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GENETICALLY ENGINEERED AND GENOME EDITED LARGE ANIMAL MODELS FOR NEURONAL CEROID LIPOFUSCINOSES – A REVIEW

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SUMMARY

The neuronal ceroid lipofuscinoses (NCL) are a group of fatal neurodegenerative inherited diseases. Ovine models have been instrumental to advance the understanding of the genetics and the underlying disease mechanism, but most importantly are crucial for the development of therapeutic interventions. We have commenced to use CRISPR/Cas9 technology to generate an ovine model for the so-called Turkish variant of late-infantile neuronal ceroid lipofuscinosis (CLN7), a relatively common disease variant in humans for which currently no ovine model exists. Other groups have created genome edited and genetically engineered models for CLN1 and CLN3 variants, respectively. We summarise information about naturally occurring variants of NCL in animals and review the limited information about genome edited and genetically engineered non-laboratory animal models for NCL.

INTRODUCTION

Neuronal ceroid lipofuscinoses (NCLs/Batten disease) are a group of lysosomal storage disorders affecting humans and animals. Common characteristics of these diseases include distinctive auto-fluorescent storage bodies in neurons and many other cells and progressive brain and retinal atrophy leading to loss of vision, mental and motor deterioration, epileptic seizures and premature death. In humans, NCL variants have been categorized based on the disease causing genes, i.e. CLN1/*PPT1*, CLN2/*TPP1*, CLN3/*CLN3*, CLN4/*DNAJC5*, CLN5/*CLN5*, CLN6/*CLN6*, CLN7/*MFSD8*, CLN8/*CLN8*, CLN10/*CTSD*, CLN11/*GRN*, CLN12/*ATP13A2*, CLN13/*CTSF*, CLN14/*KCTD7* (Warrier *et al.* 2013). Despite the identification of the disease-causing genes, the links between protein defects, lysosomal storage and pathogenesis are not well understood (Cooper *et al.* 2015). There is no cure, but enzyme replacement therapy (ERT) has shown to attenuate the progression of the CLN2 variant of disease; and research in animal models and human clinical trials suggest that promising results can be achieved with both ERT and gene therapy for variants that are caused by mutations in genes coding for the soluble proteins PPT1, TPP1, CLN5, CTSD, GRN, CTSF (Kohlschütter *et al.* 2019; Mole *et al.* 2019). However, effective therapeutic interventions for variants that are caused by mutations in genes coding for the membrane proteins CLN3, DNAJC5, CLN6, MFSD8, CLN8, ATP13A2 and KCTD7 are lacking.

NON-LABORATORY ANIMAL MODELS FOR NCL

Naturally occurring NCL diseases have been described in many animal species (Table 1) and both naturally occurring, and genetically engineered animal models have been crucial in research efforts to improve our understanding of the genetics and the underlying disease mechanism. Such animal models of NCL disease are required for safety and proof of concept studies for therapeutic interventions (Bond *et al.* 2013). Non-laboratory animal models, such as dogs and sheep, are of specific interests due to their comparatively large and complex brains, long lifespan and the spectrum of clinical signs with

which they present. Considerable progress has come from studying sheep with naturally occurring CLN5 and CLN6 forms of disease by the Batten Animal Research Network (BARN) (Palmer *et al.* 2015; Mitchell *et al.* 2018). However, naturally occurring models are not available for all variants of NCL disease (Table 1) and very few non-laboratory animal models have been maintained as research populations. Recently ovine and porcine models for the NCL variants CLN1 and CLN3 have been developed using homologous recombination followed by somatic cell nuclear transfer as well as CRISPR/Cas9 genome editing methods (Table 2; Beraldi *et al.* 2016; Eaton *et al.* 2019).

Table 1. Natural occurring NCLs in animals. NCL variants, genes, species, OMIA/MGI ID, and breed are shown (OMIA: https://omia.org/home/; MGI: http://www.informatics.jax.org)

NCL variant/*gene*	Species (OMIA or MGI ID: breed)
CLN1/*PPT1*	• *Canis lupus familiaris* (001504-9615: Miniature Dachshund; Italian Cane Corso)
CLN2/*TPP1*	• *Canis lupus familiaris* (001472-9615: Longhaired Dachshund)
CLN5/*CLN5*	• *Bos taurus* (001482-9913: Devon) • *Canis lupus familiaris* (001482-9615: Border Collie, Australian Cattle Dog; Golden Retriever) • *Ovis aries* (001482-9940: Borderdale)
CLN6/*CLN6*	• *Canis lupus familiaris* (001443-9615: Australian Shepherd) • *Mus musculus* (MGI:2159328) • *Ovis aries* (001443-9940: Merino)
CLN7/*MFSD8*	• *Canis lupus familiaris* (001962-9615: Chinese Crested Dog, Chihuahua) • *Macaca fuscata* (001962-9542: Japanese macaque)
CLN8/*CLN8*	• *Canis lupus familiaris* (001506-9615: English Setter, Australian Shepherd, Alpenlaendische Dachsbracke, Saluki) • *Mus musculus (*MGI:1856959)
CLN10/*CTSD*	• *Canis lupus familiaris* (001505-9615: American Bulldog) • *Ovis aries* (001505-9940: Swedish Landrace)
CLN12/*ATP13A2*	• *Canis lupus familiaris* (001552-9615: Tibetan Terrier)
n.d./*ARSG*	• *Canis lupus familiaris* (001503-9615: American Staffordshire Terrier)
n.d./n.d.	• *Agapornis roseicollis* (000181-60468) • *Anas platyrhynchos* (000181-8839) • *Bos taurus* (000181-9913: Holstein, Beefmaster) • *Canis lupus familiaris* (000181-9615: American Pit Bull Terrier, Cocker Spaniel, Dalmatian, Japanese Retriever, Labrador Retriever, Minature Schnauzer, Polish Owczarek Nizinny, Saluki, Welsh Corgi) • *Capra hircus* (000181-9925: Nubian) • *Equus caballus* (000181-9796: Aegidienberger) • *Felis catus* (000181-9685: domestic short-haired, Siamese) • *Macaca fascicularis* (000181-9541) • *Mustela putorius furo* (000181-9669) • *Ovis aries* (000181-9940: Rambouillet) • *Sus scrofa* (000181-9823:Vietnamese pot-bellied)

