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The homeobox gene *Gsx2* controls the timing of oligodendroglial fate specification in mouse lateral ganglionic eminence progenitors

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SUMMARY

The homeobox gene *Gsx2* has previously been shown to be required for the specification of distinct neuronal subtypes derived from lateral ganglionic eminence (LGE) progenitors at specific embryonic time points. However, its role in the subsequent generation of oligodendrocytes from these progenitors remains unclear. We have utilized conditional gain-of-function and loss-of-function approaches in order to elucidate the role of *Gsx2* in the switch between neurogenesis and oligodendrogenesis within the embryonic ventral telencephalon. In the absence of *Gsx2* expression, an increase in oligodendrocyte precursor cells (OPCs) with a concomitant decrease in neurogenesis is observed in the subventricular zone of the LGE at mid-stages of embryogenesis (i.e. E12.5-15.5), which subsequently leads to an increased number of *Gsx2*-derived OPCs within the adjacent mantle regions of the cortex before birth at E18.5. Moreover, using *Olig2*^{cre} to conditionally inactivate *Gsx2* throughout the ventral telencephalon with the exception of the dorsal (d)LGE, we found that the increase in cortical OPCs in *Gsx2* germline mutants are derived from dLGE progenitors. We also show that *Ascl1* is required for the expansion of these dLGE-derived OPCs in the cortex of *Gsx2* mutants. Complementing these results, gain-of-function experiments in which *Gsx2* was expressed throughout most of the late-stage embryonic telencephalon (i.e. E15.5-18.5) result in a significant decrease in the number of cortical OPCs. These results support the notion that high levels of *Gsx2* suppress OPC specification in dLGE progenitors and that its downregulation is required for the transition from neurogenesis to oligodendrogenesis.

KEY WORDS: Cell-fate specification, Oligodendrocyte precursor cell, Olig2, Pdgfrα, Sox10, Telencephalon, Mouse

INTRODUCTION

The vast assortment of neuronal subtypes that exist in the mature central nervous system (CNS) has driven a considerable amount of research to focus on the developmental mechanisms that contribute to this diversity (see e.g. Hobert et al., 2010). As a result, much has been learned about neurogenesis; however, the molecular mechanisms that control the switch between neurogenesis and gliogenesis (e.g. oligodendrogenesis) within neural progenitors remain unclear.

Until recently, it was thought that oligodendrocytes were generated by oligodendrocyte precursor cells (OPCs) that arise exclusively from neural progenitors in the ventral half of the CNS (Kessaris et al., 2001; Tekki-Kessaris et al., 2001). However, subsequent work has suggested that OPCs are generated in a ventral-to-dorsal temporal manner at spinal cord and hindbrain (Fogarty et al., 2005; Vallstedt et al., 2005) as well as telencephalic levels (Kessaris et al., 2006). In the telencephalon, it appears that OPCs are first generated from neural progenitors in the ventralmost region, the medial ganglionic eminence (MGE) domain around embryonic day (E) 12.5, followed by the lateral ganglionic eminence (LGE) at later embryonic stages (i.e. E15 and onward) and finally the cortical germinal zone around birth. Thus, according to this model, neural progenitors located at different positions along the dorsoventral (DV) axis of the telencephalon would transition from a neurogenic to a gliogenic state at specific developmental time points. However, the molecular mechanisms underlying the temporal control of such fate changes remain largely unknown.

Kessaris et al. (Kessaris et al., 2006) utilized a genetic fate map of *Gsx2*-expressing cells to follow LGE progenitors and demonstrated that many of these cells give rise to OPCs throughout the telencephalon. Gsx2 (also known as Gsh2) is expressed in a high-dorsal to low-ventral gradient in ventricular zone (VZ) multipotent progenitors of the LGE and MGE, respectively (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001; Waclaw et al., 2009). This gradient is refined over time as *Gsx2* downregulates in more ventral regions and gets confined largely to the dorsal LGE (dLGE) by E18.5. A temporal requirement for *Gsx2* in the specification of LGE-derived neuronal subtypes has recently been described (Waclaw et al., 2009). Despite the well-documented fact that *Gsx2* is required for neurogenesis, the results from the above-mentioned genetic fate map (Kessaris et al., 2006) indicate that many *Gsx2*-expressing progenitors ultimately give rise to OPCs at later stages of development. What remains unclear is the role of *Gsx2* in this transition from neurogenesis to oligodendrogenesis. A previous study has shown that *Gsx2* mutants exhibit ectopic expression of the OPC marker *Pdgfra* within the VZ of the ventral telencephalon at E12.5 (Corbin et al., 2003). It is important to note that expression of Pdgfrα, as well as Olig2, within VZ cells is in multipotent progenitors and not necessarily indicative of OPCs. It is not until cells expressing these markers reach the subventricular zone (SVZ) and mantle regions that they are considered to be OPCs (Woodruff et al., 2001). Interestingly, the refinement of *Gsx2* expression to the dLGE during embryogenesis (Waclaw et al., 2009)

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correlates temporally and spatially with the ventral-to-dorsal emergence of OPCs (Kessaris et al., 2006). These observations suggest that high levels of *Gsx2* may negatively regulate oligodendrogenesis, and thus we decided to further explore this possibility.

By manipulating *Gsx2* expression, we have demonstrated that in addition to promoting neurogenesis, *Gsx2* also inhibits OPC specification and thus controls the timing of oligodendrogenesis in LGE progenitors. Specifically, our results show that loss of *Gsx2* results in a transient respecification of dLGE progenitors at E14-15 towards an OPC fate. These ectopic OPCs in the dLGE then require *Ascl1* to expand, which leads to increased LGE-derived OPCs in the adjacent mantle regions of the cortex at birth. Conversely, misexpression of *Gsx2* in telencephalic progenitors from E15 to birth resulted in a significant reduction in cortical OPCs. From these results, we propose that the timing of OPC specification from LGE progenitors is dependent on the downregulation of *Gsx2* at late embryonic/early postnatal stages.

MATERIALS AND METHODS

Animals

The *enhanced green fluorescent protein* (*EGFP*) allele of *Gsx2* was genotyped as described previously (Wang et al., 2009). $Gsx2^{EGFP/RA}$ mutant embryos were generated by breeding $Gsx2^{EGFP/+}$ mice with $Gsx2^{RA/+}$ mice. $Gsx2^{RA/+}$, $Gsx2^{flox}$ embryos were genotyped as previously described in (Waclaw et al., 2009). $Olig2^{Cre/+}$ (Dessaud et al., 2007) mice were provided by Y. Yoshida (Children's Hospital Medical Center, Cincinnati, OH, USA) with permission from T. Jessell (Columbia University, New York, NY). The $Olig2^{Cre}$ allele was identified using the primers Jcre5, 5′-GCGGTCTG-GCAGTAAAAACTATC-3′ and Jcre3, 5′-CCATGAGTGAACGAAC-CTGG-3′. $Olig2^{Cre/+}$ and $Gsx2^{flox/flox}$ mice were crossed to produce $Olig2^{Cre/+}$; $Gsx2^{flox/+}$ double heterozygotes, which were then crossed with $Gsx2^{flox/flox}$ mice to generate $Olig2^{Cre/+}$; $Gsx2^{flox/flox}$ conditional mutants. *Ascl1* mice (Guillemot et al., 1993) were genotyped as previously described (Casarosa et al., 1999). Both $Gsx2^{EGFP/+}$ mice and $Gsx2^{RA/+}$ mice were bred with $Ascl1^{+/-}$ mice to generate $Gsx2^{EGFP/+}$; $Ascl1^{+/-}$ and $Gsx2^{RA/+}$; $Ascl1^{+/-}$ double heterozygotes. These double heterozygotes were then bred with each other in order to generate $Gsx2^{EGFP/RA}$; $Ascl1^{-/-}$ double mutant embryos. $Foxg1^{tTA/+}$ (Hanashima et al., 2002), and *tet-O-Gsx2-IRES-EGFP*(*IE*) embryos and adults were generated and genotyped as described previously (Waclaw et al., 2009). Doxycycline hyclate (Dox, Sigma) was used in the drinking water of pregnant females at 0.02 mg/ml beginning at E7 and removed 4 days later (i.e. E11), resulting in repression of the transgene ($Foxg1^{tTA/+}$; *tetO-Gsx2-IE*) until ~E15.

Embryos were staged by designating the morning of vaginal plug detection as E0.5. At least three embryos of each genotype were analyzed at each stage for every marker used. Animal protocols were approved by the Cincinnati Children's Hospital Medical Center Institutional Animal Care and Use Committee in accordance with NIH guidelines.

