Author/s:
Truong, PH;Ciccotosto, GD;Merson, TD;Spoerri, L;Chuei, MJ;Ayers, M;Xing, YL;Emery, B;Cappai, R

Title:
Amyloid precursor protein and amyloid precursor-like protein 2 have distinct roles in modulating myelination, demyelination, and remyelination of axons

Date:
2019-03-01

Citation:
Truong, P. H., Ciccotosto, G. D., Merson, T. D., Spoerri, L., Chuei, M. J., Ayers, M., Xing, Y. L., Emery, B. & Cappai, R. (2019). Amyloid precursor protein and amyloid precursor-like protein 2 have distinct roles in modulating myelination, demyelination, and remyelination of axons. GLIA, 67 (3), pp.525-538. https://doi.org/10.1002/glia.23561.

Persistent Link:
https://hdl.handle.net/11343/284850

Ciccotosto Giuseppe (Orcid ID: 0000-0003-2512-1047)

Title: Amyloid precursor protein and amyloid precursor-like protein 2 have distinct roles in modulating myelination, demyelination, and remyelination of axons

Running title: APP and APLP2 modulate axon myelination

Full name of Authors: Phan H. Truong[1,2,3@], Giuseppe D. Ciccotosto[1,2,3@], Tobias D. Merson[4¶], Loredana Spoerri[1,2#], Mun Joo Chuei[1,2], Margaret Ayers[1], Yao Lulu Xing[4¶], Ben Emery[4,5^] and Roberto Cappai[1,2,3*]

[@] **These authors contributed equally to the work.**

Author Affiliations: [1]Department of Pathology, [2]The Bio21 Molecular Science and Biotechnology Institute, [3]Department of Pharmacology and Therapeutics, , [4]The Florey Institute of Neuroscience and Mental Health, [5]Department of Anatomy and Neuroscience, The University of Melbourne, Melbourne, Victoria 3010, Australia.

***Current addresses**: [¶]Australian Regenerative Medicine Institute, Monash University, Clayton, Victoria 3168, Australia. [#]The University of Queensland Diamantina Institute, Woolloongabba, QLD 4102. Australia. ^Jungers Center for Neurosciences Research, Oregon Health and Science University, Portland, Oregon 97239-3098, USA.*

Corresponding author: Roberto Cappai. Tel: +61-3-83442556, email: r.cappai@unimelb.edu.au

Acknowledgments: We thank Anna Freidhuber and Cong Ho (Department of Pathology, The University of Melbourne) and Jill Dane (Peter Mac Callum Cancer Centre) for assisting with the electron microscopy. We thank the School of Biomedical Sciences animal facility staff for assisting with animal care and handling. We thank Prof Hui Zheng and Prof Sangram Sisodia for the APP and APLP2 knockout mice respectively. R.C. received funding from the Australia National Health and Medical Research Council.

Abbreviations: Amyloid Precursor Protein (APP); Amyloid Precursor-Like Protein 1 (APLP1); Amyloid Precursor-Like Protein 2 (APLP2); beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1); central nervous system (CNS); Myelin basic protein (MBP); Myelin

oligodendrocyte glycoprotein (MOG); Neuregulin 1 (NRG1); peripheral nervous system (PNS); proteolipid protein (PLP); wildtype (WT)

Competing Interests: The authors have declared that no competing interests exist

Word count

Abstract	**250**
Introduction	**393**
Materials and Methods	**1490**
Results	**2208**
Discussion	**2548**
Total	
References	**1581**
Figure legends	**892**

Main Points:

- APP and APLP2 play important and distinct roles in CNS/PNS myelination, demyelination and re-myelination.
- APP-KO and APLP2-KO mice have significantly less myelinated axons.
- APP-KO mice are less susceptible to cuprizone-induced demyelination, and have impaired remyelination.

Abstract

The identification of factors that regulate myelination provides important insight into the molecular mechanisms that coordinate nervous system development and myelin regeneration after injury. In this study, we investigated the role of Amyloid Precursor Protein (APP) and its paralogue Amyloid Precursor-Like Protein 2 (APLP2) in myelination using APP and APLP2 knockout (KO) mice. Given that BACE1 regulates myelination and myelin sheath thickness in both the peripheral and central nervous systems, we sought to determine if APP and APLP2, as alternate BACE1 substrates, also modulate myelination, and therefore provide a better understanding of the events regulating axonal myelination. In the peripheral nervous system, we identified that adult, but not juvenile KO mice, have lower densities of myelinated axons in their sciatic nerves while in the central nervous system, axons within both the optic nerves and corpus callosum of both KO mice were significantly hypomyelinated compared to wildtype controls. Biochemical analysis demonstrated significant increases in BACE1 and myelin oligodendrocyte glycoprotein and decreased NRG1 and proteolipid protein levels in both KO brain tissue. The acute cuprizone model of demyelination/remyelination revealed that whereas axons in the corpus callosum of wildtype and APLP2-KO mice underwent similar degrees of demyelination and subsequent remyelination, the myelinated callosal axons in APP-KO mice were less susceptible to cuprizone-induced demyelination and showed a failure in remyelination after cuprizone withdrawal. These data identified APP and APLP2 as modulators of normal myelination and demyelination/remyelination conditions. Deletion of APP and APLP2 identifies novel interplays between the BACE1 substrates in the regulation of myelination.

Key words: Amyloid Precursor Protein, Amyloid Precursor-Like Protein, knockout, myelin, cuprizone

Main Points:

1 INTRODUCTION

Myelination of axons in the vertebrate nervous system enables rapid and efficient transmission of impulses by facilitating saltatory conduction (Bakhti et al. 2014). A range of secreted, transmembrane and intracellular factors have been identified to regulate myelination in both the peripheral nervous system (PNS) and central nervous system (CNS) (Emery 2010). One such factor is the beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1). BACE1 knockout mice exhibited hypomyelination in both the PNS and CNS. This phenotype was postulated to result from the failure of BACE1 to cleave Neuregulin 1 (NRG1), a major regulator of axonal myelination in the PNS (Hu et al. 2006; Willem et al. 2006). Apart from NRG1, another well-known BACE1 substrate is the Amyloid Precursor Protein (APP), a protein widely known for its association with Alzheimer's disease (Kandalepas and Vassar 2014). APP is a type 1 transmembrane glycoprotein that together with its mammalian paralogues, amyloid precursor-like protein 1 (APLP1) and 2 (APLP2), form part of the APP gene family (Muller et al. 2017). Both APP and APLP2 are expressed in the CNS and PNS (for detailed review, see (Muller et al. 2017)). APP is expressed in the myelin sheath in rodents (Ikeda and Tomonaga 1990; Otsuka et al. 1991) and humans (Sapirstein et al. 1994), and APP mRNA was detected in white matter structures of the rat brain (Sola et al. 1993). Given the hypomyelination phenotype of BACE1 knockout mice, we sought to determine if APP and APLP2, as alternate BACE1 substrates, also modulate myelination, and therefore provide a better understanding of the events regulating axonal myelination.

We first investigated myelin integrity in both the PNS and CNS in APP (APP-KO) and APLP2 (APLP2-KO) knockout mice. Both APP-KO and APLP2-KO mice exhibited PNS and CNS hypomyelination compared to wild-type mice with a significant reduction in the density of myelinated axons present in the sciatic nerves, optic nerves and corpus callosum regions of adult mice, as well as changes in the expression levels of proteins associated with myelination. We also examined the role of APP and APLP2 under pathological conditions using the cuprizone model of demyelination/remyelination and found significant changes in the extent of both demyelination and remyelination between the APP-KO, APLP2-KO and wildtype mice. Collectively, our data identify APP and APLP2 as novel regulators of myelination in the PNS and CNS with shared as well as distinct mechanisms of action.

