loss-of-function and dominant mutations. We outline genetic manipulation used to develop animal models and specifically focus on the suitability of the described models for testing gene therapy approaches.

Animal models of Loss-of-function diseases

The first section describes loss-of-function diseases caused by mutations leading to a deficiency in the functional protein (Fig. 1). Therapeutic approaches for this type of diseases combine gene replacement aimed at delivery of functional protein coding sequence, splicing modulation for restoration of protein expression from the endogenous gene, or gene/base editing for removal of the causative mutation. Animal models of LoF diseases may have a deletion or knockout of the target gene (Table 1). These models are well suited for gene replacement therapy testing. Antisense therapy, such as exon-skipping, requires a similar exon–intron structure and humanization of nucleotides adjacent to mutations. The same goes for precise gene editing methods based on nucleotide sequence recognition. Classic examples of LoF pathology, such as Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA), are the most common neuromuscular disorders. These well-known disease pathologies and their high incidence allowed for the identification of disease-causing mutations and launched the development of gene therapy approaches. Not surprisingly, the first gene therapy drugs were approved for treating these diseases. Neurological disorders with vision loss are also often associated with LoF mutations. The development of eye-targeted gene therapy is attractive because of the immune privilege and limited organ volume. In this section, we describe the selection and creation of animal models for testing gene therapies of neuromuscular (i.e., DMD and SMA) and neurological (i.e., Leber's congenital amaurosis) disorders.

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a severe neuromuscular disease caused by mutations in the *DMD* gene, encoding dystrophin, and has an X-linked recessive inheritance pattern. Dystrophin acts as a linker between the contractile apparatus and sarcolemma in muscle cells, and its deficiency leads to membrane instability, cell necrosis, and impaired calcium homeostasis. Patients suffer from progressive muscular weakness and dystrophy (Duan et al.

Table 1. Examples of animal models for muscle and neurological disorders caused by loss-of-function mutations

Disorder	Mutated gene	Animal model	Technology	Brief description
Duchenne muscular dystrophy (DMD)	*DMD*	*mdx* mice (Bulfield et al. 1984)	Spontaneous mutation	Carries a nonsense mutation in exon 23
		GRMD dogs (Sharp et al. 1992)	Spontaneous mutation	Carries a splice site point mutation (transition A > G) in intron 6 leading to exon 7 skipping
		mdx52 mice (Araki et al. 1997)	Homologous recombination in ES cells	Carries the deletion of exon 52, which was replaced with a neomycin resistance cassette
		Del52hDMD/*mdx* mice (Veltrop et al. 2018)	Homologous recombination in ES cells/TALEN	Carries both murine and human *DMD* genes with deletion of exon 52 of the human gene and nonsense mutation in exon 23 of the murine gene
		Δ43 DMD, Δ45 DMD, and Δ52 DMD (Min et al. 2020)	CRISPR/Cas9 genome editing	Carries frameshift mutations with skipping of exon 43, 45, and 52.
Spinal muscular atrophy (SMA)	*SMN1*	*SMNΔ7*; *SMN2*; *Smn-/-* mice (Le et al. 2005)	Homologous recombination in ES cells (*Smn-/- SMN2*) Transgenesis (*SMNΔ7*) Further interbreeding of *Smn-/- SMN2* and *SMNΔ7* mice	Carries two human copies of *SMN2* in one tandem repeat and *SMNΔ7* insertion into the genome and knockout of the murine *Smn* gene
		SMA piglets (Duque et al. 2015)	Knocking down gene expression using shRNA	Carries *SMN* knockdown in postnatal motoneurons by intrathecal delivery of an scAAV9-shRNA directed at the porcine *SMN* (*pSMN*) gene
Inherited retinal disease (IRD)	*RPE65*	*RPE65-/-* mice (Redmond et al. 1998)	Homologous recombination in ES cells	Carries a substitution in the 5'-region containing the first three exons on the PGK-neo genome
		RPE65-deficient dogs (Veske et al. 1999)	Spontaneous mutation	Carries a homozygous deletion (485delAAGA) in putative exon 5 of the canine *Rpe65* gene
	CEP290	*CEP290* humanized mice (Garanto et al. 2013)	Homologous recombination in ES cells	Carries human exon 26, intron 26 (with or without the LCA mutation), and exon 27 inserted into the murine *Cep290* gene

2021). Due to the long length of the *DMD* gene (2.4 MB and 79 exons), a wide variety of disease-causing mutations were identified. The most prevalent mutations are deletions of one or several exons. Moreover, mutations are concentrated around exons 45–55 and 2–20, which are defined as mutation hot spots (Bladen et al. 2015). Usually, exon deletion induces a shift in the reading frame and termination of protein expression. Some mutations represent large deletions in the *DMD* gene that retains expression of the dystrophin lacking a central domain. Such in-frame mutations can be identified in patients with a milder form of disease, Becker muscular dystrophy (BMD). Based on this feature, two main gene therapy approaches were proposed: gene replacement therapy with shortened dystrophin sequences and exon-skipping therapy aimed at reading frame restoration at the transcription level. Up to 13% of DMD patients have nonsense mutations. For these patients, another therapy – stop codon readthrough – can be applied (Duan et al. 2021).

The first animal models of DMD were identified in natural populations. Among them is the most popular murine model, *mdx* (Bulfield et al. 1984), and the canine GRMD (golden retriever muscular dystrophy) model (Valentine et al. 1988), which better resemble patients' phenotype. *Mdx* mice bear spontaneous nonsense mutation within exon 23 and are helpful for testing various gene-targeted approaches including Ataluren (Translarna, PTC124) for stop-codon ignoring (Welch et al. 2007), antisense oligonucleotides (ASOs) for exon 23 skipping, and gene replacement therapies encoding mini- and micro-dystrophin and others (McGreevy et al. 2015). For example, one drug approved for the treatment of DMD, Ataluren, was tested in *mdx* mice and showed significant phenotypic improvements after 2–8 weeks of post-intraperitoneal injections, including increased protection against contraction-induced injury of skeletal muscles, reduction in serum creatine kinase, elevated levels of dystrophin-associated membrane glycoproteins, and partial restoration of dystrophin in skeletal muscles, diaphragm, and heart (Welch et al. 2007).