Histological analysis

Embryos were fixed in 4% paraformaldehyde at 4°C overnight, thoroughly rinsed in PBS, and cryoprotected in 30% sucrose in PBS before sectioning on a cryostat at 12 μm. Immunostaining was performed on slide-mounted sections. Primary antibodies were used at the following concentrations: guinea pig anti-Ascl1 (1:10,000, provided by J. Johnson, UT Southwestern Medical Center, Dallas, TX, USA), rabbit anti-Dlx (1:500, provided by J. Kohtz), rabbit anti-GFP (1:1000, Invitrogen), goat anti-GFP (1:3000, Abcam), rabbit anti-Gsx1/2 (1:2000, provided by M. Goulding, Salk Institute, La Jolla, CA, USA), rabbit anti-Gsx2 (1:5000; Toresson et al., 2000), rabbit anti-Olig2 (1:2000, Millipore), goat anti-Pax6 (1:200, Santa Cruz), rabbit anti-Pdgfrα (1:200, Santa Cruz), goat anti-Sox10 (1:200, Santa Cruz), goat anti-Sp8 (1:8000, Santa Cruz), rabbit anti-Tbr1 (1:2000, Millipore). A 2-hour incubation in secondary antibodies used for fluorescent immunostaining were: donkey anti-goat antibodies conjugated to Cy2, Cy3 or Cy5 (1:200, Jackson ImmunoResearch) and donkey anti-rabbit antibodies conjugated to Cy2, Cy3 or Cy5 (1:200, Jackson ImmunoResearch). A tyramide amplification kit (Invitrogen, T20932) was used to detect EGFP protein in all EGFP stains associated with the $Gsx2^{EGFP/+}$ allele.

Quantification

Sox10, Olig2 and Pdgfrα OPCs within the cortex were quantified manually using a cell counter in images of three adjacent rostrocaudal sections of E18.5 embryos. To account for any variation in cortical size, areas were measured and OPC numbers were normalized and compared as cells per millimeter squared (mm^2). In fact, a recent study by Teissier et al. (Teissier et al., 2012) showed that *Gsx2* mutants have a slightly thicker cortex than those of controls. OPCs double labeled with EGFP and Pdgfrα or Olig2 or Sox10 were quantified in the same manner, while switching between red and green channels in Photoshop. At least three embryos were analyzed for each genotype.

RESULTS

Altered expression of neurogenic and gliogenic factors in the *Gsx2* mutant LGE

The loss of *Gsx2* is known to reduce neurogenesis of LGE-derived striatal projection neurons and olfactory bulb interneurons (Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001; Waclaw et al., 2009). It has been demonstrated that some of the dorsalmost mutant LGE progenitors are respecified towards ventral pallial neuronal fates (e.g. lateral amygdala) (Yun et al., 2001; Waclaw et al., 2010). This may partly explain the reduced LGE neurogenesis; however, another possibility would be that in the absence of *Gsx2* LGE progenitors switch from a neurogenic to gliogenic potential, specifically oligodendroglia.

In order to address the possibility of a neuronal to oligodendroglial fate switch, we examined both neurogenic and gliogenic factors within the *Gsx2* mutant LGE at E12.5. For these studies, $Gsx2^{EGFP/+}$ mice, which have an IRES-EGFP interrupting the first exon of *Gsx2*, were used, thus replacing *Gsx2* expression with EGFP (Wang et al., 2009). Gsx2 expression is restricted to the VZ; however, the EGFP protein perdures in the progeny of these cells, thus providing short-term lineage tracing of *Gsx2* progenitors into the SVZ and surrounding mantle regions (Wang et al., 2009). $Gsx2^{EGFP/+}$ mice were bred with mice containing a null allele of Gsx2 ($Gsx2^{RA/+}$) (Waclaw et al., 2009) in order to generate $Gsx2^{EGFP/RA}$ germline mutants. $Gsx2^{EGFP/+}$ embryos from the same litters were used as controls. Having only one allele of EGFP in both the control and *Gsx2* mutant ensures that any differences in the levels of EGFP expression are not due to the number of copies of the allele.

Using EGFP to visualize *Gsx2* and its progeny, both Gsx2 and Ascl1 are normally expressed in the majority of LGE progenitors up to the pallio-subpallial boundary (Fig. 1A,B, green arrows), which is bordered on its dorsal side by Pax6 expression in the ventral pallium (Fig. 1A,B, white arrows). In the *Gsx2* mutant LGE, Pax6 is expanded ventrally into the *Gsx2* expression domain (Fig. 1E,F, white arrows) altering some of these cells to a pallial fate (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001; Waclaw et al., 2010). In the remaining LGE progenitors, however, there is a reduction of Ascl1 (Fig. 1E,F) and Dlx proteins (data not shown), leading to a depletion of neurogenic factors in the remainder of the mutant LGE. Therefore we next examined the expression of gliogenic factors Olig2 and Pdgfrα in the E12.5 mutant LGE. In control LGE progenitors, Olig2 is found in fewer cells than Ascl1, particularly in the dLGE region (Fig. 1C), whereas Pdgfrα is not detectable in the LGE (Fig. 1D). Conversely, in the *Gsx2* mutant, Ascl1 expression is largely absent in LGE progenitors whereas Olig2 expression remains in the VZ (Fig. 1G). Additionally, Pdgfrα

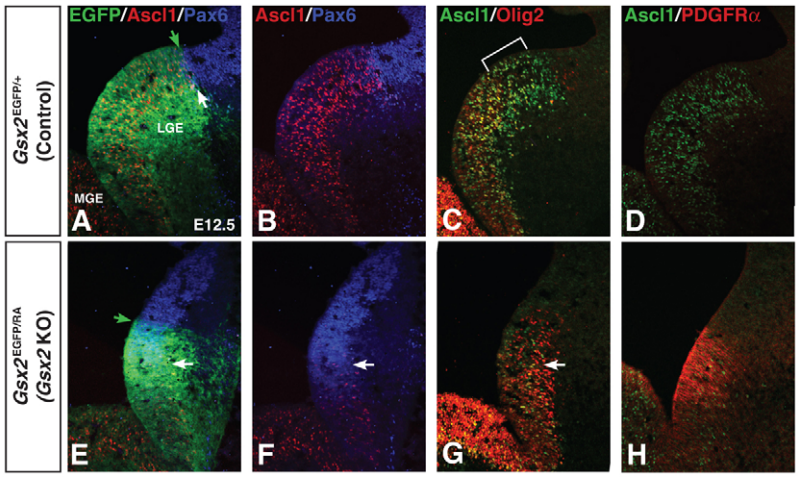

Fig. 1. Increased expression of oligodendroglial factors corresponds with a deficiency in neurogenic factors in the E12.5 *Gsx2* mutant LGE. (**A**,**B**,**E**,**F**) In control LGE, the dorsal edge of Gsx2 (green arrow) as well as Ascl1 expression create the pallial-subpallial boundary with the ventral border of Pax6 expression (white arrow) (A). In the *Gsx2* mutant LGE, the Pax6 boundary expands ventrally (white arrow) into the mutant *Gsx2* expression domain (B). *Gsx2* mutants have a drastic reduction in neurogenic factors such as Ascl1 (F) and thus *Gsx2* mutant progenitors that are not reached by the Pax6 expansion are highly deficient in neurogenic factors. (**C**,**D**,**G**,**H**) OPC marker Olig2 is normally weakly expressed in the LGE compared with Ascl1 (C, bracket indicates dLGE); however, in the *Gsx2* mutant this is reversed, with high levels of Olig2 and only scattered Ascl1 in the LGE (G). Pdgfrα, which is normally not expressed within VZ progenitors (D), is also ectopically expressed within the LGE of *Gsx2* mutants (H).

is ectopically expressed throughout the VZ of the mutant LGE and MGE (Fig. 1H), as previously described (Corbin et al., 2003). As OPCs are considered to be secondary progenitors derived from VZ progenitors (Woodruff et al., 2001), these findings suggest that in the E12.5 *Gsx2* mutant LGE, a proportion of the VZ progenitors have aberrantly adopted molecular aspects of early oligodendroglial specification.

Previous work by Petryniak et al. (Petryniak et al., 2007) has shown that loss of *Dlx1/2* gene function leads to increased production of OPCs specifically within the MGE at E12.5. By contrast, *Gsx2* mutants exhibit dramatic reductions of *Dlx* genes within the LGE but not the MGE (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). This is also true for Ascl1, as its expression remains relatively normal in the E12.5 *Gsx2* mutant MGE compared to controls (data not shown). Thus to determine if there is an effect specifically on the MGE-derived OPCs we examined the expression of OPC markers within the MGE of *Gsx2* mutants. Unlike the *Dlx1/2* mutants, no obvious increase in Olig2 or Pdgfrα staining within the SVZ or mantle of the MGE was observed in the *Gsx2* mutants at E12.5 (data not shown), indicating that specification of OPCs in the MGE is not significantly affected in the *Gsx2* mutant at this stage.