2 MATERIALS AND METHODS

2.1 Antibodies

Primary antibodies: anti-BACE1 (B0681, rabbit polyclonal 1:1000, Sigma, Australia), anti-MOG (1:50 mouse IgG supernatant, from R. Reynolds, Imperial College), anti-neuregulin-1α/β1/2 (sc-348, rabbit polyclonal 1:500, Santa Cruz, Australia), anti-MBP (AB980, rabbit polyclonal 1:1000, Millipore, Australia), anti-PLP (PA3-150, rabbit polyclonal 1:1000, Pierce, Australia), anti-RIP (MAB1580, mouse monoclonal 1:300, Millipore, Australia). Loading control antibodies for Western blots were anti-Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (rabbit polyclonal, Cell Signaling, Australia) and anti-β-tubulin (rabbit polyclonal, Cell Signaling, Australia).

2.2 Animals

The APP-KO and APLP2-KO mice are global knockouts and their generation was previously described (von Koch et al. 1997; Zheng et al. 1995). APP-KO and APLP2-KO mice were backcrossed more than fourteen generations to the C57BL/6J background ensuring greater than 99.99 percent purity.Both the APP-KO and APLP2-KO mice do not show any significant behavioral changes or deficits compared to the wildtype (WT) C57BL/6J mice. The respective male and female KO mice were fertile and used for mating and expanding the mice colony used in this study. The WT mice (C57BL/6J), which were not litter mates of the KO mice, were used as controls and were age matched for these studies. Genotypes were determined by PCR using

the previously described primer sets (von Koch et al. 1997). Mice were housed with a 12h light/12h dark cycle and had ad libitum access to standard rodent chow and tap water. All animal procedures were approved by The University of Melbourne, Institutional Animal Ethics Committee.

2.3 Cuprizone treatment

CNS demyelination was induced by supplementing the diet of 5 week old mice with 0.2% (w/w) cuprizone (bis (cyclohexanone) oxaldihydrazone) in powdered rodent chow (Cate et al. 2010; Stidworthy et al. 2003). Mice were fed a cuprizone supplemented diet for 5 weeks, and the rodent chow (8g/mouse/day) was replaced daily. For the remyelination period, the cuprizone fed mice after 5 weeks were returned to normal chow for 2 weeks. Untreated control mice were fed normal crushed chow (replaced daily) for 7 weeks.

2.4 Electron microscopy and immunohistochemistry

Harvesting tissue. Mice were deeply anaesthetized with an intraperitoneal injection of ketamine (100mg/kg)/xylazine (20mg/kg) cocktail in saline solution before being transcardially perfused with PBS followed by 4% paraformaldehyde/PBS. Brains were removed and bisected midsagittally to separate the right and left hemispheres for transmission electron microscopy (TEM) and immunohistochemistry respectively (Fig. S1a) while optic nerves and sciatic nerves were harvested for TEM.

Transmission electron microscopy. Dissected optic nerves, sciatic nerves, and right hemisphere were harvested and prepared for TEM. Since the brain caudal region of the corpus callosum was the region of interest, the midsagittal cross section of the body-caudal region (bregma: -0.7 to -2.0) was selected and processed with three areas of the body-caudal region imaged for quantification (at 10,000X magnification). All tissues were cut into 1 mm slices and fixed in glutaraldehyde buffer (2.5% glutaraldehyde, 2% paraformaldehyde, 0.1M cacodylate buffer) for 24 hours at 4°C. Fixed tissue specimens were rinsed 3 times with PBS and postfixed in solution containing 1% osmium tetroxide and 1.5% potassium ferrocyanide (v/v in distilled water) for 2 hours followed by a final rinse with distilled water and stored overnight at 4°C. On the following day, tissues were dehydrated through a series of ethanol and acetone buffers, followed by infiltration and embedding in Spurr's resin and polymerised overnight in a 70°C oven. Semi-thin sections (0.5 micron thick) were stained with toluidine blue dye and examined under light microscopy to identify regions of interest for analysis. Ultrathin sections (90 nm thick) were cut and stained with uranyl acetate and lead citrate buffers then examined using a TEM (JEOL1101, Inc., USA) instrument. Images of nerve sections were taken using a Megaview III FW camera (Olympus Soft Imaging Solutions, Münster, Germany) mounted on the TEM.

Immunohistochemistry. Left hemispheres were post-fixed in 4% paraformaldehyde/PBS for 2 hours on ice, rinsed with PBS and cryoprotected in 30% sucrose overnight at 4°C. Each hemisphere was embedded in Tissue-Tek O.C.T compound (Sakura FineTek, USA), incubated on ice for 1 hour then frozen down in a bath of 2-methyl butane precooled in liquid nitrogen and

stored at -80°C until sectioning. Coronal cryosections (10 microns thick) from forebrain region spanning the middle to caudal regions of the corpus callosum (bregma -0.7 to -2.4mm) were cut using a cryostat (HM 560 CryoStar, Thermo Scientific, Australia). These sections were collected onto polysine treated microscope adhesion slides (Thermo Scientific, Australia), air-dried at room temperature for 1 hour then stored at -80°C until staining. For staining procedures, frozen tissue sections were warmed to room temperature then permeabilised in PBST buffer (0.3% TritonX-100, 10% goat serum in PBS buffer, v/v) then incubated in blocking buffer (10% goat serum in PBS, v/v) for 1 hour. Tissue sections were covered with primary anti-RIP or anti-MBP antibodies (in PBST buffer) and incubated in a humidifier chamber overnight. The tissue sections were washed with PBS buffer three times (10 minutes/wash) then incubated with secondary antibodies, goat anti-mouse or goat anti-rabbit tagged with an Alexa fluorophore (AlexaFluor 488, Invitrogen, Australia) diluted in PBST buffer (1:500 dilution) for 1 hour followed by PBS washes (3 times, 10 minutes/wash). Antifade mounting medium (Prolong Gold, Invitrogen, Australia) containing DAPI (used to identify cell nuclei) was placed on top of the tissue sections and a glass coverslip placed on top of the mounting media then allowing the media to dry for at least 24 hours before imaging. Stained tissue sections were visualised through a 20X objective using a Zeiss Axioplan2 microscope and images taken using an Axiocam HRc camera and Zen2 software (Zeiss). Captured images were taken using the same exposure settings between treatments and exported in a tiff image file format, and the fluorescence intensity levels quantified using ImageJ software (NIH ver 1.48, USA).

2.5 Western blotting

Whole brain homogenates were prepared from the cortex region of mice as described previously (Vella and Cappai 2012). Briefly, cortex tissue specimens were weighed and PBS buffer added in a 9:1 v/w ratio and passaged several times successively through a series of 18-, 22-, and 26-gauge needles. Tissue homogenates were lysed by adding NET lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, and protease inhibitors (Roche, Australia)) in a 1:1 v/v ratio. The samples were incubated on ice for 20 minutes and then centrifuged for 5 minutes at 10,000 g. The insoluble material (pellet) was discarded and the protein concentration of the supernatant quantified by bicinchoninic acid assay (Pierce, Rockford, IL, USA). Equal amounts of brain protein samples were separated on SDS-PAGE gels (4-12% Bis-Tris, Invitrogen, Australia) and resolved under reducing and denaturing conditions. For PLP detection, samples were prepared under native conditions by omitting the heating step after adding the sample buffer. The resulting gels were electroblotted onto nitrocellulose membranes (Bio-Rad, Australia). Membranes were cut into strips and placed in blocking buffer (5% w/v milk in PBS) and probed with primary antibodies (prepared in blocking buffer) overnight at 4°C. On the following day, membranes were washed in PBST 3 times (10 minutes/wash) then incubated with a secondary antibody (conjugated to horseradish peroxidase, diluted 1:5000) for 2 hours at room temperature. Membranes were then washed in PBST 3 times (10 minutes/wash) and immunoreactivity was detected using the enhanced chemiluminescence reagents (ECL-plus, GE Healthcare, UK) and imaged on a Microchemi digital imaging system (DNR BIO-Imaging System). Densitometric

analysis of band intensities was quantified using ImageJ Software (NIH ver 1.48, USA) and normalised for protein loading across samples.

