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Neuroserpin expression during human brain development and in adult brain revealed by immunohistochemistry and single cell RNA sequencing

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Abstract

Neuroserpin is a serine-protease inhibitor mainly expressed in the CNS and involved in the inhibition of the proteolytic cascade. Animal models confirmed its neuroprotective role in perinatal hypoxia-ischaemia and adult stroke. Although neuroserpin may be a potential therapeutic target in the treatment of the aforementioned conditions, there is still no information in the literature on its distribution during human brain development. The present study provides a detailed description of the changing spatiotemporal patterns of neuroserpin focusing on physiological human brain development. Five stages were distinguished within our examined age range which spanned from the 7th gestational week until adulthood. In particular, subplate and deep cortical plate neurons were identified as the main sources of neuroserpin production between the 25th gestational week and the first postnatal month. Our immunohistochemical findings were substantiated by single cell RNA sequencing data showing specific neuronal and glial cell types expressing neuroserpin. The characterization of neuroserpin expression during physiological human brain development is essential for forthcoming studies which will explore its involvement in pathological conditions, such as perinatal hypoxia-ischaemia and adult stroke in human. **Key words:** human brain; neurodevelopment; neuroserpin; subplate.

Introduction

Neuroserpin belongs to the family of serine-protease inhibitors (Osterwalder et al. 1996; Vivien & Buisson, 2000). It is predominantly expressed in the central nervous system

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Accepted for publication *3 December 2018* Article published online *15 January 2019* (CNS) (Hastings et al. 1997; Krueger et al. 1997; Teesalu et al. 2004) but recent studies have also revealed extracerebral production (Chéret et al. 2012; Matsuda et al. 2016). Neuroserpin binds tissue plasminogen activator, blocking the conversion of plasminogen to plasmin and thus inhibiting the proteolytic cascade (Vivien & Buisson, 2000; Lee et al. 2017). This function makes neuroserpin a promising therapeutic target in conditions where the proteolytic homeostasis of the CNS is impaired, such as hypoxia-ischaemia (Millar et al. 2017).

There is still a dearth of knowledge on neuroserpin production and its interaction with molecular pathways in the human brain. This greatly hinders the translation of its already discovered neuroprotective roles in animal models (Yepes et al. 2000; Zhang et al. 2002; Lebeurrier et al. 2005; Ma et al. 2012; Li et al. 2017) towards developing pharmacological strategies and eventually bedside

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treatment. Our work aims to lay the foundations for future studies by discovering the spatiotemporal expression patterns of neuroserpin during physiological human brain development.

The current paper focuses on the pallial part of the developing telencephalon. Our special region of interest was the subplate, a transient developmental structure subjacent to the cortical plate. The precise role of this layer is still largely unknown, but there are data suggesting its important function in directing neuronal migration, the ingrowth of thalamocortical axons and the formation of associative corticocortical connections (Kostovic & Rakic, 1980, 1990; Kostovic & Judas, 2002). Some of the earliest synapses are formed in this layer during cortical development (Kostović & Judas, 2007). Moreover, the subplate represents the main efferent output from the early midfoetal cortex since development of cortical plate efferents is somewhat delayed and they have not yet reached the pons, medulla oblongata (Ip et al. 2011) or the spinal cord (Eyre et al. 2000). During the ingrowth of thalamocortical afferents to the cortical plate, these fibers are accumulated in the subplate (Krsnik et al. 2017). The thalamocortical projections have a complex interplay with the subplate and establish transient circuits during this period, once considered the 'waiting' period. The subplate has a very unique ECM composition which is particularly abundant in acidic glycosaccharides and therefore can be visualised by PAS-Alcian Blue staining (Kostovic & Rakic, 1990), which was also utilised in our study.

In this study, we identified stages of neuroserpin expression based on layer- and cell type- specific immunohistochemical detection of neuroserpin in the developing human cortical plate and subplate. These results were corroborated by single cell RNA sequencing data confirming the neuronal and glial production of neuroserpin (*Serpini1*) and revealing changing co-expression patterns between younger (6–15th gestational week) and older (15–22nd gestational week) cases.

In the first part of this paper, we describe the detailed distribution pattern of neuroserpin immunoreactive cells based on immunohistochemistry. In the second part, we provide an in-depth analysis of *Serpini1* co-expression networks and specify the clusters responsible for its production.