Transient increase in OPC specification in the *Gsx2* mutant LGE

We next wanted to determine if the *Gsx2* mutant LGE progenitors at E12.5 which were aberrantly expressing early OPC markers in the VZ did, in fact, lead to increased OPCs within the SVZ at later time points. Looking at E15.5 we found that this is the case, as *Gsx2* mutants displayed a large expansion of Olig2-positive cells within the SVZ of the dLGE of *Gsx2* mutants (Fig. 2D, arrows) compared with controls, which normally have few, if any, Olig2-positive cells in this region (Fig. 2A). Sp8 is normally expressed in olfactory bulb and amygdalar interneuron precursors in the dLGE SVZ (Fig. 2B); however, these cells are largely missing in the dLGE of *Gsx2* mutants (Fig. 2E) (Waclaw et al., 2006). Interestingly, the location of the ectopic Olig2-expressing cells in the *Gsx2* mutant dLGE SVZ complements the reduced Sp8 staining (compare Fig. 2C,F), suggesting that these cells are switching from a neuroblast to an oligodendroglial fate. Although these ectopic Olig2-positive cells in the mutant SVZ were not observed to express Sox10 at this stage, they did co-express Pdgfrα (inset in Fig. 2F), suggesting that they are early OPCs arising from mutant dLGE progenitors. At later stages of embryogenesis the reduced neurogenesis and altered molecular specification in the *Gsx2* mutant LGE begins to recover, largely because of the dorsal expansion of *Gsx1*, and return of *Ascl1* and *Dlx* expression to LGE progenitors (Toresson and Campbell, 2001; Yun et al., 2003; Wang et al., 2009). Thus, we next examined mutant LGE progenitors at E18.5 and found that the ectopic clump of Olig2-positive cells in the dLGE SVZ was no longer obvious (Fig. 2J) compared with the controls (Fig. 2G). Moreover, the Sp8-expressing interneuron precursors in the dLGE are increased

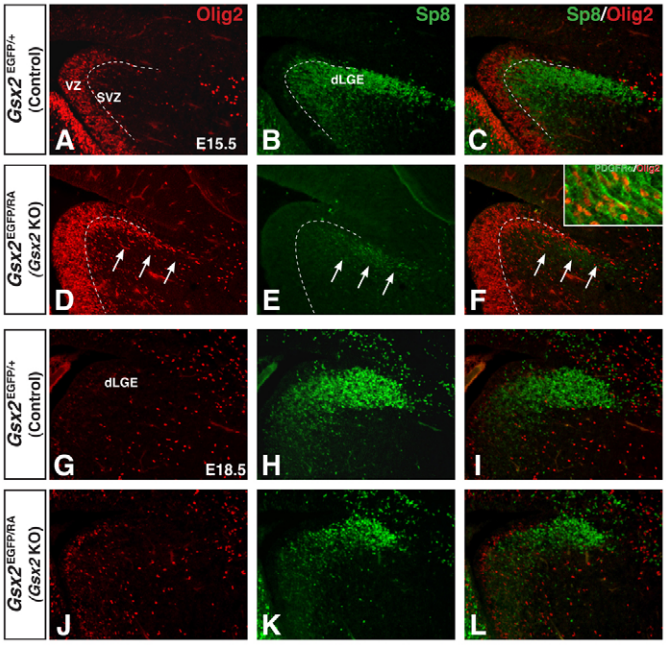

Fig. 2. *Gsx2* mutants display a transient expansion of OPCs in the dLGE. (**A-F**) At E15.5 *Gsx2* mutants have an expansion of OPC specification, as indicated by increased Olig2 cells in the dLGE SVZ and adjacent mantle regions (D, arrows) while controls show only scattered Olig2 cells in this region (A). Sp8 is severely depleted in *Gsx2* mutant LGE (E, arrows, compared with B). The upregulation of Olig2 cells corresponds with the severe reduction of Sp8 within the same region (F, arrows, compared with C). The ectopic Olig2 cells also co-express Pdgfrα (F, inset). (**G-L**) By E18.5 there is no longer an increase in Olig2 cells within the mutant dLGE SVZ (J) compared with control (G). At this stage, Sp8 expression is improved in the mutant (K), similar to the control (H). Thus, the altered ratio of Olig2 to Sp8 cells in the *Gsx2* mutant is almost back to normal at E18.5 (L compared with I).

(Fig. 2K) compared with 3 days earlier (Fig. 2E). Thus, in contrast to *Gsx2* mutants at E15.5, by E18.5 the distribution of Olig2- and Sp8-positive cells in the mutant LGE SVZ appears similar to the controls (Fig. 2L compared with 2I). Pdgfrα, however, remains upregulated within the VZ of the *Gsx2* mutant dLGE at E18.5 (Fig. 3G, arrowheads). This ectopic Pdgfrα expression is in multipotent progenitors, suggesting that the increase in OPC specification requires not only ectopic Pdgfrα expression, but also the absence of Ascl1 and Dlx proteins. Taken together, these results imply that the loss of *Gsx2* leads to a transient respecification of neurogenic LGE progenitors to an oligodendroglial fate.

Increased numbers of *Gsx2*-derived OPCs in the adjacent cortex at birth

OPCs are a highly proliferative and migratory population of progenitors (Woodruff et al., 2001; Kessaris et al., 2006). Thus it is likely that the transient increase in Olig2 and Pdgfrα cells seen in mutant LGE SVZ progenitors at E15.5 may have migrated into surrounding mantle regions (e.g. cortex adjacent to the dLGE) by E18.5. As mentioned above, the expression of EGFP from the *Gsx2* locus (Wang et al., 2009) provides a short-term fate map of the progeny from *Gsx2*-expressing progenitors. Accordingly, we observed many more EGFP-positive (i.e. *Gsx2*-derived) cells in the E18.5 cerebral cortex immediately adjacent to the dLGE in *Gsx2* mutants (Fig. 3E) compared with controls (Fig. 3A). Examining the expression of Sox10, Pdgfrα and Olig2 within the same region, there appeared to be increased numbers of OPCs. Quantification of these cells found a 42% increase in Sox10-positive (Fig. 3F,O), a 59% increase in Pdgfrα-positive (Fig. 3G,O) and a 37% increase in Olig2-positive cells (Fig. 3H,O), respectively, within the *Gsx2*-mutant cortex compared with control (Fig. 3B-D). In order to confirm the identity of these cells as OPCs, we looked at the co-expression of multiple OPC markers. In both control and mutant embryos we found that the vast majority of Sox10- and Pdgfrα-positive cells were co-labeled (supplementary material Fig. S1C,G). Moreover, most Sox10 cells also co-expressed Olig2 (supplementary material Fig. S1A,E). Unlike the *Gsx2* mutant cortex, no significant differences in cells expressing OPC markers were found in the striatum and forming external capsule (data not shown).

In order to determine whether the increased Sox10-, Pdgfrα- and Olig2-positive cells in the cortex originate specifically from *Gsx2* mutant progenitors, we used EGFP to short-term lineage trace *Gsx2*-derived progenitors and double (or triple) stained with OPC markers. Again, we observed extensive co-existence of EGFP/Sox10/Pdgfrα (supplementary material Fig. S1D,H) and EGFP/Sox10/Olig2 (supplementary material Fig. S1B,F). When considering only the EGFP-positive cells, we observed a much larger increase in OPCs between the *Gsx2* mutants and controls. Specifically, the mutants showed a 130% increase in EGFP/Sox10 (Fig. 3L,P), 102% increase in EGFP/Pdgfrα (Fig. 3M,P) and a 138% increase in EGFP/Olig2 double-labeled cells (Fig. 3N,P) compared with the controls (Fig. 3I-K,P). This represents more than a doubling in the number of *Gsx2*-derived OPCs in the mutant and thus it appears that the overall increase in OPCs is largely if not exclusively from the *Gsx2* mutant progenitors. Thus the increased production of

