CNS myelination is dependent on astrocyte lipid metabolism

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Abstract:

Myelination requires massive incorporation of lipids into the expanding glial cell membrane, yielding these cells very active in lipid metabolism. Whether myelin membrane formation fully relies on endogenous lipid synthesis or also requires uptake of extracellular lipids is not known. We recently reported that mice carrying a deletion of sterol regulatory binding-element (SREBP) cleavage-activating protein (SCAP) in astrocytes have compromised brain lipid composition accompanied by microcephaly and a set of neurological dysfunctions including progressive motor deficits and reduced sensorimotor gating. Here we show that in the brains of these mice myelinated fibers were reduced in number, had thinner myelin sheaths, and accordingly, had fewer fast conducting fibers. Interestingly, a high-fat diet, rich in cholesterol and mono-unsaturated fatty acids, rescued the impaired conduction velocity and CNS myelination. These data demonstrate for the first time that myelin membrane synthesis by oligodendrocytes is supported by extracellular lipids, supplied by astrocytes under healthy conditions, or derived from the circulation under conditions of compromised astrocyte lipid metabolism. The dependence of CNS myelination on astrocyte lipid metabolism and the ability of high fat diet to ameliorate myelination when this process is impaired may have important implications for the understanding and treatment of myelin pathologies that are associated with compromised brain lipid metabolism.
Introduction

The integrity of the myelin membrane is crucial for proper functioning of the brain and the peripheral nervous system. Myelin acts as an insulator by increasing the electrical resistance across the cell membrane and decreasing membrane capacitance, thereby preventing the electrical current from leaving the axon and ensuring the fast conduction of action potentials between nodes of Ranvier over long distances (for review see Hartline, 2008). Moreover, in the brain myelinating oligodendrocytes protect axons from degeneration, independently of their role as axon insulator (Nave, 2008; Kassmann et al. 2008; Quintes et al. 2010).

A prominent biochemical characteristic of myelin is its high lipid-to-protein ratio. Lipids account for at least 70% of the dry weight of myelin membrane (Norton and Poduslo, 1973), which is twice that of other plasma membranes (Chраст et al. 2011). The high lipid content of the myelin membrane makes it vulnerable for lipid metabolism disorders, and makes lipid availability rate limiting for myelination (Verheijen et al. 2009). Myelinating glia has been suggested to be autonomous in lipid metabolism (Saher et al. 2005; Verheijen et al. 2009; Chраст et al. 2011). However, it was shown that genetic impairment of endogenous lipid synthesis in Schwann cells (SC) (Verheijen et al. 2009), does hamper myelin membrane formation in the PNS, but uptake of extracellular lipids by these cells did partially rescue myelination (Verheijen et al. 2009, Saher et al. 2011). Similarly, Saher et al (Saher et al. 2005) showed that mice carrying an oligodendrocyte-specific deletion of squalene synthase (SQS), an enzyme required for cholesterol synthesis, have CNS hypomyelination that becomes close to normal after 3 months. It was suggested that horizontal cholesterol transfer to cholesterol-deficient oligodendrocytes, via lipoprotein receptor dependent uptake, might underlie this, however, whether extracellular lipids also contribute to myelination by oligodendrocytes under healthy conditions, and what is the origin of these lipids remained to be determined.