Methods

Subjects

Anonymised cases were received from the Oxford Brain Bank (OBB), the Netherlands Brain Bank (NBB) and the hGENESIS collection, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen. All material has been collected from donors from whom written informed consent had been obtained by the OBB or NBB for brain autopsy and use of material and clinical information for research purposes. We included samples in the study with available clinical information and postmortem neuropathological diagnoses confirming minimal hypoxic insults in the CNS. The identifier and age of cases are shown in Table 1. Prenatal ages were described using gestational weeks, which refer to the date of the last menstruation bleeding occurring before pregnancy. This usually precedes conception by 2 weeks (Bannister et al. 1995).

Immunohistochemistry

Serial sections (6 μ m thick) were cut in the coronal plane from paraffin-embedded blocks containing the frontal and temporal cortices and mounted on slides. Our immunohistochemical analysis was done as described in detail in an earlier study (Adorjan et al. 2017). Briefly, the following four primary anti-neuroserpin antibodies were applied: (1) rabbit, Abcam ab33077, 1 : 200; (2) mouse, Abcam ab55587, 1 : 200; (3) rabbit, Sigma HPA 001565, 1 : 200; (4) rabbit, Sino Biological 11107-RP02, 1 : 500. Application on adjacent (serial) sections gave similar staining patterns. In order to recognise the layers in developing cortex, staining with Nissl and PAS-Alcian Blue were done, as described previously (Kostovic et al. 2002).

Table 1 List of cases included in the immunohistochemical analysis

| Identifier | Age | Source |
|------------|-----------|----------|
| Z3379/124 | 7 gw | hGENESIS |
| 1317/196 | 9 gw | hGENESIS |
| 1425/270 | 10 gw | hGENESIS |
| 1265/94 | 13 gw | OBB |
| 146/11 | 14 gw | OBB |
| 1248/94 | 16 gw | OBB |
| 1120/95 | 16 gw | OBB |
| 41/14 | 18 gw | OBB |
| B5349 | 19 gw | OBB |
| 153/12 | 19 gw | OBB |
| 121/5 | 19 gw | OBB |
| 155/10 | 21 gw | OBB |
| 85/06 | 21 gw | OBB |
| 65/08 | 22 gw | OBB |
| 59/07 | 25 gw | OBB |
| 57/09 | 25 gw | OBB |
| 193/04 | 25 gw | OBB |
| 144/12 | 30 gw | OBB |
| 72/14 | 33 gw | OBB |
| 1054/91 | 38 gw | OBB |
| C2196 | 38 gw | OBB |
| C3492 | 40 gw | OBB |
| C2450 | 3 pw | OBB |
| C1951 | 3 pw | OBB |
| C3175 | 4 pw | OBB |
| 24/14 | 3 pm | OBB |
| 143/13 | 1 year | OBB |
| 77/13 | 1 year | OBB |
| 105/15 | 1.5 years | OBB |
| 1/15 | 2 years | OBB |
| 86/15 | 7 years | OBB |
| S11/081 | 55 years | NBB |
| S12/002 | 55 years | NBB |
| S12/071 | 57 years | NBB |
| \$10/196 | 60 years | NBB |
| S11/096 | 70 years | NBB |
| \$12/059 | 78 years | NBB |

Image analysis

Slides were digitised using slidescanners (AperioScanScope AT Turbo, Leica Biosystems; 3DHistech) at $20 \times$ and $40 \times$ magnification and stored on a server (msdlt-slide.dpag.ox.ac.uk). The regions of interest were outlined using the IMAGESCOPE programme (Aperio, v11.2.0.780) and the longest diameter of every neuroserpin-immunopositive (neuroserpin-ip) cell body in the telencephalic wall was manually measured. Heatmaps were visualised based on the coordinates of neuroserpin-ip cells using QGIS 3.2.2 software (Ic6 line).

Single cell RNA sequencing in developing human

Single cell sequencing was performed and analysed as described in Nowakowski et al. (2017). In addition to the analyses provided in that paper, we performed analyses of *Serpini1* (neuroserpin) across ages and cell types to identify populations with enriched expression. To do this, we performed correlations and violin plots from the normalised sequencing read counts per million with the metadata properties of interest. The threshold for considering a gene to be expressed was a count per million greater than 0 in at least 30 cells in the dataset. A count of transcripts per million of 2 lies in the bottom quartile of expression for all genes.