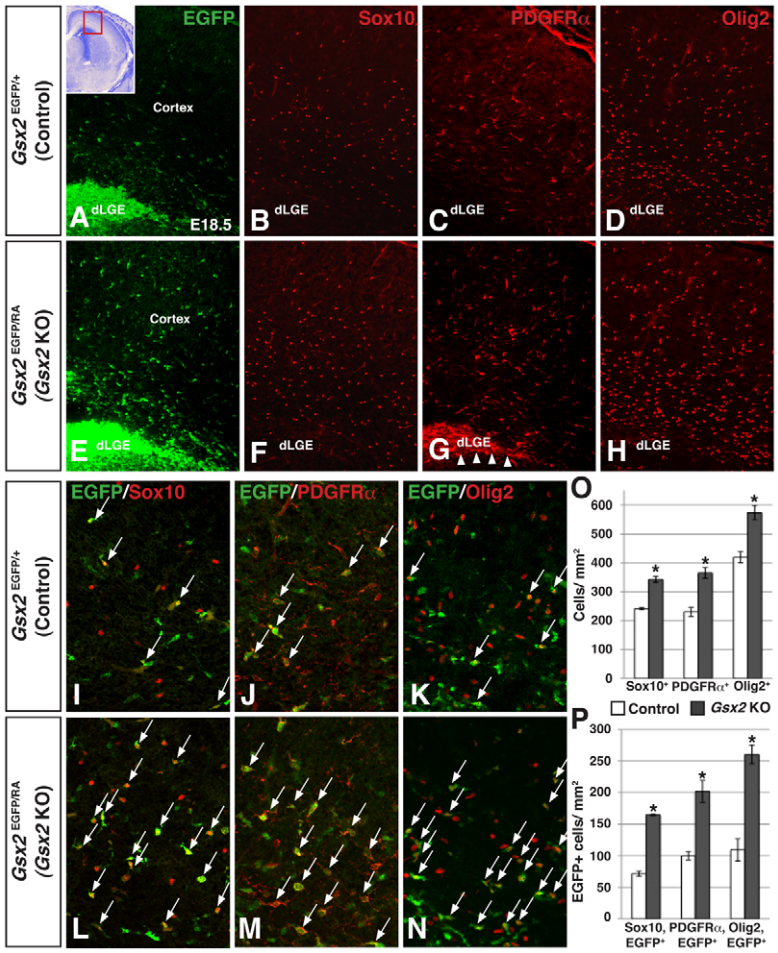

Fig. 3. *Gsx2* mutant progenitors give rise to increased numbers of OPCs within the cortex. (**A-H**,**O**) More *Gsx2*-derived (EGFP+) cells are seen migrating from the dLGE into the cortex in *Gsx2* mutants (E) compared with controls (A). This corresponds with increased Sox10 (control 241.4±3.6; mutant 342.9±10.2 cells/mm^2), Pdgfrα (control 230.7±15.4; mutant 365.9±18.4 cells/mm^2), and Olig2 (control 420.3±19.2; mutant 574.2±24.2 cells/mm^2) OPCs within the cortex of *Gsx2* mutants at E18.5 (F-H,O). (**I-N**,**P**) Considering only the population of cortical OPCs that originate from *Gsx2* progenitors (co-labeled with EGFP), there is a much larger increase in Sox10 (control 71.6±3.9; mutant 164.4±1.4 cells/mm^2), Pdgfrα (control 99.7±6.6; mutant 201.7±17.4 cells/mm^2), and Olig2 (control 109.1±17.7; mutant 260.1±14.8 cells/mm^2) (L-N, arrows, compared with I-K, arrows). In fact, *Gsx2* mutants have more than a doubling in the number of Gsx2-derived OPCs within the cortex (P). Data shown in O and P represent the mean±s.e.m.; **P*<0.005, significance determined by Student's *t*-test.

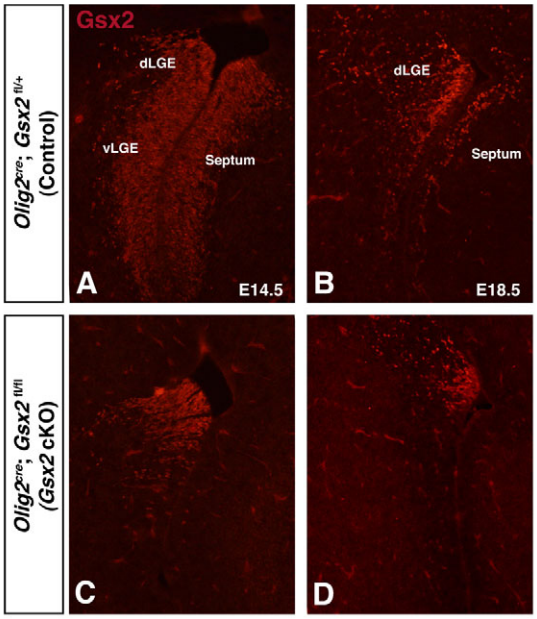

Fig. 4. Conditional knockout of *Gsx2* using *Olig2*^{Cre} mice. (**A**,**B**) The normal expression pattern of Gsx2 is in the VZ of the LGE, MGE, and septum in a dorsal to ventral gradient, with highest levels of Gsx2 in the dLGE. (**C**,**D**) When *Gsx2* is conditionally inactivated using *Olig2*^{Cre} it results in a deletion of Gsx2 everywhere throughout the ventral telencephalon except for in the dLGE, where expression remains largely intact.

OPCs observed in the SVZ of the *Gsx2* mutant dLGE at earlier embryonic time points leads to increased numbers of OPCs in the adjacent perinatal cortex.

One limitation in studying germline *Gsx2* mutants is that they die at birth, and OPCs do not fully mature into oligodendrocytes until early postnatal time points. However, to determine whether the increased OPCs in *Gsx2* mutants are accompanied by precocious differentiation, we looked at oligodendrocyte markers Mbp, Plp (Plp1 – Mouse Genome Informatics), and Cc1 (Rb1cc1 – Mouse Genome Informatics) at E18.5 and found no ectopic expression (data not shown). Thus it appears that the loss of *Gsx2* has an effect on the initial specification of OPCs but not their subsequent maturation.

Conditional inactivation of *Gsx2* using *Olig2*^{cre} mice

The findings described above suggest that the increased OPCs observed in the *Gsx2* mutant arise from the dLGE; however, it is not possible to conclude this definitively, as *Gsx2* is normally expressed in the ventral LGE (vLGE) and MGE as well. To determine if these expanded OPCs are in fact dLGE-derived we wanted to inactivate *Gsx2* throughout the ventral telencephalon with the exception of the dLGE. If our notion is correct, then these embryos should not display the increase in oligodendroglial markers described above in germline mutants. To test this further, we deleted *Gsx2* using *Olig2*^{cre} mice (Dessaud et al., 2007). These *Olig2*$^{cre/+}$; *Gsx2*$^{flox/flox}$ conditional knockouts (cKOs) showed a complete loss of Gsx2 within the septum, MGE and vLGE but left expression largely intact within the dLGE at both E14.5 (Fig. 4C) and E18.5 (Fig. 4D). This recombination pattern is roughly in line with the expression of *Olig2*, which is high throughout the ventral telencephalon except for in the dLGE, where its expression tapers off and is much weaker (Takebayashi et al., 2000; Nery et al., 2001) (see also Fig. 1C).

Unlike the *Gsx2* germline mutants, which have increased markers of early OPC specification and a concomitant decrease in neurogenesis (e.g. Sp8) within the SVZ of the dLGE (see Fig. 2F), the *Gsx2* cKO mutants (Fig. 5E) were indistinguishable from the controls at E15.5 (Fig. 5A). Indeed, the ratio of Sp8- to Olig2-expressing cells appears the same in both control and *Gsx2* cKO mutants. Interestingly, the *Gsx2* cKO mutants exhibited ectopic Pdgfrα in multipotent VZ progenitors of the vLGE, but not the dLGE (Fig. 5F) in accordance with the loss of Gsx2 expression (see Fig. 4). Furthermore, at E18.5, no significant difference in the number of Sox10- or Pdgfrα-positive cells within the cortex was