Whereas neurons have a poor capacity to synthesize lipids (Vance et al. 1994; Nieweg et al. 2009; Saito et al. 2009; Pfrieger and Ungerer, 2011), astrocytes are highly active in metabolism and secretion of lipids (Boyles et al. 1985; Bernoud et al. 1998; Mauch et al. 2001; Moore, 2001; Innis and Dyer, 2002; Medina and Tabernero, 2002; Nieweg et al. 2009; Pfrieger and Ungerer, 2011). We recently provided the first in vivo evidence that astrocyte lipid metabolism is required for proper brain function
We showed that mice with obliterated astrocyte lipid synthesis develop severe motor dysfunction and die prematurely. Astrocytes pathology, such as Alexander disease, where glial fibrillary acidic protein gene is mutated, is characterized by degeneration of the white matter, suggesting a link between astrocyte dysfunction and myelin integrity. A number of recent in vitro studies have shown that astrocytes have a positive effect on myelination in neuron-oligodendrocyte co-cultures (for review see Nash et al. 2010). Astrocytes promote myelination of dorsal root ganglion neurons in response to electrical activity by releasing the cytokine leukemia inhibitory factor (Ishibashi et al. 2006). Moreover, astrocytes, but not olfactory ensheathing cells or Schwann cells, support myelination of dissociated spinal cord cultures (Sorense et al. 2008). In addition, Watkins and coworkers showed that astrocytes, when co-cultured with purified retinal ganglion neurons and oligodendrocyte precursors (OPC), specifically enhance myelin growth rather than affecting OPC proliferation or survival (Watkins et al. 2008). These studies showed that astrocytes are able to support myelination in vitro, however whether they actively support myelination in vivo and what is the mechanism behind this remained to be determined. We have recently demonstrated that lipogenesis in Schwann cells and in astrocytes relies on sterol regulatory element binding proteins (SREBPs) (Verheijen et al. 2009; Camargo et al. 2012). SREBPs are transcription factors that regulate lipid metabolism, and crucially rely on post-translational activation by the sterol sensor SCAP. When sterol levels are low, SCAP escorts SREBP from the ER to the Golgi, where it is activated and subsequently translocates to the nucleus where it binds genes containing sterol regulatory elements. We previously showed that conditional deletion of SCAP in Schwann cells seriously affected myelin membrane synthesis causing neuropathy, and that SCAP mutant Schwann cells were able to slowly synthesize myelin, in an external lipid-dependent fashion (Verheijen et al. 2009). In addition, we recently found that mice carrying a deletion of SCAP in astrocytes show progressive motor deficits, behavioral dysfunction and premature death (Camargo et al. 2012). Here, we show that reduction in brain volume of the SCAP mutant mice is a consequence of reduced numbers of myelinated fibers and thinner myelin sheaths.

Our results demonstrate that compromising lipid metabolism in astrocytes dramatically affects CNS myelination, however, that this can be ameliorated by a lipid-enriched diet. In particular, treatment with a lipid-enriched diet improved
integrity and function of the myelin tracts in SCAP mutants, consistent with overall improvement of neurological function in these animals. This may have important implications for the understanding and treatment of myelin diseases that are associated with compromised brain lipid metabolism, such as Smith-Lemli-Opitz syndrome, and with leukodystrophies, such as Alexander disease, which is directly associated with defective astrocyte function.

**Results**

**Reduced white matter tracks in the CNS of SCAP mutants**

To determine the role of astrocyte-derived lipids in myelination we analyzed mice in which SCAP is deleted from astrocytes by cre-recombinase technology (Camargo et al. 2012). Using magnetic resonance imaging (MRI) we scanned brains of 1-year-old SCAP mutant animals and their wild-type littermates (Fig. 1A, and Supplementary Fig. S1A). Analysis of these brains revealed a large decrease in white matter volume of mutants (to less than 60% of the wild-type volume), whereas grey matter volume was only reduced by 10% (Fig. 1B). 3D reconstructions of the MRI scanned brains showed the most pronounced reduction in mutant white matter in the corpus callosum (Supplementary Fig. S1B). Using diffusion tensor imaging we found a lower degree of anisotropy of the main tracks in mutant brains compared to wild-type (Fig. 1C). Higher white matter anisotropy implies more organized axonal pathways, likely reflecting the myelination of axons forming tightly packed fiber bundles (Pierpaoli et al. 1996; Nistri et al. 2000). Reduction in anisotropy in mutants likely reflects the disorganization in the architecture of the tracks. In line with this, histological staining of lipid-rich structures, using Sudan Black impregnation, showed smaller white matter structures, evident in corpus callosum and internal capsula (Fig. 1D). In line with previous observations, the size and structure of hippocampal and cortical regions were normal (Camargo et al. 2012). Thus, SCAP deletion in astrocytes leads to reduced and disorganized white matter tracks in the CNS.

**Mature myelin protein levels are decreased in SCAP mutant brains**

In line with the reduced adult white matter, we found the levels of mature myelin proteins, as myelin basic protein (MBP) and myelin-associated glycoprotein (MAG),
Figure 1. SCAP mutants have reduced white matter volume. A). Depicted is a selection of MRI scans of brains of 1-year-old PFA-fixed mutant and wild-type mice as well as the contrast between the two groups. The brains were scaled and the difference in area size is indicated with colors, increasing from red to yellow when the brain of the mutants is smaller than that of the wild-type. See Fig. S1 for complete scans. B) Volumes of the whole brain and the white and grey matter of SCAP mutant and wild-type mice, as determined with MRI. Volumes are normalized to wt levels in which the wt levels were set to 1. Data is shown as mean ± SEM (t-test **= p < 0.01). C) Diffusion tensor imaging (DTI) of same brains as in A, showing reduced anisotropy in the SCAP mutant brains. Arrowheads show examples of the most affected white matter regions, e.g., corpus callosum and internal capsule. D) Sudan Black staining of sagittal sections of 8-month old mutants and wild-type animals, showing reduced white matter in the mutant brains. Arrowheads show the most affected regions, i.e., corpus callosum and internal capsule. Bar in D, 200 μm.