Single cell RNA sequencing data analysis in adult human

Violin plots for *Serpini1* expression were generated from human cortex single nucleus dataset available in Lake et al. (2016). First, neuronal nuclei from the temporal cortex were isolated from the whole dataset, resulting in 1406 single nucleus transcriptomes. Classification of neuronal clusters into excitatory (Ex) and inhibitory (In) subtypes was done as in Lake et al. (2016). The level of gene

expression was estimated using transcript per million mapped reads (tpm), and the threshold of tpm \geq 10 was used for Serpini1 to be considered expressed.

Results

Our immunohistochemical analysis, which spanned from the 7th gestational week (gw) until adulthood and was based on 37 cases, revealed five different patterns in terms of the distribution of neuroserpin immunoreactive cells in the developing human cortex:

Neuroserpin begins to be produced in the first trimester (7–10th gw)

Neuroserpin was expressed in the telencephalon as early as the 9th gw (Fig. 1A). Neuroserpin immunoreactivity was localised in cells with elongated morphology and cell processes in the upper part of the developing cortical plate adjacent to the marginal zone (for a detailed description of lamination see Kostović & Judas, 2007). The immunopositive (ip) cells were restricted to the developing ventrolateral pallium. No ip cells were seen in the developing diencephalon, mesencephalon or rhombencephalon at this stage. The aforementioned neuroserpin-ip cell population in the ventrolateral pallium was also present in the 10th gw (Fig. 1B). Besides, neuroserpin-ip perikarya and processes appeared in the ventricular zone of the anterior thalamic neuroepithelium (Fig. 1B). Although no telencephalic expression was detected in the 7th gw, there was a conspicuous neuroserpin-ip population situated in the rhombencephalon (Fig. 1C).

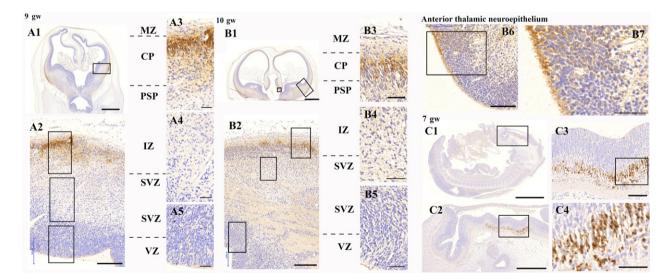


Fig. 1 Neuroserpin expression in the first trimester of human brain development. A1–A5: neuroserpin is expressed in the upper cortical plate of the ventrolateral pallium in the 9th gw. B1–B5: neuroserpin is expressed in the upper cortical plate of the ventrolateral pallium in the 9th gw. B1–B5: neuroserpin is expressed in the upper cortical plate of the ventrolateral pallium in the 10th gw. B6–B7: the anterior thalamic neuroepithelium also contained neuroserpin-ip cells with long processes. C1–C4: neuroserpin is expressed by a distinct neuronal population in the rhombencephalon in the 7th gw. No telencephalic expression was observed at this stage of development. MZ, marginal zone; CP, cortical plate; PSP, pre-subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. Scale bars: (A1) 1000 μ m, (A2, B2) 200 μ m, (A3–A5) B3–B6, (C3) 100 μ m, (B1, C1) 2000 μ m, (B7, C4) 50 μ m, (C2) 500 μ m.

The early second trimester (13th–16th gw)Neuroserpin immunoreactivity was localised in migrating neurons through the whole cortical plate and subplate.

By the second trimester, neuroserpin immunoreactivity was localised in cortical plate and subplate cells with long processes showing migratory morphology (Fig. 2). These were present through the whole thickness of the cortical plate. Production in the ventricular zone, however, showed regional differences. In general, cortical, LGE and CGE ventricular zones contained neuroserpin-ip cells, whereas MGE did not (data not shown).

The middle of the second trimester (18th–22nd gw)Neuroserpin immunoreactivity was localised in migrating/maturing neurons of the middle and deep cortical plate and subplate.

