

A crucial role for *Olig2* in white matter astrocyte development

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The mechanisms underlying astrocyte heterogeneity in the developing mouse brain are poorly understood. The bHLH transcription factor *Olig2* is essential for motoneuron and oligodendrocyte formation; however, its role in astrocyte development remains obscure. During cortical development, *Olig2* is transiently expressed in immature developing astrocytes at neonatal stages and is progressively downregulated in astrocytes at late postnatal stages. To assess the function of *Olig2* in astrocyte formation, we conditionally ablated *Olig2* in a spatiotemporally controlled manner. In the *Olig2*-ablated cortex and spinal cord, the formation of astrocytes in the white matter is severely compromised. Temporally controlled mutagenesis revealed that postnatal *Olig2* function is required for astrocyte differentiation in the cerebral white matter. By contrast, astrocytes in the cortical gray matter are formed, but with sustained GFAP upregulation in the superficial layers. Cell type-specific mutagenesis and fate-mapping analyses indicate that abnormal astrocyte formation is at least in part attributable to the loss of *Olig2* in developing astrocytes and their precursors. Thus, our studies uncover a crucial role for *Olig2* in white matter astrocyte development and reveal divergent transcriptional requirements for, and developmental sources of, morphologically and spatially distinct astrocyte subpopulations.

KEY WORDS: Astrocyte differentiation and heterogeneity, Oligodendrocyte lineage, bHLH transcription factors, *Olig2*, Cortex, Spatiotemporally specific knockout, mouse

INTRODUCTION

Astrocytes, the most numerous neural cell type in the brain, play a crucial role not only in neuronal development and function, but also in brain integrity and remodeling after injury or disease (Ridet et al., 1997; Shu and Richards, 2001; Sofroniew, 2005; Ullian et al., 2001). Cortical astroglial cells are derived from neural progenitors, including radial glia present in the ventricular zone (VZ) and subventricular zone (SVZ) of the developing brain at embryonic and early postnatal stages (Goldman, 2004; Miller and Reynolds, 2004) that migrate into the cortex, where they differentiate and become mature astrocytes at adulthood (Sauvageot and Stiles, 2002; Temple, 2001). Astrocytes in the cortex exhibit heterogeneous morphologies and are subdivided into at least two subpopulations – white and gray matter astrocytes – based on their location. White matter astrocytes harbor abundant glial filaments in their processes, whereas gray matter astrocytes exhibit many branched processes and few glial filaments. These spatially distinct astrocyte subclasses are commonly defined by expression of glial fibrillary acidic protein (GFAP) (Kimelberg, 2004; Reichenbach and Wolburg, 2005; Walz, 2000). Although many signaling pathways involved in astrocyte differentiation *in vitro* have been identified (Mehler, 2002; Sauvageot and Stiles, 2002; Sun et al., 2003), at present it is unclear whether the formation of spatially and morphologically heterogeneous astrocytes is subject to identical or distinct transcriptional regulatory mechanisms.

The basic helix-loop-helix (bHLH) transcription factor *Olig2* is essential for the formation of motoneurons and oligodendrocytes in the developing spinal cord (Lu et al., 2002;

Takebayashi et al., 2002; Zhou and Anderson, 2002); however, its functions in astrocyte development remain elusive, particularly in the developing brain. *Olig2* expression can be detected in cortical neural progenitors including radial glial cells at embryonic stages and in GFAP⁺ progenitor/stem cells in the postnatal SVZ (Furusho et al., 2006; Malatesta et al., 2003; Setoguchi and Kondo, 2004). Recent studies with a dominant-negative form of *Olig2* indicate that *Olig2* directs cortical astrocyte differentiation from neural progenitors in the SVZ of the neonatal brain (Marshall et al., 2005), whereas *in vitro* evidence suggests that *Olig2* inhibits astrocyte differentiation (Fukuda et al., 2004; Gabay et al., 2003). *In vivo* characterization of the function of *Olig2* in cortical astroglial differentiation, which occurs largely at postnatal stages (Sauvageot and Stiles, 2002), has been hampered by the early embryonic lethality of *Olig2*-null mice (Lu et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002). At present, it is not clear how *Olig2* regulates the formation of distinct astrocyte subpopulations in the developing brain.

In this study, we show that *Olig2* is transiently expressed in immature developing astrocytes neonatally and its level is downregulated progressively in mature astrocytes at late postnatal stages. We utilize a spatiotemporally specific conditional ablation approach to examine *Olig2* function in astrocyte development in the postnatal CNS. We uncover a crucial role of *Olig2* in white matter astrocyte differentiation in the brain and the spinal cord. By contrast, in the gray matter, *Olig2* ablation leads to abnormal GFAP upregulation in a population of astrocytes located in superficial cortical layers. Cell type-specific ablation and fate-mapping analyses indicate that abnormal development of cortical astrocytes is at least in part attributable to the loss of *Olig2* function. Thus, our studies using conditional *in vivo* mutagenesis indicate an important dual role of *Olig2* in regulating gray and white matter astrocyte development and reveal distinct transcriptional requirements for the formation of diverse astrocyte subpopulations during CNS development.

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MATERIALS AND METHODS

Generation of tissue-specific *Olig2* conditional mutant mice

Olig2 conditional knockout (*Cko*) mice and Cre deleter lines including hGFAP-Cre, CNP-Cre and Syn1-Cre, as well as the RosaYFP reporter line, were maintained on a C57BL/6J and 129 SVJ hybrid background. *Olig2Cko* mice were bred with a Cre line and/or a reporter line to generate tissue-specific *Olig2*-knockout mice. To generate temporally regulated *Olig2*-knockout mice, *Olig2Cko* mice were bred with tamoxifen (TM)-inducible *CAGGS-CreERTM* mice (Hayashi and McMahon, 2002). To induce *Olig2* ablation, 0.3 mg TM per 4 g body weight was injected intraperitoneally into neonatal pups and their foster mother as described previously (Hayashi and McMahon, 2002). All protocols involving the use of animals were approved by the Institutional Animal Care and Research Advisory Committee (IACRAC) at the UT Southwestern Medical Center at Dallas.

RNA in situ hybridization and immunohistochemistry

RNA in situ hybridization and probes, as well as immunostaining methods with tissue sections from mouse brains, were as described previously (Lu et al., 2002). Double immunostaining was performed by simultaneous incubation with the relevant antibodies. The following antibodies were used: *Olig2* (gift from Chuck Stiles, Harvard Medical School, Boston, MA); GFAP (Dako), NG2 and NeuN (Chemicon), BrdU, GFAP, S100 β , NF200, Tau-1, Gap43 (Sigma), Ki67 (1:500, Swant) and rat PDGF α R (1:500, BD Bioscience). For 5'-bromo-2'-deoxyuridine (BrdU) immunostaining, perinatal control and mutant littermates were injected with 100 mg BrdU (Sigma, St Louis, MO) per kg body weight 4 hours prior to sacrifice. Goat anti-mouse, rat and rabbit secondary antibodies conjugated to Cy2 and Cy3 (Jackson ImmunoResearch) were used for double-labeling experiments. Microscopy was performed on a Zeiss LSM 510 laser scanning confocal microscope.

RESULTS

Transient *Olig2* expression in developing gray and white matter astrocytes at early postnatal stages

To determine the relationship between *Olig2* and astrocyte differentiation in the postnatal cortex, we examined the expression of *Olig2* and the astrocytic marker GFAP at different developmental stages. At early neonatal stages such as postnatal day 5 (P5), scattered GFAP⁺ astrocytes were detected in the cortical gray matter, the majority of which expressed a high level of *Olig2* in the nucleus (Fig. 1A,B-B''). Their identity was confirmed by the fact that these GFAP⁺ astrocytes expressed another astrocyte marker, glutamine synthetase (GS) (Stanimirovic et al., 1999) (Fig. 1C-E). Whereas GFAP expression became undetectable in the cortex at perinatal stages such as P14 (Figs 2 and 3), a low level of *Olig2* expression was detectable in GS⁺ cortical astrocytes (Fig. 1H,I, arrowheads), as compared with intense *Olig2* expression in early-developing astrocytes (Fig. 1F,G). At this stage, *Olig2*⁺ GS⁻ cells with a high level of *Olig2* expression were only identified as oligodendrocyte lineage cells (Fig. 1H,I, arrows; data not shown). *Olig2* expression was completely absent from GS⁺ cortical astrocytes of young adult mice by P21 (Fig. 1J,K). Similarly, in the corpus callosum – a developing cerebral white matter tract – of wild-type mice, *Olig2* expression was detected in a population of developing GFAP⁺ astrocytes at P5 (Fig. 1L-O), but was absent in mature astrocytes by adulthood (data not shown). Thus, *Olig2* appears to be transiently expressed in immature developing GFAP⁺ astrocytes at early postnatal stages, whereas it undergoes downregulation in mature astrocytes at late postnatal stages and adulthood.

Abnormal astrocyte development in the *Olig2*-ablated cortex

To assess the function of *Olig2* in cortical astrocyte formation, we used a hGFAP-Cre deleter line (Zhuo et al., 2001) to ablate *Olig2* in the developing cortex by generating *Olig2Cko*;hGFAP^{Cre} (*Cko^G*)

mice. The hGFAP promoter drives Cre recombinase expression in the majority, if not all, embryonic multipotent cortical progenitors, which give rise to neural cell types including neurons, oligodendrocytes and astrocytes in the cortex (Malatesta et al., 2003; Zhuo et al., 2001). In addition, the Cre activity persists in developing astrocytes and SVZ precursors at postnatal stages (Malatesta et al., 2003; Zhu et al., 2005).