GRMD dogs bear a splice site mutation in intron 6 of the *DMD* gene, leading to the loss of exon 7 and a reading frame shift. This model was derived from the natural mutation of the breed (Valentine et al. 1988; Sharp et al. 1992). GRMD dogs were used to test gene replacement therapies and various ASO chemistries for exon-skipping. AAV-mediated systemic delivery of canine and human microdystrophins under constitutive and muscle-specific promoters demonstrated effective restoration of dystrophin expression, stabilizing clinical symptoms in studies performed on GRMD dogs. Systemic intravenous administration without immunosuppression resulted in significant and sustained levels of microdystrophin in skeletal muscles and reduced dystrophic symptoms for more than 2 years. No toxicity or adverse immune consequences of vector administration were observed (Le Guiner et al. 2017; Duan 2018). These studies form the basis of the ongoing clinical trials of SGT-001 by Solid Biosciences (NCT03368742).

Unlike the universal gene replacement approach, exon-skipping drugs should be developed for each group of patients individually and, therefore, tested in animal models with similar deletions. For this, dozens of animal models representing various exon deletions were created (re-

viewed in Egorova et al. 2021). For example, the mdx52 murine model was created to reproduce the exon 52 deletion, which is common in patients with DMD (Araki et al. 1997). The authors created a targeting vector from the murine genomic fragment. They replaced exon 52 with a neomycin resistance and thymidine kinase genes. A linearized vector was used for ES cell transfection, and antibiotic and pyrimidine analog addition allowed for the selection of positive clones. Modified ES cells were used for transfer into blastocysts. Female mice developed from these embryos were chimeric for the desired mutations and used for line derivation (Araki et al. 1997). Resulting murine model was used for in vivo testing of the first Food and Drug Administration (FDA) approved exon-skipping drug Eteplirsen (Aoki et al. 2010). Initially Eteplirsen was designed to target the DMD exon 51 splice site and tested on cellular models and hDMD mice bearing the normal human DMD gene (Arechavala-Gomeza et al. 2007). However, due to the expression of normal human and murine dystrophins, the hDMD mouse model does not represent any disease features necessitating additional testing. The utilization of mdx52 mice allowed researchers to demonstrate the effectiveness of the approach without drug candidate toxicity. The skipping of exon 51 resulted in mRNA frame restoration and the expression of shortened dystrophin after intramuscular, single systemic, and weekly systemic drug injections in mdx52 mice. The obtained dystrophin expression was sufficient for muscle strength recovery and the amelioration of histological signs of the disease. For testing on mdx52 mice, ASO was redesigned for adaptation to murine sequence followed by rescreening of candidates in a cell model (Aoki et al. 2010).

The most helpful for antisense and gene editing therapies testing DMD models bear the human *DMD* gene. These models allow binding of human mutation-directed oligonucleotides to the target site in a sequence-specific manner. For example, the hDMDdel52/*mdx* murine model was created for testing exon-skipping approaches (Veltrop et al. 2018; Yavas et al. 2020). ES cells derived from hDMD mice (Veltrop et al. 2013) were transfected with a vector plasmid containing fragment of human *DMD* gene with the deletion of exon 52 and TALEN construct to facilitate directed integration. Selected ES cells were injected into blastocysts and positive mice born from this experiment crossed with *mdx* mice (Veltrop et al. 2018). The Del52hDMD/*mdx* model was used to demonstrate the effectiveness of human sequence-targeting ASO for skipping exons 51 and 53 upon intramuscular delivery (Veltrop et al. 2018). Myoblasts derived from these mice were used to test CRISPR/Cas9 genome editing complexes for reframing in exons 51 and 53 during lentiviral delivery (Lyu et al. 2020).

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is a severe neuromuscular disorder that leads to a progressive loss of motor function and reduced life expectancy. SMA manifests as a broad spectrum of severities and is classified into clinical types based on the age of onset and the highest motor milestone achieved. Early-onset Type 1 SMA patients are never able to sit independently, and Type 4 patients achieve all motor milestones and develop symptoms in adulthood (Annoussamy et al. 2021). SMA is caused by a mutation in the *SMN1* gene, which normally produces the SMN (survival motor neuron) protein. Besides the *SMN1* gene, which is placed in the telomere, the survival motor neuron protein is encoded by *SMN2*, located in the centromere. Single nucleotide variation in exon 7 of *SMN2* leads to a high degree of exon 7 skipping, resulting in a non-functional protein (Annoussamy et al. 2021). Patients lacking the expression from *SMN1* gene have at least one copy of *SMN2* and traces of extremely important protein produced from this gene. Species other than humans have only one copy of the *Smn* gene.

The first attempts to create an animal model of SMA resulted in the early embryonic lethality of mice with the *Smn-/-* genotype (Schrank et al. 1997). The authors created targeting vectors in which the *LacZ* sequence was fused in frame with murine *Smn* exon 2 and the *Neo*^{r} gene under an independent promoter. Following transfection into ES cells and neomycin selection, homologous integration into the *SMN* locus was verified in five independent ES clones. Two independent lines of heterozygous mice were generated following blastocyst injection. The intercrossing of mice never resulted in homozygous offspring. Moreover, the authors suggested no implantation of such embryos, indicating the early embryonic lethality of complete *Smn* knockouts (Schrank et al. 1997). Next, these mice were crossed with transgenic mice containing two tandem copies of human *SMN2*. Intercrossing of the offspring resulted in mice *Smn-/- SMN2* with a short lifespan. Among the 56 newborn mice from the first experiment, 42 died within the first 6 h after birth and another 14 died at 4–6 days of age. Mice with a high copy number of *SMN2* on *Smn*$^{-/-}$ background did not demonstrate any disease symptoms (Monani et al. 2000). Although these mice can be used for studying pathogenesis and testing therapeutic approaches, including gene replacement therapy and splicing modulation, the short life expectancy hinders the experimental outline.