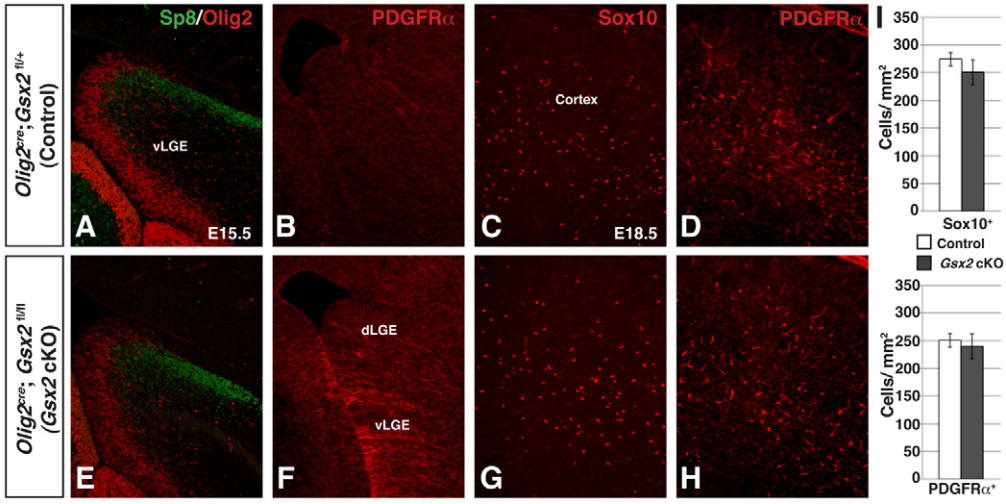

Fig. 5. Maintained Gsx2 expression exclusively in the dLGE is sufficient for normal OPC specification. (**A-H**) At E15.5, the dLGE of *Gsx2* cKOs looks nearly identical to control with a stream of Sp8 neuroblasts from the dLGE and only scattered Olig2 cells (E compared with A). The ectopic VZ expression of Pdgfrα is absent within the dLGE of the *Gsx2* cKO; however, it remains in the vLGE of these mutants (F compared with B). This expression pattern complements the Gsx2 expression remaining in the dLGE of *Gsx2* cKO embryos (see Fig. 4). (C,D) Additionally, at birth no differences in cortical Sox10 (control 274.4±12.1; cKO 250.7±22.6 cells/mm^2) and Pdgfrα (control 250.7±12.7; cKO 239.9±13.5 cells/mm^2) cells were found between *Gsx2* cKO (G,H) and control (C,D) embryos. (**I**) The quantification of cortical OPCs. Data represent the mean±s.e.m. $P>0.2$, significance determined by Student's *t*-test.

seen between *Gsx2* cKO (Fig. 5G-I) and control embryos (Fig. 5C,D,I). These data indicate that the increased OPCs in the germline *Gsx2* mutant cortex do, in fact, originate from mutant dLGE progenitors.

Gsx2 is sufficient to repress the specification of telencephalic OPCs

The loss-of-function studies described above suggest that maintained *Gsx2* expression in dLGE progenitors is required to prevent precocious specification of OPCs from this region. We next wanted to determine whether high *Gsx2* expression throughout early telencephalic progenitors is sufficient to repress OPC specification. To do so, we used the doxycyclin (Dox)-regulated binary transgenic system described in Waclaw et al. (Waclaw et al., 2009) to temporally misexpress *Gsx2* throughout the developing telencephalon.

Foxg1^{tTA}; *tetO-Gsx2-IE* double transgenic (DT) embryos have been shown to misexpress EGFP and Gsx2 throughout early telencephalic progenitors from E9.5 onward causing severe morphological defects (Fig. 6A) (Waclaw et al., 2009). Dox treatment from E7-9 delays transgene expression until around E13.5, resulting in slightly improved morphology (Fig. 6A) (Waclaw et al., 2009). To avoid these morphological defects, we have further delayed the misexpression of *Gsx2* by administering Dox from E7-11, which results in transgene expression first emerging around E14.5 and fully expressed throughout the telencephalon by E15.5. The timing of this misexpression coincides with the emergence of OPCs from *Gsx2*-expressing progenitors within the LGE (Kessaris et al., 2006), and furthermore, these brains appear relatively normal morphologically. Therefore, this Dox-treatment paradigm is better suited to study the role of *Gsx2* in OPC specification.

To determine the effect of *Gsx2* misexpression on OPC specification, we generated E18.5 *Gsx2* DT and control embryos with Dox administration from E7-11. Both *tetO-Gsx2-IE* and *Foxg1*$^{tTA/+}$ single transgenic mice were used as controls, and both contained normal numbers of OPCs. Despite the improvement in morphology, the *Gsx2* DT embryos still show an upregulation of Ascl1 and Dlx proteins within the dorsal telencephalon (Fig. 6C,D; data not shown). Accordingly, Sp8 expression was significantly increased in the dorsal telencephalon and a concomitant reduction in the dorsal telencephalic regulator Tbr1 was observed within the SVZ and intermediate zone of the *Gsx2* DT cortex, compared with control (Fig. 6E-J). In line with our previous studies where Gsx2 misexpression was delayed (Waclaw et al., 2009), these DT embryos do not show an upregulation of the vLGE marker Isl1 (data not shown). A substantial reduction in Sox10-, Pdgfrα- and Olig2-expressing cells was observed in the cortex of the *Gsx2* DT embryos (Fig. 7D-F) compared with the controls (Fig. 7A-C). Specifically, we found there to be a 46% decrease in Sox10-positive cells, a 47% decrease in Pdgfrα-positive cells and a 37% decrease in Olig2-positive cells at E18.5 (Fig. 7G). Remarkably, the few Sox10-, Pdgfrα- or Olig2-expressing cells that do remain in the *Gsx2* DT cortex were never observed to co-express EGFP (Fig. 7H-J′) and thus are not derived from progenitors overexpressing Gsx2. These gain-of-function studies, together with the loss-of-function studies above, provide further evidence for *Gsx2* playing a negative role in the specification of OPCs: perhaps through the direct promotion of dLGE neurogenesis (i.e. Ascl1, Dlx, Sp8).

Ascl1 is required for the expansion of OPCs in *Gsx2* mutants

To further understand the mechanisms underlying the increased OPC generation in *Gsx2* mutants, we decided to examine the role of

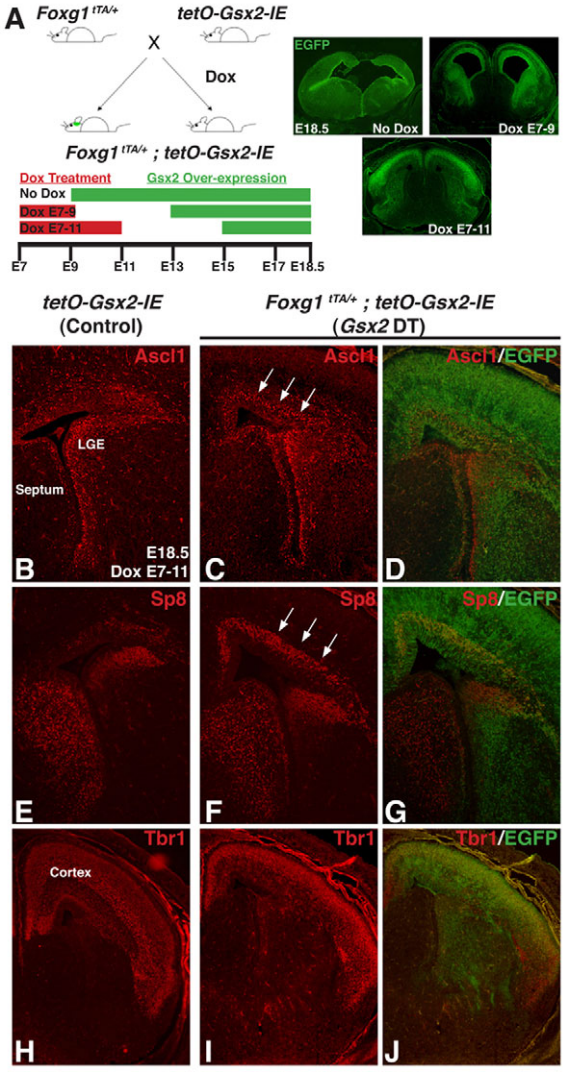

Fig. 6. Dox-regulated binary transgenic system used to misexpress Gsx2. (**A**) Breeding scheme and Dox treatment schedule for temporal overexpression of Gsx2. Dox treatment from E7-11 delays expression of the transgene until around E15, which drastically improves the morphology of the telencephalon compared with a shorter delay (i.e. Dox E7-9) or no Dox treatment. (**B-J**) Misexpression of Gsx2 from E15 onward promotes dLGE neurogenesis, with Ascl1 and the dLGE marker Sp8 ectopically expressed throughout the SVZ of the dorsal telencephalon (C,D,F,G, arrows, compared with B,E). Decreased expression of Tbr1 in the late-generated cortical cells of the *Gsx2* DT embryos (I,J compared with H).

