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Characterization of *Glcci1* expression in a subpopulation of lateral ganglionic eminence progenitors in the mouse telencephalon

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Abstract

The lateral ganglionic eminence (LGE) in the ventral telencephalon is a diverse progenitor domain subdivided by distinct gene expression into a dorsal region (dLGE) that gives rise to olfactory bulb and amygdalar interneurons and a ventral region (vLGE) that gives rise to striatal projection neurons. The homeobox gene, *Gsx2*, is an enriched marker of the LGE and is expressed in a high dorsal to low ventral gradient in the ventricular zone (VZ) as development proceeds. Aside from Gsx2, markers restricted to the VZ in the dorsal and/or ventral LGE remain largely unknown. Here we show that the gene and protein expression of *Glucocorticoid-induced transcript 1* (*Glcci1*) has a similar dorsal to ventral gradient of expression in the VZ as Gsx2. We found that *Glcci1* gene and protein expression are reduced in *Gsx2* mutants, and are increased in the cortex after early and late *Gsx2* misexpression. Moreover, *Glcci1* expressing cells are restricted to a subpopulation of Gsx2 positive cells on the basal side of the VZ and co-express Ascl1, but not the subventricular zone (SVZ) dLGE marker, Sp8. These findings suggest that *Glcci1* is a new marker of a subpopulation of LGE VZ progenitor cells in the *Gsx2* lineage.

Introduction

The lateral ganglionic eminence (LGE) is a progenitor domain located in the ventral telencephalon that gives rise to diverse neuronal subtypes (reviewed in Campbell, 2003; Marin and Rubenstein, 2003). Based on gene expression, the LGE has been proposed to contain multiple distinct progenitor domains (Flames et al., 2007) that can be broadly defined in two domains; a ventral (vLGE) domain that produces striatal projection neurons, and a dorsal (dLGE) domain giving rise to olfactory bulb and amygdala interneurons (Yun et al., 2001; Stenman et al., 2003; Waclaw et al., 2006, 2010; Cocas et al., 2011; Ehrman et al., 2013). The homeobox gene *Gsx2* is the earliest enriched marker of the LGE and is required for the normal development of all major LGE neuronal subtypes (Szucsik et al., 1997; Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001, 2003; Carney et al., 2006; Waclaw et al., 2009). Gsx2 protein is largely expressed in

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ventricular zone (VZ) progenitor cells, and is downregulated as cells progress to secondary progenitors in the subventricular zone (SVZ) and post-mitotic cells in the mantle zone (MZ) (Toresson et al., 2000). Gsx2 has a dynamic expression pattern during LGE development with nearly uniform VZ expression at E10.5 that resolves into a high dorsal to low ventral gradient of expression as development proceeds (Toresson et al., 2000; Waclaw et al., 2009). From E12.5 to E18.5, the gradient of Gsx2 expression helps define the vLGE (low Gsx2) and dLGE (high Gsx2) in the VZ. These domains are further defined by SVZ genes with restricted expression to either the vLGE (*Isl1*, *Zfp503*, *Ebf1*, and *Sox8*) or the dLGE (*Er81*, *Sp8*, *Tshz1*, and *Pax6*) (Garel et al., 1999; Stenman et al., 2003; Caubit et al., 2005; Waclaw et al., 2006; Long et al., 2009; Ko et al., 2013; Merchan-Sala et al., 2017). Other ventral genes in the telencephalon including Ascl1 and Dlx2 do not follow the Gsx2 expression gradient and are expressed in a more uniform manner in both the vLGE and dLGE of the VZ (Yun et al., 2002). An extensive catalog of LGE genes in the VZ, SVZ, and MZ has been described by Long et al., (2009), however, few markers enriched in the dorsal or ventral VZ have been identified in the LGE, other than the temporal gradient expression of Gsx2.

Glucocorticoid-induced transcript 1 (*Glcci1*) was originally identified as a steroid induced transcript in thymocytes (Chapman et al., 1995) with described expression in the testis, kidney, and somites during development (Ishikawa et al., 2004; William et al., 2007; Nishibori et al., 2011). In zebrafish, reduction of *Glcci1* expression using morpholinos has been shown to alter kidney glomerular development (Nishibori et al., 2011). Moreover, a recent report suggests that the expression of Glcci1 in the glomerulus can be regulated by the PI3 Kinase pathway under hyperglycemic conditions (Kim et al., 2016). Despite these reports, the normal regulation of *Glcci1* expression and the *in vivo* roles for *Glcci1* remain largely unexplored. Little is known about *Glcci1* in the central nervous system other than the recent identification that it is expressed in the medial ganglionic eminence (MGE) progenitor region of the ventral forebrain (Au et al., 2013, Sandberg et al., 2016). No detailed expression analysis exists for this gene in the adjacent LGE progenitor domain or in other regions of the telencephalon.