measured by immunoblotting, were reduced in mutants at P120 (Fig. 2A). Smaller differences were found at P14 where only MAG levels were slightly reduced in mutants. During myelination, oligodendrocyte precursor cells (OPC) differentiate via immature oligodendrocytes into myelinating oligodendrocytes. The level of olig2, a transcription factor expressed in cells of the oligodendrocyte lineage, was not different between mutants and wild-types at P14 nor at P120. In addition, we
visualized cells of the oligodendrocyte lineage in the corpus callosum by olig2 immunostaining (Fig. 2C), and found that SCAP mutants had a higher density of olig2-positive cells than wild-types (Fig. 2D). This likely relates to the reduced overall volume of the corpus callosum in mutants and suggests that survival and proliferation of these cells is not affected. Furthermore, NeuN protein levels were similar between wild-types and mutants, indicating that also the number of neurons is not affected (Fig. 2A). Together, these data suggest that SCAP mutants have lower numbers of fully myelinating OLs.

Figure 2. Reduced myelin proteins levels in SCAP mutant mice. A) Protein levels of Olig2 (oligodendrocyte marker), MBP and MAG (myelin proteins), NeuN (neuronal marker), and β–act (loading control) were determined by immunoblotting of total brain extracts of wild-type (wt) and mutant (mt) animals at P14 and P120. B) Bars show quantification of protein levels that were first corrected for equal loading using coomassie staining, and subsequently normalized to wt levels at P14, in which the wt levels were set to 100%. Data represent the mean ± SEM of at least triplicate samples. C) Staining of nuclei (Hoechst; left panel) and oligodendrocytes (anti-Olig2; right panels) in corpus callosum (P21) for mt and wt as depicted, right panel: enlarged view of the dashed square as present in the middle figure. White lines delineate the corpus callosum; Bars left and middle 50 µm; right (enlarged) 16.5 µm. D) Quantification of Olig2-positive cells in the corpus callosum of wt and mt animals. Data represent the mean ± SEM of at least triplicate samples. Bars represent the density of cells as a percentage of wt values (Y-axis). (t-test *= p < 0.05; **= p < 0.01).
Reduced white matter volume is due to hypomyelination of the CNS
We analyzed the ultrastructure of the corpus callosum tracks with electron microscopy, and found less myelinated fibers in SCAP mutants compared to wild-types (Fig. 3A). In particular, this was due to the absence of myelin around the small diameter axons (< 0.5 μm) in SCAP mutants (Fig. 3A, B). Moreover, myelin of the large diameter axons was thinner as depicted by a higher G-ratio in mutants (Fig. 3B and C). Together these results indicate that axons in the corpus callosum of SCAP mutants are hypomyelinated.

We previously reported that the cerebellum of SCAP mutant animals was significantly reduced in size (Camargo et al. 2012). In this study we corroborated this finding with MRI (Fig. 1A). Immunostaining of myelin for MBP, and Purkinje cells for calbindin, showed that all axons in wild-type mouse white matter were fully myelinated, whereas mutant animals had a large number of non-myelinated axons (Fig. 4A). Moreover, mutants showed a disturbed arrangement of axons and swellings of non-myelinated axonal segments. In addition, immunostaining revealed increased levels of GFAP expression in astrocytes of cerebellar white matter in SCAP mutants, indicating astrocyte activation (Nash et al. 2010) (Fig. 4B). Together, these results show that deletion of SCAP in astrocytes causes CNS hypomyelination and activation of astrocytes.

Figure 3. Hypomyelination of axons in the corpus callosum of SCAP mutants. A) Electron microscopy analysis of the corpus callosum of 3 months-old mice reveals a large number of non-myelinated axons in mutant mice compared to wild-types. B) Morphometric analysis of myelinated axons in the corpus callosum of SCAP mutant and wild-type mice, showing g-ratio (axon diameter: myelinated fiber diameter). C) Shown is the average of the g-ratio of wild-types (wt) and mutants (mt). Data is presented as mean ± SEM. (t-test **= p < 0.01).
CNS hypomyelination and impaired conduction velocity of SCAP mutants are partially rescued by a high fat diet.