During the middle of the second trimester, neuroserpin immunoreactivity was localised to cortical and subplate cells with migratory morphology, suggesting its presence mostly

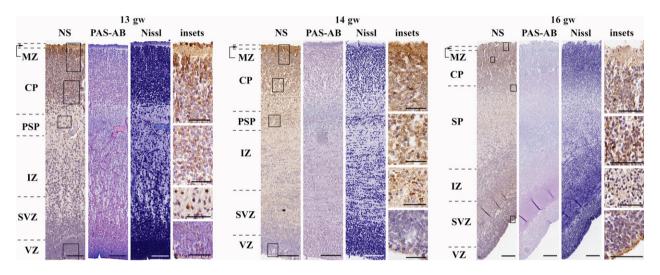


Fig. 2 Neuroserpin expression in the early second trimester (frontal lobe). Neuroserpin-ip cells were present through the cortical plate and subplate. They had long processes and elongated perikarya showing migratory morphology. Minimal expression was seen in the marginal zone. Neuroserpin-ip cells were observed in the ventricular zone of the lateral and caudal ganglionic eminences. MZ, marginal zone; CP, cortical plate; PSP, pre-subplate; SP, subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. Scale bars: (13th gw) NS, PAS-AB, Nissl: 100 μm, insets: 50 μm; (14th–16th gw) NS, PAS-AB, Nissl: 200 μm, insets: 50 μm.

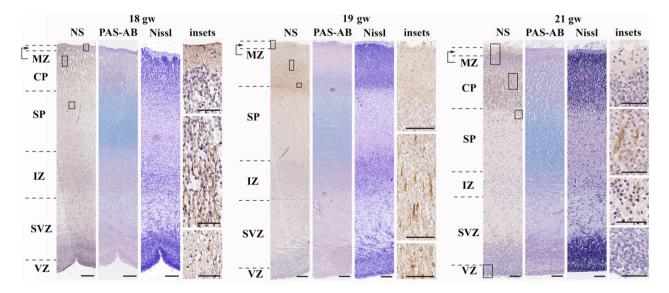


Fig. 3 Neuroserpin expression in the middle second trimester in the human telencephalon (frontal lobe). Neuroserpin-ip cells with elongated perikarya and long processes directed to the pial surface were mainly seen in the middle and deep cortical plate and subplate. No neuroserpin-ip cells were observed in the upper cortical plate. MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. Scale bars: (18th gw) NS, PAS-AB, Nissl: 100 µm; (19th–21st gw) NS, PAS-AB, Nissl: 200 µm, insets: 50 µm.

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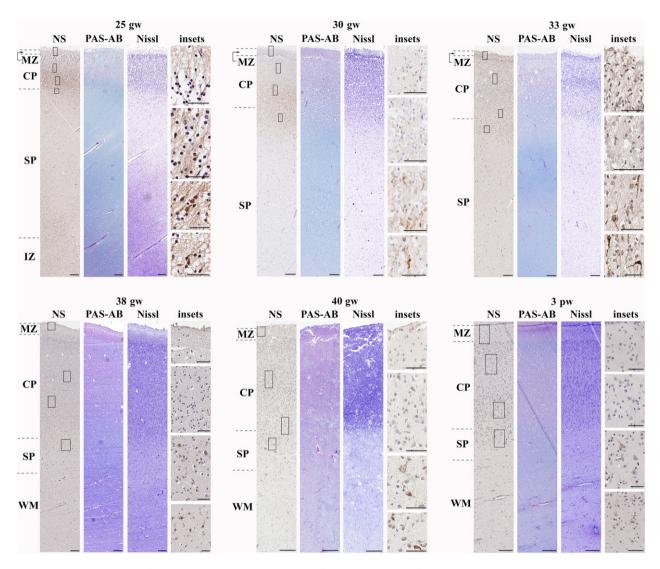


Fig. 4 Neuroserpin expression from the late second trimester until the first postnatal month (frontal lobe). Neuroserpin-ip cells were situated to the deep cortical plate and subplate with a pyramidal and elongated shape. The upper and middle regions of the cortical plate were devoid of neuroserpin immunoreactivity. MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; WM, white matter. Scale bars: (25th gw– 3rd pw) NS, PAS-AB, Nissl: 200 μm, insets: 50 μm.

in migrating neurons (Fig. 3). In contrast to the previous stage, neuroserpin-ip cells were distributed in the middle and deeper cortical plate but not in the upper cortical plate. Interregional differences were seen in case of the VZ, as neuroserpin-ip cells were usually contained in dorsal regions but not ventral ones (data not shown). No neuroserpin immunoreactivity was seen in the VZ from the 21st gw (Fig. 3).

