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Research Report

Progressive retinal degeneration and accumulation of autofluorescent lipopigments in Progranulin deficient mice

Brian P. Hafler[a,*,1], Zoe A. Klein[b,1], Z. Jimmy Zhou[a,c], Stephen M. Strittmatter[b]

[a]Department of Ophthalmology and Visual Science, Yale School of Medicine, New Haven, CT 06510, USA
[b]Cellular Neuroscience, Neurodegeneration and Repair Program, Departments of Neurology and Neurobiology, Yale School of Medicine, New Haven, CT 06510, USA
[c]Departments of Cellular and Molecular Physiology and Neurobiology, Yale School of Medicine, New Haven, CT 06510, USA

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ABSTRACT

Prior investigations have shown that patients with neuronal ceroid lipofuscinosis (NCL) develop neurodegeneration characterized by vision loss, motor dysfunction, seizures, and often early death. Neuropathological analysis of patients with NCL shows accumulation of intracellular autofluorescent storage material, lipopigment, throughout neurons in the central nervous system including in the retina. A recent study of a sibling pair with adult onset NCL and retinal degeneration showed linkage to the region of the progranulin (*GRN*) locus and a homozygous mutation was demonstrated in *GRN*. In particular, the sibling pair with a mutation in *GRN* developed retinal degeneration and optic atrophy. This locus for this form of adult onset neuronal ceroid lipofuscinosis was designated neuronal ceroid lipofuscinosis-11 (*CLN11*). Based on these clinical observations, we wished to determine whether *Grn*-null mice develop accumulation of autofluorescent particles and retinal degeneration. Retinas of both wild-type and Progranulin deficient mice were examined by immunostaining and autofluorescence. Accumulation of autofluorescent material was present in Progranulin deficient mice at 12 months. Degeneration of multiple classes of neurons including photoreceptors and retinal ganglion cells was noted in mice at 12 and 18 months. Our data shows that $Grn^{-/-}$ mice develop degenerative pathology similar to features of human CLN11.

Abbreviations: NCL, neuronal ceroid lipofuscinosis; CLN11, neuronal ceroid lipofuscinosis-11; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer

*Corresponding author. Present address: Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114, USA.
E-mail address: brian_hafler@meei.harvard.edu (B.P. Hafler).

[1]Contributed equally to this work.

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1. Introduction

Neuronal ceroid lipofuscinoses (NCL) are a group of inherited, neurodegenerative disorders in which there is abnormal lipopigment accumulation in the lysosome (Bartsch et al., 2013; Boustany, 2013). They belong to a broader group of lysosomal storage disorders (Ballabio and Gieselmann, 2009; Cotman et al., 2013). The lysosome is an important cellular organelle involved in the breakdown of macromolecules that can be transported back into the cytoplasm (de Duve, 2005). In NCL, mutations in genes involved in lysosomal function lead to the accumulation of autofluorescent storage material known as lipopigment within lysosomes (Mink et al., 2013). The abnormal accumulation of lipopigment within neurons may lead to eventual neuronal death (Bartsch et al., 2013; Katz et al., 2008).

Clinically, patients with NCL display symptoms of visual loss, motor dysfunction, seizures, and often early death (Boustany et al., 1988; Warrier et al., 2013). Understanding the pathogenesis of lysosomal storage disorders has led to new treatments including Cerezyme for treating Type 1 Gaucher disease, Fabrazyme for treating Fabry disease, and most recently Lumizyme for treating late-onset Pompe disease (Boustany, 2013; Lidove et al., 2010; Schoser et al., 2008); however, there are over 50 inherited diseases of lysosomal dysfunction for which there is no currently available treatment (Hodges and Cheng, 2006; Platt and Lachmann, 2009).

Recent analysis of two siblings with NCL showed linkage to the region of the *GRN* locus and a novel, homozygous mutation in the progranulin gene (*GRN*) was identified (Smith et al., 2012). The sibling pair who were Progranulin deficient developed visual loss, seizures, and cerebellar ataxia. Retinal photography of the siblings showed retinal dystrophy (Smith et al., 2012). Optic atrophy was also noted consistent with of a loss of retinal ganglion cells, the retinal cell type that gives rise to the optic nerve. This neurologic disease was termed CLN11 (Smith et al., 2012).