We previously reported that GFAP-SCAP mutant mice show progressive motor deficits and premature death, and that treatment with a high fat diet (HFD) ameliorated these phenotypes (Camargo et al. 2012). Here we show that treatment of these mutant mice with HFD also rescued hypomyelination, in particular small diameter axons that were devoid of myelin in SCAP mutant mice, fed with standard diet (SD) (Fig. 5A-C). Moreover, HFD also increased the levels of myelin proteins, e.g. MAG, PLP, CNP and MBP in brains of SCAP mutants, however, not of wild-type animals (Fig. 5D). To determine whether treatment with HFD also led to functional recovery of myelin tracks in mutants, we measured conduction velocity (CV) in the corpus callosum. The corpus callosum is the principal commissural pathway of the CNS that links the two cerebral hemispheres and contains both myelinated and non-myelinated axons. Evoked action potentials were measured using two different recording electrodes (Supplementary Fig. S2A). The distance between the electrodes, and the time for each wave to reach the electrodes (Supplementary Fig. S2B) were used to determine the action potential velocity. Action potentials evoked in the corpus...
Figure 5. Effects of a high fat diet on myelination, myelin protein levels and conduction velocity in the corpus callosum. A) Electron microscopy analysis of corpus callosum myelination in cross-sections of mutant mice (P100) on either standard diet (SD) or high fat diet (HFD). B) Morphometric analysis of myelinated axons of the corpus callosum of mutants fed with standard diet (mt-SD) and mutants fed with HFD (mt-HFD), showing g-ratio (axon diameter: myelinated fiber diameter). C) Shown is the average of the g-ratio of mutants fed with standard diet (mt-SD) and mutants fed with high fat diet (mt-HFD). D) Immunoblot against MAG, CNP, MBP and PLP and protein levels of total brain extracts of mutant and wild-type animals (P90) fed with SD or HFD. E) Quantification of myelin proteins MAG, CNP, MBP and PLP in mutant and wild-type mice fed with SD or HFD (n=3). Coomassie staining was used for normalization. Data is presented as mean ± SEM, in which wt-SD levels were set to 100% (t-test p *< 0.05; **= p < 0.01). F) Example of compound action potential waveforms in the corpus callosum for a wild-type mouse on standard diet (wt-SD), and a mutant mouse on standard diet (mt-SD) or high fat diet (mt-HFD). G) Individual plots of conduction velocity (CV) measurements in the corpus callosum of mutants (mt) and wild-types fed with standard diet (SD) or high fat diet (HFD) (n=12-17).
The callosum showed responses generated by myelinated axons (fast wave) and non-myelinated axons (slow wave) (Fig. 5F). These two waves could be discriminated by the kinetics of the curves representing the paired stimulus-response values for increasing stimulus intensity (Fig. S2C) and they were both abolished when tetrodotoxine (TTX), a selective blocker of voltage-gated sodium channels, was applied to the bath (Fig. S2D), showing that the response is due to neuronal transmission. We found that both fast and slow waves were present in more than 95% of the measured nerves of all tested wild-type animals (Fig. 5F, G). However, in mutant animals the fast wave was absent in more than 95% of the measured nerves of all slices, whereas the slow wave remained intact (Fig. 5F, G). Interestingly, treatment with HFD increased the number of fast responses in mutant animals from 5% (in SD) to 50%, whereas no effect on CV was observed in wild-type animals. Thus, a HFD partially rescues both myelination and conduction velocity of the SCAP mutant. Altogether, our results show that SCAP deletion in astrocytes leads to CNS hypomyelination, which can be overcome structurally and functionally by a high fat diet.

Discussion

Deletion of SCAP in astrocytes caused CNS hypomyelination

We previously reported that GFAP-SCAP mutant mice have microcephaly without changes in neuronal and astrocyte density or cell size (Camargo et al. 2012). Here we show that volume reduction was mostly pronounced in the white matter. Cells of the oligodendrocyte lineage were not reduced in density; instead the formation of fully developed myelin membranes was lower in mutant, in particular around small diameter axons, resulting in a functional loss of fast conduction myelinated fibers. We conclude that SCAP mutant mice have white matter atrophy that is caused by hypomyelination of mostly small diameter axons.