From the late second trimester until the end of the first postnatal month (25th gw–1st pm)Neuroserpin was found in migrating/maturing neurons of the deep cortical plate and subplate.

During the late second trimester and the whole third trimester, neuroserpin-ip cells were localised to the deep cortical plate and subplate (Fig. 4). Their morphology suggested migrating and maturing neurons, predominantly of pyramidal type. This pattern remained characteristic of neuroserpin expression until the first postnatal month. The VZ was devoid of neuroserpin-ip cells at this stage. Their presence in the upper and middle cortical plate was minimal (Fig. 4). *From the third postnatal month until adulthood (3rd pm-adult)*

After the 3rd postnatal month, the expression of neuroserpin was propagated towards the upper cortical layers (Fig. 5). By the second year of age, all cortical layers consisted of neuroserpin-ip cells, most of them with pyramidal morphology. This pattern remained characteristic of childhood and adulthood as well.

Heatmaps based on the co-ordinates and density of neuroserpin-ip cells helped to distinguish the distribution

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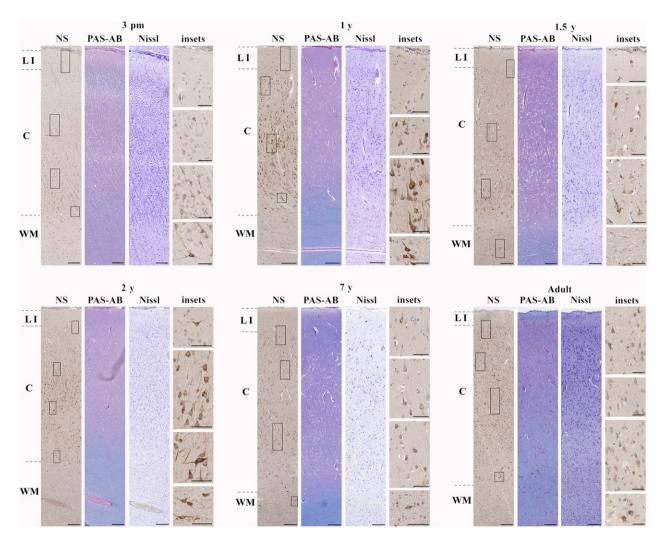


Fig. 5 Neuroserpin expression from the third postnatal month until adulthood in the cortex (frontal lobe). Neuroserpin-ip neurons were observed throughout layers of the cortex with the exception of layer 1, where minimal immunoreactivity was present. The majority of neuroserpin-ip neurons had a pyramidal morphology. LI, layer 1; C, cortical layers 2–6; WM, white matter. Scale bars: (3 pm–adult) NS, PAS-AB, Nissl: 200 µm, insets: 50 µm.

patterns of these cells and identify the above-described five characteristic stages during human cortical development (Fig. 6 and Supporting Information Fig. S1).

Single cell RNA sequencing in embryonic brain revealed pyramidal neurons and migrating neurons as the main sources of Serpini1

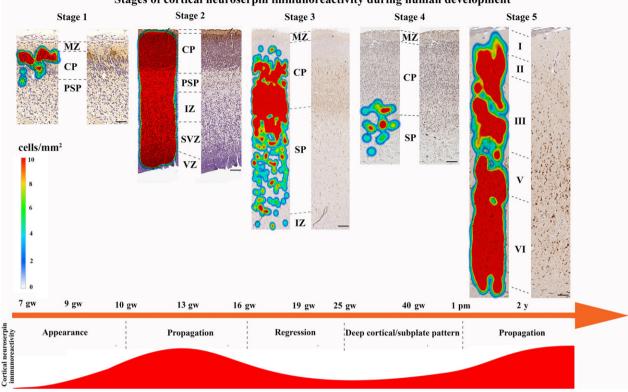
Single cell RNA sequencing made possible the detailed cell-type analysis of neuroserpin-expressing cells. This work was based on correlation levels of *Serpini1* to more than 20 000 other transcripts within a dataset already clustered to different cell types during human brain development (Nowakowski et al. 2017). This approach confirmed *Serpini1* expression in post migratory pyramidal neurons and migrating neurons, and the scarcity of neuroserpin production by neural stem/progenitor cells, oligodendrocytes and microglia (Fig. 7). Also, our results on co-expression patterns highlighted the possible astrocytic- (*Glul, Fabp7, Slc1a2*) and

subplate-specific (*Bcl11b*, *Cplx2*, *Map1b*) cell-type origin of neuroserpin production (Fig. 7).