Another murine model of SMA with *SMNΔ7* insertion into the genome was obtained by transgenesis during direct injection of linearized vector into one-cell embryos and crossing with *Smn-/- SMN2* mice and further interbreeding (Le et al. 2005). The resulting murine line *SMNΔ7*;*SMN2*;*Smn-/-* on an FVB/N background (*SMNΔ7*) demonstrated prolonged survival for 15 days on average. These mice were used in many preclinical trials for developing SMA therapeutics including splicing modulators based on ASO (Porensky et al. 2012; Osman et al. 2014), small molecules (Ratni et al. 2016; Pinard et al. 2017), and AAV-based *SMN1* delivery (Passini et al. 2010; Armbruster et al. 2016; Bowerman et al. 2017). For example, intracerebroventricular (ICV) injection of morpholino oligomer against the ISS-N1 silencing site

within intron 7 to newborn *SMNΔ7* pups resulted in survival markedly increasing from 15 days to >100 days. ICV treatment upregulated full-length *SMN2* transcript as well as SMN protein in neural tissue but only minimally in the periphery. Prolonged analysis showed a decrease in alternative splice modification over time (Porensky et al. 2012).

Viral vectors were utilized for SMA modeling in pigs (Duque et al. 2015). The authors generated scAAV encoding short-hairpin RNA (shRNA) under the control of H1 promoter for targeting pig *Smn* transcript by RNA interference (RNAi). The AAV vectors were administered into motor neurons via intrathecal injections into piglets at postnatal day 5. The scAAV9-shSMN-treated animals showed a 30% reduction in SMN protein in lumbar spinal cord lysates compared to non-injected controls. Three to four weeks following vector administration, piglets developed progressive muscle weakness followed by the loss of the ability to stand without help. The authors evaluated the effect of scAAV9-SMN intracisternal administration in scAAV9-shSMN piglets at onset of symptoms. The neuropathology of the lumbar spinal cord and corresponding ventral root tissues of animals treated at the onset of symptoms showed marked improvement, with fewer chromatolytic motoneurons and degenerative motor axons observed. Indeed, a 39% increase in motoneuron count was observed in the onset-treated group compared to the untreated animals as well as an increase in the number of motor axons per ×1000 μm^2 (Duque et al. 2015).

Years of developing gene therapy approaches for SMA and validation in a variety of animal models have resulted in gene therapy drug registration and beneficial treatment of patients with Nusinersen (Spinraza, Ionis), AVXS-101 (Zolgensma, Avexis), and Risdiplam (Evrysdi, Roche).

Inherited retinal diseases

Inherited retinal diseases (IRDs) are a group of disorders that lead to progressive loss of vision and are associated with retinal degenerative changes. IRDs include such diseases as retinitis pigmentosa (RP), Leber congenital amaurosis (LCA), and early-onset severe retinal dystrophy (EOSRD). These disorders are characterized by heterogeneity in genetic causes and phenotypic manifestation and can be caused by mutations in more than 250 genes (Aoun et al. 2021).

Up to 6% of RP patients and up to 16% of LCA/EOSRD patients bear mutations in the *RPE65* gene. In most cases, RPE65-dependent IRDs are autosomal recessive diseases. The gene product – retinal pigment epithelium (RPE)-specific 65 kDa protein or retinoid isomerohydrolase – is an enzyme involved in the regeneration of visual pigment in photoreceptor cells of the retina. The absence of RPE65 causes a decrease in 11-*cis*-retinol levels and the accumulation of retinyl esters in the RPE. In RPE65 deficiency, photoreceptor cells do not regenerate their visual pigment, and vision is not maintained (Cideciyan et al. 2008; Aoun et al. 2021). Most patients with RPE65 deficiency have symptoms of the disease from birth in the form of deep night blindness, progressive loss of light sensitivity, nystagmus, and weak pupillary reflex (Kumaran et al. 2017).

The eye is a convenient site for administration of gene-directed drugs because of its immune privileged status and compartmentalized structure, which allows for targeted drug delivery to intraocular cells (Gao et al. 2020). RPE65 deficiency has an advantage over other IRDs owing to the availability of small and large animal models enabling expanded preclinical testing of gene therapy. The canine model of RPE65 deficiency was identified in a natural population of a highly inbred kinship of Swedish briard/briard–beagle dogs (Veske et al. 1999). The affected dogs had a homozygous deletion (485delAAGA) in putative exon 5 of the canine *RPE65* gene that resulted in progressive retinal dystrophy. A murine model of the RPE65 deficiency was described in 1998 (Redmond et al. 1998). Transgenic *RPE65-/-* animals were obtained using homologous recombination technology in ES cells. The 5'-region containing the first three exons was replaced with a genetic construct consisting of the PGK-neo gene for the selection of modified cells. Microinjection of selected ES cells into C57Bl6 blastocysts resulted in chimeric mice with a disrupted *RPE65* gene (Redmond et al. 1998). *RPE65-/-* mice showed a lack of rhodopsin, characteristic electroretinography (ERG) changes, retinal dysfunction, and loss of photoreceptors (Caruso et al. 2010).

Both canine and murine animal models were used in preclinical trials of *RPE65* gene replacement therapy (Gao et al. 2020). Animals received a subretinal injection of AAV2 carrying a murine- or canine-functional *Rpe65* genes. Following subretinal injections, cone and rod ERGs improved and visual function was partially restored in both model *RPE65-/-* animals (Acland et al. 2001; Lai et al. 2004). Subsequent dose-response studies identified a potentially safe and effective starting dose for human trials, which ultimately led to the FDA approval of neparvovec voretigene (Luxturna, Spark Therapeutics) in 2017 for the treatment of hereditary retinal disorders associated with RPE65.