2.6 Axonal counts and *g*-ratio analysis

At least six TEM micrograph images were taken of the optic and sciatic nerves for analysis per animal. To examine the number of myelinated, unmyelinated and remyelinated axons, three TEM images were taken at the midline region of the corpus callosum. The number of myelinated, unmyelinated and remyelinated axons (cuprizone treatment study only) were counted from each image (Fig. S1b). The data were expressed as a total number of axons per field and percentage of myelinated axons per field was determined. Axonal counts for cuprizone treated and untreated mice were quantified by a researcher blinded to the identity of each sample. The thickness of the myelin sheath surrounding myelinated axon was determined by calculating the *g*-ratio, which is defined as the numeric ratio between the diameter of the axon and the outer diameter of the myelinated fibre where a larger *g*-ratio indicates thinner myelin (hypomyelination). For both the optic nerve and corpus callosum region, *g*-ratios were calculated from at least 140 axons per animal per region. For analysis of sciatic nerves, *g*-ratios were calculated from more than 50 axons per nerve. Axon and fibre diameters were measured using ROI manager in ImageJ analysis software (NIH ver 1.48, USA) with the aid of a handheld electronic tablet.

2.7 Statistics

Graphical presentation and statistical analysis were performed using Prism 7software (GraphPad Software, Inc., San Diego, CA, USA). Statistical analysis of differences between animal groups was performed using one-way or two-way analysis of variance with the Tukey posthoc-test and significant difference shown. Data are represented as mean ± sem.

3 RESULTS

3.1 APP and APLP2 have redundant roles in myelination of the sciatic nerve in juvenile mice.

To determine if genetic deletion of APP or APLP2 influences myelination in the PNS of juvenile mice, the sciatic nerve of postnatal day 14 (P14) from WT, APP-KO and APLP2-KO mice were examined by TEM (Fig 1a) to compare the quantity of axons and extent of axon myelination. There was no significant difference between the three genotypes in the combined myelinated plus non-myelinated axons (total axons/field, Fig. 1b) or the percentage of myelinated axons (Fig. 1c). When the overall size distribution of axon diameter was plotted, we observed a tendency toward an increase in the proportion of smaller axonal calibres in the APP-KO mice compared to WT, but this was not statistically significant (Fig. 1d). The distribution between APLP2-KO mice and WT mice was also similar (Fig. 1e). The calculated *g*-ratio, a measure of myelin thickness, was determined by measuring the axon and fibre diameters of individual myelinated axons and the axon/fibre diameter ratio calculated. When plotting the *g*-ratio as a function of axon diameter, no differences in myelin thickness between the WT and either APP-KO (Fig. 1f) or APLP2-KO (Fig. 1g) were observed. Together, these data indicate that APP and APLP2 genes have a redundant role in sciatic nerve myelination in juvenile mice.

3.2 APP and APLP2 expression play a critical role in the myelination of axons in the optic nerve of juvenile mice.

To investigate whether deletion of APP or APLP2 affected CNS myelination in juvenile mice, we examined the optic nerve in P14 mice by TEM (Fig 2a). While there was no significant difference in the quantitation of total axons/field between the three genotypes (Fig 2b), the percentage of myelinated axons/field was significantly lower in the optic nerve for both the APP-KO and APLP2-KO mice compared to WT mice (Fig 2c). To evaluate whether axons of a certain calibre were more severely affected in the optic nerves of the KO juvenile mice, we plotted the relative size distribution of axon diameter against the frequency of myelinated axons and again, there was no statistical difference in the overall distribution of axonal size between the genotypes (Fig. 2d and 2e). Despite the significant reduction in the percentage of myelinated axons in both APP-KO and APLP2-KO, the calculated *g*-ratio revealed no difference in myelin sheath thickness between the KO and WT juvenile mice (Fig. 2f and 2g).

3.3 APP and APLP2 modulate axon myelination in the central and peripheral nervous system in adult mice.

To determine whether the reduction in myelination as seen in the optic nerve of juvenile APP-KO and APLP2-KO mice represented a developmental delay or whether it was sustained in adults, we investigated axonal myelination in adult mice. We performed TEM analysis on PNS (sciatic nerves) and CNS (optic nerves and corpus callosum) tissue taken in the adult mouse at P77 (Fig 3a). Although quantification of total axons/field showed no differences between the three genotypes (Fig. 3b), the proportion of myelinated axons was significantly lower in the sciatic and optic nerves and corpus callosum of both APP-KO and APLP2-KO compared to WT

in adult P77 mice (Fig 3c). Analysis of the calculated *g*-ratio revealed no difference in myelin sheath thickness between KO and WT adult P77 mice (Fig 3d-i).

3.4 The levels of myelin-associated proteins are altered in the brains of APP-KO and APLP2-KO mice during development.

To investigate both the permanency and the molecular basis for the CNS hypomyelination phenotype in APP-KO and APLP2-KO mice, we analysed the expression of key myelin and myelin regulatory proteins in whole brain lysates from both juvenile (P14) and adult ages (P30 and P60) mice by western blotting (Fig. 4a). BACE1 and NRG1 have a key association with axon myelination (Hu et al. 2006; Willem et al. 2006) and we found BACE1 protein expression levels were significantly elevated at P14 and P30 ages for both APP-KO and APLP2-KO mice, while at P60, BACE1 levels were significantly increased only in APP-KO mice by 2.5-fold but no change was detected in the APLP2-KO compared to WT mice (Fig 4b). Interestingly, BACE1 expression was significantly higher in APLP2-KO compared to APP-KO at P30, but at P60, the opposite effect was seen (Fig. 4b). We used the anti- NRG1 antibody sc-348, which recognizes an epitope mapping to the carboxyl terminus of NRG1 isoform, to identify the cleaved ~45 kDa NRG1 C-terminal fragment (NRG1 ctf) by western blot and its expression levels at P14 were significantly decreased for both APP-KO and APLP2-KO compared to WT mice (Fig. 4c). However at P30, NRG1 ctf levels were similar across all genotypes while at P60, NRG1 ctf was significantly higher in APP-KO mice compared to both WT and APLP2-KO mice (Fig. 4c). When we compared the BACE1 and NRG1 ctf protein

expression levels within each genotype, and across the different age groups, we found that both BACE1 and NRG1 ctf expression levels were significantly lower in the adult aged mice compared to the juvenile mice in all three genotypes (Fig. S3).

