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Lack of Niemann–Pick type C1 induces age-related degeneration in the mouse retina

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ABSTRACT

Niemann–Pick type C (NPC) disease is an inherited lysosomal storage disease and caused by mutations in Npc1 or Npc2, which mediate cooperatively the egress of cholesterol from lysosomes. The disease entails progressive neurodegeneration, whose cause is poorly understood. Here, we report that Npc1 is distributed in distinct layers of the mouse retina and that its deficiency causes striking retinal degeneration in 2-month-old mice with signs of age-related maculopathies. This includes impaired visual function, accumulation of lipofuscin in the retinal pigment epithelium layer, degeneration of photoreceptor outer segments, disruption of synaptic layers and an increase in autophagy markers in the ganglion cell layer. Moreover, the lack of Npc1 results in the upregulation of proteins that mediate cellular cholesterol release in the retina. Our findings suggest that Npc1 is required for normal retinal function and that its absence may serve as model to study age-related degeneration of the retina.

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Introduction

Niemann–Pick type C (NPC) disease (OMIM no. 257220) is a rare, and ultimately fatal, autosomal recessive lysosomal storage disorder (Pentchev, 2004) and caused by mutations in the Npc1 (95% of cases) or Npc2 gene (Vanier and Millat, 2003; Ikonen and Holtta-Vuori, 2004; Karten et al., 2009). NPC patients present visceral and neurological symptoms (Vanier and Millat, 2003; Garver et al., 2007) with remarkable heterogeneity in age of onset, severity and life span (Imrie et al., 2007; Sevin et al., 2007; Spiegel et al., 2009). The neurologic signs, which include dementia, ataxia and dystonia, are caused by progressive degeneration of specific neuronal cell types, in particular cerebellar Purkinje cells (Higashi et al., 1993; Walkley and Suzuki, 2004). The genes mutated in NPC disease encode for ubiquitously expressed components of the endosomal–lysosomal system. Npc1 is a transmembrane glycoprotein (Carstea et al., 1997) and Npc2 is a water-soluble lysosomal protein (Naureckiene et al., 2000; Liu et al., in press; Storch and Xu, 2009). Both proteins cooperate to mediate the exit of lipoprotein-derived cholesterol from lysosomes (Sleat et al., 2004; Kwon et al., 2009).

So far, it remains unclear why defects in these proteins cause neurodegeneration. Our previous studies (Mauch et al., 2001; Goritz et al., 2005; Nieweg et al., 2009) provoked the hypothesis that deficiency in Npc1 or Npc2 impairs a lipoprotein-mediated uptake of glia-derived cholesterol by neurons and thereby causes neuronal dysfunction and loss (Pfrieger, 2003). In this context, we have studied retinae of a well-established mouse model for NPC (Morris et al., 1982; Loftus et al., 1997). The retina of Npc1-deficient mice has not been analysed, although the retina per se offers several advantages, namely a defined set of neurons and glial cells, a layered structure and the opportunity to monitor morphological and functional properties in vivo. Our results show that Npc1 is concentrated in specific layers of the mouse retina and that its deficiency causes severe defects in retinal morphology and function at 2 months of age that mimic signs of age-related maculopathy.

Results

In this study, we characterized the distribution of Npc1 in the mouse retina and determined whether its absence causes structural and functional changes.

Distribution of Npc1 in the mouse retina

Immunohistochemical staining revealed that Npc1 was concentrated in distinct layers of the adult mouse retina (Fig. 1). The strongest staining appeared in the outer plexiform layer (OPL), which contains synapses between photoreceptors and bipolar cells, and in

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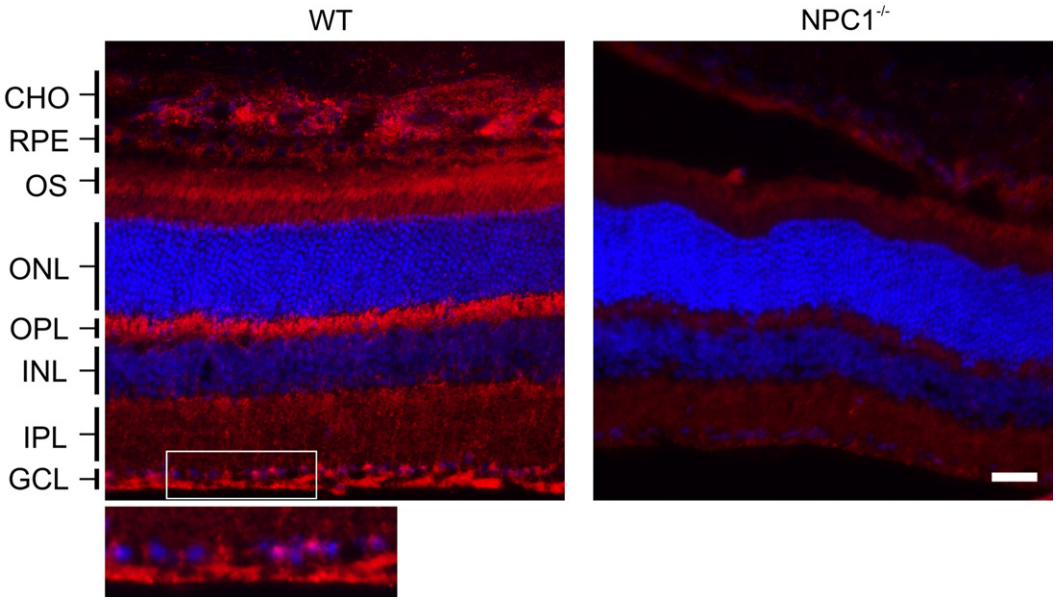