Another IRD-causing gene is *CEP290*, which encodes 290 kDa centrosomal protein and plays an important role in cilia and centrosome development. Loss-of-function mutations in this gene are associated with a frequent form of LCA called LCA10. The most prevalent LCA-causing mutation in *CEP290*, accounting for up to 15% of LCA cases, is the *CEP290* c.2991+1655A>G point mutation located within intron 26. This mutation generates a splice donor site, resulting in the insertion of a 128 base pair cryptic exon X with a premature stop codon into CEP290 transcripts (Den Hollander et al. 2006). In order to clarify the pathophysiology of the intronic *CEP290* mutation c.2991+1655A>G, researchers generated two humanized mouse models in which human exon 26, intron 26 (with or without the LCA mutation), and exon 27 were inserted into the murine *Cep290* gene via homologous recombination in ES cells (Garanto et al. 2013). A detailed

characterization of these mice at the transcriptional level revealed unexpected splicing of *CEP290* mRNA that was only partially in line with the aberrant splicing observed in patients with *CEP290*-associated LCA (Garanto et al. 2013). In vivo genome editing therapy based on AAV5 encoding of S.a. Cas9 and two guides directed to intron 26 (EDIT-101) was tested in this model (Maeder et al. 2019).

Animal models of dominant diseases

The pathogenic mechanism of dominant diseases is often associated with the toxic function of protein or RNA product due to the fact of mutation in the causative gene (Fig. 1). Dominant mutations enhance or alter gene product activity (gain-of-function mutations, GoF) or confer dominant-negative properties (adverse effects of the mutant gene product on the wild-type counterpart). Examples of dominant mutations include repeat expansion inducing aggregate formation of the gene product, synthesis of enzymes with enhanced or uncontrolled activity, and increase in the copy number of the dosage-sensitive genes. Animal models for dominant disorders can be generated by adding a transgenic causative gene or its fragment with a toxic mutation into the host genome by transgenesis methods. Alternative strategies include editing the endogenous gene by introducing a deleterious mutation. A gene therapy strategy for dominant diseases is to suppress the expression of the mutated gene in order to prevent the production of the malfunctioning product. Non-viral technology for gene suppression is ASO-induced cleavage of the mutant transcript by RNase H. Mutant RNA can be also downregulated by an RNA interference (RNAi) mechanism triggered by AAV-delivered RNAi effectors and siRNA. Other technologies, such as splicing modulation and gene editing, can also be applied to dominant disorders as therapeutic strategies. This section describes examples of animal model generation (Table 2) and gene therapy development for selected dominant forms of muscular (i.e., myotonic dystrophy), neurological (i.e., Parkinson's disease and Huntington's disease), and neuromuscular (i.e., amyotrophic lateral sclerosis and Charcot-Marie-Tooth disease) disorders.

Myotonic dystrophy type 1

Myotonic dystrophy (DM) is the most common adult-onset muscular dystrophy with an estimated prevalence of 1:8000. The disease leads to progressive myopathy and myotonia, muscle weakness, atrophy of various muscle groups, and multiorgan disorders. There are two types of myotonic dystrophy. The first type (DM1) develops during the expansion of trinucleotide repeats in the 3'-UTR of *DMPK* gene; the second type (DM2) is characterized by the presence of repeats in the *ZNF9* gene. The frequency of occurrence of DM1 in Yakutia (Russia) is especially high: 21.3 cases per 100,000 inhabitants (Zabnenkova et al. 2018). DM2 is, on average, five times less frequent than DM1, but local prevalence data are available in Germany and Finland, where DM2 is as common as DM1 (Suominen et al. 2011). There is no cure for myotonic dystrophy. Patients receive symptomatic medical treatment in the case of respiratory symptoms, dysphagia, cardiac contractility disorders, endocrine disorders, etc.

The *DMPK* transcripts with expanded repeats accumulate in the cell nucleus, interfering with at least two antagonistic protein families that regulate alternative splicing throughout development: the muscleblind-like (MBNL) and CUGBP/Elav-like family (CELF) proteins. MBNL1 function is lost due to the sequestration by ribonuclear aggregates or foci. On the contrary, CELF1 is upregulated through protein stabilization that is mediated by hyperphosphorylation. MBNL1 sequestration and CELF1 upregulation result in aberrant expression of embryonic splicing profiles of MBNL1- and/or CELF1-regulated transcripts in adult skeletal muscle, heart, and other tissues, leading to multiorgan dysfunction and subsequent pathology (Gomes-Pereira et al. 2011). One of the possible gene therapy approaches is the RNAi for suppression of allele with expanded repeats.

Table 2. Mice models of dominant neurological and muscle disorders exemplified in the review

Disorder	Genetic cause	Animal model	Technology	Brief description
Myotonic dystrophy type 1	CTG repeat expansion in the 3'-untranslated region of the *DMPK1* gene	Tg26 mice (Jansen et al. 1996)	Transgenesis	Carries >20 copies of the human DMPK gene fragment with short CTG repeats
		HSA^{LR} mice lines (Mankodi et al. 2000)	Transgenesis	Contains genomic fragment of human skeletal actin (HSA) with ~250 CTG repeats placed in the 3'UTR of the transgene
Parkinson's disease	*LRRK2* with gain-of-function mutation G2019S	FLAG-Lrrk2-G2019S mice (Li et al. 2010)	BAC transgenesis	Contains the entire mouse Lrrk2 genomic sequence with G2019S substitution
Huntington's disease	Repeat expansion in exon 1 of the *HTT* gene	Hu97/18 (Southwell et al. 2013) and Hu128/21 mice (Southwell et al. 2017)	YAC and BAC transgenesis	Contains mutant and wild-type full-length genomic human HTT heterozygous for polymorphisms
Amyotrophic lateral sclerosis	*SOD1* with gain-of-function point mutations	SOD1 G93A mice (B6SJL-Tg(SOD1*G93A)1Gur/J) (Gurney et al. 1994)	Transgenesis	Contains several copies of human full-length SOD1-G93A
Charcot-Marie-Tooth disease, type 1A	Duplication of the *PMP22* gene	C22 mice (Robaglia-Schlupp et al. 2002)	YAC transgenesis	Contains seven copies of the PMP22 genomic region
Charcot-Marie- Tooth disease, type 2D	*GARS* with gain-of-function mutations	*Gars*$^{\Delta ETAQ/huEx8}$ mice (Morelli et al. 2019)	CRISPR/Cas9 Genome editing	Bears 12 bp deletion in exon 8 of murine *Gars* and humanized exon 8 in another allele