We next examined three major myelin proteins of the CNS, the proteolipid protein (PLP) and myelin basic protein (MBP), as well as the myelin structural protein, myelin oligodendrocyte glycoprotein (MOG) (Johns and Bernard 1999; Lees and Brostoff 1984; Nave et al. 1987). In the juvenile P14 group, the PLP expression level was similar across the three genotypes while in the adult P30 and P60 age group, PLP levels were unchanged in the APLP2-KO mice compared to WT mice, while PLP levels in the APP-KO mice were 20% lower at P30 and 50% lower at P60 and statistically different compared to both WT and APLP2-KO mice (Fig. 4d). We then used a different antibody to detect both PLP and its smaller splice variant, DM20 (missing 35 residues) (Nave et al. 1987) in the P60 mice brain lysates and again saw significantly lower levels of PLP expression in APP-KO mice compared to both WT and APLP2-KO mice while the protein expression level of DM20 was lower in both KO mice but it was not statistically different (Fig. S4). The DM20 isoform was not detectable in the P14 and P30 brain lysates and was therefore not examined further (data not shown). In the WT mice, the PLP protein expression level significantly increased from P14 to the P30 and P60 age groups by 37- and 58-fold respectively, but these large increases were not seen in the KO mice. In both APP-KO and APLP2-KO mice, PLP levels were significantly increased from P14 to P30 but only by 13- to 15-fold (Fig. S3). The PLP levels in the APP-KO mice decreased significantly by 5-fold from P30 to P60, and to

near juvenile levels. While in the APLP2-KO mice, PLP levels at P60 were still similar to the P30 values (Fig. S3).

We detected four MBP isoform bands by western blotting and the MBP expression levels (Fig 4a), which represents the sum total of MBP1 to MBP4 (Fig 4e), displayed a similar profile as PLP (Fig. 4d). At P14, MBP protein expression level was similar across all three genotypes. However, in the adult tissue, changes in MBP levels were most prevalent in the APP-KO mice compared to both WT and APLP2-KO mice with MBP levels in APP-KO brains reduced at P30 and significantly lower at P60 compared to WT. When compared to APLP2-KO levels, they were significantly lower in both the P30 and P60 age groups. Like PLP, the MBP protein expression level displayed a large 20-fold increase in the WT mice from P14 to P30 age groups but unlike PLP, the MBP level did not change from P30 to P60 and MBP levels were significantly higher at P30 and P60 compared to P14 for the WT mice (Fig S3). Again, the large increases in MBP expression seen in the WT mice were not seen in the KO mice with a smaller, 5-fold increase in MBP expression from P14 to P30 that were statistically different (Fig. S3). Like PLP, the MBP levels in APP-KO mice decreased significantly from P30 to P60, and approached juvenile levels, while in the APLP2-KO mice, MBP levels at P60 remained similar to the P30 values (Fig. S3).

Analysis of MOG expression levels in the juvenile P14 mice brain lysates was associated with a significantly higher level in both KO compared to WT mice (Fig 4f). Unlike PLP and MBP, MOG protein expression levels were unchanged in the brain lysates of adult mice, across

the three genotypes. Interestingly, the pattern of MOG expression levels during aging differed between different genotypes – in WT mice, MOG was significantly elevated at P30 compared to P14 and P60. While in APP-KO, MOG levels decreased at P30 and were significantly lower at P60 compared to P14. In APLP2-KO mice, MOG levels were unchanged across the three age groups (Fig. S3). Taken together, these results demonstrate that APP and APLP2 gene expression have an important role in modulating the protein expression levels of myelin associated proteins in the CNS during development.

3.5 APP and APLP2 have distinct effects on demyelination and remyelination in the cuprizone model.

We next determined whether the hypomyelination phenotype observed in APP-KO and APLP2-KO mice could be related to alterations in myelin degeneration and/or regeneration in the context of nerve injury. To do this, we utilized the cuprizone model to induce acute demyelination (Fig. 5a), where provision of 0.2% cuprizone in the mouse diet over 5 weeks results in substantial demyelination, particularly in the caudal corpus callosum region of the brain (Matsushima and Morell 2001). In the cuprizone group, weight gain over the 5 week treatment period was 5 to 8% lower compared to the Control 5 week group fed a normal diet (data not shown) and they did not display any abnormal behavior. As expected, mice in the cuprizone group displayed a clear loss of compact myelin in the corpus callosum region compared to the Control 5 week group across all three genotypes (Fig. 5b). To determine the

extent of demyelination, TEM images from the corpus callosum were examined and the percentage of unmyelinated axons present in cuprizone Treatment group and Control 5 week group (Treatment/Control) was quantitated for each genotype and then normalised to the WT Control 5 week group (Fig. 5c). The cuprizone treated WT and APLP2-KO mice showed similar levels of unmyelinated axons with only 23.8 ± 3.1% and 31.8 ± 3.4% of axon remaining myelinated respectively. The extent of demyelination was less severe for the APP-KO mice with 52.7 ± 4.3% of axons still remaining myelinated after cuprizone treatment, which was significantly different to both the WT and APLP2-KO values (Fig. 5c). Immunohistochemical analysis of the corpus callosum region with antibodies to MBP and CNPase/RIP (2', 3'-cyclic nucleotide 3'-phosphodiesterase), markers for myelin and oligodendrocytes respectively, showed a significant decrease in staining intensity for MBP (WT and APP-KO) and RIP (WT and APLP2-KO) in the cuprizone groups compared to the Control 5 week group. These observations support the significant demyelination was caused by cuprizone treatment model (Fig. S5).

To investigate the role of APP and APLP2 in remyelination, a cohort of the cuprizone treated groups were allowed to recover by changing their diet to normal chow for a further two weeks (termed the Recovery group) and the data compared to the Control 7 week group who were on a normal diet of chow (Fig. 6a). The TEM images of the corpus callosum region from the Recovery group displayed an increase in newly formed and notably thinner (compared to those of non-treated mice) myelin sheaths (Fig. 6b), a defined characteristic associated with remyelination occurring (Fig. S1c) (Matsushima and Morell 2001). The total number of axons

per field (Fig 6c) through the midline region of the corpus callosum was unchanged following cuprizone treatment for all three genotypes. Clear changes were observed when determining the percentage of myelinated axons normalised to the level of myelination in the WT Control groups at 5 and 7 weeks respectively (Fig. 6d). There was a significant decrease in the percentage of myelinated axons in the cuprizone group compared to the Control group across all genotypes. While both the WT and APLP2-KO mice showed an increase in the proprotion of myelinated axons between cuprizone and the recovery groups (Fig. 6d), the APP-KO mice did not, suggesting a failure of remyelination in APP-KO mice. Quantitation of the *g-ratio* showed that cuprizone treatment did not affect the *g*-ratio value in any group, indicating the cuprizone treatment in these mice caused a total removal of myelin from the axons sheaths. However, the *g*-ratio average of the Recovery group was significantly higher compared to the Control 7 week group for all genotypes, indicating remyelination occurred during this recovery period and these axons were therefore displaying a hypomyelination phenotype (Fig 6e). To further quantitate this remyelination change in the Recovery group, a frequency distribution of the *g*-ratio data was plotted and compared to the Control 7 week group for each genotype respectively (Fig 6f-h). We observed that the remyelinated axons had a much thinner myelin sheath, as seen by the statistically significant shift in the frequency distribution to a larger *g*-ratio (i.e.: to the right of the x-axis) in the Recovery group compared to the Control 7 week group for all three genotypes.