The GFAP-Cre transgenic mouse line has been described to predominantly target cre-mediated recombination in the majority of astrocytes (Bajenaru et al. 2002), whereas only minor neuronal populations in the hippocampus (Fraser et al. 2004), cortex (Fraser et al. 2004), and cerebellum (Komine et al. 2007) and a small population (<6%) of oligodendrocytes (Bottelbergs et al. 2012) may also be affected.
Our observations that white matter of mutant mice does contain many axons, albeit without or with thinner myelin, and contains normal density of oligodendrocytes, argues against a substantial involvement of these minor cell populations that are potentially carrying a SCAP deletion. In addition, mature neurons, unlike astrocytes, have a low activity in lipid metabolism (Pfrieger, 2003) and glial cholesterol can support neurons defective in cholesterol synthesis (Funfschilling et al. 2007; 2012). Taken together, we conclude that deletion of SCAP in astrocytes causes hypomyelination of CNS axons.

**Full myelin membrane synthesis by oligodendrocytes is dependent on astrocyte-derived lipids**

Whereas adult mutant mice (P120) showed clear CNS hypomyelination, the level of CNS myelination of younger mutant animals (P14) was close to normal, based on myelin protein levels, and previously reported cholesterol levels (Camargo et al. 2012). This suggests that early postnatal myelination is not much affected in SCAP mutants, and that depletion of astrocyte lipids becomes only limiting after the first stage of myelination. In line with this, oligodendrocytes produce large amounts of cholesterol during the peak of myelination but thereafter, cholesterol synthesis occurs mainly in astrocytes (Pfrieger et al. 2003; Dietschy, 2004). A role of astrocytes in later stages of myelination is not unprecedented, as Watkins showed that astrocytes support myelin membrane growth *in vitro*, rather than OPC differentiation or initial myelin membrane wrapping. Extracellular supplied lipoproteins or cholesterol were recently shown to increase myelin membrane growth of Schwann cells *in vitro* (Verheijen et al. 2009). Here we show that oligodendrocytes require astrocyte lipid metabolism or circulating lipids *in vivo*, suggesting a role for extracellular supplied lipids in full myelin membrane growth by oligodendrocytes. Our observation that small diameter axons are completely devoid of myelin whereas large diameter axons present at least a certain degree of myelination, might be related to the finding that large axons are the first to be myelinated (Nave and Trapp, 2008). Therefore, under conditions that lipid supply is limited, e. g., when astrocyte-derived lipid supply is compromised, oligodendrocytes that enwrap large axons are in favor to use the small amount of lipids initially available, presumably self-produced, whereas small
diameter axons are devoid of myelin as a consequence of reduced extracellular lipid supply by astrocytes.

Saher (Saher et al. 2005) showed that mice carrying an oligodendrocyte-specific deletion of squalene synthase, which is required for cholesterol synthesis, have CNS hypomyelination that becomes close to normal after 3 months. The recovery in myelination in adult CNP (2’,3’-cyclic nucleotide 3’-phosphodiesterase)-SQS mice was suggested to be a consequence of compensatory mechanisms, in which oligodendrocytes take up lipids from the extracellular environment, possibly derived from astrocytes, but only in the condition where they are unable to produce their own lipids (for review see Saher, 2011). Here we show that endogenous lipid synthesis by oligodendrocytes is not sufficient for full myelination. Our data suggest that CNS myelination is dependent on lipid synthesis in both oligodendrocytes and astrocytes, with oligodendrocyte lipid synthesis being sufficient for the first stage of early myelination, and astrocyte derived lipids required for the synthesis of a fully mature myelin membrane.

**Implications for understanding and treatment of white matter diseases**

The observed hypomyelination and reduced fiber conduction velocity are likely to underlie the motor deficits of SCAP mutant mice, as reported in many myelin defective disorders (Kaye, 2001; Gold et al. 2006), which includes paroxysmal dyskinesia and tremors, as also observed in humans with multiple sclerosis or white matter injuries (Shields et al. 2012; Shields et Waxman, 2012). Our data imply that deficits in astrocyte lipid metabolism might underlie white matter diseases as found in humans. Importantly, astrocyte dysfunction is indeed often accompanied by myelin defects (e. g Alexander disease, multiple sclerosis) and changes in astrocyte phenotype are a hallmark of diseased myelin tracks. Whether changes in astrocyte phenotype or reactivity are beneficial to myelin repair is controversial. In a recent review the contribution of reactive astrocytes to myelin repair has been discussed and the authors concluded that mild astrocyte reactivity would enhance myelination whereas very reactive astrocytes would secrete inhibitory signal that are not permissive to myelin repair (Nash et al. 2010).