To investigate the consequences of *DMPK* overexpression, transgenic lines carrying multiple copies of the human gene with a short $(CTG)_{11}$ repeat were generated (Jansen et al. 1996). For this purpose, authors injected one-cell embryos with linear DNA representing human DNA fragment encoding the *DMPK* gene and upstream sequence up to the last exon of the preceding gene. Founder mice and their offspring carried >20 copies of the *DMPK* gene, and the highest expression of the Dmpk protein was found in the Tg26 mouse strain, exceeding expression in wild-type animals by 5–10-fold. This mouse model did not demonstrate any disease symptoms indicating that simple gain-of-expression of *DMPK* is not the only crucial requirement for the disease development.

In 2000, a more suitable mouse model was created (Mankodi et al. 2000). The authors used a genomic fragment containing the human skeletal actin (*HSA*) gene to express an untranslated CUG repeat (CUGexp) in the muscle of transgenic mice. An expanded (×250) repeat was placed midway between the termination codon and the polyadenylation site. Mice that expressed the long-repeat transgene developed histologically defined myopathy. Six of the seven lines expressing long repeats (LR) showed a consistent pattern of muscle histopathology including increases in the central nuclei and ring fibers and variability in fiber size. Higher levels of HSA^{LR} expression were associated with more severe pathology. Although abundant central nuclei, variability in fiber size, and ring fibers can each be observed in other disorders, this constellation of features in the absence of muscle fiber necrosis is suggestive of DM. Fluorescent in situ hybridization analysis of muscle cryosection of these mice helped to define intracellular localization of long-repeat containing transcripts in the nucleus in multiple discrete foci. These mice were utilized for proof-of-concept studies of gene-specific knockdown application for DM1 treatment (Wheeler et al. 2012). The systemic administration of ASOs caused a rapid degradation of CUGexp RNA in skeletal muscle, correcting the physiological, histopathologic, and transcriptomic features of the disease. The effect was sustained for up to one year after treatment was discontinued. These results provided a general strategy to correct RNA gain-of-function caused by expanded repeats with prolonged nuclear residence. The authors demonstrated that nuclear-retained transcripts containing expanded CUG repeats are extraordinarily sensitive to antisense silencing (Wheeler et al. 2012).

Parkinson's disease

Parkinson's disease (PD) is a common neurodegenerative disorder affecting 7–10 million people worldwide, and its prevalence increases with age. The clinical manifestations include impairment of voluntary motor control (tremors, rigidity, slowness of movement, and postural instability) and non-motor symptoms including behavior disorders, cognitive changes, constipation, and sleep disturbances. Disease progression is caused by the death of dopamine-producing neurons in the midbrain area called substantia nigra.

The etiology of most PD cases is complex, combining environmental and genetic factors. Monogenic forms of PD account for 5–10% of cases. Among high-risk genetic factors for PD are pathogenic variants of the *LRRK2* gene encoding leucine-rich repeat kinase 2. LRRK2 is a large protein with GTPase and kinase domains. Pathogenic mutations induce abnormal gain in LRRK2 kinase activity which, in part, promotes aggregation of α-synuclein protein triggering the onset of the dominant form of PD (Seegobin et al. 2020).

Numerous therapeutic approaches are directed at lowering LRRK2 activity, ranging from small molecule inhibitors to RNA-targeting therapies (reviewed in Wojewska and Kortholt 2021). For instance, Ionis Pharmaceuticals developed ASOs to reduce overall production of LRRK2 in neurons (Zhao et al. 2017). Sequences of two *LRRK2*-targeted ASOs were homologous to human and mouse *Lrrk2* gene, which allowed for their preclinical testing in wild-type C57BL6/C3H mice. Co-injection of α-synuclein pre-formed fibrils mimicked PD-like conditions. ASOs achieved long-lasting suppression of endogenous *Lrrk2* and reduced the formation of pathogenic α-synuclein inclusions. Ionis's lead candidate ION859 (BIIB094) is currently in the phase 1 clinical trial REASON for adults with PD (NCT03976349). Another ASO-based approach suggests modulating the splicing of *LRRK2* transcripts to produce protein without a portion of the kinase domain thus abolishing its hyperactivity (Korecka et al. 2020).

Mutation-specific approaches attempt to suppress *LRRK2* transcript with G2019S substitution, which is the most common PD-associated mutation (4–5% in familial and 1% in sporadic cases). In vitro studies demonstrated robust discrimination of the mutant and wild-type alleles with RNAi effectors (shRNA and siRNA) downregulating LRRK2–G2019S (Sibley and Wood 2011). These pilot studies have shown the promise of developing AAV-RNAi strategies for the treatment of *LRRK2*-G2019S-associated PD. Shape Therapeutics aims at editing mutations in the *LRRK2* transcript using the RNA-dependent adenosine deaminase ADAR (RNAfix™ platform), which can be delivered into neurons with AAV vectors. Both RNAi and RNA editing technologies require animal models with the *LRRK2*-G2019S variant for in vivo testing (reviewed in Seegobin et al. 2020).