4 DISCUSSION

This is the first study to directly test if APP and its homologue APLP2 have discernable roles in regulating the process of myelination and remyelination in both the CNS and PNS of juvenile and adult mice. We previously demonstrated a clear functional difference between the roles of APP and APLP2 in the adult retina (Dinet et al. 2016). The data presented in this current study identifies another non-redundant function for these two paralogues in the CNS involving axon myelination and remyelination when using the cuprizone induced demyelination model. During juvenile development, which is the most active period for myelinogenesis in the young mouse, the percentage of myelinated axons in the optic nerve, but not sciatic nerve, was significantly decreased in both APP-KO and APLP2-KO mice when compared to WT mice. However, we observed that the total number of axons per field area for both the sciatic and optic nerves was similar across the three genotypes. In the adult mouse, the loss of either APP or APLP2 caused a significant decrease in the percentage of myelinated axons for the sciatic and optic nerves and within the corpus callosum region compared to WT. The g-ratio, which reflects the degree of axon myelination, and the total number of axons per unit area was not significantly changed across all the age groups examined. These results are in agreement with previous studies using young adult C57Bl/6 mice less than 10 weeks of age having no significant change in the number of axons following demyelination and recovery phases (Irvine and Blakemore 2006). Nor was there any significant change when plotting the g-ratio as a function of axon diameter. These findings suggest that the lack of either APP or APLP2 expression does not contribute to a

hypo- or hypermyelination phenotype of the whole nerve. Rather, we propose that APP and APLP2 expression affects the myelination process of a subset of nerve axons since a lack of expression caused total, rather than partial demyelination.

We found that cuprizone treatment of APLP2-KO and WT mice caused dramatic axonal demyelination effects of 68% and 76% respectively, followed by 15% remyelination after the two week recovery period. In contrast, the myelinated callosal axons in APP-KO mice were far less susceptible to cuprizone-induced demyelination with a smaller proportion of axons (47%) identified to be unmyelinated, an amount that is 20 to 30% less compared to the WT and APLP2-KO mice respectively. Secondly, unlike WT and APLP2 mice, the two week recovery period resulted in no detectable remyelination in the APP-KO mice suggesting that there is a delayed onset in remyelination or possibly, no remyelination will occur in mice lacking APP. Cuprizone treatment in mice is a long established model for inducing axonal demyelination and it has been shown that total remyelination can take up to 6 weeks to occur (Matsushima and Morell 2001). However, at least 77% of remyelination occurs within the first two week recovery period (Lindner et al. 2009) suggesting rapid and substantial remyelination occurs once the toxic agent is removed from the diet. Taken together, this data allows us to differentiate the functional roles for APP from APLP2 in modulating myelination during normal physiological development as well as during cuprizone-induced demyelination/remyelination conditions.

Myelination of axons is mediated by Schwann cells in the PNS and by the oligodendrocytes in the CNS and it occurs in an orderly and predictable manner with synthesis

beginning in brain stem before reaching the centrum semioval which is located superior to the lateral ventricles and corpus callosum (Inder and Huppi 2000). In the mouse, Schwann cells and oligodendrocytes (in the cervical spinal cord) initiate the myelination process at birth and are well underway by postnatal day 10 to 14 and peaking at approximately 20 days postnatally, which coincides with the detectable expression of the mature oligodendrocyte markers MBP and PLP (Dib et al. 2011; Morello et al. 1986; Verity and Campagnoni 1988). APP and APLP2 have essential roles in the PNS and CNS and were required to mediate neuromuscular transmission, spatial learning, and synaptic plasticity (Weyer et al. 2011). APP protein expression occurs in CNS grey matter areas of the spinal cord oligodendrocytes along with myelin proteins like PLP and MOG (Bauer et al. 2002) and at nodes of Ranvier in the myelinated CNS axons, but not in the PNS (Xu et al. 2014). Overexpressing human APP family members in *Drosophila* induced Notch gain of function phenotypes during the development of the adult PNS (Merdes et al. 2004). Our studies in APP-KO and APLP2-KO identified non-redundant roles for these proteins in the retina, with APP being important for modulating normal neuronal development of mouse retina (Dinet et al. 2016; Ho et al. 2012) and APLP2 required for retinal synaptogenesis (Dinet et al. 2016). We hypothesised APP and APLP2 would have a role during the most active period of myelinogenesis in the optic and sciatic nerves, and while no differences in myelination were observed in the juvenile sciatic nerve, we did identify a significant decrease in the axon diameter of the myelinated nerve fibres in both KO mice compared to WT. These results contrast with the observations seen in the optic nerve as the proportion of myelinated axons were significantly

lower for both KO mice compared to WT, but the axon diameter of these myelinated nerve fibres was unchanged. Therefore, our results support the model that APP and APLP2 have essential roles in the PNS and CNS development even though they do not have a role in modulating the extent of myelination since the g-ratio was unchanged between the genotypes.

BACE1 expression is highest at time points that are important for nerve myelination, while deficiency of BACE1 was associated with hypomyelination of juvenile (P8) and adult sciatic nerves (Hu et al. 2006; Willem et al. 2006). NRG1, like APP and APLP2, is a BACE1 substrate and is a key regulator of peripheral myelination (Taveggia et al. 2005). How APP and APLP2 regulate myelination during development is unclear but based on our western blot data analysis, there were significant changes in the expression pattern of key proteins known to be important for regulating myelination in APP-KO and APLP2-KO brains. The juvenile P14 brain lysate from APP and APLP2-KO mice displayed an increase in BACE1 protein expression and a concomitant decrease in NRG1 ctf protein expression relative to WT mice. Surprisingly, NRG1 ctf protein expression was increased along with BACE1 protein expression in the P60 APP-KO mice relative to WT mice, but no changes were observed for P60 APLP2-KO. This could explain the contrasting phenotypes between APP-KO and APLP2-KO and BACE1-KO mice. BACE1 deletion caused an increase in full-length NRG1 levels and a concomitant decrease in the NRG1 ntf cleavage product in adult mouse brain extracts (Hu et al. 2006). APP and APLP2 are also cleaved by α-secretases such as ADAM10 (a disintegrin and metalloproteinase 10) and ADAM17 (Luo et al. 2011; Vingtdeux and Marambaud 2012). Interestingly, ADAM17

expression affected nerve myelination and ADAM17 inactivation in motor neurons caused a hypermyelination phenotype (La Marca et al. 2011). ADAM10, and to a lesser extent ADAM17, cleaves NRG1 (Luo et al. 2011) and this suggested the balance between BACE1 and ADAM17 activities may regulate the timing and extent of PNS myelination (La Marca et al. 2011).

Since reduced cleavage of NRG1 can result in altered activation of the downstream pathways such as phosphatidylinositol-3-OH and serine/threonine kinase, and these changes will affect the expression of MBP and PLP (Hu et al. 2006; Hu et al. 2013; Luo et al. 2011). Analysis of myelin protein expression in adult mice supported the functional differences between APLP2 and APP in CNS myelination. In contrast to the significant reductions in PLP and MBP expression in APP-KO, APLP2-KO mice showed no significant differences. Taken together, the lack of APP and APLP2 expression in adult and juvenile mice affected both brain BACE1 and NRG1 protein expression levels supporting a novel interplay between the BACE1 substrates, NRG1 and APP / APLP2 in the regulation of axon fibre myelination.