Due to the large amount of research conducted on naturally occurring ovine CLN5 and CLN6 variants, creation of additional ovine models of NCL disease is of particular interest. Direct comparison of natural disease history across these different ovine models would be possible. Standardised assessments of the disease progression as well as gene therapy methods that have been developed for the ovine CLN5 and CLN6 research flocks in Australia and New Zealand (Palmer *et al.* 2015;

Mitchell *et al.* 2018) could be directly transferred to newly developed ovine models for NCL variants for which there is currently no non-laboratory research population.

OVINE CRISPR/CAS9 CLN7 MODEL

Until recently there were no non-laboratory animals diagnosed with CLN7 disease (MIM # 610951), which is the 5th most common variant of NCL disease in humans (NCL-Resource https://www.ucl.ac.uk/ncl-disease/mutation-and-patient-database). It is unclear if CLN7 research populations can be established from the recently reported Chihuahua (Ashwini *et al.* 2016) and macaque (McBride *et al.* 2018) cases. We have therefore commenced to develop a CRISPR/Cas9 genome edited CLN7 sheep model (Table 2; Tammen *et al.* 2019) that mimics one of the 39 known human *MFSD8* mutations and will allow direct comparison to the existing natural occurring ovine variants of NCL disease. We have confirmed that our chosen electroporation approach modified from Kaneko *et al*. (2013) is an efficient way to deliver CRISPR/Cas9 components to *in vitro* produced embryos. We identified sgRNAs and donor template that create the desired genome edit. However, regulatory uncertainties have delayed this work as the current requirement to maintain CRISPR/Cas9 genome edited sheep as genetically modified organisms (GMO) substantially increases the costs for the planned research. However, amended regulations, which consider animals that are created using CRISP/Cas9 and Cas9-induced non-homologous end joining (NHEJ) as non-GMO, will take effect in October 2019 in Australia and will allow us to proceed with this research.

Table 2. Genetically engineered and genome edited non-laboratory animal models for CLN3, CLN1 and CLN7 variants of NCL disease

NCL variant	CLN3/*CLN3*	CLN1/*PPT1*	CLN7/*MFSD8*
Number of human patients / families with disease variant*	432 / 401	230 / 177	104 / 88
Protein location**	late endosomal/ lysosomal membrane, presynaptic vesicles	lysosomal matrix	lysosomal membrane
Protein function**	unknown	palmitoylthioesterase	predicted transporter
GE model species	*Sus scrofa*	*Ovis aries*	*Ovis aries*
Targeted gene / mutation	*CLN3* $^{\Delta ex7\text{-}8/\Delta ex7\text{-}8}$	*PPT1* p.Arg151Ter	*MFSD8* c.103C>T
Methodology	homologous recombination in fetal fibroblasts & somatic cell nuclear transfer	CRISPR/Cas9 HDR via microinjection of *in vitro* derived embryos	CRISPR/Cas9 HDR & NHEJ via electroporation of *in vitro* derived embryo
Animals with targeted mutation	yes	yes (3 Indel, 6 heterozygous HDR and 3 homozygous HDR)	embryos only
Clinical signs/ histopathology characteristic of NCL disease	yes	yes	unknown
Reference	Beraldi *et al*. 2016; Johnson *et al*. 2019	Eaton *et al.* 2019	Tammen *et al*. 2019

* NCL-Resource: https://www.ucl.ac.uk/ncl-disease/mutation-and-patient-database
** Kollman *et al*. (2013)

CONCLUSIONS

Variants of NCL have been described in many animal species and the identification of disease-causing mutations and development of DNA diagnostics allows for effective management of these diseases in companion animals and livestock. Non-laboratory animal models for NCL have been instrumental in increasing our understanding of this devastating group of diseases in humans and are of particular importance for safety and proof of concept studies for therapeutic interventions. CRISPR/Cas9 technology is an efficient method to develop new animal models for human disease and can be used to validate the effect of predicted disease-causing mutations in animals. Changes to the regulation relating to the use of CRISPR/Cas9 technology will make it easier to create animal models for human disease.

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