In this study, we examined the expression of *Glcci1* in the developing LGE. Similar to Gsx2, *Glcci1* has a dynamic expression pattern in the LGE that resolves into a high dorsal to low ventral gradient as development proceeds. Both *Glcci1* gene and protein expression are reduced in the LGE of *Gsx2* mutants. Moreover, we find that Glcci1 expression is increased after both an early and a late misexpression of Gsx2 in the developing cortex. We characterized the cell types expressing *Glcci1* in the LGE and found it coincides with a subpopulation of Gsx2 positive cells in the basal half of the VZ. Interestingly, Glcci1 expression is enriched in Ascl1 positive cells of the VZ, but not in Sp8 expressing cells of the dLGE SVZ. Our data suggests that Glcci1 is a new marker of a subpopulation of Gsx2 positive progenitor cells in the basal half of the VZ, which will be useful for future studies examining the maturation of LGE progenitors from the apical to the basal VZ, as well as the subsequent transition to the SVZ.

Results/Discussion

Temporal and regional characterization of Glcci1 expression in the LGE

Glcci1 was recently identified in a RNA microarray analysis of *Nkx2.1* regulated genes in the MGE at E13.5 (Sandberg et al., 2016). In addition to the reported MGE expression of *Glcci1*, we noticed robust expression of *Glcci1* in the VZ of the LGE at this stage (see Supp. Figure 1 in Sandberg et al., 2016). The LGE expression of *Glcci1* was confirmed using the Eurexpress database, which catalogs gene expression in the entire embryo at E14.5 (Diez-Roux et al., 2011). Since a detailed expression analysis of *Glcci1* in the LGE has not been reported, we set out to carefully analyze *Glcci1* expression throughout LGE development.

After confirming expression of *Glcci1* in the VZ of the MGE at E13.5 as previously described in Au et al., (2013) and Sandberg et al., (2016), we found that *Glcci1* remains expressed in the MGE or MGE remnant at all embryonic stages examined (E11.5–E18.5 in Fig. 1). At E11.5, the expression of *Glcci1* is widespread in the MGE, and found only in scattered cells of the LGE (Fig. 1A). This is unlike Gsx2 expression, which is observed a day earlier at E10.5 throughout the LGE (Corbin et al., 2000, Torreson and Campbell, 2000; Waclaw et al., 2009). By E13.5 and E16.5, *Glcci1* is robustly expressed in a high dorsal to low ventral gradient of expression in the LGE (Fig. 1B and 1H), which is maintained at rostral and caudal LGE levels (Fig. 1G–H). At E18.5, the *Glcci1* expression gradient becomes restricted and largely confined to the dLGE (Fig. 1C). The dLGE is proposed to be the major source of embryonic olfactory bulb interneurons (Stenman et al., 2003, Waclaw et al., 2006; Wang et al., 2009). Genes of the dLGE, such as Er81, Sp8, and Tshz1 are expressed in the SVZ and in the maturing embryonic olfactory bulb interneurons (Stenman et al., 2003; Waclaw et al., 2006; Allen et al., 2007; Saino-Saito et al., 2007; Ragancokova et al., 2014). We analyzed olfactory bulb levels for *Glcci1* expression at E16.5 and E18.5 (Fig. 1E–F and data not shown). Interestingly, very few *Glcci1* cells are detected at rostral olfactory bulb levels (Fig. 1E). We observed the strongest expression in the ventral region of the olfactory bulb at caudal levels (Fig. 1F). Our data suggests that *Glcci1* may downregulate as dLGE cells migrate to the olfactory bulb and differentiate into olfactory bulb interneurons. At all examined stages, we noticed weak or absent expression in the ventral most region of the vLGE, located immediately dorsal to the MGE or MGE remnant expression of *Glcci1* (see arrows in Fig. 1B–D and 1H). The progressive gradient of *Glcci1* expression (high dorsal and low ventral) in the LGE is like the temporal dynamics of Gsx2 expression (Yun et al., 2001; Waclaw et al., 2009). Given that the LGE is a progenitor domain for the neurogenic niche in the postnatal SVZ (Young et al., 2007), we analyzed P7 brains and found similar restricted staining in the dorso-lateral SVZ region (Fig. 1D–D'), which also corresponds to where Gsx2 is highly expressed (Lopez-Juarez et al., 2013). In addition to characterizing the LGE expression of *Glcci1*, we also detected novel developmental expression at E16.5 in the cortex (Fig. 1G–H), olfactory epithelium (Fig. 1E), and dorsal septum at rostral levels (Fig. 1G).