Ascl1 within *Gsx2* mutants. *Ascl1* is known for its role in neurogenesis and is required for normal development of the ventral telencephalon and its neuronal derivatives, including striatal and olfactory bulb interneuron development (Casarosa et al., 1999; Horton et al., 1999; Yun et al., 2002). Additionally, *Ascl1* has been implicated in oligodendrogenesis (Parras et al., 2007; Kim et al., 2008). Moreover, *Ascl1* is a downstream target of *Gsx2*, as *Gsx2* mutants display a loss of Ascl1 at early stages (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). Interestingly, by E16.5 *Ascl1* has fully recovered throughout the dLGE of *Gsx2* mutants, and there even appears to be a slight increase in *Ascl1*-positive cells adjacent to the dLGE [see figure 2I in Wang et al. (Wang et al., 2009)], which correlates well with the increased Olig2- and Pdgfrα-expressing

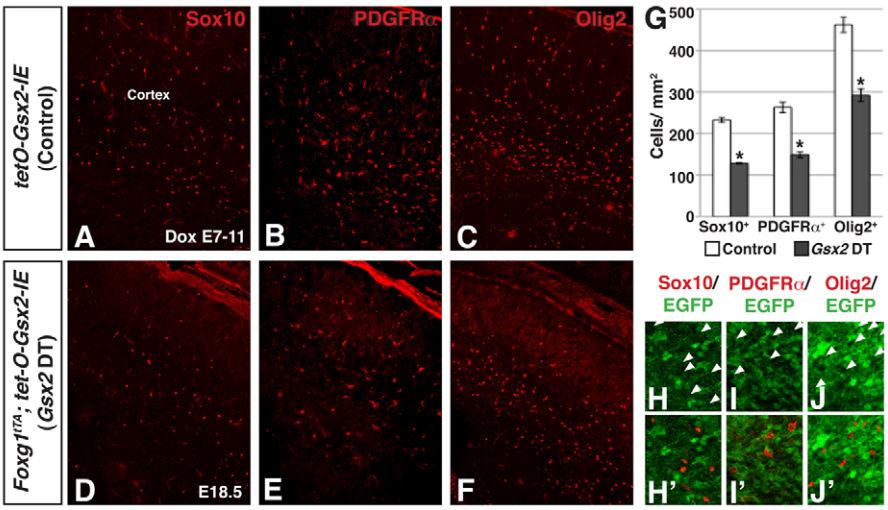

Fig. 7. Misexpression of Gsx2 is sufficient to inhibit OPC specification. (**A-G**) Sox10 (control 232.5±5.7; DT 128.7±1.7 cells/mm^2), Pdgfrα (control 262.8±12.7; DT 149.2±7.0 cells/mm^2), and Olig2 (control 462.1±18.6; DT 292.2±15.6 cells/mm^2) cells within the cortex are severely reduced at E18.5 in *Gsx2* DT (D-F compared with control, A-C); quantification in G. (**H-J'**) When stained with EGFP (H-J) in combination with OPC markers (H'-J'), co-labeling was not observed, suggesting that misexpressing Gsx2 inhibits OPC specification. Data in G represent the mean±s.e.m. **P*<0.005, significance determined by Student's *t*-test.

OPCs observed in *Gsx2* mutants (Fig. 2F). Thus we next looked at the requirement of *Ascl1* in the increased generation of OPCs within *Gsx2* mutants. To do so we studied *Gsx2*$^{EGFP/RA}$; *Ascl1*$^{-/-}$ double mutant embryos, which contain the EGFP allele, again providing a short-term lineage trace of *Gsx2*-progenitors.

We first examined the dLGE of E15.5 *Gsx2*$^{EGFP/RA}$; *Ascl1*$^{-/-}$ double mutants to determine if *Ascl1* is required for the increased OPCs in *Gsx2* mutants at E15.5. We found that these embryos showed increased Olig2-positive cells accompanied by a decrease in Sp8-positive neuroblasts in the dLGE SVZ (Fig. 8G-I), similar to the *Gsx2* single mutants (Fig. 8D-F). *Ascl1* single mutants show an Sp8/Olig2 ratio in the E15.5 dLGE SVZ (Fig. 8J-L) similar to controls (Fig. 8A-C). Thus in the absence of *Gsx2*, the initial misspecification of dLGE progenitors to an oligodendroglial fate does not involve *Ascl1*. In *Gsx2* mutants, this transient misspecification of dLGE progenitors at E15.5 results in an increase in OPCs in the adjacent cortical mantle regions by E18.5 (Fig. 3 and Fig. 8O,P,U). Remarkably, when quantifying OPCs (using Sox10 or Pdgfrα) within the cortex of E18.5 *Gsx2*$^{EGFP/RA}$; *Ascl1*$^{-/-}$ embryos, we found normal numbers of cortical OPCs (Fig. 8Q,R) compared to controls (Fig. 8M,N,U). No differences were found in *Ascl1* single mutant cortex at E18.5 (Fig. 8S-U) (Parras et al., 2007). Similarly, when considering only the EGFP-positive population of cells, only the *Gsx2* single mutant has an increase in OPCs derived from *Gsx2*-expressing progenitors (Fig. 8V).

Overall, these results suggest that *Ascl1* does not play a role in the early neurogenic to oligodendrogenic fate change of *Gsx2* mutant dLGE progenitors; however, it is required for the subsequent expansion of this population of cortical OPCs.

DISCUSSION

The molecular mechanisms that regulate the specification of OPCs (e.g. Olig, Nkx and Sox genes) are beginning to be elucidated (reviewed in Guillemot, 2007; Wegner, 2008). Oligodendrocytes arise from neural progenitors located along multiple levels of the DV axis (Vallstedt et al., 2005; Kessaris et al., 2006). Thus these progenitors are endowed with distinct positional identities that may interact with OPC specification pathways to regulate aspects of their development such as timing of their appearance. Our results indicate that *Gsx2*, which is known to regulate DV patterning in the lateral telencephalon (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001; Waclaw et al., 2009) also controls the timing of OPC specification within LGE progenitors. Specifically, it appears that *Gsx2* must be downregulated in LGE progenitors for lineage progression towards OPC and ultimately oligodendrocyte generation.

In the *Gsx2* mutant telencephalon there is an expansion of ventral pallial identity into the LGE, which accordingly leads to an increase in ventral pallial-derived lateral amygdala projection neurons and a concomitant reduction in dLGE-derived interneurons of both the olfactory bulb and amygdala (Stenman et al., 2003; Waclaw et al., 2010). This expansion of dorsal telencephalic identity in the *Gsx2* mutant, however, does not encompass and respecify the entire LGE. A pallial-subpallial boundary appears to be re-established at a more ventral location, and at later stages LGE molecular identity is, at least partially, restored (Toresson and Campbell, 2001; Yun et al., 2003). However, before this restoration occurs, the progenitors in the mutant dLGE largely lack a neurogenic program (e.g. Ascl1, Dlx and Sp8) and instead express markers of OPC specification (e.g. Olig2 and Pdgfrα). This suggests that in the absence of *Gsx* gene function mutant dLGE VZ progenitors are biased to generate oligodendroglial rather than neuronal progeny.

While OPCs are probably beginning to be specified within the VZ, they typically transition to the SVZ and mantle regions because of their migratory capacity (Woodruff et al., 2001). Accordingly, when we examined *Gsx2* mutant LGEs at E15, a time when the molecular identity of the mutant LGE is beginning to be restored, we found a large increase in Olig2-positive cells in place of the usual Sp8-expressing neuroblasts in the SVZ of the dLGE. These ectopic Olig2 cells also expressed Pdgfrα and thus appeared to be OPCs and probably arose from the VZ before the molecular restoration of the dLGE. Interestingly, they were not increased in germinal regions at late stages of embryogenesis (e.g. E18.5) when Sp8-expressing neuroblasts were beginning to reseed the dLGE. This is presumably the result of the *Gsx1*- and *Ascl1/Dlx*-led restoration of LGE identity (Toresson and Campbell, 2001; Yun et al., 2003; Wang et al., 2009), which appears to restore neurogenesis and halt ectopic OPC specification. Despite this apparent normalization of VZ progenitors, these transiently generated *Gsx2*-derived OPCs migrate from the SVZ into the adjacent cerebral cortex and expand. These findings indicate that OPCs detected in the *Gsx2* mutant dLGE indeed originate from Gsx2-expressing progenitors, and that their transient misspecification at E15.5 leads to considerable increases in OPC numbers within the adjacent cortex by birth.