According to our data, *Serpini1* was expressed in 11.37% of total neurons (170/1494 across all developmental ages examined. Almost half of them (36.47%, 62/170) had acquired the excitatory neuron-specific neurofilament *Nefm*. Only a handful of them belonged to the calbindinor calretinin-interneurons (2.35% and 2.35%, 4/170 and 4/ 170, respectively). Radial glial clusters were identified as an early source of low levels of *Serpini1* expression that peaked around the 15th gw (Fig. 8). However, no robust neuroserpin-ip radial glial pattern was observed by our immunohistochemical analysis (Figs 1–3).

In adult cortex, neuroserpin is ubiquitously expressed in pyramidal neurons and GABAergic interneurons

To describe expression pattern of *Serpini1* gene across neuronal subtypes in the adult human cortex, we utilised a



Stages of cortical neuroserpin immunoreactivity during human development

Fig. 6 Heatmaps showing the characteristic stages of neuroserpin immunoreactivity in the developing human cortex based on the density of neuroserpin-ip cells. MZ, marginal zone; CP, cortical plate; PSP, pre-subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. Scale bars: (stage 1) 50 μm, (stage 2) 80 μm, (stage 3) 130 μm, (stage 4) 140 μm, (stage 5) 150 μm.

previously published dataset of the temporal cortex (Lake et al. 2016). Of 1406 excitatory and inhibitory neurons sequenced, > 80% of neurons expressed Serpini1 with tpm > 10 that was set as a threshold of expression (Fig. 9A,B). All subtypes of pyramidal neurons except deep layer Ex6 and Ex8 had ~ 90% or more neurons expressing Serpini1. On average, ~50% of GABAergic interneurons expressed Serpini1 above the threshold. However, a few groups of GABAergic interneurons expressed higher levels of Serpini1; in particular, almost 100% of In6 cluster that can be assigned as parvalbumin-positive interneurons expressed Serpini1. Among other interneurons expressing higher levels of Serpini1 are In4 (reelin and Ndnf-double positive, but vasoactive intestinal peptide-negative interneurons) and In8 (somatostatin and Nos1-double positive interneurons).

Discussion

The regulation of the proteolytic homeostasis by neuroserpin in the human brain may be of crucial importance in the reaction to hypoxia-ischaemia (Millar et al. 2017). To understand its role in brain pathology, however, first its tissue distribution and cell type-specific production should be thoroughly analysed using various methods. Our study revealed hitherto unknown patterns of neuroserpin production during human brain development.

The importance of our work is underscored by the fact that there is a scarcity of descriptive morphological studies done in human. These works have been eclipsed by the boom of molecular biological approaches and the advent of big data in recent decades (Hawrylycz et al. 2015; Dillman et al. 2017). However, without descriptive morphological studies in human there is no prospect of linking molecular biological data to distinct neural circuits and eventually understanding the function of the investigated molecules in a spatial-dependent context.

The first telencephalic expression of neuroserpin was observed in the 9th gw when the developing pallium is formed as a trilaminar structure (marginal zone, cortical plate and pre-subplate, Kostović & Judas, 2007). Based on the morphology of neuroserpin-ip cells, most likely they belong to migrating neurons reaching the interface between the marginal zone and cortical plate. No neuroserpin-ip cells were seen in the deeper pallial layers or in the ventricular zone at this early stage of brain development.

The expansion of neuroserpin-ip cells throughout layers of the telencephalic wall was observed between the 13rd and 18th gw. The identity of these cells as migrating neurons and maturing excitatory neurons was confirmed by