The first mouse model of PD with the LRRK2-G2019S mutation was generated using the BAC technique and overexpressed murine transgene (Li et al. 2010). To produce transgene, a BAC clone containing the entire wild-type mouse *Lrrk2* genomic sequence (RP23-312I9) was genetically engineered by inserting FLAG epitope after start codon ATG and introducing G2019S substitution. The BAC DNA was injected into fertilized oocytes. Repeated genetic crosses of BAC transgenic mice with C57BL/6J were performed to generate the FLAG-Lrrk2-G2019S mouse line. A PD mouse model overexpressing Lrrk-G2019S was used for detailed characterization of pathogenic effects due to the increased

kinase activity and showed an age-dependent decrease in striatal dopamine content (Li et al. 2010). Application of Lrrk2-G2019S mice for testing RNA-targeting therapeutics for PD is limited due to the differences in nucleotide sequence of mouse and human *LRRK2* transcripts. Therefore, several humanized mouse and rat models have been generated using BAC (RP-11 568G5) or cDNA insertion to overexpress pathogenic variants of human *LRRK2* (reviewed in Baptista et al. 2013; Seegobin et al. 2020). These models recapitulate the hallmarks of PD to different extents and could be potentially used for testing RNA-targeting therapies such as ASO, AAV-RNAi, and ADAR-mediated editing.

Huntington's disease

Huntington's disease (HD) is a dominant, progressive brain disorder caused by mutations in the *HTT* gene, encoding huntingtin. The symptoms usually start at 30–50 years of age and include involuntary hyperkinetic movements, emotional problems, and cognitive impairment. There is no therapy to slow down disease progression. HD leads to total physical and mental deterioration and death within 12–15 years after the manifestation. This disorder affects an estimated 5–10 per 100,000 people worldwide.

The molecular pathology of HD is associated with pathogenic expansion of CAG repeats (polyQ tract) over the normal range (>36 CAGs) in the exon 1 of the *HTT* protein-coding sequence. Mutant huntingtin acquires neurotoxic function leading to the degeneration of striatum (Marxreiter et al. 2020).

Next-generation therapies are directed towards lowering the content of the mutant HTT in neuronal tissues (reviewed in Marxreiter et al. 2020). The straightforward approach is to reduce total HTT levels in a non-selective manner by applying gene suppression tools. UniQure Biopharma demonstrated promise of this approach for AAV5-delivered miRNA targeting mutant and wild-type *HTT* transcripts (Miniarikova et al. 2016) with their lead candidate AMT-130 reaching clinical trials (NCT04120493). Similar technology was adopted by Voyager Therapeutics in the development of their HD gene therapy candidate VY-HTT01. Ionis Pharmaceuticals developed *HTT*-targeting ASO (HTTRx) which demonstrated dose-dependent reductions in concentrations of mutant HTT following intrathecal administration to patients with early HD (Phase I/IIa clinical trials, NCT02519036) (Tabrizi et al. 2019). However, the same ASO termed Tominersen did not confer any benefit to patients in a Roche-sponsored phase III clinical trial (NCT03761849).

Allele-selective suppression of the mutant *HTT* is considered a potentially safer disease-modifying strategy compared to the nonselective approach, as it leaves normal transcript unaffected. Discrimination of mutant and wild-type transcripts can be achieved by targeting single-nucleotide polymorphisms (SNPs) in the *HTT* that are linked to CAG expansion in the population. Attempts to develop allele-selective HTT-lowering therapies were undertaken using ASO, AAV-miRNA, and CRISPR–Cas9 gene editing platforms (Southwell et al. 2014; Miniarikova et al. 2016; Shin et al. 2016).

Several humanized HD mouse models have been generated suitable for the preclinical evaluation of the efficacy and safety of *HTT*-targeting therapies including SNP-dependent approaches (reviewed in Miniarikova et al. 2018). The recently developed models, Hu97/18 and Hu128/21, are of particular interest, as they do not express murine *Htt* and contain copies of the mutant and wild-type human *HTT* gene, thus genetically resembling HD patients. For generation of these models transgenic mouse lines with full-length human *HTT* gene were used. All mice lines express transgene from the human *HTT* promoter and vary in the number of CAG (Q) repeats. The model Hu97/18 was generated by intercrossing mice BACHD (97Q) and YAC18 (18Q) on the *Hdh*$^{-/-}$ background (Southwell et al. 2013). A companion model, Hu128/21, was obtained by interbreeding YAC128 and BAC21 mice on the *Hdh*$^{-/-}$ background. While the Hu97/18 model contains mutant *HTT* with SNPs from populations of Caucasian descent (A haplogroup), the Hu128/21 model more closely resembles haplogroup C polymorphisms associated with CAG expansion in HD patients of East Asian descent (Southwell et al. 2017). Both models exhibit HD-like phenotypes including motor, behavioral, cognitive disturbances, and striatal gene expression changes. These transgenic mouse models were helpful for evaluating the efficacy and tolerability of gene therapy drugs inducing allele-selective HTT suppression (Southwell et al. 2014; Miniarikova et al. 2016).

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disorder in adults with a prevalence of approximately six cases per 100,000 (Talbott et al. 2016). The disease is characterized by neurodegeneration of motor neurons in the brain and spinal cord affecting voluntary muscle actions. As the disease progresses, patients become totally paralyzed. No cure is available for this disorder, and the average life expectancy does not exceed 3–4 years from the onset of symptoms.

The genetic markers of ALS have been identified in familial (hereditary) and sporadic forms of the disease. To date, over 30 mutated genes (e.g., *SOD1*, *FUS*, *TARDBP*, *C9orf72*, *HNRNPA1*, *SQSTM1*, and *OPTN*) are linked to ALS, and most of these genes confer a dominant inheritance (reviewed in Renton et al. 2013). The ALS hallmark triggered by mutated genes comprises cytoplasmic inclusions of TDP-43 (TAR DNA-binding protein 43) in motor neurons as well as mislocalization of proteins FUS (fused in sarcoma) and hnRNPs (heterogeneous nuclear ribonucleoproteins) to the cytoplasm.