In complementary experiments, we found that overexpression of Gsx2 in early telencephalic progenitors from E15 onwards results in

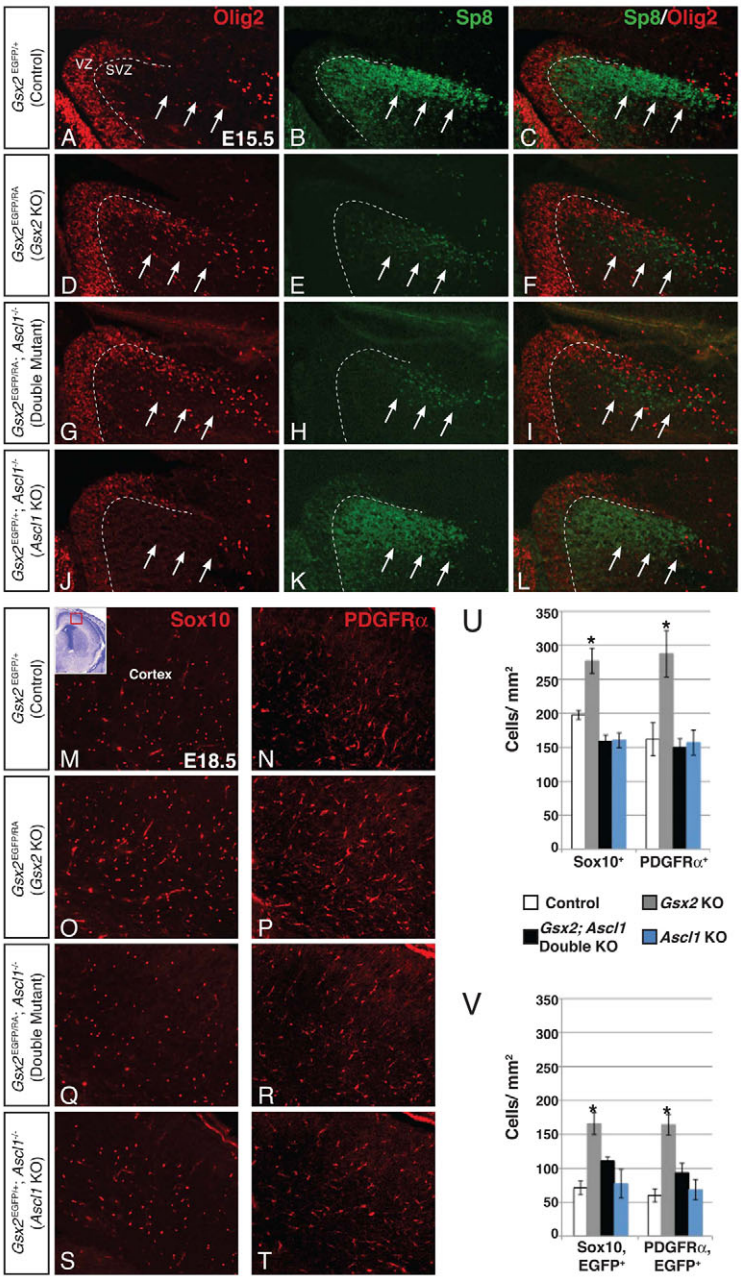

Fig. 8. Requirement of *Ascl1* for expansion of ectopic OPCs in *Gsx2* mutants. (**A-L**) Altered specification from Sp8-positive neuroblasts to Olig2-positve OPCs in E15.5 dLGE progenitors still occurs when *Ascl1* is lost in addition to *Gsx2* (G,H). Indeed, the distribution of Sp8 and Olig2 cells in *Gsx2*; *Ascl1* double mutants (G-I) appears comparable to *Gsx2* mutant dLGE progenitors (i.e. increased Olig2 and decreased Sp8) (D-F). This early misspecification of dLGE progenitors does not occur in *Ascl1* single mutants (J-L), as many Sp8-positive cells are seen streaming from the dLGE (K) with only scattered Olig2 cells (J), which is similar to control embryos (A-C). (**M-V**) No significant differences were found in Sox10- (control 197.8±6.7; *Gsx2*; *Ascl1* KO 159.1±9; *Ascl1* KO 160.4±10.9 cells/mm^2) and Pdgfrα- (control 162.1±24.4; *Gsx2*; *Ascl1* KO 150±12.9; *Ascl1* KO 157±18.4 cells/mm^2) positive OPCs within the cortex of E18.5 *Gsx2*; *Ascl1* double mutants (Q,R,U) and *Ascl1* single mutants (S-U) comparing these also to *Gsx2* single mutants (O,P), significant increases in Sox10 (277.1±18.3 cells/mm^2) and Pdgfrα (287.5±34.2 cells/mm^2) OPCs were again observed (U), confirming results from earlier experiments (see Fig. 3O). Considering only the *Gsx2*-EGFP population of cortical OPCs, there is still no difference in Sox10 (control 71.3±10; *Gsx2*; *Ascl1* KO 110.9±6; *Ascl1* KO 77.4±21.2 cells/mm^2) and Pdgfrα (control 60.1±9.6; *Gsx2*; *Ascl1* KO 93.6±13.7; *Ascl1* KO 68.6±14.9 cells/mm^2) OPCs within *Gsx2*; *Ascl1* double mutants and *Ascl1* single mutants (V). Again, *Gsx2* single mutants demonstrate more than a doubling of Sox10 (165.9±16) and Pdgfrα (164.3±15.1) OPCs derived from EGFP-positive *Gsx2* mutant progenitors (V), once more verifying earlier results (see Fig. 3P). Data in U,V represent the mean±s.e.m. *P<0.01 compared to control, *Ascl1* and *Gsx2*; *Ascl1* mutants, significance determined using a one-way ANOVA with a Tukey post-hoc test.

a significant decrease in the number of OPCs in the cerebral cortex concomitant with upregulation of neurogenic factors Ascl1 and Dlx proteins. Interestingly, none of the cells expressing the *Gsx2* transgene were ever observed to co-express any of the OPC markers used. This suggests that the small number of OPCs present in the overexpressing embryos were probably generated before Gsx2 misexpression (~E15) and derive from the MGE (Kessaris et al., 2006). These results are in accordance with loss-of-function studies indicating that *Gsx2* negatively regulates the specification of OPCs in LGE progenitors (Corbin et al., 2003) (present findings). Furthermore, these results suggest that the downregulation of *Gsx2* in LGE progenitors is imperative for the transition from neuronal to oligodendroglial specification.

As we observed previously (Waclaw et al., 2009; Pei et al., 2011), the misexpression of Gsx2 from E13 onward promoted the specification of dLGE (i.e. Sp8) and not vLGE (i.e. Isl1) neuronal fate. This was also observed in our misexpression from E15 onward, suggesting that any late embryonic Gsx2 misexpression (i.e. E13 and on) will promote dLGE over vLGE neurogenesis. Thus it is possible that the negative regulation of OPC specification exhibited by *Gsx2* could simply be due to its strong effect on promoting neuronal fates (via Ascl1 and Dlx factors). However, it also remains possible that *Gsx2* represses aspects of OPC specification directly. Corbin et al. (Corbin et al., 2003) previously showed that *Pdgfra* expression is upregulated within the VZ of both the MGE and LGE in *Gsx2* mutants. This upregulation of Pdgfrα remains in the LGE VZ even at E18.5 (Fig. 3G), when the ectopic OPCs in the dLGE SVZ are no longer present. In fact, it appears that loss of *Gsx2* invariably leads to an increase in VZ Pdgfrα expression, even when the OPC phenotype is not present. Likewise, the *Olig2^{cre}* recombination, which removes *Gsx2* in the vLGE, shows an increase in Pdgfrα specifically within the VZ of the vLGE, despite the fact that cortical OPC numbers are not different from controls. Thus Gsx2 may directly repress *Pdgfra* expression. This ectopic

Pdgfrα expression in the VZ of *Gsx2* mutants is not sufficient to turn on a full specification program for OPCs; however, its increased expression in combination with the loss of factors required for neurogenesis (e.g. Ascl1 and Dlx proteins) probably contribute to the respecification of dLGE progenitors towards OPCs.