In control mice, *Gfap* was readily detected in the cerebral white matter, the hippocampus and at the subpial surface. However, expression of *Gfap*, as assessed by in situ hybridization, was essentially undetectable in the cortical gray matter from postnatal stage P7 to adulthood (Fig. 2A,C,G), although a population of GFAP⁺ cells was detected in the cortex at early neonatal stages (Fig. 1). These observations are consistent with previous observations that most gray matter astrocytes contain little, if any, GFAP (Connor and Berkowitz, 1985).

By contrast, in the *Olig2* conditional mutant (*Cko^G*) mice, intense *Gfap* expression was initially observed in cells scattered throughout the cortex at neonatal stage P7 (Fig. 2B), and increased dramatically from postnatal week 2 (Fig. 2D). High *Gfap* expression persists throughout adulthood (Fig. 2H). This increase in expression was not uniform in the *Olig2*-ablated cortex. The highest level of *Gfap* expression was seen in superficial cortical layers II-IV and the stratum lacunosum-moleculare of the hippocampus (Fig. 2D,F), a region anatomically akin to the superficial layers of the cortex (Forster et al., 2006), whereas *Gfap* expression in the deep layers of the cortex (layers V-VI) was essentially undetectable (Fig. 2D,H). Strikingly, in contrast to *Gfap* upregulation in the gray matter, *Gfap* expression in the white matter was significantly downregulated in *Olig2* mutants at perinatal and adult stages (Fig. 2F,H compared with E,G, arrowheads). The lower level of in situ signal could be due to fewer astrocytes or reduced GFAP expression in each cell. Nonetheless, these observations suggest that the effect of *Olig2* ablation on *Gfap* expression is region-specific within the cortex.

Astrocyte formation deficit in the white matter of the *Olig2*-ablated cortex

In the cerebral white matter of *Olig2*-ablated mice, we observed marked downregulation not only of *Gfap* mRNA but also at the protein level, as compared with the control (Fig. 3A-D, arrowhead). When *Olig2*-ablated cortices were examined by Hematoxylin and Eosin staining for cell morphology, there was a substantial reduction of cell number in the corpus callosum (Fig. 3F compared with E). To determine whether the formation of astrocytes is compromised in this region, we examined expression of another astrocyte marker, S100 β . In the white matter of *Olig2* mutants, the number of S100 β ⁺ cells was severely reduced, in contrast to the high density of these cells in the control (Fig. 3G,H,O). Since S100 β could reflect a population of oligodendrocyte lineage cells, we examined expression of an additional astrocyte marker, GS. The number of GS-expressing cells in the corpus callosum of *Olig2* mutants (Fig. 3M,N) was markedly reduced to approximately 21% of wild-type and control hGFAP-Cre (*Ctrl^G*) mice (Fig. 3I-L,O). Expression of the astrocyte markers GS and GFAP in the white matter of wild-type and hGFAP-Cre-expressing control mice (*Ctrl^G*) (Fig. 3I,J compared with K,L) was comparable (Fig. 3O), suggesting that expression of hGFAP-Cre does not exert detectable toxic effects on the formation of white matter astrocytes. A disproportionate reduction in S100 β ⁺ cells as compared with GS⁺ cells (Fig. 3O) is likely to be due to a population of S100 β ⁺ cells marking early-differentiated oligodendrocytes (Deloulme et al., 2004; Rickmann and Wolff, 1995) that are lost in the *Olig2* mutant (Yue et al., 2006). Thus, the

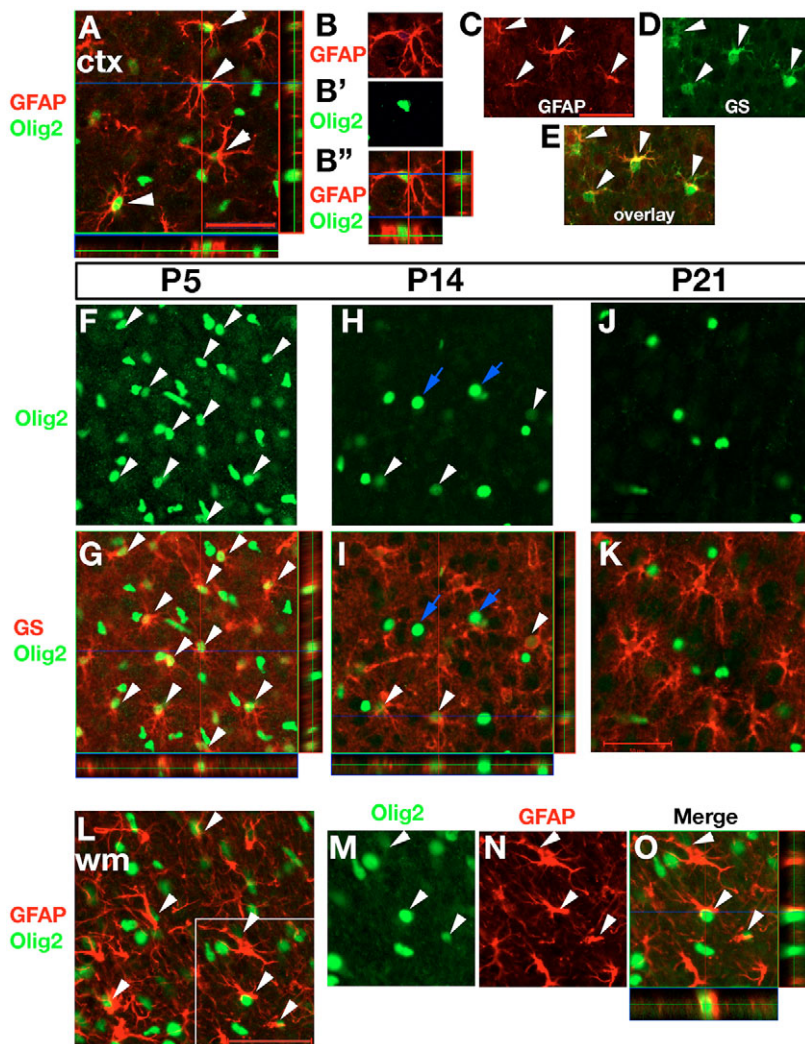


Fig. 1. Transient Olig2 expression in developing cortical astrocytes. (A-E) Sagittal cortical sections from wild-type mice at P5 were immunostained with antibodies against GFAP, Olig2 or glutamine synthetase (GS). (A) GFAP⁺ cells (red) at this stage expressed a high level of Olig2 (green) as judged by staining intensity. Orthogonal reconstructions of confocal images at z-axis level are shown in side panels (along the right-hand edge and beneath). Arrowheads indicate GFAP⁺ Olig2⁺ double-positive cells. A GFAP⁺ Olig2⁺ cell, marked by cross-lines, is shown at a higher magnification in B-B''. (C-E) Co-expression (E) of GFAP (C, red) and GS (D, green) was observed in the astrocytes of the neonatal cortex. (F-K) Sagittal cortical sections of wild-type mice at P5 (F,G), P14 (H,I) and P21 (J,K) were immunostained with antibodies against Olig2 and GS. Confocal images at z-axis level are shown in side panels of G and I. (F,G) GS⁺ astrocytes (red) express a high level of Olig2 (green) at P5. Arrowheads indicate GS⁺ Olig2⁺ double-positive cells. (H,I) At P14, Olig2 expression in GS⁺ astrocytes (arrowheads) is reduced to a low level as compared with the intense Olig2 expression in GS⁻ cells (blue arrows). (J,K) At P21, Olig2 expression (green) is undetectable in GS⁺ astrocytes (red) in the cortex. (L-O) The corpus callosum region of wild-type mice at P5 was immunostained with antibodies against GFAP and Olig2. Arrowheads indicate GFAP⁺ Olig2⁺ double-positive cells in this developing white matter. The boxed area in L is shown at a higher magnification in M-O. Orthogonal reconstructions of confocal images at z-axis level are shown for a GFAP⁺ Olig2⁺ cell marked by crosslines in O. Scale bars: 50 μm in A,F-L.

reduction in cell number and the expression of different astrocyte markers suggest that Olig2 is required for formation and differentiation of an astrocyte subpopulation in the cerebral white matter.

Postnatal Olig2 function is required for white matter astrocyte formation

hGFAP-Cre activity is not only detected in radial glia and cortical progenitors at embryonic stages (Malatesta et al., 2003; Yue et al., 2006), but also persists postnatally in GFAP⁺ developing astrocytes in the white matter and in GFAP⁺ progenitors in the SVZ (Fig. 4A-D). It has been suggested that astrocyte subtypes in the optic nerve have distinct temporal maturation patterns (Miller and Raff, 1984). To determine the critical stage at which Olig2 function is needed for cerebral white matter astrocyte formation, we ablated Olig2 at early postnatal stages by breeding Olig2 conditional mutant mice with an inducible Cre line (CAGGS-CreERTM) (Hayashi and McMahon, 2002), in which tamoxifen (TM)-inducible Cre activity is controlled by a ubiquitous cytomegalovirus promoter.

The control and compound Olig2Cko;CreERTM animals were administered TM at neonatal stages for 4 days, from P2-P5, and their brains harvested at P13 for in situ hybridization analysis. Olig2 expression in the cortex was essentially eliminated by TM-induced Cre activity (Fig. 4F). Since Olig2 is required for

adequate postnatal myelination (Yue et al., 2006), expression of the myelin gene *Mbp* and of the mature oligodendrocyte marker CC1 were accordingly significantly downregulated in the gray and white matter of the Olig2-ablated cortex, as compared with the TM-injected control (Fig. 4G,H,M). Residual *Mbp*⁺ cells in the white matter are likely to result from the continued maturation of developing oligodendrocytes formed prior to TM-induced Olig2 ablation in these cells. In the inducible Olig2 mutant, *Gfap* expression was severely downregulated in the cerebral white matter as compared with the otherwise robust expression in this tract, whereas *Gfap* expression persisted in the SVZ and subpial surface at a level comparable to the control (Fig. 4J,L compared with I,K).