Mutations of Cu/Zn superoxide dismutase 1 (SOD1) were the first to be discovered in association with ALS (Rosen et al. 1993). The *SOD1* mutations account for

~12–20% of familial ALS and ~2% of all cases. Point mutations in *SOD1* cause conformational instability of the protein and lead to the accumulation of the misfolded SOD1 with neurotoxic properties in the motor neurons.

Mice models with the toxic gain-of-function mutation G93A in *SOD1* have become extensively used to address ALS pathology and for preclinical studies. The most common mouse SOD1-G93A line (B6SJL-Tg(-SOD1*G93A)1Gur/J) was generated in 1994 by classic transgenesis (Gurney et al. 1994). To obtain transgene, the region of the human *SOD1* exon 4 with mutation was amplified from the DNA of individuals with the familial ALS and cloned into plasmid containing the entire genomic sequence of *SOD1*. A linear 14.5 kb fragment containing the *SOD1* gene including promoter sequences was excised from the recombinant plasmid and microinjected into fertilized eggs of C57BL6 × SJL crosses. The founder mice were bred on a mixed B6SJL genetic background, and their progeny were subsequently analyzed. One of the G93A transgenic lines (G1) demonstrated a high expression level of the human *SOD1* mRNA in the brain due to the presence of multiple copies of transgene and developed a stereotyped syndrome suggestive of motor neuron disease (Gurney et al. 1994). G1 mice histopathological analysis revealed a severe loss of choline acetyltransferase (ChAT)-containing spinal motor neurons with the most pronounced changes in the ventral spinal cord. The muscles showed severe loss of myelinated axons from the intramuscular nerves and consequent reinnervation of muscle fibers by primarily nodal sprouts.

The described SOD1-G93A mouse model has been utilized to test various gene therapy approaches for treating SOD1-linked forms of ALS in preclinical studies (reviewed in Amado and Davidson 2021). Similar to other dominant disorders, SOD1-targeting gene therapies aim to downregulate production of the toxic mutant protein. Next-generation ASOs jointly developed by Biogen, Ionis, and Harvard Medical School researchers potently reduced SOD1 mRNA and protein in SOD1-G93A mice after a single intraventricular injection and extended animal survival by almost 40 days (McCampbell et al. 2018). Reduction of *SOD1* expression induced by AAV-RNAi was attempted by several biotech companies including Novartis Gene Therapies (project previously developed by AveXis) and Voyager Therapeutics (reviewed in Cappella et al. 2021). The therapeutic potential of AAV-based therapies has been demonstrated in a SOD1-G93A mouse model (Iannitti et al. 2018; Keeler et al. 2020). Alternative molecular strategies utilize AAVrh10-delivered modified U7 small-nuclear RNA to induce exon-skipping. Following combined intravenous and intracerebroventricular delivery, AAVrh10-U7-hSOD substantially reduced mutant SOD1 in the spinal cord, prevented weight loss and preserved motor function in SOD1-G93A mice (Biferi et al. 2017). Gene therapy company ApicBio has advanced development of the "dual-function" AAV vector for ALS that will allow silencing of the disease-causing gene and replacing a normal gene product (THRIVE™ Platform). AAV-delivered CRISPR-Cas9 was used to disrupt mutant *SOD1* in the G93A-SOD1 mouse model which led to a ~25% increase in survival of treated animals (Gaj et al. 2017). Finally, AAV9-vectors with CRISPR base editors were developed to introduce nonsense-coding substitution into a mutant SOD1 gene. Intrathecal injection of AAV9 encoding base editors reduced by 40% the abundance of SOD1-containing inclusions in the spinal cord, improved neuromuscular function, and prolonged survival of the SOD1-G93A mice (Lim et al. 2020). While SOD1-G93A mice do not contain the most common disease-causing gene variant in humans and do not reproduce the whole spectrum of ALS symptoms, this model has been extremely useful for illustrating in vivo therapeutic potential of gene therapy approaches for SOD1-linked forms of ALS.

Charcot-Marie-Tooth disease

Charcot-Marie-Tooth disease (CMT) is one of the most frequent neurological inherited disorders. It manifests in the second decade of life as distal muscle wasting and weakness, decreased tendon reflexes, and peripheral, usually symmetrical, desensitization. The severity of the disease varies greatly, depending more on the underlying genetic defect (Theadom et al. 2019). Forms of CMT with autosomal dominant, autosomal recessive, and X-linked inheritance have been described. It is classically separated into a demyelinating form (CMT1) and an axonal form (CMT2).

Subtype 1A, the most common form of the disease, is inherited as an autosomal dominant trait. This disease has been associated with the duplication in chromosome 17 spanning 1.5 Mb and including the *PMP22* gene. The CMT1A phenotype in humans most frequently results from a dosage effect caused by the duplication rather than point or other mutations of the *PMP22* gene. The duplication has been found in up to 92% of families with CMT1 (Huxley et al. 1996). A mouse model with *PMP22* overexpression was obtained by YAC-mediated transgenesis technique (Robaglia-Schlupp et al. 2002). The authors created YAC carrying the 40 kb human *PMP22* gene flanked by about 100 kb of upstream sequences and about 300 kb of downstream sequences. C22 mouse line derived from a male founder bearing 7 copies of *PMP22* demonstrated a strong neuropathy phenotype. The authors detected a 2.1-fold *PMP22* upregulation at the transcription level. The C22 mice demonstrated progressive severe demyelination with occasional onion bulb formations, occasional axonal hypertrophy, early disease manifestation and shortened lifespan (Huxley et al. 1996; Robaglia-Schlupp et al. 2002). In C22 mice, ASO-mediated reduction of *PMP22* mRNA levels markedly improved and even reversed several neuropathy end points such as motor, electrophysiology, pathology, and transcriptomic changes (Zhao et al. 2018). The authors demonstrated efficient *PMP22* downregulation after weekly subcutaneous injections of ASO1 which targets the 3' UTR of the human gene. All tested doses induced recovery of sciatic nerve conduction velocity to normal rates (Zhao et al. 2018).