It is well known that the patterning defects in *Gsx2* mutants are confined to LGE progenitors despite the expression of *Gsx2*, albeit at a lower level, in the MGE (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). In line with this, we did not observe any changes in MGE-derived OPCs, which is in contrast to those observed in the *Dlx1/2* (Petryniak et al., 2007) and *Ascl1* mutants (Parras et al., 2007). Although these developmental regulators are downstream of Gsx2, *Dlx1/2* mutants show a significant increase in OPCs within the MGE, whereas *Ascl1* mutants exhibit a reduction in this telencephalic region. It is interesting to note that Petryniak et al. (Petryniak et al., 2007) found that the loss of both *Ascl1* and *Dlx1/2* leads to increased OPC generation in other ventral telencephalic regions such as the LGE and caudal ganglionic eminence (CGE). This is in line with our results because *Gsx2* mutants are known to lack *Ascl* and *Dlx* gene expression in the mutant dLGE until around E15 (Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001; Yun et al., 2003). Thus, the loss of both of these factors in the *Gsx2* mutant may be a major cause of the respecification of dLGE progenitors towards an OPC fate. Furthermore, the misexpression of Gsx2 was found to upregulate Ascl1 and Dlx proteins throughout the telencephalon, effectively promoting neuronal fates (i.e. Sp8) and limiting OPC specification. Thus in addition to the well-known role of *Gsx2* in regulating DV patterning, it also appears to repress OPC specification and therefore play an important role in controlling the timing of LGE oligodendrogenesis. Similar mechanisms are likely to be utilized at multiple levels of the neuraxis in order to regulate neuronal versus glial specification. In fact, Pax6, another factor involved in regulating DV patterning, has been suggested to regulate the timing of glial specification in the spinal cord (Sugimori et al., 2007). This study demonstrated that together with Ascl1, Pax6 promotes neurogenesis but in the absence of Pax6, Olig2 and Nkx2.2 cooperates with Ascl1 to promote precocious OPC specification in the developing spinal cord.

Although Kessaris et al. (Kessaris et al., 2006) showed that LGE-derived OPCs first appear at the pallial-subpallial boundary around E15, they did not determine whether these OPCs arise simultaneously from both the vLGE and dLGE. Unlike in the dLGE, Gsx2 normally begins downregulating in the vLGE already by E12-13 (Waclaw et al., 2009). Thus it is likely that LGE-derived OPCs are first generated by vLGE progenitors in control animals. The dLGE seems to be largely concerned with neurogenesis (giving rise to about 20% of all olfactory bulb interneurons) at embryonic time points (Hinds, 1968; Bayer, 1983). Moreover, the dLGE has been suggested to ultimately give rise to a significant proportion of the postnatal SVZ (Stenman et al., 2003), and Gsx2 expression in this structure is much reduced from that seen in the embryo (Parmar et al., 2003). This postnatal downregulation of Gsx2 correlates with a burst of OPC generation from the postnatal SVZ (Levison and Goldman, 1993; Luskin and McDermott, 1994). Thus dLGE-derived SVZ cells do not normally transition to gliogenic progenitors until Gsx2 downregulation at some point after birth. In any case, our data show that the loss of *Gsx2* results in precocious OPC specification from dLGE progenitors, ultimately leading to increased numbers of these gliogenic progenitors in the adjacent cortex.

We examined *Gsx2*; *Ascl1* double mutants to determine any role of *Ascl1* in generating the ectopic OPCs observed in *Gsx2* mutants. Intriguingly, we found that the misspecification in VZ/SVZ dLGE progenitors at E15.5 still occurs, indicating that *Ascl1* is not required for the initial misspecification of ectopic OPCs in the *Gsx2* mutants. However, in E18.5 cortical mantle regions we found that *Gsx2*; *Ascl1* double mutants have normal numbers of OPCs. This suggests that *Ascl1* is required for the subsequent expansion of the ectopic OPCs derived from the mutant dLGE. This is in line with recent studies that demonstrate that *Ascl1* is required for the proliferation of intermediate progenitors (including OPCs) within the SVZ (Castro et al., 2011). Thus, it appears that within the dLGE Ascl1 is playing a role in the expansion of intermediate progenitors and not their initial specification. As mentioned above, OPCs are not normally specified from this region until postnatal stages, and therefore the loss of *Ascl1* alone does not normally have an effect on OPCs at late embryonic times. The observed requirement for *Ascl1* in *Gsx2* mutants may, however, indicate a postnatal role for *Ascl1* in the subsequent expansion of postnatal SVZ-derived OPCs that are normally generated following the downregulation of *Gsx2*.

In summary, *Gsx2* regulates the timing of OPC production from dLGE progenitors probably by biasing them towards neurogenesis, and only after its downregulation can OPC specification from these progenitors proceed. These OPCs uniquely require *Ascl1* to further expand in the adjacent mantle regions (e.g. cortex). Thus *Gsx2* represents a factor normally associated with DV patterning that clearly interacts with the OPC specification program to regulate the timing and number of OPCs generated from a distinct telencephalic region.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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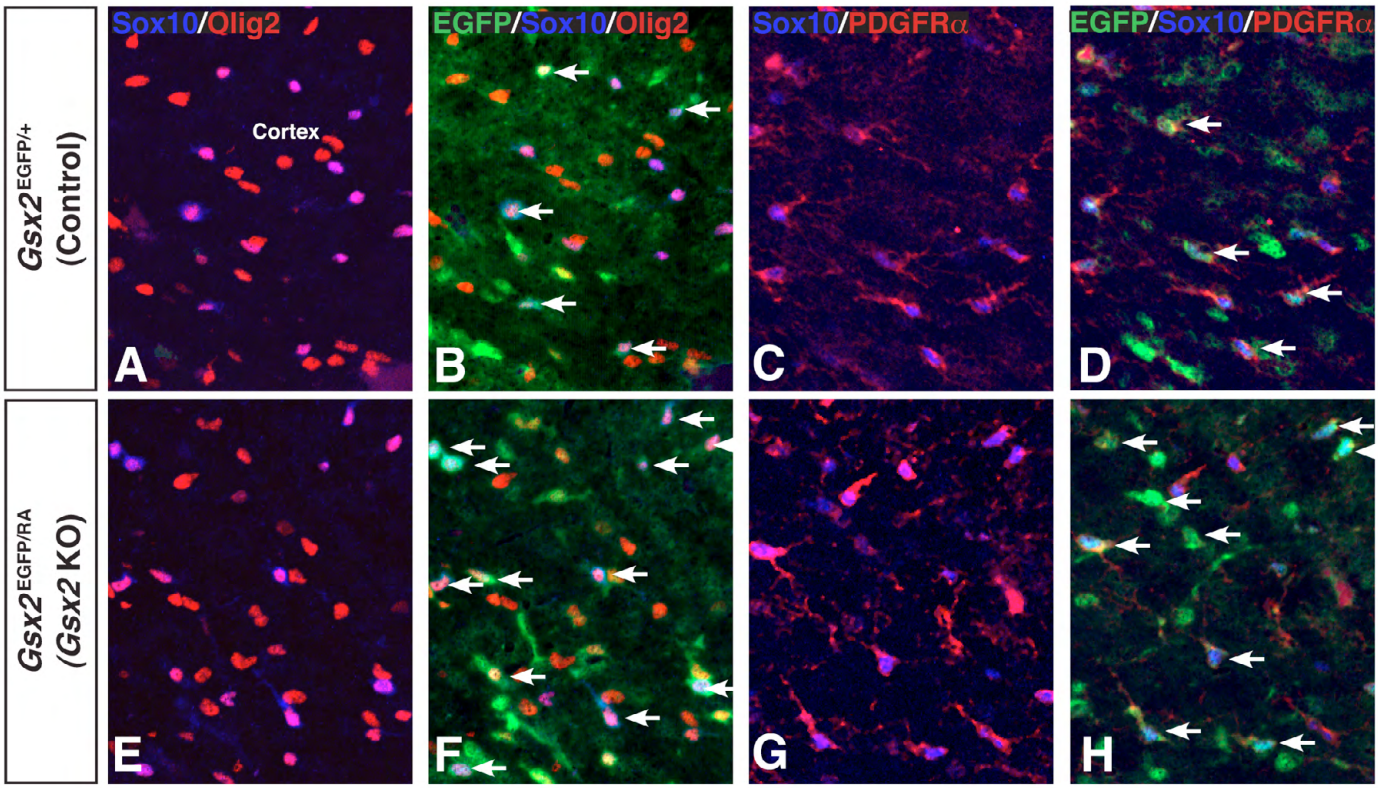

Fig. S1. *Gsx2*-derived cortical cells express multiple markers of OPCs. Nearly all Sox10-positive cells coexpress Olig2 within the E18.5 cortex in both control (**A**) and *Gsx2* mutants (**E**). More of these double-labeled OPCs are seen coming from EGFP-positive mutant progenitors (**F**, arrows) compared with control (**B**, arrows), which is in accordance with our observations of cells expressing single oligodendroglial markers. The majority of Sox10- and Pdgfrα-expressing cells were co-labeled in both controls (**C**) and mutants (**G**). Again, many more of these cells were *Gsx2* derived (i.e. EGFP-expressing) in mutant embryos (**H**, arrows) compared with controls (**D**, arrows).