Downregulated expression of other astrocyte markers such as GS was also observed in the cerebral white matter of the TM-treated Olig2 mutant mice (Fig. 4M). By contrast, *Gfap* expression was not perturbed in the cortical gray matter (Fig. 4J) and there was no significant difference in terms of the number of GS⁺ astrocytes in the cortical areas when Olig2 was ablated postnatally (Fig. 4M). These results suggest that TM-induced Olig2 deletion at neonatal stages results in a severe deficit in the formation of white matter astrocytes. Thus, the results from inducible Olig2 ablation suggest that Olig2 plays an important role in postnatal white matter astrocyte formation.

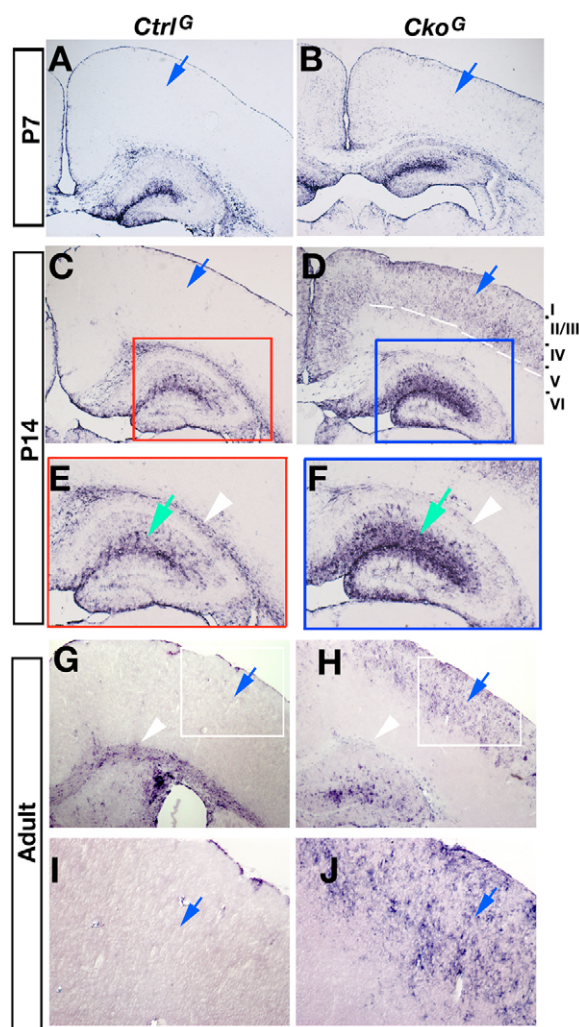


Fig. 2. Alteration of GFAP expression in the developing and adult *Olig2*-ablated cortex. Expression of *Gfap* was analyzed by in situ hybridization on coronal brain sections of control (*Olig2*^{Ctrl};*hGFAP*^{Cre} or *Ctrl*^G) and *Olig2* mutant mice (*Olig2*^{Cko};*hGFAP*^{Cre} or *Cko*^G) at P7 (A,B), P14 (C-F) and adult (P79; G-J). Neocortical *Gfap* expression is indicated by blue arrows. Red, blue and white boxed areas in C,D and G,H, representing the hippocampus and the neocortex from control and *Olig2*-ablated mice, are shown at high magnification in E,F and I,J, respectively. Arrowheads and arrows in E,F indicate *Gfap* expression in the white matter tract and stratum lacunosum-moleculare of the hippocampus, respectively. Cortical lamination layers are indicated on the right-hand side of D.

***Olig2* function is required for white matter astrocyte formation in the spinal cord**

A recent fate-mapping study indicated that *Olig2*-expressing cells could give rise to astrocytes in the spinal cord (Masahira et al., 2006). In light of the profound deficit in white matter astrocyte formation in the brain of *Olig2*-ablated mice (*Cko*^G) mediated through *hGFAP*-Cre, we examined the spinal cord from these mutants to determine whether white matter astrocytes in this caudal region of the CNS are affected in the absence of *Olig2*. Strong *Gfap* expression was observed in the white matter region of the wild-type spinal cord at P14, as was expression of the other astrocyte markers, S100 β and GS (Fig. 5). By contrast, in the spinal white matter of

Olig2 mutants, we observed marked downregulation not only of *Gfap* mRNA, but also of protein expression (Fig. 5B,E,F), despite relatively normal GFAP expression in the gray matter of the spinal cord. In *Olig2*-mutant mice, the number of cells expressing S100 β and GS in the white matter of the spinal cord was markedly reduced (Fig. 5I-J,M-N), representing approximately 18% and 25% of that in the controls, respectively (Fig. 5O). Thus, the reduction of astrocyte marker expression in the spinal white matter of the *Olig2* mutant suggests that *Olig2* is required for the formation of white matter astrocyte subpopulations in the spinal cord.

***Olig2* ablation results in sustained GFAP expression in the superficial layers of the cortical gray matter**

In contrast to the situation in the cerebral white matter, excessive GFAP expression was observed in the cortical gray matter of *Olig2* mutants (Fig. 3). This could be due to an increase in the number of cortical astrocytes or to upregulation of GFAP expression in glial cells. Hematoxylin and Eosin staining of cortical cells indicated that there was no significant alteration in total cell numbers in the gray matter of the *Olig2*-ablated cortex (Fig. 6A,B). To examine astrocyte formation in the gray matter, we analyzed expression of GS, which is independent of GFAP expression in astrocytes (Stanimirovic et al., 1999). The number of GS⁻ cells in the superficial layers of the *Olig2*-ablated cortex was comparable to the control (Fig. 6C,D,J), suggesting that there is no significant alteration in the extent of cortical astrocyte formation in the absence of *Olig2*. Intriguingly, GS⁺ cells in the *Olig2*-ablated cortex became GFAP⁺, in contrast to the low level or absence of GFAP expression in the control (Fig. 6F compared with E). Similarly, when examining the expression of another astrocyte marker, S100 β , which co-expresses with *Olig2* in the superficial cortical layers (Fig. 6G), we observed that a population of S100 β cells acquired a high level of GFAP expression in the *Olig2*-ablated cortex, but not in the control (Fig. 6I compared with H). As in the white matter, a reduction in S100 β ⁺ cells was observed in the gray matter (Fig. 6J); however, this is likely to result from the loss of a subset of S100 β ⁺ oligodendroglial lineage cells (Deloulme et al., 2004) in the *Olig2* mutant (Yue et al., 2006).

To further determine if there is an alteration in astrocyte proliferation, we examined the cortex of *Olig2* mutant (*Cko*^G) mice at perinatal stage P14 for BrdU incorporation and expression of the proliferation marker Ki67 (Maeda et al., 2001; Torp, 2002). In the *Olig2* mutant cortex, the number of BrdU⁺ proliferating cells at early postnatal stages P7 and P14 after a 4-hour pulse of BrdU was similar to the control, despite the presence of excessive GFAP⁺ cells (Fig. 6L compared with K; Fig. 6N). Similarly, these cortical GFAP⁺ cells did not express Ki67 (Fig. 6M), suggesting that the high GFAP-expressing cells in the cortex were not in the cycling state at perinatal stages. In addition, the Ki67 and BrdU labeling suggested that there was no significant alteration of proliferation among SVZ progenitor cells (Fig. 6O,P). Together with a normal number of cortical astrocytes, an absence of local proliferation of excessive GFAP⁺ cells suggests that GFAP is upregulated in astrocytes of the superficial layers in the *Olig2*-ablated cortex.

Absence of detectable oligodendrocyte-to-astrocyte conversion in the *Olig2*-ablated cortex

Since oligodendroglial (or O2A) precursors isolated from optic nerves can adopt an astrocytic fate in vitro under certain culture conditions (Raff et al., 1983), we examined whether cortical oligodendroglial cells could acquire ectopic GFAP expression in