Glcci1 is downstream of *Gsx2* in the LGE

Gsx2 mutants show dorso-ventral patterning defects that lead to reductions in both dLGE and vLGE markers in the LGE (Corbin et al., 2000; Toresson et al., 2000; Toresson and

Campbell, 2001; Yun et al., 2001, 2003; Carney et al., 2006; Waclaw et al., 2006; Wang et al., 2013). Given that *Glcci1* expression emerges later than Gsx2 expression in the LGE, and that it resolves into a similar high dorsal to low ventral gradient of expression by E18.5, it represents a potential *Gsx2* regulated downstream candidate gene. We performed a comparative analysis of *Glcci1* gene and protein expression in the LGE of $Gsx2^{RA/RA}$ null mutants (Waclaw et al., 2009). At E13.5, both *Glcci1* gene and protein expression are lost in *Gsx2* mutant LGE (Compare Fig. 2B,D to 2A,C), which is similar to the result observed with other dLGE SVZ genes such as Er81 and Sp8 (Stenman et al., 2003; Waclaw et al., 2006). There is a partial recovery of LGE identity at late embryonic stages in *Gsx2* mutants (after E14.5) when the closely related family member *Gsx1* expands into the LGE and compensates for the loss of *Gsx2* (Toresson and Campbell, 2001; Yun et al., 2003). In line with this, we detect weak *Glcci1* gene and protein expression in the LGE of late stage (E18.5) *Gsx2* mutants compared to controls (Compare Fig. 2F,H to Fig.2E,G). Therefore, our data suggests that *Glcci1* expression in the LGE is dependent on *Gsx* gene function.

There is no change in *Glcci1* gene and protein expression in the MGE of *Gsx2* mutant as compared to controls suggesting that *Gsx2* regulation of *Glcci1* is specific to the LGE. However, this is not surprising given that a MGE phenotype has not been described in either *Gsx2* or *Gsx1/2* mutants (Corbin et al., 2000; Toresson and Campbell, 2001; Yun et al., 2003; Wang et al., 2013). A recent study showed that *Glcci1* expression is reduced in the MGE of *Nkx2.1* mutants, which show defects in the generation of GABAergic neurons derived from the MGE (Sandberg et al., 2016). Therefore, *Glcci1* expression is regulated by different homeodomain transcription factors in the ventral telencephalon, with *Gsx2* controlling LGE expression and *Nkx2.1* directing MGE expression.

The temporal dynamics of Gsx2 expression in the developing LGE reflects a changing role in regulating vLGE/striatal specification or dLGE/interneuron specification (Waclaw et al., 2009). We have previously shown that the transgenic misexpression of Gsx2 in the developing cortex (*$Foxg1^{tTA/+}$;tetOGsx2* referred to as *Gsx2-GOF*) results in the downregulation of dorsal identity genes (Pax6, Tbr1) and an upregulation of LGE identity genes (Ascl1, Dlx1/2/5/6) including markers of vLGE/striatal identity (i.e. Isl1) (Waclaw et al., 2009). In addition, doxycycline (Dox) treatment of *Gsx2-GOF* from E7–E9 or E7–E11 delays the onset of transgenic Gsx2 expression but still results in a downregulation of dorsal identity genes and an increase in LGE identity genes (Ascl1, Dlx1/2/5/6). However, instead of an increase in vLGE genes (i.e. Isl1), the Dox delayed embryos show an increase in dLGE genes (i.e. Sp8) in the cortex, and suggests temporal role for Gsx2 in regulating vLGE and dLGE identity (Waclaw et al., 2009; Chapman et al., 2013). We used this *Gsx2-GOF* strategy to examine Glcci1 after early Gsx2 misexpression (onset of transgene expression at E9.0 with no Dox treatment) or late Gsx2 misexpression (Dox treatment E7–E11, resulting in the onset of transgenic Gsx2 expression after E11.5). We found that embryos with early misexpression of Gsx2 (compare Fig. 3B,B' to 3A,A') show increased Glcci1 immunoreactivity in the developing cortex. The early misexpression of Gsx2 disrupts the normal cortical morphology; however, the abnormal morphology can be improved by using Dox treatment from E7–E11 to delay the onset of transgene expression until E11.5 (Waclaw et al., 2009; Chapman et al., 2013). Glcci1 expression remained increased in the developing cortex even after Dox treatment from E7–E11 in Gsx2-GOF embryos (compare Fig. 3D to

3C). This data suggests that the timing of Gsx2 misexpression in the cortex does not change the activation of Glcci1 expression, which reflects the behavior of more uniformly expressed LGE identity genes like Ascl1 or Dlx genes and not the later expressed SVZ markers of vLGE/striatal (Isl1) or dLGE/interneuron (Sp8) identity. Thus, the expression of Glcci1 may represent a more mature LGE VZ progenitor in the *Gsx2* lineage.