Fig. 1. Distribution of Npc1 in the retina of adult mice. False-color fluorescence micrographs of retinal sections from adult (2-month-old) wild-type (left) and Npc1-deficient (right) mice subjected to immunohistochemical staining with a polyclonal antibody directed against Npc1 (red) and nuclear staining with DAPI (blue). White rectangle on the left indicates area of the GCL shown at a two-fold higher magnification. Retinal layers are indicated. Scale bar: 50 μm. CHO, choroid; RPE, retinal pigment epithelium; OS, outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

the ganglion cell layer (GCL), where label was detected in processes that surround ganglion cells and extend in the IPL, which may represent Müller glia. Moreover, label appears in the nerve fiber layer below the GCL (Fig. 1). Npc1 was also present at the level of photoreceptor outer segments (OS), in retinal pigment epithelium (RPE) cells and in the choroid (CHO). Retinae from 2-month-old mice that were homozygous for the mutated allele ($Npc1^{-/-}$), hereafter referred to as Npc1-deficient or mutant mice, showed only weak background staining supporting the specificity of the antibody staining (Fig. 1).

Cholesterol-related changes in retinae from Npc1-deficient mice

A hallmark of Npc1 deficiency is intracellular accumulation of unesterified cholesterol (Sokol et al., 1988; Liscum et al., 1989). To determine whether this occurs in retinal cells from mutant mice, we stained retinal sections with the sterol-binding drug filipin (Sokol et al., 1988). For orientation, we also stained sections with antibodies against synaptophysin and calretinin, which label the OPL and IPL, respectively. In mutant mice filipin staining occurred in the RPE layer, whereas no signal was detected in retinae from wild-type mice ($n=3$ animals each) (Fig. 2a). Filipin-positive material below the GCL (Fig. 2a) was observed in a few sections. Surprisingly, the synaptic staining revealed folding of the synaptic layer and a focal loss of synaptophysin staining in Npc1-deficient mice (Fig. 2a).

The intracellular accumulation of cholesterol may affect cellular components involved in cholesterol trafficking. Therefore, we studied the levels of selected proteins in retinal lysates by immunoblotting. Control blots confirmed the presence and absence of Npc1 in wild-type and mutant mice, respectively (Fig. 2b). The levels of ApoA1 and Abca1, which mediate the release of cholesterol from cells, were strongly upregulated in retinae from mutant mice compared to wild-type mice (Fig. 2b), whereas Abca2 and ApoE were unchanged (data not shown). Low retinal expression of ApoA1 in adult wild-type mice has also been reported previously (Kurumada et al., 2007). Immunohistochemical staining of the cholesterol transporter Abca1 in wild-type mice revealed strong labelling of photoreceptor inner segments and at the level of the OPL, the GCL and the RPE layer (Fig. 2c) consistent with a recent report (Duncan et al., 2009). In mutant mice, the staining intensity increased in the RPE, IPL and GCL (Fig. 2c), indicating that the protein was upregulated in a layer-specific manner.

Impaired retinal function in Npc1-deficient mice

To test whether lack of Npc1 affects retinal function in vivo, we recorded full-field electroretinograms (ERGs), which represent light-evoked electrical responses of distinct retinal cells (Peachey and Ball, 2003) (Fig. 3). Under scotopic (dark-adapted) conditions, a large fraction of the mutant mice had smaller single-flash ERG responses than wild-type littermates (Figs. 3a and b). Overall, amplitudes of both, a- and b-waves, which represent signals from photoreceptors and their postsynaptic neurons, respectively, were reduced in mutant mice compared to wild-type animals. The difference in b-wave amplitudes reached statistical significance at nearly all stimulus intensities tested (Fig. 3b). Latencies of a- and b-waves from mutant mice were not different from those of wild-type mice (data not shown). The selective effect on amplitudes indicated that the number of photoreceptors contributing to the light-evoked responses was decreased, whereas the response kinetics was unaltered. Notably, mutant mice showed significant differences in b-wave amplitudes between the left and right eye ($p=0.000475$; paired t-test), which were absent in wild-type mice ($p=0.654$). This suggested that in individual mice, the decline of retinal function due to Npc1 deficiency occurred independently in each eye. Oscillatory potentials, which reflect responses from the inner retina, were also significantly smaller in mutant compared to wild-type animals (Figs. 3c and d). Next, we performed photopic (light-adapted) ERGs to isolate the response of the cone pathway. Under these conditions, single-flash responses were reduced in some mutant mice compared to wild-type controls, but the difference did not reach statistical significance (Figs. 3e and f). Flicker stimulation allows to isolate cone responses from the rod component based on the lower temporal resolution of rod responses (Peachey and Ball, 2003). As shown in Figs. 3a and b, the size of potentials evoked at 10 and 15 Hz varied widely among individual retinae and showed no significant difference between wild-type and mutant animals. However, the relative size of flicker responses of individual eyes at 15 Hz compared to 10 Hz was much

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