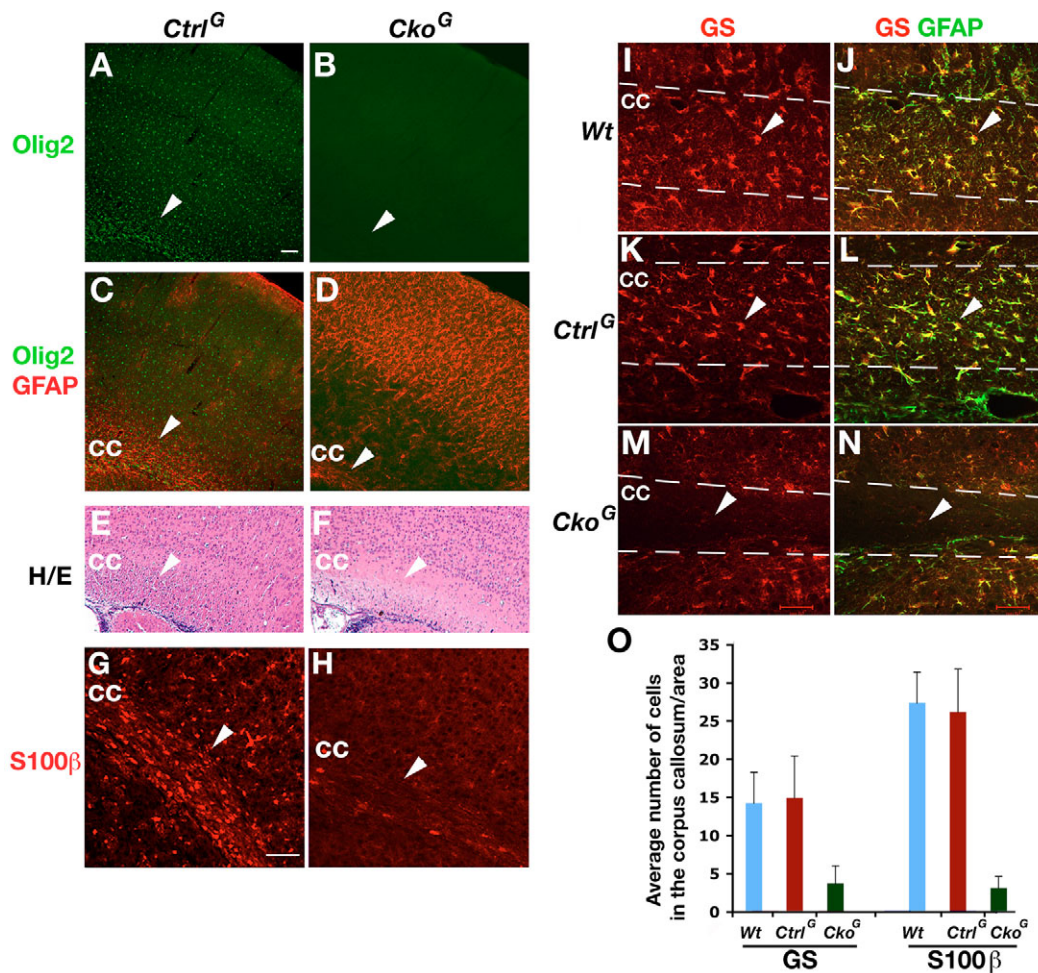


Fig. 3. Astrocyte differentiation deficit in the white matter of the *Olig2*-ablated cortex. (A-D) Expression of GFAP (Red) and *Olig2* (green) was analyzed by immunohistochemistry on coronal brain sections of control (A,C) and *Olig2*-ablated (B,D) mice at P14. Arrowheads indicate white matter. (E,F) Hematoxylin and Eosin (H/E) staining of sagittal sections of control (*Ctrl^G*) and *Olig2*-ablated (*Cko^G*) cortices at P14. (G,H) Cortices of control (*Ctrl^G*) and *Olig2* mutant (*Cko^G*) mice at P14 were immunostained with an anti-S100β antibody. Arrowheads in E-H indicate the corpus callosum (CC). (I-N) Double immunostaining of glutamine synthetase (GS, red) and GFAP (green) in the corpus callosum (arrowheads) of wild-type (I,J), *Ctrl^G* (K,L) and *Olig2* mutant (*Cko^G*; M,N) mice. GS⁺ and GS⁺ GFAP⁺ cells are shown in I,K,M and J,L,N, respectively. (O) Bar chart showing the average number of GS⁺ and S100β⁺ cells per unit area (0.1 mm²) in the corpus callosum of wild-type (wt), *Ctrl^G* and *Olig2* mutant (*Cko^G*) mice (>300 cells counted, *n*=3); bars indicate s.d. Scale bars: 100 μm in A-D; 100 μm in G,H; 50 μm in I-N.

the absence of *Olig2*. Although formation of myelinating oligodendrocytes is virtually abolished, oligodendrocyte precursors (OPCs) that express PDGFαR (Woodruff et al., 2001) and NG2 (also known as *Cspg4* – Mouse Genome Informatics) (Nishiyama et al., 1996) are maintained throughout the *Olig2*-ablated cortex (Yue et al., 2006). They are present within the domain of excessive GFAP expression. When double immunostaining of PDGFαR or NG2 with GFAP was performed in the *Olig2*-ablated cortex at P14 (Fig. 7A,B), we did not detect GFAP expression in PDGFαR⁺ OPCs by confocal imaging (Fig. 7A-A’). Similarly, expression of NG2 was clearly distinct from that of GFAP (Fig. 7B-B’). These observations indicate that OPCs are either not the source of GFAP-expressing cells or in so becoming, they lose their classic OPC markers.

To further determine whether expression of *Gfap* is the consequence of oligodendrocyte lineage cells of a certain stage acquiring ectopic *Gfap* expression in the absence of *Olig2*, we ablated *Olig2* using an oligodendrocyte lineage-specific CNP-Cre knock-in line (Lappe-Siefke et al., 2003), in which Cre is expressed

in all stages of oligodendrocytes including their precursors. *Olig2* ablation by CNP-Cre activity resulted in severe cortical myelination deficit (Fig. 7D compared with C), but without affecting OPC formation or causing significant cell death (data not shown). There was, however, an absence of GFAP upregulation in the cortex at P14 and adulthood in this *Olig2* mutant (Fig. 7E,F), suggesting that persistent cortical GFAP expression is not due to oligodendroglial lineage cells acquiring ectopic GFAP expression in the absence of *Olig2*.

In addition, although *Olig2* ablation by CNP-Cre activity leads to a deficit in oligodendrocyte myelination in the white matter, there was no apparent alteration in white matter astrocyte formation in the mutant mice, as indicated by a comparable number of GFAP⁺ or GS⁺ cells in the white matter in the control and *Olig2 Cko^C* mutant mice (Fig. 7H compared with G). This suggests that the myelination deficit that resulted from *Olig2* ablation in committed oligodendrocyte lineage cells does not lead indirectly to a defect in white matter astrocyte formation.

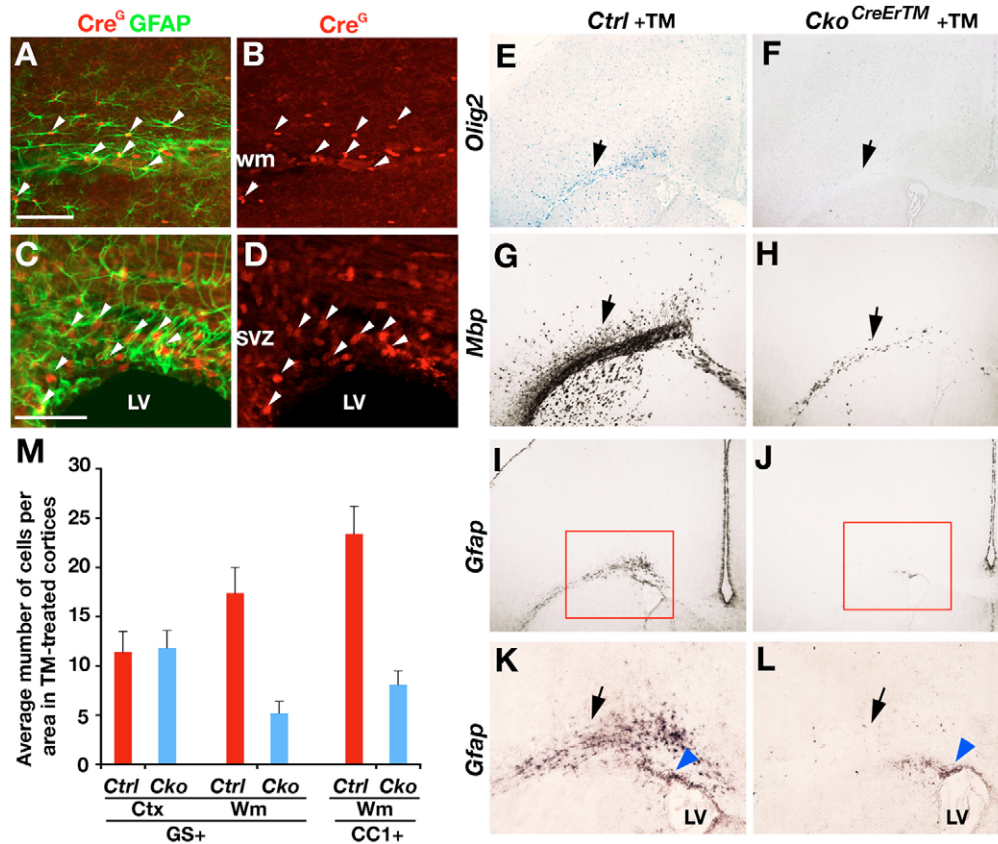


Fig. 4. Effects of neonatal *Olig2* ablation on white matter astrocyte development. (A-D) Cre and GFAP expression in the cerebral white matter (A,B) and SVZ (C,D) of hGFAP-Cre mice at P14 was examined by immunohistochemistry. Arrowheads indicate Cre⁺ cells (B,D) and Cre⁺ GFAP⁺ cells (A,C). LV, lateral ventricle; wm, white matter. (E-L) Control (*Olig2*C/+;CAGGS^{CreErTM}) and *Olig2*Cko;CAGGS^{CreErTM} pups were administered with tamoxifen (TM) for 4 days from P2 to P5, and their brains were harvested at P13. Expression of *Olig2* (E,F), *Mbp* (G,H) and *Gfap* (I,J) was examined by in situ hybridization on coronal sections of the cortex. Boxed areas in I,J are shown at high magnification in K,L, respectively. Arrows and arrowheads indicate the white matter and the SVZ, respectively. (M) Bar chart showing the average number per unit area (0.1 mm²) of GS⁺ cells in the cortex (Ctx) and the white matter (Wm) and CC1⁺ cells in the white matter of TM-treated control (*Olig2*C/+;CAGGS^{CreErTM}) and *Olig2*Cko;CAGGS^{CreErTM} animals (n=3); bars indicate s.d. Scale bars: 100 μm in A for A,B, and C for C,D.

Absence of significant neuronal deficit in the *Olig2*-ablated cortex

In view of the neurological abnormalities in *Olig2*-ablated mutants (Yue et al., 2006), we examined the effects of *Olig2* deletion on cortical neurogenesis, as reactive astrocytes exhibiting elevated GFAP expression can be induced by abnormal neuronal formation and function. For cortical neurons in the *Olig2*-ablated cortex, cortical lamination and the expression of the pan-neuronal marker NeuN (also known as Neuna60 – Mouse Genome Informatics) were comparable with that of control mice (Fig. 8A,B). Since multipotent *Olig2*⁺ progenitor cells are capable of generating certain neuronal populations, including GABAergic interneurons, in the developing cortex (Furusho et al., 2006; He et al., 2001), we further examined interneuron subtype development in the *Olig2*-ablated cortex. Immunoreactivity for the neurotransmitter GABA, reflecting GABAergic interneurons and subcortical afferents, appeared to be normal in the *Olig2* mutant (Fig. 8C,D). In addition, we were unable to detect robust changes in calbindin-expressing interneurons, which are present in superficial layers of cortex within the domain of GFAP upregulation (Fig. 8C,D). Furthermore, there is a lack of marked apoptosis in the cortex of *Olig2* mutant mice as assessed by TUNEL and caspase 3

immunoreactivity (data not shown). Thus, our observations indicate that *Olig2* ablation does not obviously alter cortical neurogenesis.