Glcci1 is expressed in a subpopulation of cells enriched in the dLGE at the VZ/SVZ boundary

To further characterize *Glcci1* in the LGE, we performed double labeling with Gsx2 at E16.5 when both genes show a gradient of expression enriched in the dLGE. Since both antibodies to Glcci1 and Gsx2 were generated in rabbit, we performed in situ hybridization for *Glcci1* followed by immunohistochemistry for Gsx2 at E16.5. We found overlap of *Glcci1 in situ* signal in the Gsx2 positive domain of the LGE (Fig. 4A–B). Interestingly, the most apical Gsx2 positive cells (brown signal) in the VZ are *Glcci1* negative (Fig. 4B). *Glcci1* expression appears to overlap with Gsx2 in the basal half of the VZ near the VZ/SVZ boundary. Ascl1 is another marker of the VZ that is downstream of Gsx2 and accumulates in the basal half of the VZ near the VZ/SVZ boundary (Yun et al., 2002; Wang et al., 2009 and 2013; Chapman et al., 2013). Double immunofluorescence indicates that Glcci1 is cytoplasmic (Fig. 4D–E) and largely overlaps with Ascl1 in the dLGE and less so in the vLGE (Fig. 4D). Finally, double labeling either *Glcci1* gene or protein reveals minimal overlap with dLGE SVZ marker Sp8 (Fig. 4C and 4F). Our expression characterization suggests that Glcci1 represents a subpopulation of Gsx2 positive cells in the basal half of the VZ near the VZ/SVZ boundary region in the LGE. However, unlike Ascl1 which is expressed throughout the LGE, Glcci1 is enriched in regions of high Gsx2 expression, including the dLGE.

In summary, Glcci1 represents a unique marker that exhibits dynamic temporal expression, which resolves into a high dorsal to low ventral gradient in the LGE. *Glcci1* is downstream of Gsx2 and labels a subpopulation of progenitors in the basal half of the VZ near the VZ/SVZ boundary (Fig. 4G). Glcci1 gene or protein expression provides a useful tool to identify VZ progenitors at a more mature stage during the development and progression from a Gsx2 positive cell in the VZ to an Isl1 (vLGE) or Sp8 (dLGE) positive cell in the SVZ.

Experimental Procedures

Animals

The morning of vaginal plug detection was designated as day 0.5 for timed mouse embryo collections. *Gsx2* mutant embryos at E13.5 and E18.5 were generated with $Gsx2^{RA/+}$ mutant allele (Waclaw et al., 2009) maintained on CD-1 outbred mouse strain. *Gsx2-GOF* embryos ($Foxg1^{tTA/+};tetOGsx2$) were generated and Doxycycline (Sigma) treatment was given as previously described (Waclaw et al., 2009; Chapman et al., 2013). Processing of embryonic and postnatal brain tissue was completed as previously described (Waclaw et al., 2010). At least 3 *Gsx2-GOF* or *Gsx2* mutant embryos were stained for each stage analyzed. Animal

protocols were approved by the Cincinnati Children's Hospital Medical Center Institutional Animal Care and Use Committee in accordance with NIH guidelines.

In Situ Hybridization

In situ hybridization was essentially carried out as described (Toresson et al., 1999) except levamisole was added to blocking solution on day 2 and followed with PBT washes before the addition of Anti-Digoxigen antibody (1:2500, Roche). A *Glcci1* cDNA fragment (914bp) identical to the probe used in the Eurexpress database (Diez-Roux et al., 2011), was amplified from E14.5 mouse embryo forebrain cDNA with the following primer pair: 5'-ACGGGACCCTCATGTTCA-3' and 5'-CCAATTAACCCTCACTAAAGAGTGTTGCCAGAGCCGAG-3'. However, a T3 RNA polymerase sequence was added to the 3' Glcci1 primer for the generation of Digoxigenin-labeled antisense probes using T3 RNA polymerase (Roche). For combinatory *in situ* hybridization/IHC with *Glcci1* RNA probe and the rabbit-Gsx2 antibody (kind gift from K. Campbell- Cincinnati Children's Hospital Medical Center, 1:3000), slides were washed in PBS following the *in situ* hybridization development in BM purple (Roche) and IHC was completed as described below.