Astrocyte reactivity has been reported to correlate with altered ApoE expression in neurodegenerative diseases such as Parkinson, Alzheimer and some
types of dementia (Gallardo et al. 2008), suggesting that lipid synthesis and secretion by astrocytes is altered when their phenotype changes. We found that SCAP mutant astrocytes in white matter showed increased GFAP expression, which is indicative for increased reactivity. This type of reaction may be related to a response from astrocytes to degenerating neurons.

We previously reported that a fat-enriched diet could partially rescue some of the motor deficits observed in SCAP mutants (Camargo et al. 2012). Here, we found that the same diet also counteracted hypomyelination. Accordingly, the diet led to a functional recovery of the myelin tracks. The exact mechanisms by which this diet improved myelination remains to be determined, but may involve the close vicinity of astrocytes end-feet to blood capillaries, and thereby the uptake of circulating lipids by astrocytes and subsequent delivery to oligodendrocytes. It should be noted that a cholesterol-enriched diet did not improve myelination for CNP-SQS KO animals (Saher et al. 2005), probably because oligodendrocytes do not have the same access to circulating lipids as astrocytes. Our results indicate that the requirement of exogenous lipid uptake by oligodendrocytes for myelin membrane synthesis may have been underestimated. Without astrocyte lipid synthesis, oligodendrocytes are unable to finalize CNS myelination, leading to hypomyelinated and slower conducting fibers in adulthood. These data may have important implications in the understanding and treatment of myelin diseases. Some of the myelin defective phenotypes are known to benefit from dietary supplemented lipids, (SLOS, X-linked Adrenoleukodystrophy), however, with controversial effects in different patients, which calls for optimization and detailed understanding of the underlying mechanisms (Elias, 1997; Moser, 2007). Considering the need of lipids for myelination and remyelination, our findings show that oligodendrocytes depend on astrocyte lipid metabolism and also from lipids supplemented in the diet, which might be instrumental for the development of novel strategies aimed at restoring loss of function in myelin diseases.

**Materials and methods**

**Animals**

All experimental procedures were approved by the local animal research committee and complied with the European Council Directive (86/609/EEC). SCAP-floxed mice
were from the Jackson Laboratory and have been described (Matsuda et al. 2001). The hGFAP-Cre-IRES-LacZ transgenic mice have been described (Bajenaru et al. 2002) and are referred to as GFAP-Cre. Both of the mouse lines were maintained on a C57Bl6 background. In subsequent generations, we obtained mice with a genotype SCAPloxP/loxP, which are referred to as SCAP mutants (Camargo et al. 2012). Littermates with genotypes SCAPloxP/loxP, SCAPlox/wt or wild-type are referred to as ‘wild-types’. Unless indicated otherwise, mice were housed with their littermates of the same gender in Macrolon cages on sawdust bedding, for the purpose of animal welfare, after weaning (three weeks after birth). Food (Harlan Teklad, Madison, WI, USA) and water were provided ad libitum. Housing was controlled for temperature, humidity and light-dark cycle (7 AM lights on, 7 PM lights off).

**Diets**

Pregnant mice, on day 14 of gestation, were randomly separated in 3 groups. Group 1 received the standard diet (Teklad diets, Harlan Laboratories, Madison, WI, USA), group 2 received a fish oil-enriched diet containing 22% of fish oil (TD.08323, Teklad diets, Harlan Laboratories) and group 3 received a high fat diet containing 60% fat calories diet (1% cholesterol, 31% lard and 3% soybean oil, TD.09167, Teklad diets, Harlan Laboratories). Fatty acid content has been described in Camargo et al. 2012. These diets were given to the pregnant mice during the last week of gestation and during the lactation period until weaning (3 weeks after birth). At weaning, animals were separated, housed by gender, and continued to receive the same diet.