To examine whether *Olig2* ablation alters neuronal functions in the cortex, potentially accounting for increased GFAP expression in astrocytes, we ablated *Olig2* specifically in early-postmitotic cortical neurons using a synapsin I promoter-driven Cre (Syn1-Cre) transgenic line (Luikart et al., 2005; Zhu et al., 2001). The synapsin I promoter drives Cre recombinase expression in early-postmitotic neurons as early as embryonic day (E) 12.5, the onset of cortical neurogenesis (Luikart et al., 2005; Zhu et al., 2001). Ablation of *Olig2* in early-postmitotic neuronal cells directed by Syn1-Cre did not lead to GFAP upregulation or alter the number of NeuN⁺ neurons in the *Olig2* mutant (*Cko*^S) cortex (Fig. 8F compared with E). Furthermore, when examining axonal proteins such as neurofilament 200 (NF200; also known as Nefh – Mouse Genome Informatics), Tau-1 (Ifne1 – Mouse Genome Informatics) and Gap43, in the cortex of wild-type and *Olig2*-ablated *Cko*^G mice (Fig. 8G-N), we observed normal formation of axonal filaments in *Olig2*-ablated brains. Thus, there is an absence of significant axonal protein deficit in both the gray and white matter of *Cko*^G mice as compared with control mice.

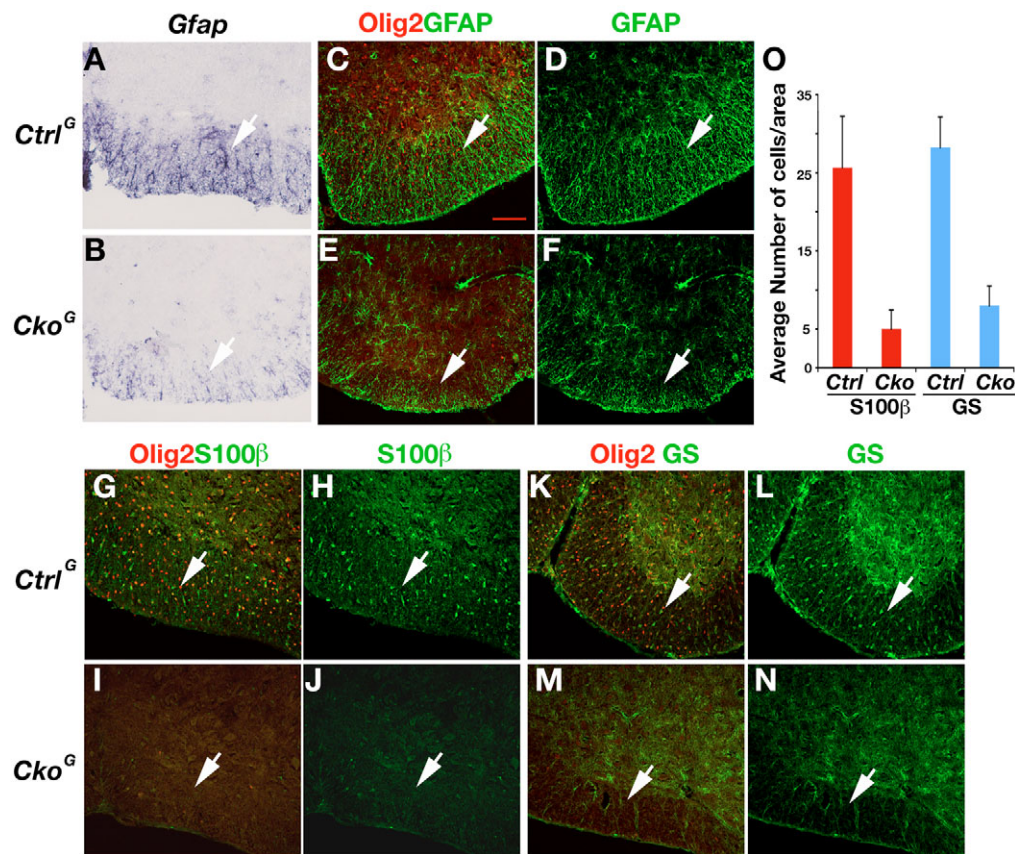


Fig. 5. White matter astrocyte formation deficiency in the *Olig2* mutant spinal cord. In situ hybridization and immunostaining of *Olig2*, GFAP, S100β and GS were performed on frozen spinal sections of *Ctrl*^G and *Cko*^G mice at P14. Arrows indicate the white matter region of the spinal cord. (A,B) mRNA expression of *Gfap* was examined in the control (A) and *Olig2* mutant (B) spinal cord by in situ hybridization. (C-F) Expression of *Olig2* and GFAP was analyzed by immunohistochemistry in the control (C) and *Olig2* mutant (E) spinal cord. GFAP expression in C,E is shown in D,F, respectively. GFAP is strongly expressed in the spinal white matter of the control, but only weakly in the mutant (arrows). (G-N) Expression of *Olig2* and S100β (G,I), and *Olig2* and GS (K,M), were analyzed by immunohistochemistry in the spinal cord of control (G,H,K,L) and *Olig2* mutant (I,J,M,N) mice. Staining of S100β and GS is shown in H,J and L,N, respectively. S100β- and GS-expressing cells are barely detectable in the white matter of *Olig2* mutants (J,N) as compared with controls (H,L). (O) Bar chart showing the average number of S100β⁺ and GS⁺ cells per unit area (0.06 mm²) in the spinal white matter of control and *Olig2* mutant mice ($n=3$); bars indicate s.d. Scale bar: 100 μm in A-N.

A subset of cortical astrocytes with sustained GFAP upregulation is derived from *Olig2*-ablated cells

Since *Olig1* has been shown to be co-expressed with *Olig2* in the same cells (Zhou et al., 2000), we examined *Olig1* expression in the *Olig2*-ablated cortex. *Olig1*⁺ cells were present throughout the gray matter and in numbers comparable to control mice at perinatal stage P8 (Fig. 9A,B). At P14, however, *Olig1* expression in the superficial cortical layers was downregulated compared with the control (Fig. 9D compared with C), coincident with the appearance of *Gfap* upregulation (Fig. 9F compared with E). Intriguingly, *Olig1* expression was maintained in the deep cortical layers (Fig. 9D,F), but markedly reduced in the white matter (Fig. 9B,D, arrowheads). This reciprocal pattern of *Gfap* and *Olig1* expression in the cortex was confirmed by double in situ hybridization labeling (Fig. 9F). These observations suggest that the absence of *Olig2* affects the expression of *Olig1*, either directly or indirectly, and that *Olig1* may cooperate with *Olig2* in regulating cortical astrocyte development.

To further examine whether the cortical astrocytes displaying GFAP upregulation are derived from *Olig2*-ablated cells, we crossed hGFAP-Cre mice with the floxed *Olig2* mutant mice on a

RosaYFP reporter background (Srinivas et al., 2001). Cre-mediated *loxP* site excision simultaneously leads to *Olig2* ablation and activates YFP expression (Fig. 9G). Indeed, in the cortex of triple-transgenic mice (*Olig2*^{Cko};*hGFAP*Cre;*RosaYFP*) at P14, GFAP expression was observed in YFP-expressing cells, indicating that cortical astrocytes with GFAP upregulation or high GFAP expression are derived from *Olig2*-ablated cells exhibiting YFP reporter expression (Fig. 9H). Furthermore, to map the fate of *Olig2*-ablated cells in the cortex, we examined expression of the *Olig2* mRNA 3' untranslated region (3' UTR) outside the floxed *Olig2* coding sequence (Fig. 9G). Expression of the *Olig2*-3' UTR, transcribed from the *Olig2* promoter, represents the cells in which endogenous *Olig2* is normally expressed. In the *Olig2* mutant cortex at P14, essentially all *Olig2*-3' UTR⁺ cells expressed *Gfap* in the superficial cortical layers but not in deep layers (Fig. 9I,J), suggesting that this population of cortical astrocytes with *Gfap* upregulation is derived from presumptive *Olig2*-expressing cells. We observed that not all *Gfap*⁺ cells express *Olig2*-3' UTR. This might be due to the downregulation of the *Olig2* promoter activity in mature astrocytes (Gabay et al., 2003). Alternatively, it is possible that a