Immunohistochemistry and Immunofluorescence

Rabbit polyclonal anti-Glcci1 was obtained from Sigma-Aldrich (HPA001674) and used at 1:200 dilution for IHC and IF. Citrate retrieval was necessary to detect the Glcci1 signal. Boiling citrate retrieval buffer was added to slides in a coplin jar and covered for 30–40 minutes at room temperature. IHC and IF were carried out using standard techniques as described previously (Waclaw et al., 2010). A tyramide amplification kit (Invitrogen-ThermoFisher Scientific) was used to detect the fluorescent Glcci1 signal. Goat polyclonal anti-Sp8 (Santa Cruz, 1:3000) and Guinea-Pig anti-Ascl1 (kind gift from J. Johnson at UT-Southwestern, 1:10,000) were used for fluorescent double stains with the rabbit anti-Glcci1 antibody. Secondary antibodies were purchased from Jackson Immunoresearch (Cy3 or Cy5 conjugated Donkey-anti-Goat or Cy3 conjugated Donkey-anti-Guinea-Pig used at 1:200 dilution). Bright-field images were taken on a Leica DM2500 upright microscope with Leica DFC-500 camera using Leica acquisition software (Leica Microsytems). Fluorescent images were taken on a Nikon C2-Confocal Microscope using Nikon Elements software (Nikon Instruments Inc.).

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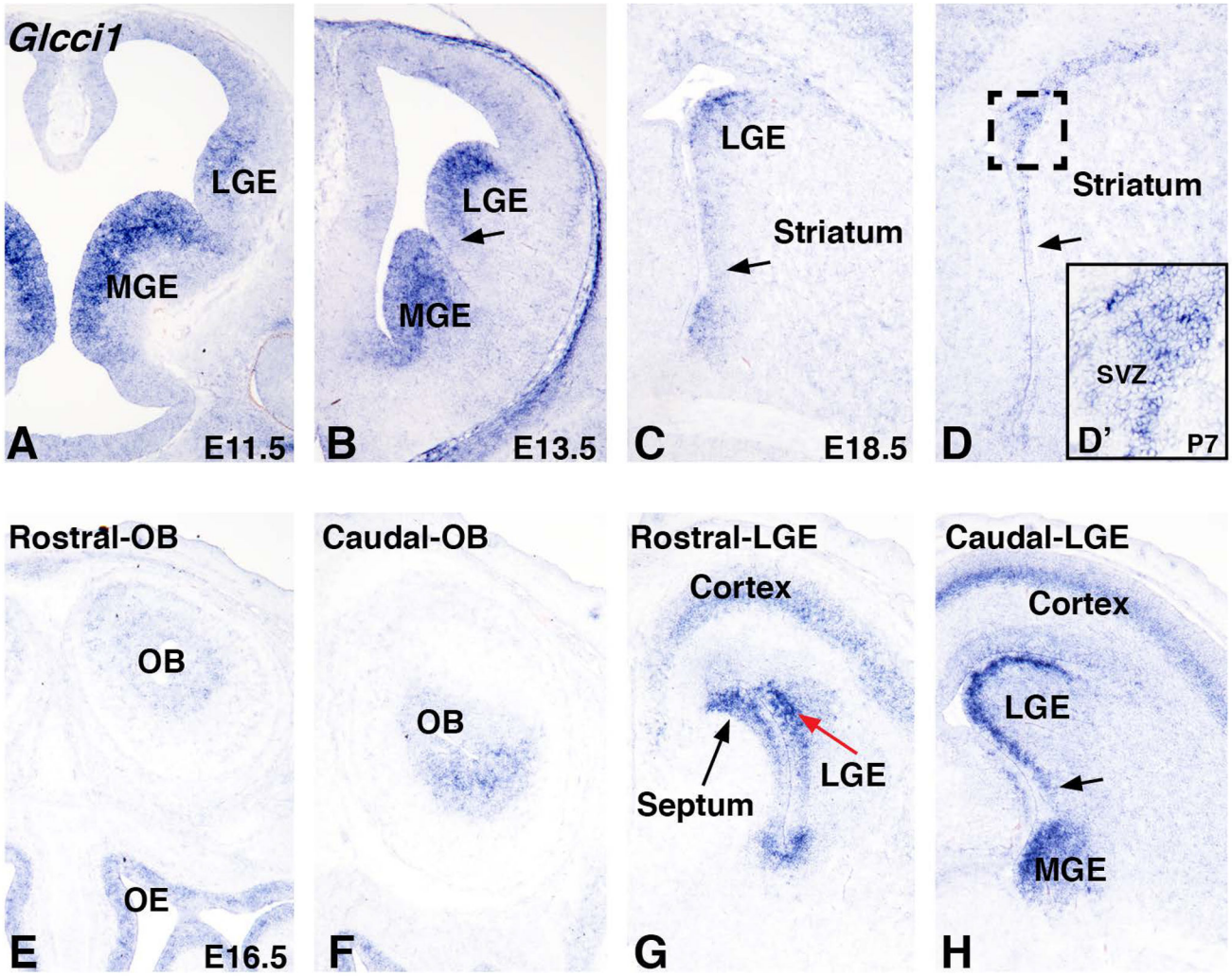