Progranulin is a secreted glycoprotein containing seven and a half granulin repeats (Hu et al., 2010; Zhu et al., 2002). Progranulin binds to neurons through its receptor Sortilin, which mediates endocytosis of Progranulin to the neuronal lysosome (Andersen et al., 2005; Hu et al., 2010). Ablation of Sortilin in $Grn^{+/-}$ mice results in the rescue of Progranulin levels in brain and serum (Hu et al., 2010). Furthermore, reducing Sortilin levels through a small-molecule binder results in an increase in extracellular Progranulin (Lee et al., 2014). Previous studies have shown that Progranulin deficiency leads to lysosomal dysfunction through accumulation of abnormal autofluorescent lipopigments (Ahmed et al., 2010; Petkau et al., 2012); however, the mechanism of Progranulin function within lysosomes is not well understood. Electron microscopy of Progranulin deficient mice showed NCL pathology with evidence of storage granules in a rectilinear complex characteristic of most types of NCL (Smith et al., 2012). A recent study suggested that Progranulin deficiency may lead to lysosomal dysfunction through decreased activation of lysosomal genes through binding to the transcription factor EB (Tanaka et al., 2013); however, no investigation of the retinas in the mice of $Grn^{-/-}$ has been performed.

We examined the retinas of Progranulin deficient mice in order to address whether the accumulation of storage of abnormal autofluorescent lipopigment in the brains of $Grn^{-/-}$ mice is present in the retina and whether this may lead to retinal degeneration. $Grn^{-/-}$ mice were examined for autofluorescence using fluorescence microscopy at age 12 months and retinal degeneration using immunohistochemistry at age 12 and 18 months. Analysis of retinal autofluorescence revealed a significant accumulation of particles in $Grn^{-/-}$ mice retinas compared to controls by 12 months. Further, $Grn^{-/-}$ mice exhibit photoreceptor and retinal ganglion cell degeneration at 12 and 18 months of age. To our knowledge, these studies are the first to show that $Grn^{-/-}$ mice develop age-related retinal degeneration concurrent with accumulation of autofluorescent material in the retina. Given that homozygous mutations in the *GRN* gene lead to CLN11 in humans, these mice may be of use for further study of NCL pathogenesis.

2. Results

2.1. *Grn expression in the mature retina*

Progranulin was previously reported to be detected in neurons in the mouse brain using immunohistochemistry (Daniel et al., 2000); however, its expression has not been previously reported in the retina. Immunohistochemistry was performed for Progranulin protein in the mature retina. In adult wild-type mice, Progranulin protein was detected in the outer nuclear layer where photoreceptors reside. Progranulin protein was also detected in the inner nuclear layer, and in the ganglion cell layer, where retinal ganglion cells are located (Fig. 1A). As expected, Progranulin protein was not detected in $Grn^{-/-}$ mice (Fig. 1B).

It has been previously reported that Progranulin co-localizes with a lysosomal marker protein Lamp1 (Almeida et al., 2011; Hu et al., 2010; Tanaka et al., 2013). Given the association of NCL with lysosomal storage defects, we performed immunofluorescence on primary cortical neuronal cultures for Progranulin and Lamp1. As expected, immunostaining showed that intracellular Progranulin co-localized with Lamp1 (Fig. 1C), consistent with a portion of Progranulin present in the lysosomes of cortical neurons.

2.2. *Retinal autofluorescence is detected in the retinas of Progranulin deficient mice*

Given that Progranulin deficiency is associated with increased autofluorescent material in the hippocampal neurons in $Grn^{-/-}$ mutant mice, fluorescence microscopy was used to detect autofluorescence in the retinas of $Grn^{-/-}$ mice at 12 months. Autofluorescent particles were visualized at multiple excitation wavelengths including 488 nm and 543 nm. As expected in control adult mice, little to no autofluorescence was observed in retinal neurons, including retinal ganglion cells or photoreceptors (Fig. 2A). Interestingly, by 12 months age in $Grn^{-/-}$ mice, substantial accumulation of autofluorescent material was detected throughout the retina using 543 nm excitation (Fig. 2B) as well as 488 nm