**Magnetic resonance imaging and diffusion tensor imaging**

Wild-types (n=4) and SCAP mutant mice (n=5) of 1 year old, under deep anesthesia were perfused transcardially with 20 ml of PBS 0.1 M, pH 7.4 containing 0.1% heparin followed by 100 mL of freshly prepared cold fixative solution composed of 4% paraformaldehyde in 0.1M PBS, pH 7.4. Brains were removed, postfixed overnight in fixative solution at 4 °C, and cryoprotected with 30% sucrose for 2–3 days at 4 °C. *Ex vivo* brains were fixated in a syringe filled with perfluoro polyether (Fomblin®, Solvay Solexis) to prevent susceptibility artifacts at the borders of the brain. High resolution DTI was conducted on a 9.4T Varian MR scanner (eight-shot...
EPI; TR/TE = 6000/32 ms; FOV = 25 x 25 mm; matrix = 128 x 128; zero-filled to 256 x 256; 91 coronal slices with slice thickness of 0.2 mm; isotropic voxel resolution = 0.2 x 0.2 x 0.2 mm³; 4 images without diffusion-weighting; diffusion-weighted images in 60 directions with b = 2871.50 s/mm² and b= -2871.50 s/mm² (Δ/δ=13/6 ms); total acquisition time = 13.25 h). The diffusion tensor for each voxel was calculated based on the eigenvectors and eigenvalues using multivariate fitting and diagonalization. Derived FA maps were further analyzed using unbiased whole brain tract-based spatial statistics (TBSS) (Smith et al., 2006). Image-based registration was performed with Elastix (http://elastix.isi.uu.nl). FA maps of all animals were first aligned to a 3D reconstruction of the Paxinos & Watson rat brain atlas (Paxinos and Watson, 1998) using non-linear registration with limited degrees of freedom preceded by affine-only registration. A skeleton of white matter tracts shared across subjects was obtained by thresholding the mean FA maps at 0.2. Subject FA maps were registered with a perpendicular search algorithm starting from the skeleton towards individual tracts, and subsequently stacked into a sparse skeletonized 4D image. Permutation tests with threshold-free cluster enhancement (Smith et al., 2006) were conducted for each point at the mean FA skeleton to assess statistically significant differences between mutant and control groups.

Conduction velocity

Conduction velocity was analyzed in 400 μm thick acutely prepared brain slices from wild-types and SCAP mutants fed with standard diet or high fat diet. Mice were decapitated and the brains were rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF; containing NaCl 129 mM, KCl 3 mM, MgSO4 1.8 mM, CaCl2 1.6 mM, glucose 10 mM, NaH2PO4 1.25 mM, NaHCO3 21 mM; pH 7.4) carboxygenated with 5% CO2 and 95% O2. Coronal slices were prepared for recordings from the frontal cortex including hippocampus. After sectioning, slices were maintained at 21 °C and recorded at room temperature (20-22 °C) in a similar solution. Field currents were recorded with two Heka EPC-8 amplifiers (D-67466 Lambrecht/Pfalz, Germany). The ACSF-filled glass microelectrodes were voltage clamped at 0 mV. The measurements were done at three different locations along the corpus callosum with a platinum/iridium electrode (FHC, Bowdoin, ME 04287, USA). Data were low-pass filtered at 5 kHz, digitized at 20 kHz, with an instrutech
ITC-16 and pulse software (D-67466 Lambrecht/Pfalz, Germany) and analyzed off-line with Igor Pro (Wavemetrics, 10200 SW Nimbus, G-7, Portland, USA).

**Immunoblotting**

Total brain extracts preparation. Brains were dissected from SCAP mutants and wild-type animals (P14 and P120 for time point experiments and P90 for diet experiments), separated from the spinal cord and medulla oblongata and cleaned from the meninges. Brains were rapidly frozen in iso-pentane and kept at -80°C until used. Upon use, the tissue was homogenized in 5 mL of homogenisation buffer (5 mM of HEPES/NaOH pH 7.4, 0.32 M sucrose and complete EDTA-free protease inhibitory cocktail (Roche applied sciences), containing 0.016 U/mL of RNAse inhibitor, Invitrogen). Whole-brain extracts were mixed with SDS and heated to 90 °C for 5 min. Proteins were separated by SDS-PAGE in a Mini-Protean Electrophoresis system and electroblotted overnight onto PVDF membranes. Membranes were probed with primary antibodies rabbit anti-Olig2 (1:500, Millipore), mouse anti-MBP (1:2000, Santa Cruz), goat anti-Mag2 (1:1000, Santa Cruz), mouse anti-CNP (1:1000, Sigma), mouse anti-NeuN (1:2000, Millipore) and mouse anti-ß-actin (Millipore, 1:1000); followed by an alkaline phosphatase-conjugated secondary antibody (1:1000, Dako). After reaction with the ECF substrate (GE Healthcare) membranes were scanned with an FLA instrument (Fujifilm). Quantification was done using the Quantity One software (Biorad).