Figure 1.

Glcci1 expression in the LGE of the developing telencephalon. Scattered *Glcci1* expression is detected in the LGE at E11.5 (A). *Glcci1* is expressed in a high dorsal to low ventral gradient in the LGE at E13.5 (B), E16.5 (G,H), and E18.5 (C). By Postnatal day 7 (P7), *Glcci1* is enriched in the dorso-lateral SVZ (D). The dashed box in D represents a high magnification image of the SVZ (D'). Note the weak or lack of expression in the ventral most regions of the LGE (arrows in B-D, H) which is immediately dorsal to the robust staining in the MGE (B, H) or MGE remnant (C). Rostral to caudal examination of *Glcci1* at E16.5 in the olfactory bulb (E–F) and LGE (G–H). Rostral levels of the LGE (G) reveal staining on the medial side in the septum (black arrow) and lateral side in the LGE (red arrow). OB-olfactory bulb, OE-olfactory epithelium.

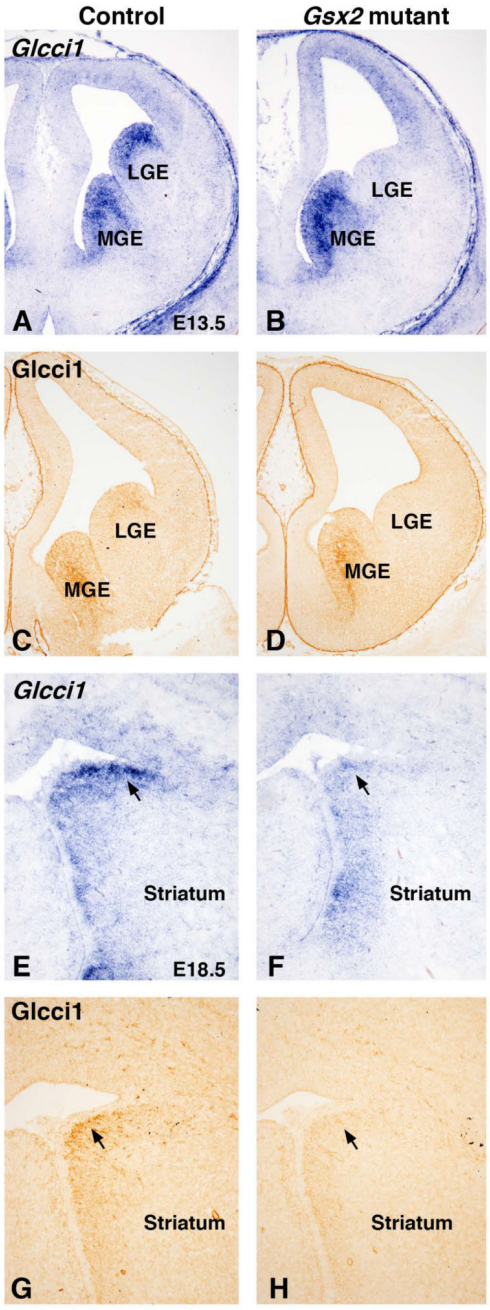

Figure 2.

Glcci1 gene and protein expression are reduced in the LGE of *Gsx2* mutants. At E13.5, *Gsx2* mutants show severe reduction in both *Glcci1* gene (compare B to A) and protein (compare D to C) expression in the LGE. Note that the expression of *Glcci1* remains in the MGE of *Gsx2* mutants (A–D). *Glcci1* gene (compare F to E) and protein (compare H to G) expression remains reduced in the LGE in late stage Gsx2 mutants (E18.5).