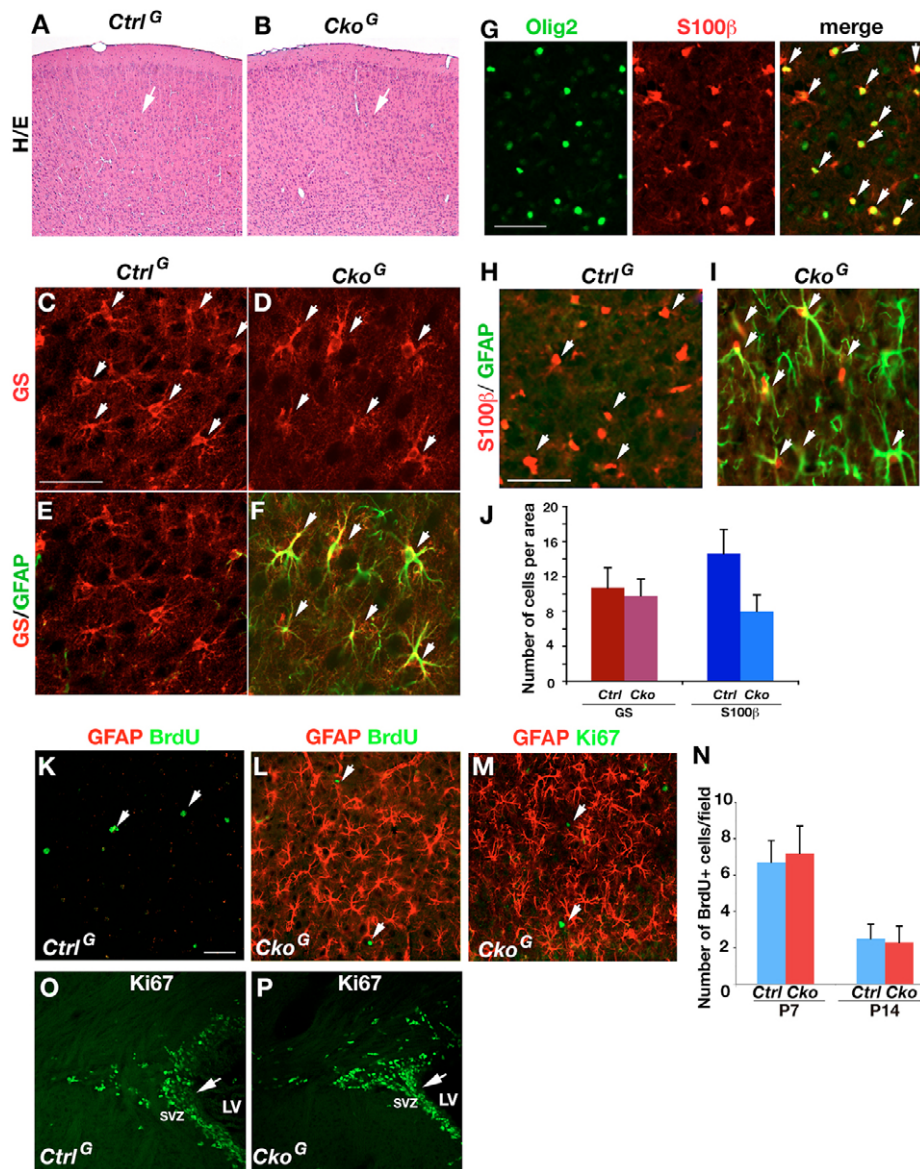


Fig. 6. Formation of astrocytes with GFAP upregulation in the superficial layers of the *Olig2*-ablated cortex. (A,B) Hematoxylin and Eosin (H/E) staining of sagittal sections of control (*Ctrl*^G) and *Olig2*-ablated (*Cko*^G) cortices at P14. Arrows indicate the superficial cortical layers. (C-F) Cortices of control and *Cko*^G mice at P14 were immunolabeled with antibodies against GS and GFAP. Arrows indicate GS⁺ cells (C,D) and GS GFAP co-expressing cells (F). (G-I) Cortices of control (G,H) and *Cko*^G (I) mice at P14 were immunostained with Olig2, S100β and GFAP. Regions from superficial layers of the cortex are shown. Arrows in G indicate cells co-labeled with Olig2 and S100β. Arrows in H,I indicate S100β⁺ in the control and S100β GFAP co-expressing cells in the *Olig2* mutant, respectively. (J) Bar chart showing the average number of GS⁺ and S100β⁺ cells per unit area (0.1 mm²) in the superficial layers of control and *Olig2* mutant (*Cko*) mice (>400 cells counted, *n*=3); bars indicate s.d. (K-M) Cortices of control and *Cko*^G animals at P14 were immunolabeled with GFAP (K-M), Ki67 (M) and BrdU (K,L) after a 4-hour pulse of BrdU administration before sacrifice. Arrows indicate BrdU⁺ and Ki67⁺ cells in the cortex, respectively. (N) Bar chart indicating the gross number of BrdU⁺ cells per field (0.4 mm²) in the superficial layers of control and *Olig2*-ablated (*Cko*) cortices (>200 cells counted, *n*=3) at P7 and P14; bars indicate s.d. (O,P) Immunostaining of Ki67 in the SVZ of the control (O) and *Olig2* mutant (P) brains. Scale bars: 100 μm.

subset of GFAP⁺ cortical astrocytes such as those being generated ventrally would never have expressed *Olig2* during their development. Nonetheless, co-expression of GFAP with *Olig2*-3' UTR and the YFP reporter in the *Olig2*-ablated cortex suggests a cell-autonomous function of *Olig2* in cortical astrocyte development.

DISCUSSION

Astrocytes comprise a large and heterogeneous group of neural cells in the CNS. This diversity is underscored by their different structural, biochemical and electrophysiological properties, which are related to their functions (Kimelberg, 2004; Reichenbach and Wolburg, 2005; Walz, 2000). At present, the regulatory mechanisms behind gray and white matter astrocyte development are not fully understood. With tissue-, cell type-, and temporal-specific *in vivo* mutagenesis, our present studies unveil a novel and crucial role for *Olig2* in white matter astrocyte formation, about which very little is known, while illuminating divergent transcriptional regulatory mechanisms and developmental sources for gray and white matter astrocytes in the developing brain.

Cell-autonomous function of *Olig2* in cortical astrocyte development

Previous studies indicate that *Olig2* is initially expressed in a specialized domain (pMN) of the embryonic ventral spinal cord that gives rise to oligodendrocytes and motoneurons (Lu et al., 2000; Takebayashi et al., 2000; Zhou et al., 2000). In the adult brain, *Olig2* is colocalized with markers for oligodendrocytes but not astrocytes, suggesting that *Olig2* is not expressed in mature astrocytes of the cortex in adulthood (Lu et al., 2000; Zhou et al., 2000). Although most astrocytes do not express a detectable level of GFAP in the adult cortex, GFAP expression can be detected in a population of immature cortical astrocytes at early postnatal stages. In the present study, however, we found that immature GFAP⁺ astrocytes in the developing cerebral gray and white matter at early neonatal stages express a high level of *Olig2*. *Olig2* expression subsequently decreases in astrocytes beginning at the perinatal stage and is completely absent from mature astrocytes at adulthood. Thus, *Olig2* appears to be expressed transiently in developing immature astrocytes at early postnatal stages but is downregulated progressively as astrocytes mature in the cortex (Fig. 9K).

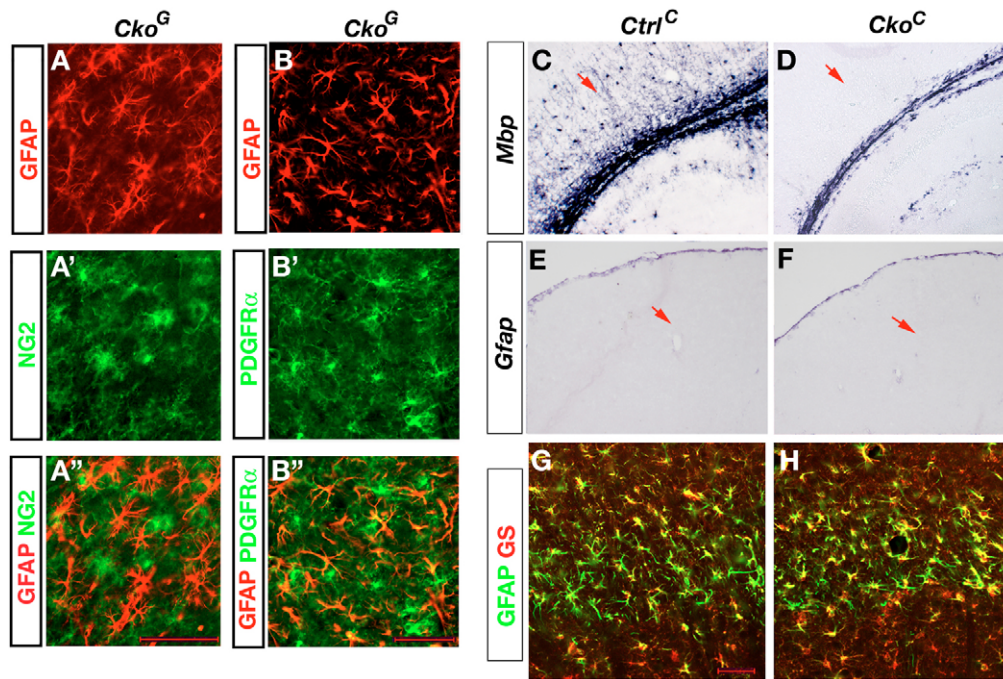


Fig. 7. Absence of ectopic GFAP expression in oligodendroglial precursors of the *Olig2*-ablated cortex. (A-B'') The *Olig2* (*Cko^G*) mutant mouse cortex at P14 was immunostained with GFAP and OPC markers NG2 (A-A'') and PDGFR α (B-B''). Regions of the superficial layers of *Olig2*-ablated cortices are shown. Expression of GFAP is distinct from that of OPC markers. **(C-F)** Coronal sections of control (*Ctrl^C*) and *Olig2**Cko*; *CNP^{Cre}* (*Cko^C*) mutant cortices at P14 were subjected to in situ hybridization labeling for *Mbp* (C,D) and *Gfap* (E,F). Arrows indicate the cortical gray matter. **(G,H)** The corpus callosum of control (*Ctrl^C*) and *Olig2* (*Cko^G*) mutant cortices at P14 were immunostained with antibodies against GFAP (green) and GS (red).

In the absence of *Olig2*, astrocyte formation in the cortex is severely compromised in the white matter, whereas there is a sustained upregulation of GFAP in a population of astrocytes in the gray matter (Fig. 9K). Together with *Olig2* expression in immature developing astrocytes and their progenitors in the SVZ in the developing brain, our fate-mapping study further indicates that a population of GFAP⁺ cortical astrocytes is derived from *Olig2*-ablated cells expressing the *Olig2*-3' UTR. Thus, these observations suggest a cell-autonomous function of *Olig2* in cortical astrocyte development and that abnormal astrocyte development is, at least in part, attributed to the direct loss of *Olig2* function in developing astrocytes and their progenitors.