Charcot-Marie-Tooth disease type 2D (CMT2D) is caused by dominant mutations in the glycyl-tRNA synthetase (*GARS*) gene. To date, at least 19 individual mutations in *GARS* have been identified in patients with CMT2D, all of which result in single amino acid changes (Morelli et al. 2019). Most disease-associated *GARS* variants cause impaired enzymatic activity. At the same time, protein-null alleles in mice and humans do not cause dominant neuropathy. Furthermore, overexpression of wild-type *GARS* does not rescue neuropathy in mouse models, suggesting that mutant forms of GARS adopt a toxic gain-of-function activity that the normal protein cannot outcompete (Motley et al. 2011). One of the mutations in the *GARS* gene leads to severe peripheral neuropathy and represents heterozygous 12 nucleotide deletion (*GARS* c.894-905del). This mutation results in the deletion of four amino acids in the GARS protein (p.Glu299-Gln302del or ΔETAQ). To study the pathogenesis and test appropriate gene therapy approaches, Morelli and colleagues created a murine model of CMT2D $Gars^{\Delta ETAQ/huEx8}$, bearing the patient's mutation (Morelli et al. 2019). These mice were the result of crossing two independent lines, created by targeted genome editing using CRISPR/Cas9. To create both lines, the authors microinjected genome editing complex consisting of Cas9 mRNA, sgRNA, and donor template DNA into male pronucleus and cytoplasm of zygotes at the pronuclei stage. $Gars^{\Delta ETAQ/+}$ mice were obtained after microinjections with short synthetic single-stranded DNA with 12 bp deletion in exon 8. For $Gars^{huEx8/+}$ mice creation, the donor was a 10 kb sequence containing a 2.8 kb 5' arm of homology and a 7-kb 3' arm of homology isolated from a C57BL/6J BAC library flanking the human exon 8 sequence. As a result, the mouse model of CMT2D $Gars^{\Delta ETAQ/huEx8}$ bears 12bp deletion in murine exon 8 and humanized exon 8 in another allele. $Gars^{\Delta ETAQ/huEx8}$ mice displayed overt neuromuscular dysfunction and a reduction in body weight and grip strength compared with $Gars^{huEx8/+}$ controls. Analysis of the femoral nerve cross-section revealed an overall decrease in axon number and axon diameter. Nerve conduction velocities were reduced by 62% in the sciatic nerve in mutant mice, consistent with those observed in patients with *GARS*-mediated peripheral neuropathy (Morelli et al. 2019). These mice were used for in vivo allele-specific $Gars^{\Delta ETAQ}$ downregulation studies. After intracerebroventricular injections of scAAV9-encoding miRNA constructs, mice demonstrated improvement in a wire hanging test, increased muscle-to-body-weight ratios, and improved sciatic nerve conduction velocity (Morelli et al. 2019).

Conclusion

Animal models of human disorders are a valuable tool for biomedical research. They reproduce critical clinical features and allow studying and testing drugs for disease treatment. Next-generation therapeutics such as gene therapy raise demand for animal models that also replicate the genetic context of the disease-causing mutation. For instance, testing certain therapeutic approaches requires the presence of the exact human pathogenic variant in the mouse genome, or humanized sequences surrounding the mutation site (Nair et al. 2019).

Murine models are most often used to study hereditary diseases. Compared to other mammals, mice have apparent advantages: simplicity of handling and maintenance, short gestation period, genome similar to 99% of the human one, a small dose of the drug is required for testing, which is particularly important for viral vector-based gene therapies (Gopinath et al. 2015). Moreover, there are well established protocols for creating genetically modified mice. However, murine models also have their drawbacks associated with immunity distinguished from humans. As a result, the disease phenotype may be milder in mice than in patients. In this case, studies in mice can be supplemented by experiments using large animal models, such as dogs, pigs or primates. Disease modeling as well as maintenance of genetically modified large animals is difficult and expensive. Transgene delivery using viral vectors has become an alternative approach for creating models for human diseases in rats, pigs, and non-human primates. Among other viruses, AAV vectors have proven to be an ideal tool for modeling severe neurological and neuromuscular disorders due to high tropism to neuronal tissues, skeletal and cardiac muscles (Duque et al. 2015; Huntington and Srinivasan 2021).

Over the past decades, technologies for creating animal models have evolved from transgenesis with undesired effects caused by transgene insertions at different locations in the genome, to precise genome editing using "molecular scissors" such as TALEN, ZFN, and CRISPR/Cas. The use of ES cells instead of direct manipulation of embryos made it possible to select clones with the desired mutation and further improve the accuracy of the modeling. To date, the majority of the animal models used for gene therapy preclinical testing have been generated using transgenesis, BAC and YAC techniques, and homologous recombination in ES cells. At the same time, the most convenient CRISPR/Cas method became available for genetically modified animals less than 10 years ago, and dozens of animal models including models with patient-specific mutations have already been generated (White et al. 2018; Egorova et al. 2019; Morelli et al. 2019; Min et al. 2020). Not only the creation but also the reproduction and characterization of the disease model takes a lot of time (Cong et al. 2013; Egorova et al. 2021). For rare diseases with unknown mechanisms of development, studying the physiology of animal models and their natural history is the only way to determine the molecular, biochemical and physiological features of the pathology. Thus, animals with finely edited genomes are expected to be used in preclinical studies of new gene therapy methods in the near future.

Conflict of interests

The authors declare no conflict of interests.

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