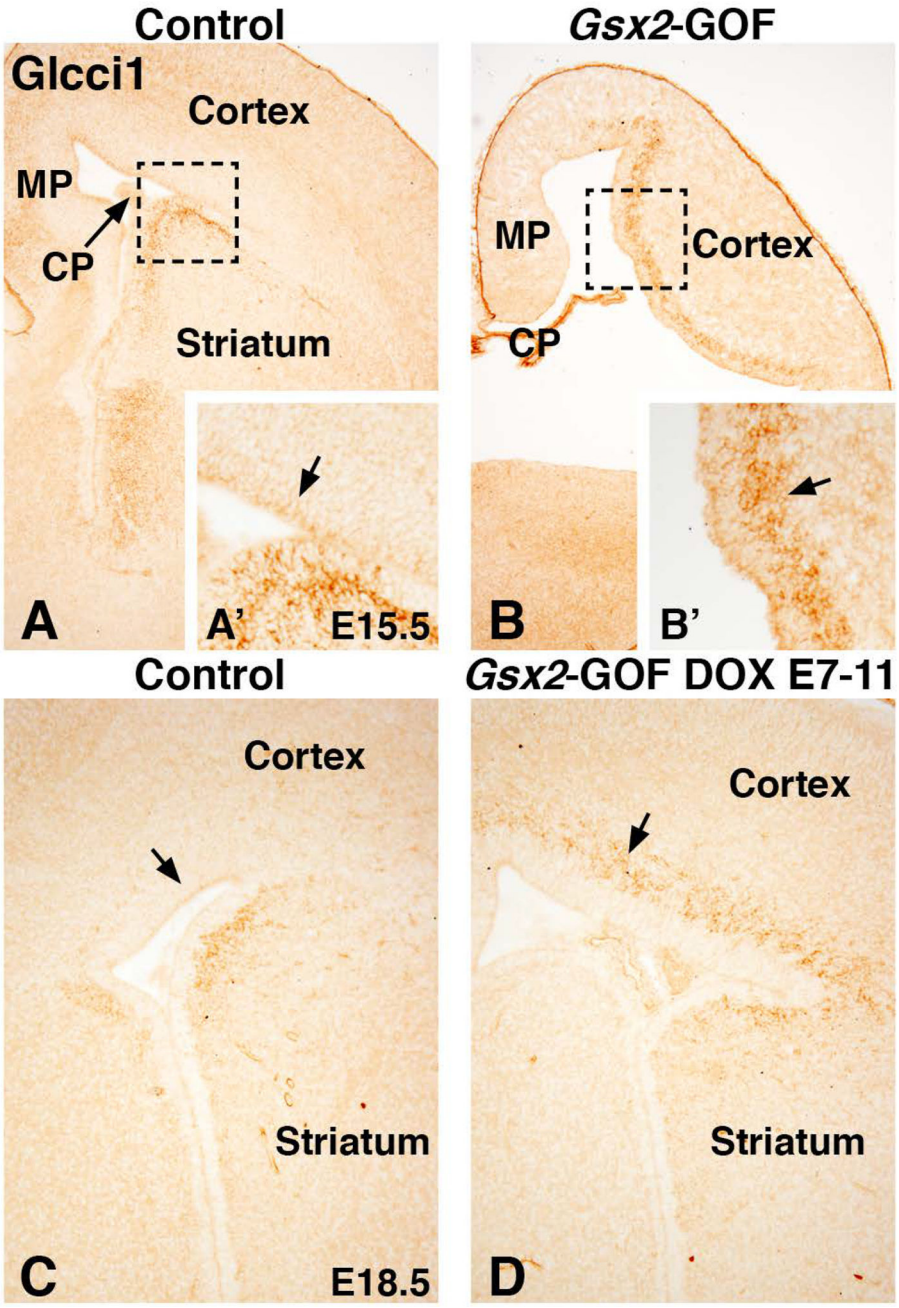

Figure 3.

Glcci1 expression is increased after early and late misexpression of Gsx2 in the cortex. At E15.5, *Gsx2-GOF* embryos show increase Glcci1 immunoreactivity in the cortex (compare B to A). Dashed boxes in A and B represent high power magnification views in A' and B'. Arrow in B' indicates ectopic expression of Glcci1 in *Gsx2-GOF*, which is not observed in the control cortex (arrow in A'). Glcci1 is ectopically expressed in the cortex of *Gsx2-GOF* embryos at E18.5 after delayed expression of the transgene using doxycycline treatment from E7–E11 (compare arrows in D to C). MP-medial pallium, CP-choroid plexus