Our data showed the APP-family had distinct actions when compared to other myelination regulatory proteins. NRG1 type III protein is a key determinant of myelination in Schwann cells (Michailov et al. 2004; Taveggia et al. 2005) and NRG1 haploinsufficient adult mice displayed hypomyelination in the corpus callosum region, whereas myelination (determined histologically and biochemically) of the optic nerve and spinal cord was normal (Taveggia et al. 2008). Similar studies looking at the conditional null mutants lacking NRG1 also demonstrated that the NRG1/ErbB signalling pathways are markedly different between

Schwann cells and oligodendrocytes (Brinkmann et al. 2008). Collectively these studies suggest there are spatiotemporal differences in the regulation of myelination, and these different proteins mediate specific signals to control myelination which can differ between the CNS and PNS. We observed significant changes in PLP and MBP expression in the brain of APP-KO mice compared to wildtype mice. A reduction in the proteolipid isoform DM-20, which has high a degree of structural similarity to PLP (Lees and Brostoff 1984), was also observed in APP-KO mice (Fig. S4). PLP is a key component of the myelin sheath in the CNS (Lees and Brostoff 1984) and mutations or alterations in PLP expression results in oligodendrocyte death (Klugmann et al. 1997). In contrast to PLP knockout mice, which do not have alterations in MBP expression, the adult APP-KO mice showed changes in both PLP and MBP expression. Therefore, we'd suggest APP expression affects both PLP and MBP levels and indicates APP could have multiple modes of action upon myelination. The composition and metabolism of myelin are similar in different regions of the adult rat brain (Smith 1973), thus these results are consistent with the reduction in myelinated axons observed in the optic nerve and the corpus callosum of adult APP-KO mice (Fig. 6C). Axon myelination in the corpus callosum is related to conduction speed and is crucial for interhemispheric communication for information such as sensation, perception and functional network (Phillips et al. 2015). The reduction in myelinated axons observed in the mid region of the corpus callosum in adult APP-KO mice, a region primarily responsible for connecting the motor and sensory areas (Wahl et al. 2007), is in line with a previous report showing APP-KO mice have decreased locomotor activity and forelimb

grip strength (Zheng et al. 1995). In addition, electrophysiological studies looking at the conduction properties of myelinated axons of APP-KO mice showed a reduction in the compound action potentials in the spinal cord of these mice (Li et al. 2016). Therefore, our data provides a mechanism to explain the basis for APP in modulating the conduction of action potentials in the CNS. We further propose that APP and APLP2 expression is essential for normal axon myelination throughout early development in the CNS and in adult PNS, but their expression does not contribute to the extent of myelination around an axon.

Axonal injury associated with demyelination and inflammation are the typical histopathological hallmarks for multiple sclerosis (Ferguson et al. 1997; Kuhlmann et al. 2002; Lassmann 2014). Remyelination in chronic multiple sclerosis lesions fails despite the presence of remyelinating oligodendrocytes (Franklin and Gallo 2014; Wolswijk 2002). The cuprizone model is typically used to mimic this disease phenotype since it causes axonal injury in the CNS with demyelination occurring during treatment and remyelination occurring following a recovery period. Cuprizone treatment caused significant demyelination in the WT, as well as the APP-KO and APLP2- mice, with significant reductions in the number of myelinated axons present following treatment. However, no changes were observed in the g-ratios when comparing between the untreated and cuprizone treated group for each genotype. The unchanged g-ratio's may be explained by axons undergoing complete demyelination rather than partial demyelination as g-ratio cannot be measured for completely denuded axons. An increase in g-ratio is expected during axon remyelination as the myelin sheath begins to wrap around the naked axon with

increasing layers. Therefore, as expected, a significant increase in the g-ratio relative to the 7 week control group was measured following the two week recovery period for both WT, APP-KO and APLP2-KO. This would indicate axonal hypomyelination. Hypomyelination and thinner myelin sheaths were also measured in the BACE1 KO mice following a recovery phase (Treiber et al. 2012). However, the WT and APLP2-KO mice differed to the APP-KO mice because they displayed a significant increase in the percentage of myelinated axons and g-ratio following the two week recovery period compared to the 5 week cuprizone treated groups. Our observations suggest that APP has a greater role in remyelination rather than in myelin maintenance. This notion is supported by the data showing APP expression was undetectable in healthy axons but accumulated in damaged axons and was significantly upregulated during the early remyelination but decreased gradually during myelination as the proportion of myelinated axons increased (Schultz et al. 2017). Other evidence supporting APP having a critical role in axon remyelination is obtained in reports showing high levels of APP immunoreactivity in premyelinating oligodendrocyte expressing cells and in demyelinating lesions (Ferguson et al. 1997; Gehrmann et al. 1995). Moreover, a recent study demonstrated that the addition of sAPPα, the α-secretase cleavage product, protected myelinated axons from demyelination while also promoting remyelination (Llufriu-Daben et al. 2018). Taken together, these data make a strong case for having a major functional role in axon remyelination. As for the therapeutic potential of targeting APP to modulate remyelination it is unclear which APP metabolite and/or signaling pathway is mediating APP's remyelination activity and therefore which to target.

Notwithstanding, the large number of drug development studies that have targeted APP metabolism as a treatment strategy for Alzheimer's disease means there is a diverse range of drug options available to pharmacologically test APP in remyelination.

The data showed that APLP2 appears to be dispensable for modulation of axon remyelination. APLP2, as a member of the APP gene family, is highly related to APP based on gene and protein sequence identity (Wasco et al. 1993). Various functions associated with APP have also been attributed to APLP2 such as regulation of synaptogenesis, neurite outgrowth and neuronal differentiation (Cappai et al. 1999; Shariati et al. 2013; Weyer et al. 2011). This current study has identified APP as a new regulator of axon remyelination in the CNS and PNS, and APLP2 to be important in regulating the extent of myelination in the CNS. Normal APP expression is a major contributing factor for normal remyelination to occur following demyelination.

We would conclude that APP and APLP2 expression play important and distinct roles in CNS myelination, demyelination and remyelination events. Unlike BACE1, which regulates the process of myelin sheath thickness, (Hu et al. 2006), APLP2 and APP do not regulate sheath thickness but rather have a more of a niche role for ensuring normal myelination and remyelination respectively in adult nerves. In addition to BACE1, we propose that APLP2 and APP represent novel therapeutic targets either collectively or separately based on their niche roles in modulating the myelination/remyelination process. This study shows that the actions of BACE1 in myelination may not be restricted to Neuregulin 1, and that the APP-family members

can also be key players in this process. Whether this extends to the other BACE1 substrates is a question that needs further investigation, as is defining the interplay between the BACE1 and its substrates in regulating myelination.

References

Bakhti M, Aggarwal S, Simons M. 2014. Myelin architecture: zippering membranes tightly together. Cell Mol Life Sci 71:1265-77.

Bauer J, Bradl M, Klein M, Leisser M, Deckwerth TL, Wekerle H, Lassmann H. 2002. Endoplasmic reticulum stress in PLP-overexpressing transgenic rats: gray matter oligodendrocytes are more vulnerable than white matter oligodendrocytes. J Neuropathol Exp Neurol 61:12-22.

Brinkmann BG, Agarwal A, Sereda MW, Garratt AN, Muller T, Wende H, Stassart RM, Nawaz S, Humml C, Velanac V and others. 2008. Neuregulin-1/ErbB signaling serves distinct functions in myelination of the peripheral and central nervous system. Neuron 59:581-95.

Cappai R, Mok SS, Galatis D, Tucker DF, Henry A, Beyreuther K, Small DH, Masters CL. 1999. Recombinant human amyloid precursor-like protein 2 (APLP2) expressed in the yeast Pichia pastoris can stimulate neurite outgrowth. FEBS Lett 442:95-8.

Cate HS, Sabo JK, Merlo D, Kemper D, Aumann TD, Robinson J, Merson TD, Emery B, Perreau VM, Kilpatrick TJ. 2010. Modulation of bone morphogenic protein signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination. J Neurochem 115:11-22.

Dib S, Denarier E, Dionne N, Beaudoin M, Friedman HH, Peterson AC. 2011. Regulatory modules function in a non-autonomous manner to control transcription of the mbp gene. Nucleic Acids Res 39:2548-58.