Differential requirement of *Olig2* function in the development of gray and white matter astrocytes in the cortex

In vitro studies indicate that *Olig2* inhibits astrocyte differentiation (Fukuda et al., 2004; Gabay et al., 2003). Our in vivo mutagenesis analysis, however, indicates that the function of *Olig2* in astrocyte differentiation does not appear to be uniform, but rather region-specific within the brain. In the gray matter, *Olig2* ablation appears to alleviate likely GFAP repression in a population of presumptive *Olig2*⁺ cortical astrocytes and leads to persistent GFAP expression throughout adulthood (Fig. 9K). Abnormal GFAP upregulation in developing astrocytes of the *Olig2* mutant cortex is consistent with a GFAP repression function of *Olig2* suggested by in vitro studies. By contrast, our data indicate that *Olig2* function is required for astrocyte formation in the white matter of the brain and the spinal cord. The mechanisms underlying the deficit of white matter astrocytes in *Olig2* mutants are not entirely clear at present. It is

possible that the astrocytes originally destined for white matter somehow end up in the cortex. This will be resolved in future cell lineage tracing studies. The requirement for *Olig2* in white matter astrocyte formation is unlikely to be due to a loss of neural progenitor/stem cells because GFAP⁺ precursor cells in the SVZ of *Olig2* mutants are maintained at a comparable level to the control. In addition, we do not observe any significant alteration in the number of proliferative cells (Ki67⁺ or BrdU⁺) or in cell death in the cortex and the white matter of *Olig2* mutants at early postnatal stages, although this does not exclude the possibility of astrocyte precursor proliferation deficit, which might be obscured amidst a significant number of proliferative OPCs and hampered by the lack of well-defined astrocyte precursor markers. Temporally specific ablation suggests that *Olig2* is required for the differentiation of neural progenitor cells or developing immature astrocyte precursors at neonatal stages into mature astrocytes in the cerebral white matter. Alternatively, hypomyelinated axons may create an environment incompatible with white matter astrocyte formation as there is a myelination deficit in the white matter of *Cko^G* mice (Yue et al., 2006). However, we do not observe significant, if any, deficit in axonal protein expression or axonal degeneration in the white matter of *Cko^G* mice. In addition, dysmyelination in the white matter caused by *CNP*-*Cre*-mediated *Olig2* ablation in oligodendrocyte lineage cells including oligodendrocyte progenitors does not cause GFAP upregulation or alter astrocyte formation, indicating that hypomyelination does not preclude formation of astrocytes and their presence in the white matter. Thus, hypomyelination alone is insufficient to account for the deficit in white matter astrocyte formation. Conversely, given that *Olig2* is required for white matter astrocyte formation, there remains the possibility that

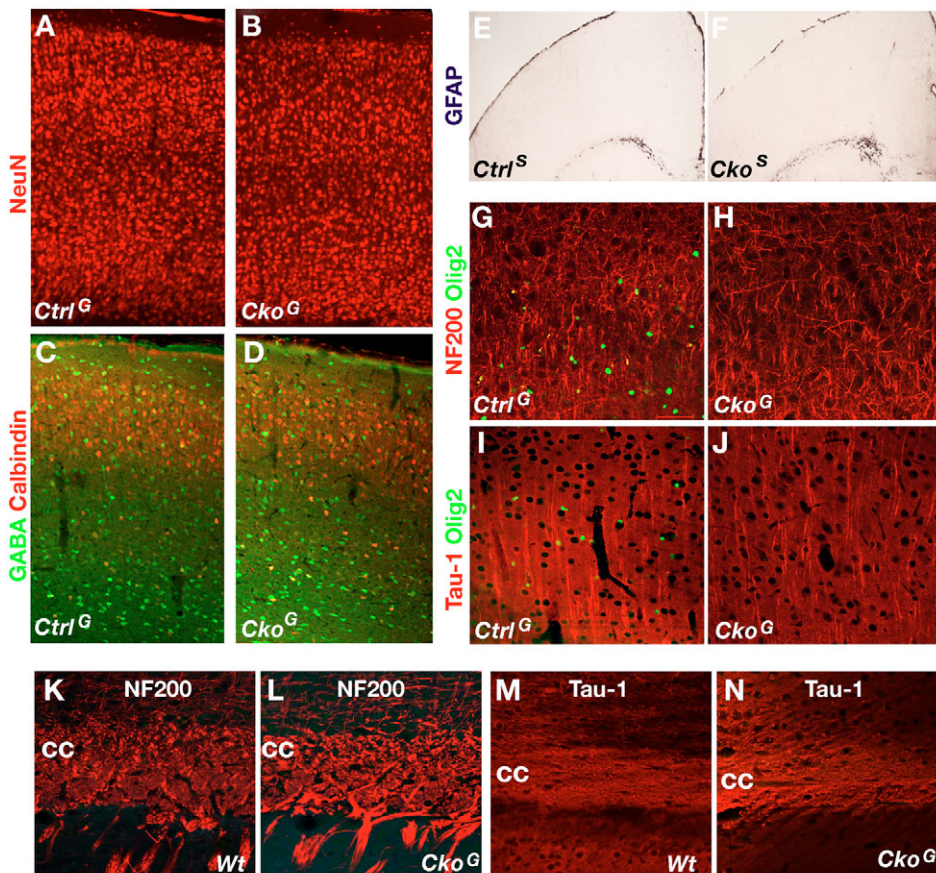


Fig. 8. Absence of significant neuronal deficit in the *Olig2*-ablated cortex. (A–D) The cortices from control (*Ctrl^G*) and *Olig2*-ablated (*Cko^G*) mice at P14 were immunostained with antibodies to NeuN (red), calbindin (red) and GABA (green), as indicated. (E, F) *Gfap* expression was examined by in situ hybridization in the forebrain of control *Olig2^{C/+};Syn1^{Cre}* (*Ctrl^S*) and *Olig2^{Cko};Syn1^{Cre}* (*Cko^S*) mice. (G–J) Immunostaining of *Olig2* (green) and axonal proteins NF200 (red) and Tau-1 (red) in the cortex of control (*Ctrl^G*; G, I) and *Olig2* mutant (*Cko^G*; H, J) mice. (K–N) Immunostaining of axonal proteins NF200 and Tau-1 in the corpus callosum (CC) of wild-type (K, M), and *Olig2* mutant (*Cko^G*; L, N) mice. The more intense staining in the *Cko^G* mice, as compared with the wild-type mice, might be due to the fact that dysmyelinating axons in the *Olig2* mutant are more accessible to the antibodies.

oligodendrocyte deficit in the *Olig2* mutant might be caused in part by the loss of white matter astrocytes, which are normally generated prior to oligodendrocytes. This possibility will be addressed further in the future by examining oligodendrocyte formation in the animals with astrocyte-specific deficits. Nonetheless, because *Olig2* expression is observed in a population of developing GFAP⁺ white matter astrocytes, the differentiation deficit of white matter astrocytes is, at least in part, attributed to the loss of *Olig2* function in developing astrocytes and their progenitors.

Although *Olig2* loss at early stages may affect the development of all cortical astrocytes, we only observed *Gfap* upregulation in the superficial layers and not in the deep layers of the *Olig2*-ablated cortex. Our observation suggests that astrocytes populating different layers differ in their responses to *Olig2* ablation in their precursors. In addition, *Olig2* ablation may differentially affect expression of *Gfap* repressors such as *Olig1* in different cortical layers. The *Gfap* upregulation appears to occur in parallel with the downregulation of *Olig1* in the *Olig2*-ablated cortex. The downregulation of both *Olig1* and *Olig2* is likely to contribute to *Gfap* upregulation in the superficial layers. The absence of *Gfap* upregulation in the deep cortical layers may be attributable to the expression of *Olig1*, which, like *Olig2*, could repress *Gfap* promoter activity in these cells (Xin et al., 2005). This is consistent with observations in the embryonic spinal cord where increased GFAP expression was only observed with both *Olig1*- and *Olig2*-null mutations (Zhou and Anderson, 2002). Therefore, our observations indicate that gray and white matter astrocytes differ not only in their spatial locations and morphologies but also in their transcriptional regulation, suggesting that astrocytes are not created equally in the developing CNS.

Temporally specific *Olig2* ablation reveals diverse developmental sources for cortical gray and white matter astrocyte subpopulations

Cortical astrocytes form from at least two different origins – namely, radial glial cells at early embryonic stages and SVZ progenitors at early postnatal stages (Goldman, 2004). *Olig2* ablation by inducible Cre activity at early postnatal stages leads to severe astrocyte differentiation deficit specifically in the white matter. This suggests that postnatal progenitor cells, which are likely to reside in the SVZ and the developing white matter, contribute significantly to the formation of white matter astrocytes in the developing brain. This observation is in keeping with the inhibitory effects of *Olig2* on astrocyte differentiation when a dominant-interfering form of *Olig2* was introduced into SVZ progenitors at postnatal stages (Marshall et al., 2005). However, Marshall et al. (Marshall et al., 2005) provided evidence that *Olig2* function is required for cortical gray matter astrocyte formation, in contrast to our present study showing that *Olig2* is required for white matter astrocyte generation. The reasons for this discrepancy are unclear. It is possible that the effects of genetic *Olig2* ablation might differ from those of the dominant-interfering form of *Olig2*, or that the timing and cell types where *Olig2* function is perturbed diverge between these two approaches.