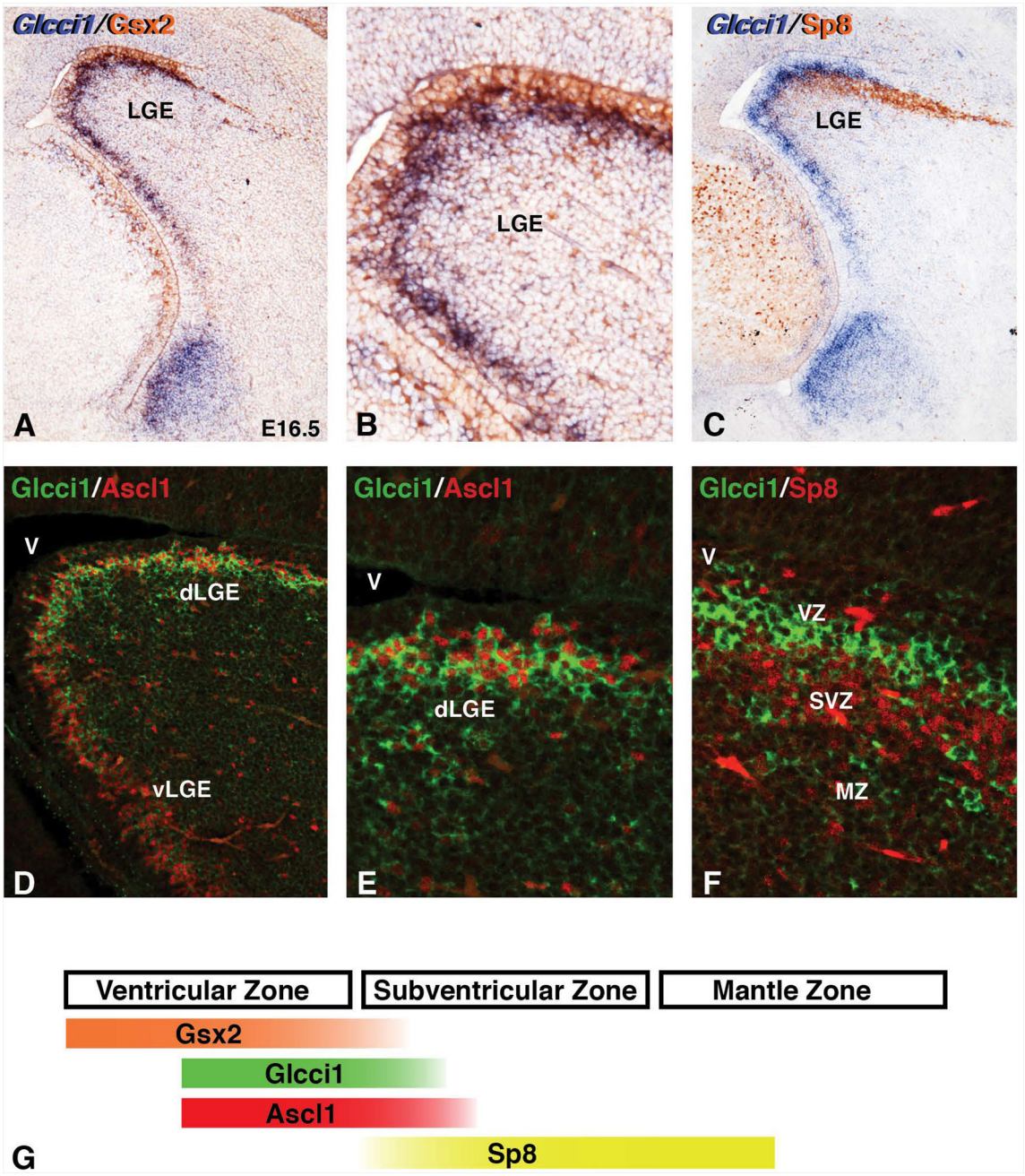

Figure 4.

Cell type characterization of *Glcci1* in the LGE at E16.5. Double labeling with *in situ* for *Glcci1* and immunohistochemistry for Gsx2 (A–B) or Sp8 (C) show that *Glcci1* is expressed in the basal half of the VZ in a subdomain of the Gsx2 positive cells in the LGE (A–B). *Glcci1* expression is largely absent from the Sp8 expressing SVZ cells in the LGE (C). B represents a higher magnification of A. Double immunofluorescence labeling of Glcci1 (green) and Ascl1(red) or Sp8(red) reveal that Glcci1 is cytoplasmic and largely overlaps with Ascl1 (D–E) at the VZ/SVZ boundary but not with Sp8 in the SVZ (F). E is a higher magnification view of D. A model of cell type specific expression of *Glcci1* in the LGE is

represented in G. V-ventricle, VZ- ventricular zone, SVZ-subventricular zone, MZ-mantle zone, vLGE-ventral LGE, dLGE-dorsal LGE.