**Immunohistochemistry**

Wild-type and SCAP mutant mice of 21 days old, under deep anesthesia, were perfused transcardially with 20 ml of PBS 0.1 M, pH 7.4 containing 0.1% heparin followed by 100 mL of freshly prepared cold fixative solution composed of 4% paraformaldehyde in 0.1M PBS, pH 7.4. Brains were removed, postfixed overnight in fixative solution at 4 °C, and cryoprotected with 30% sucrose for 2–3 days at 4 °C. Brains were rapidly frozen in powdered dry ice and sliced into 50 μm-thick sagittal sections on a cryostat (-20 °C). Free-floating sections were rinsed three times and stored in 0.1M PBS, pH 7.4, containing 0.1% NaN₃ at 4 °C or 0.1% anti-freeze solution at -20 °C until use. For immunostainings, sections were rinsed 4 times for 10 minutes in 0.1M PBS, incubated 30 minutes in 0.1M PBS containing 0.5% Triton-100 and 10% normal goat serum, and subsequently incubated with appropriate antibody
concentrations in incubating medium (0.1M PBS, 0.1% Triton-100, 2% normal goat serum) with mild shaking for 72 hours at 4 °C. The sections were washed 4 times for 10 minutes in 0.1M PBS and incubated in incubating medium containing the appropriate secondary antibody for 2 hours. Sections were washed 2 times with 0.1M PBS, 1 time with water, and subsequently DNA counterstained in Hoechst reagent 33258 solution (Sigma), washed 2 times with PBS 0.1M and mounted on glass slides. Primary antibodies were rabbit GFAP (1:1000, DAKO), rabbit Calbindin (1:10000, Swant), mouse Olig2 (1:1000, Millipore) and mouse MBP (1:1000, Santa Cruz). Secondary antibodies were goat anti-mouse Alexa 488 (1:400, Molecular probes) and donkey anti-rabbit Cy3 (1:400, Jackson laboratories).

Sections were examined on a Zeiss microscope, sampled with a Nikon video camera under fluorescent illumination and cell density was determined using Image J 1.42q software. Six sets of matched sagittal sections were obtained and the three more medial slices of matched sets were compared per antibody. Using Image J Olig2-positive cells in 3 comparable regions of the corpus callosum were counted and divided by the area of the rectangle to calculate the density. Finally, close ups of the white matter in the cerebellum were photographed using MBP and calbindin staining or GFAP and calbindin staining with a confocal microscope (Zeiss).

**Electron Microscopy and Morphometric Analysis**

Animals under deep anesthesia were perfused transcardially with 20 ml of PBS 0.1 M, pH 7.4 containing 0.1% heparin followed by 100 mL of freshly prepared cold fixative solution composed of 4% paraformaldehyde in 0.1M PBS and 2% glutaraldehyde, pH 7.4. Brains were removed, postfixed overnight in fixative solution at 4 °C, and cryoprotected with 30% sucrose for 2–3 days at 4 °C. Brains were rapidly frozen in powdered dry ice and sliced into 50 µm-thick sagittal sections on a cryostat (-20 °C). Free-floating sections were rinsed three times, dehydrated with ethanol and imbedded in Epon. Ultra-thin sections were subsequently cut, collected on formvar coated single slot grids, and stained with a 1% aqueous uranyl acetate solution for 20 min and subsequently for 1 min with lead citrate. Photographs were obtained by using a JEOL 1010 electron microscope. For each myelinated axon present the g-ratio was calculated by dividing the axonal diameter (defined by the inner limit of the myelin sheath), by the total fiber diameter (defined by the outer limit of the myelin sheath).
Supplementary figures

A- Overlay MRI images

Supplementary Figure 1. MRI scans of 1-year old SCAP mutant and wild-type mice. A) Complete MRI scans. Depicted are scans showing the contrast between wild-type and mutant mice from posterior to anterior, color shows intensity of the difference in brain volume increasing from red to yellow, of 4 SCAP mutant and 4 wild-type mice. B) 3D reconstruction of MRI scanned brains. The images show the difference between wild-types and SCAP mutants. Depicted in blue are the areas where brains of mutant mice are significantly smaller than of wild-type mice (p < 0.05, FDR-corrected), in particular, the corpus callosum.
Supplementary Figure 2. Experimental set-up of the nerve conduction velocity experiments. A) Photo showing the position of the action potential evoking electrode (labeled with ‘Stim’) and the 2 recording electrodes (‘Rec 1’, and ‘Rec 2’) B) Example of the recorded signal showing in blue the signal recorded with electrode 1, and in orange the signal recorded with electrode 2. C) Paired stimulus-response values for increasing stimulus intensity showing the different kinetics between the fast and the slow wave. Shown is the average ± SEM of 5 animals. D) Inhibition of the signal by 1 µM tetrodotoxin (TTX) applied in the bath before stimulation.

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References