Dinet V, Ciccotosto GD, Delaunay K, Borras C, Ranchon-Cole I, Kostic C, Savoldelli M, El Sanharawi M, Jonet L, Pirou C and others. 2016. Amyloid Precursor-Like Protein 2 deletion-induced retinal synaptopathy related to congenital stationary night blindness: structural, functional and molecular characteristics. Mol Brain 9:64.

Emery B. 2010. Regulation of oligodendrocyte differentiation and myelination. Science 330:779-82.

Ferguson B, Matyszak MK, Esiri MM, Perry VH. 1997. Axonal damage in acute multiple sclerosis lesions. Brain 120 (Pt 3):393-9.

Franklin RJ, Gallo V. 2014. The translational biology of remyelination: past, present, and future. Glia 62:1905-1915.

Gehrmann J, Banati RB, Cuzner ML, Kreutzberg GW, Newcombe J. 1995. Amyloid precursor protein (APP) expression in multiple sclerosis lesions. Glia 15:141-51.

Ho T, Vessey KA, Cappai R, Dinet V, Mascarelli F, Ciccotosto GD, Fletcher EL. 2012. Amyloid precursor protein is required for normal function of the rod and cone pathways in the mouse retina. PLoS One 7:e29892.

Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, Yan R. 2006. Bace1 modulates myelination in the central and peripheral nervous system. Nat Neurosci 9:1520-5.

Hu X, Schlanger R, He W, Macklin WB, Yan R. 2013. Reversing hypomyelination in BACE1-null mice with Akt-DD overexpression. FASEB J 27:1868-73.

Ikeda K, Tomonaga M. 1990. Alzheimer's disease amyloid precursor protein is present in the myelin sheath of central nervous system in rat. Brain Res 527:140-4.

Inder TE, Huppi PS. 2000. In vivo studies of brain development by magnetic resonance techniques. Ment Retard Dev Disabil Res Rev 6:59-67.

Irvine KA, Blakemore WF. 2006. Age increases axon loss associated with primary demyelination in cuprizone-induced demyelination in C57BL/6 mice. J Neuroimmunol 175:69-76.

Johns TG, Bernard CC. 1999. The structure and function of myelin oligodendrocyte glycoprotein. J Neurochem 72:1-9.

Kandalepas PC, Vassar R. 2014. The normal and pathologic roles of the Alzheimer's β-secretase, BACE1. Curr Alzheimer Res 11:441-9.

Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA. 1997. Assembly of CNS myelin in the absence of proteolipid protein. Neuron 18:59-70.

Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Bruck W. 2002. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125:2202-12.

La Marca R, Cerri F, Horiuchi K, Bachi A, Feltri ML, Wrabetz L, Blobel CP, Quattrini A, Salzer JL, Taveggia C. 2011. TACE (ADAM17) inhibits Schwann cell myelination. Nat Neurosci 14:857-65.

Lassmann H. 2014. Mechanisms of white matter damage in multiple sclerosis. Glia 62:1816-1830.

Lees M, Brostoff S. 1984. Proteins of myelin. In: Morell P, editor. Myelin: Springer. p 197-224.

Li S, Wang X, Ma Q-H, Yang W-l, Zhang X-G, Dawe GS, Xiao Z-C. 2016. Amyloid precursor protein modulates Nav1. 6 sodium channel currents through a Go-coupled JNK pathway. Scientific reports 6:39320.

Lindner M, Fokuhl J, Linsmeier F, Trebst C, Stangel M. 2009. Chronic toxic demyelination in the central nervous system leads to axonal damage despite remyelination. Neurosci Lett 453:120-5.

Llufriu-Daben G, Carrete A, Chierto E, Mailleux J, Camand E, Simon A, Vanmierlo T, Rose C, Allinquant B, Hendriks JJA and others. 2018. Targeting demyelination via a-secretases promoting sAPPa release to enhance remyelination in central nervous system. Neurobiol Dis 109:11-24.

Luo X, Prior M, He W, Hu X, Tang X, Shen W, Yadav S, Kiryu-Seo S, Miller R, Trapp BD and others. 2011. Cleavage of neuregulin-1 by BACE1 or ADAM10 protein produces differential effects on myelination. J Biol Chem 286:23967-74.

Matsushima GK, Morell P. 2001. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 11:107-16.

Merdes G, Soba P, Loewer A, Bilic MV, Beyreuther K, Paro R. 2004. Interference of human and Drosophila APP and APP-like proteins with PNS development in Drosophila. EMBO J 23:4082-95.

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave KA. 2004. Axonal neuregulin-1 regulates myelin sheath thickness. Science 304:700-3.

Morello D, Dautigny A, Pham-Dinh D, Jolles P. 1986. Myelin proteolipid protein (PLP and DM-20) transcripts are deleted in jimpy mutant mice. EMBO J 5:3489-93.

Muller UC, Deller T, Korte M. 2017. Not just amyloid: physiological functions of the amyloid precursor protein family. Nat Rev Neurosci 18:281-298.

Nave KA, Lai C, Bloom FE, Milner RJ. 1987. Splice site selection in the proteolipid protein (PLP) gene transcript and primary structure of the DM-20 protein of central nervous system myelin. Proc Natl Acad Sci U S A 84:5665-9.

Otsuka N, Tomonaga M, Ikeda K. 1991. Rapid appearance of β-amyloid precursor protein immunoreactivity in damaged axons and reactive glial cells in rat brain following needle stab injury. Brain Res 568:335-8.

Phillips KA, Stimpson CD, Smaers JB, Raghanti MA, Jacobs B, Popratiloff A, Hof PR, Sherwood CC. 2015. The corpus callosum in primates: processing speed of axons and the evolution of hemispheric asymmetry. Proc R Soc B 282:20151535.

Sapirstein V, Durrie R, Berg M, Marks N. 1994. Amyloid precursor protein is enriched in axolemma and periaxolemmal -myelin and as 37:348-358.

Schultz V, van der Meer F, Wrzos C, Scheidt U, Bahn E, Stadelmann C, Brück W, Junker A. 2017. Acutely damaged axons are remyelinated in multiple sclerosis and experimental models of demyelination. Glia 65:1350-1360.

Shariati SA, Lau P, Hassan BA, Muller U, Dotti CG, De Strooper B, Gartner A. 2013. APLP2 regulates neuronal stem cell differentiation during cortical development. J Cell Sci 126:1268-77.

Smith ME. 1973. A regional survey of myelin development: some compositional and metabolic aspects. Journal of Lipid Research 14:541-551.

Sola C, Mengod G, Probst A, Palacios JM. 1993. Differential regional and cellular distribution of β-amyloid precursor protein messenger RNAs containing and lacking the Kunitz protease inhibitor domain in the brain of human, rat and mouse. Neuroscience 53:267-95.

Stidworthy MF, Genoud S, Suter U, Mantei N, Franklin RJM. 2003. Quantifying the Early Stages of Remyelination Following Cuprizone-induced Demyelination. Brain Pathology 13:329-339.

Taveggia C, Thaker P, Petrylak A, Caporaso GL, Toews A, Falls DL, Einheber S, Salzer JL. 2008. Type III neuregulin-1 promotes oligodendrocyte myelination. Glia 56:284-93.

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P and others. 2005. Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 47:681-94.

Treiber H, Hagemeyer N, Ehrenreich H, Simons M. 2012. BACE1 in central nervous system myelination revisited. Mol Psychiatry 17:237-9.

Vella LJ, Cappai R. 2012. Identification of a novel amyloid precursor protein processing pathway that generates secreted N-terminal fragments. FASEB J 26:2930-40.

Verity AN, Campagnoni AT. 1988. Regional expression of myelin protein genes in the developing mouse brain: in situ hybridization studies. J Neurosci Res 21:238-48.