Interestingly, postnatal ablation of *Olig2* does not lead to GFAP upregulation in the gray matter. By contrast, *Olig2* ablation with hGFAP-Cre activity, which is present in cortical progenitors at both early embryonic and postnatal stages, results in not only a defect in white matter astrocyte formation, but also in GFAP upregulation in a subpopulation of cortical astrocytes. By comparison, region-specific alteration of astrocyte subpopulations suggests that there might be different sources for cortical astrocyte development, with

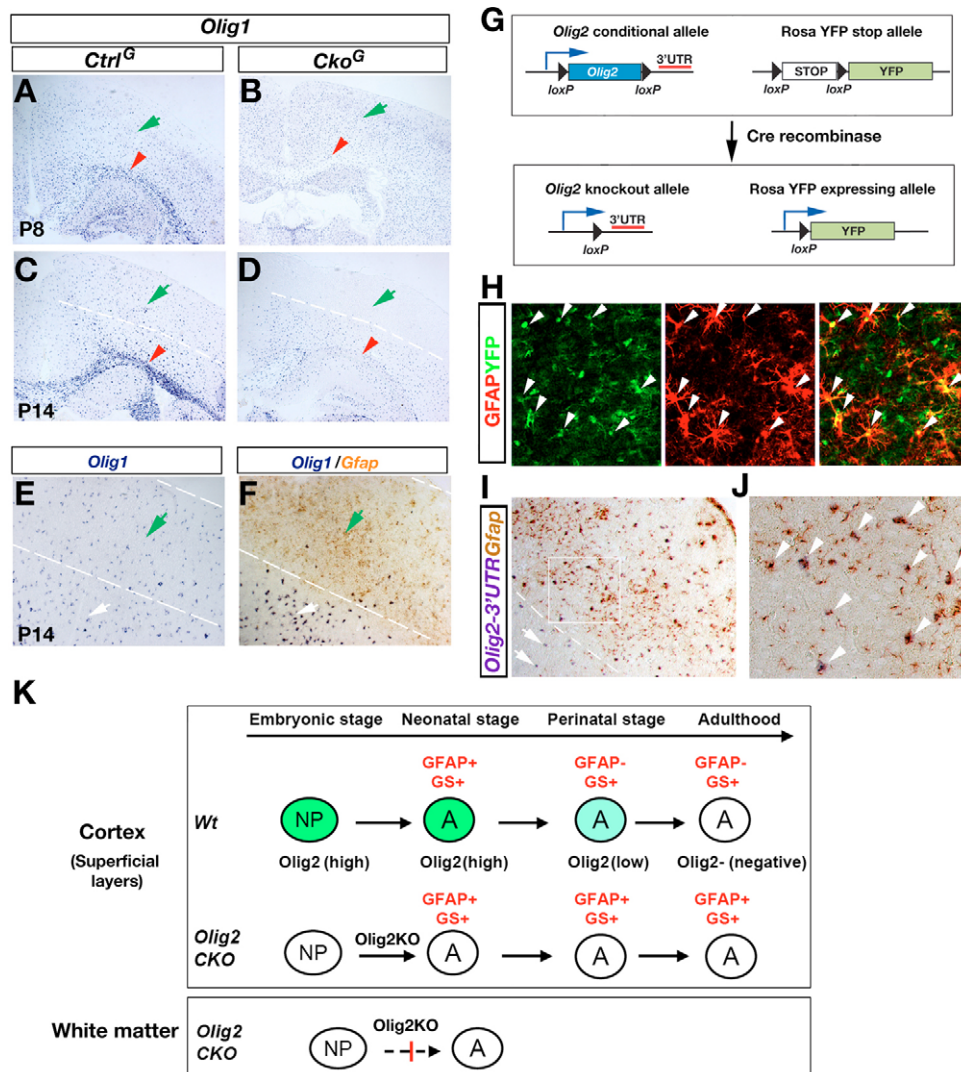


Fig. 9. A population of cortical astrocytes with GFAP upregulation is derived from *Olig2*-ablated cells. (A-D) Expression of *Olig1* was examined by in situ hybridization in the coronal section of control and *Olig2*-ablated *Cko^G* cortices at P8 (A,B) and P14 (C,D). Arrowheads above and below the dashed line in indicate the superficial layers and the white matter in the cortex, respectively. (E,F) In situ hybridization for *Olig1* and *Gfap* in the gray matter. Arrows above and below the longer dashed line indicate the superficial and deep cortical layers, respectively. (G) Schematic showing fate-mapping of *Olig2*-ablated cells after hGFAPCre-mediated recombination. Breeding of hGFAP-Cre mice with *Olig2*-floxed mice carrying the RosaYFP reporter gene (Srinivas et al., 2001) generated offspring with *Olig2* ablation and activation of YFP expression in the same cells after Cre-mediated excision of *loxP* sites (black triangles). Red line indicates *Olig2*-3' UTR. (H) Immunostaining with antibodies to YFP and GFAP in the superficial cortical layers of *Olig2*-ablated triple-transgenic mice at P14. Arrowheads indicate cells co-labeled with YFP and GFAP. (I,J) Expression of *Olig2*-3' UTR and *Gfap* was examined by double in situ hybridization on sections of the *Olig2*-ablated cortex at P14. Arrows in I indicate *Olig2*-expressing cells in the deep cortical layers. Boxed area in I is shown at high magnification in J. Arrowheads in J indicate cells co-expressing *Olig2*-3' UTR and *Gfap*. (K) Schematic depicting cortical astrocyte development in relation to *Olig2* expression. In the normal developing brain, *Olig2* is expressed in cortical neural progenitor cells (NP). At early postnatal stages, *Olig2* is expressed in immature developing astrocytes (A) at a high level. Beginning at postnatal week 2, the *Olig2* expression level is reduced in astrocytes and GFAP expression becomes undetectable. In adulthood, *Olig2* expression is absent in mature astrocytes, suggesting that the *Olig2* expression level is downregulated in cortical astrocytes during the process of astrocyte maturation in brain development. GFAP downregulation in mature astrocytes suggests that other factors also negatively regulate GFAP expression in adulthood. In the absence of *Olig2*, GFAP expression is sustained or upregulated in a population of cortical astrocytes throughout adulthood. By contrast, *Olig2* ablation results in a defect in the formation of white matter astrocyte subpopulations (lower panel).

a major contribution of early embryonic cortical progenitors to gray matter astrocytes and a significant contribution of postnatal progenitor cells to white matter astrocytes.

The presence of residual astrocytes in the white matter of *Olig2* mutant mice suggests that *Olig2* might only be required for the formation of a subset of astrocytes in the white matter. Alternatively, other sources of astrocytes might contribute to the development of

white matter astrocytes. The exact source for the astrocytes that remain in the white matter is presently unknown; however, as hGFAP-Cre activity is mainly active in dorsal cortical progenitor cells (Yue et al., 2006), ventrally derived astrocytes may migrate and contribute to astrocyte formation in the white matter. Together, our observations suggest that diverse developmental sources for gray and white matter astrocytes might exist in the developing CNS.

Olig2 function in cortical neurogenesis

Although there is a deficit in motoneuron formation in the spinal cord of *Olig2*-null mice, *Olig2* ablation in cortical progenitor cells and early-postmitotic neurons does not lead to a significant abnormality in cortical neurogenesis. The lack of any overt alteration in cortical neuronal formation is rather surprising because recent studies indicate that expression of dominant-negative forms of *Olig2* leads to an increase in neurogenesis during development (Marshall et al., 2005) and to new neuron formation in response to injury (Buffo et al., 2005). One potential explanation for this discrepancy is that *Olig2* ablation in the cortical progenitor cells might lead to an increase in neuronal formation, with newly formed neurons subsequently eliminated through programmed cell death. We do not observe, however, any appreciable increase in cell death or the alteration of neural patterning in the cortex. Alternately, the dominant-negative forms of *Olig2* used could potentially interact with other transcriptional regulators such as neural bHLH factors in addition to inhibiting *Olig2*, thereby altering cortical neurogenesis. Nonetheless, the lack of any obvious neuronal abnormality in the *Olig2*-ablated cortex suggests that elimination of *Olig2* function does not significantly alter neurogenesis in the cortex, at least under the present ablation conditions, in contrast to motoneuron formation in the developing spinal cord where *Olig2* plays an essential role.

Distinct and convergent developmental requirements for cortical oligodendroglial and astroglial lineage development

Since *Olig2* negatively regulates astrocyte differentiation in neural progenitor cells in vitro, this raises the possibility that *Olig2* might regulate an intrinsic fate switch for glial subtypes. Intriguingly, in the *Olig2*-ablated cortex, OPCs present within the domain of ectopic GFAP expression do not acquire GFAP expression. Furthermore, *Olig2* ablation in oligodendrocyte lineage cells with the oligodendrocyte-specific CNP-Cre line does not lead to upregulation of GFAP in the cortex. Together, these data suggest that committed oligodendroglial lineage cells in the cortex do not adopt an astrocytic fate in the absence of *Olig2*, although they fail to further differentiate into mature myelinating oligodendrocytes (Yue et al., 2006).

Conversely, *Olig2* ablation leads to a decrease in both oligodendrocytes and astrocytes in the white matter, suggesting that there is a common requirement for *Olig2* for glial subtype differentiation in the white matter tract. Intriguingly, OPCs isolated from the neonatal rat optic nerve tract can differentiate into fibrous astrocytes if cultured under certain conditions (Raff et al., 1983). The fact that *Olig2* is required for both oligodendrocyte and white matter astrocyte differentiation suggests that oligodendrocytes and astrocytes in the white matter might develop from the same source, although further evidence for this cell lineage connection needs to be obtained. Since astrocytes in the gray matter are formed in the absence of *Olig2*, our results suggest that the development of astrocytes in the gray and white matter are regulated by distinct developmental mechanisms, although there might be a convergent regulatory mechanism between oligodendrocytes and astrocytes in the white matter during brain development. Thus, spatiotemporal-specific *Olig2* ablation reveals divergent regulatory mechanisms for the development of gray and white astrocyte subpopulations and highlight important roles of *Olig2* in glial subtype development.

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