Vingtdeux V, Marambaud P. 2012. Identification and biology of a-secretase. J Neurochem 120 Suppl 1:34-45.

von Koch CS, Zheng H, Chen H, Trumbauer M, Thinakaran G, van der Ploeg LH, Price DL, Sisodia SS. 1997. Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. Neurobiol Aging 18:661-9.

Wahl M, Lauterbach-Soon B, Hattingen E, Jung P, Singer O, Volz S, Klein JC, Steinmetz H, Ziemann U. 2007. Human motor corpus callosum: topography, somatotopy, and link between microstructure and function. Journal of Neuroscience 27:12132-12138.

Wasco W, Gurubhagavatula S, Paradis MD, Romano DM, Sisodia SS, Hyman BT, Neve RL, Tanzi RE. 1993. Isolation and characterization of APLP2 encoding a homologue of the Alzheimer's associated amyloid β protein precursor. Nat Genet 5:95-100.

Weyer SW, Klevanski M, Delekate A, Voikar V, Aydin D, Hick M, Filippov M, Drost N, Schaller KL, Saar M and others. 2011. APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP. EMBO J 30:2266-80.

Willem M, Garratt AN, Novak B, Citron M, Kaufmann S, Rittger A, DeStrooper B, Saftig P, Birchmeier C, Haass C. 2006. Control of peripheral nerve myelination by the β-secretase BACE1. Science 314:664-6.

Wolswijk G. 2002. Oligodendrocyte precursor cells in the demyelinated multiple sclerosis spinal cord. Brain 125:338-49.

Xu DE, Zhang WM, Yang ZZ, Zhu HM, Yan K, Li S, Bagnard D, Dawe GS, Ma QH, Xiao ZC. 2014. Amyloid precursor protein at node of Ranvier modulates nodal formation. Cell Adh Migr 8:396-403.

Zheng H, Jiang M, Trumbauer ME, Sirinathsinghji DJ, Hopkins R, Smith DW, Heavens RP, Dawson GR, Boyce S, Conner MW and others. 1995. β-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. Cell 81:525-31.

Figure Legends

Fig. 1. APP and APLP2 have redundant roles in myelination of the sciatic nerve in juvenile mice. (a) TEM images of highly myelinated axons from the sciatic nerves in the WT, APP-KO and APLP2-KO mice at P14 juvenile age. **(b)** The total number of axons (myelinated plus non myelinated) per 100 μm^2 field of view and **(c)** the percentage (%) of myelinated axons are shown. The myelinated axon diameter distributions of the WT (black) with **(d)** APP-KO (red) and **(e)** APLP2-KO (green) mice. Scatter plots against the axonal diameter of the *g*-ratios from the myelinated axons in WT with **(f)** APP-KO and **(g)** APLP2-KO mice and inset bar graphs of the calculated *g*-ratio. Data represented as mean ± sem. N=6 (red text). Scale bar = 10μm.

Fig. 2. APP and APLP2 expression play a critical role in the myelination of axons in the optic nerve of juvenile mice. (a) TEM images of highly myelinated axons from the optic nerve in the WT, APP-KO and APLP2-KO mice at P14 juvenile age. **(b)** The total number of axons (myelinated plus non-myelinated) per 100 μm^2 field of view and **(c)** the percentage of myelinated axons are shown. The myelinated axon diameter distributions of the WT (black) with **(d)** APP-KO (red) and **(e)** APLP2-KO (green) mice. Scatter plots against the axonal diameter of the *g*-ratios from the myelinated axons in WT with **(f)** APP-KO and **(g)** APLP2-KO mice and inset bar graphs of the calculated *g*-ratio. Data represented as mean ± sem. N=3 (red text). *$p<0.05$, **$p<0.01$. Scale bar = 10μm.

Fig. 3. APP and APLP2 have essential roles in axon myelination in the central and peripheral nervous system in adult mice. **(a)** TEM images of highly myelinated axons from

the sciatic nerve, optic nerve, and corpus callosum region of the brain from the WT, APP-KO, and APLP2-KO mice at P77 adult age were used to calculate **(b)** total axon number per 100 μm^2 field and **(c)** percentage of myelinated axons. **(d-j**) Scatter analysis plots of axon diameter against the *g*-ratios for (**d-e**) sciatic nerve, **(f-g)** optic nerve and **(h-i)** corpus callosum were calculated from the myelinated axons in (**d-i**) WT (black) with (**d, f, h**) APP-KO (red) and (**e, g, i**) APLP2-KO (green) mice with inset bar graphs showing the calculated *g*-ratio. Data represented as mean ± sem. N=3-4 per genotype (see red text for exact numbers), ~60 axons were analyzed in sciatic nerves, 140-150 axons in optic nerves and 125 axons in the corpus callosum. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Scale bar = 10, 5, and 2 μm for sciatic nerve, optic nerve and corpus callosum respectively.

Fig 4. The levels of myelin-associated proteins are altered in the brains of APP-KO and APLP2-KO mice during development. Representative western blot images of WT (black bar), APP-KO (red bar) and APLP2-KO (green bar) mice in **(a)** P60 brain lysates and subsequent densitometric analysis of protein band intensities (normalised to WT value) at P14, P30 and P60 age groups for the myelin associated proteins- **(b)** BACE1, **(c)** Neuregulin 1 (NRG-1), **(d)** PLP, and **(e)** MBP, and **(f)** MOG. Data represented as mean ± sem. N=3-4 animals per genotype (red text). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Fig. 5. APP and APLP2 have distinct effects on demyelination in the cuprizone model. (a) Schematic diagram illustrating the experimental timeline for the mice showing the Control 5 week group (fed a normal diet) and the cuprizone group (diet supplemented with 0.2%

cuprizone) for 5 weeks before mice were killed for analysis. **(b)** Representative TEM images taken from the midline region of the brain corpus callosum of the Control 5 week group (upper images) and the cuprizone group (lower images) from WT, APP-KO, and APLP2-KO mice. **(c)** The extent of axon demyelination in the cuprizone group relative to Control 5 week group was determined from the brain corpus callosum region for the three mouse genotypes by counting the proportion of unmyelinated axons and then normalising to the WT Control 5 week group value. Data represented as mean ± sem. N=3-4 (red text), **p<0.01. Scale bar= 2μm.

Fig. 6. APP and APLP2 have distinct effects on remyelination following recovery from cuprizone treatment. (a) Schematic diagram illustrating the experimental timeline for the mice showing the Control 7 week group (fed a normal diet) and the Recovery group (diet supplemented with 0.2% cuprizone for 5 weeks followed by 2 weeks on a normal diet). **(b)** Representative TEM images of the midline region of the brain corpus callosum of the Control 7 week group (upper images) and Recovery group (lower images) from WT, APP-KO, and APLP2-KO mice. **(c)** The total axon number per 100 μm^2 field image, **(d)** percentage of myelinated axons for the Control 5 week group (grey), cuprizone group (purple), control 7 week group (black) and Recovery group (blue) for WT, APP-KO, and APLP2-KO mice (data normalised to WT Control 5 week group) and **(e)** the calculated *g-ratio* of myelinated axons. The myelinated axon diameter distribution for the Control 7 week group of **(f)** WT (black) **(g)** APP-KO (red) and **(h)** APLP2-KO (green) mice with respective cuprizone (purple) and Recovery group (blue). Data represented as mean ± sem, N=3-4 animals per genotype (red text). *p<0.05,

p<0.01, ***p<0.001, ****p<0.0001. A Chi-square test for trend analysis was performed with the χ^2 , degrees of freedom and the two-sided P value shown for graphs (f-h**), Scale bar = 2μm.