Correlation of SERPINI1 with transcripts grouped to different clusters

Interneuronal markers

| | Pyramidal | neuronal | markers | | | | |
|--------|---|--|---|--|--|--|---|
| | | СР | GZ | Deep | Upper | Deepyounger | Deepolder |
| | NEFL | 0.230192 | 0.050199 | 0.048558 | 0.098598 | 0.096149379 | -0.13960701 |
| | NEFM | 0.126429 | 0.129592 | -0.06456 | 0.14515 | -0.106441091 | 0.399236426 |
| | NEFH | -0.01716 | 0.039189 | -0.08491 | -0.00097 | -0.072689474 | -0.15992587 |
| | TBR1 | 0.090796 | 0.035297 | 0.021227 | 0.059226 | 0.02332458 | -0.01503762 |
| | FEZF2 | 0.117264 | -0.01265 | 0.071495 | 0.316356 | 0.091328613 | -0.21086247 |
| | SATB2 | 0.104655 | 0.0437 | | | -0.199789087 | |
| | CUX1 | | | | | -0.066720705 | |
| | SLC17A7 | | | 0.125222 | | | 0.061736551 |
| | SLC17A6 | | 0.014192 | | | -0.00817647 | |
| | SLC17A8 | | | | | -0.093449724 | |
| | | | | | | | |
| | POU3F3 | 0.008581 | | | | 0.06398866 | |
| | POU3F3 POU3F2 | | | | | -0.045882393 | |
| | | | -0.05775 | | | | |
| | ZFPM2 | | | | | 0.02601928 | |
| | | -0.00987 | | | -0.02159 | | NA |
| | SOX5 | | -0.01981 | | | 0.026036414 | |
| | CUX2 | 0.005371 | 0.017635 | -0.04313 | -0.04598 | -0.045383925 | NA |
| | 6 h | | | | | | |
| | Subplate n | | C 7 | - | | - | |
| | | | GZ | Deep | | Deepyounger | |
| lurr1 | NR4A2 | | | | | -0.111652323 | |
| TIP2 | BCL11B | | | | | 0.094502805 | |
| | CTGF | 0.012092 | | 0.049867 | | | |
| | CPLX2 | | | 0.109568 | | 0.096102319 | |
| | CPLX1 | | | | | -0.01797311 | |
| | CPLX3 | | | NA | | NA | NA |
| | MAP1B | 0.118334 | 0.050303 | 0.14702 | 0.130856 | 0.141154469 | 0.326281013 |
| | LPAR1 | 0.003894 | -0.0215 | NA | -0.00684 | NA | NA |
| | | | | | | | |
| | Migrating | neuroblas | t markers | | | | |
| | | CP | GZ | Deep | Upper | Deepyounger | Deepolder |
| | DCX | 0.029298 | 0.037975 | 0.116512 | 0.079762 | 0.119926105 | 0.085526752 |
| | NCAM1 | 0.093754 | 0.054322 | 0.055016 | 0.111751 | 0.029769995 | 0.226876776 |
| | NCAM2 | 0.080461 | 0.015555 | 0.035379 | 0.086519 | 0.055957546 | -0.25941112 |
| | CD24 | 0.069986 | 0.033917 | -0.03527 | 0.089707 | -0.058452947 | 0.230449888 |
| | TUBB3 | 0.0324 | -0.0085 | 0.124052 | 0.009833 | 0.130666374 | -0.13960701 |
| | MAP2 | 0.09546 | -0.01009 | 0.051781 | 0.03714 | 0.045707086 | 0.082964019 |
| | | | | | | | |
| | Radial glia | markers | | | | | |
| | - | | GZ | Deep | Upper | Deepyounger | Deepolder |
| | NES | 0.049506 | | | | -0.068739717 | |
| | VIM | | -0.01133 | | | 0.229806828 | |
| | ALDH1L1 | | | | | 0.082144635 | |
| | GLUL | | | 0.027346 | | -0.0195301 | |
| LBP | FABP7 | | | | | | |
| LDF | FADE 7 | | | | | | |
| | | 0.069712 | -0.03344 | 0.082096 | 0.151155 | 0.063326124 | 0.3035/8123 |
| | | | | | | 0.063326124 | 0.303578123 |
| | Neural ste | em cell/pro | genitor ce | II markers | | | |
| | | em cell/pro CP | genitor ce <mark>GZ</mark> | II markers Deep | Upper | Deepyounger | Deepolder |
| | SOX2 | em cell/pro CP -0.07615 | genitor ce GZ -0.03132 | II markers Deep –0.03356 | Upper -0.0347 | Deepyounger -0.035486649 | Deepolder NA |
| | SOX2 HES1 | em cell/pro CP -0.07615 0.01619 | GZ -0.03132 -0.02849 | II markers Deep -0.03356 0.106875 | Upper -0.0347 -0.02001 | Deepyounger -0.035486649 0.110969832 | Deepolder NA NA |
| | SOX2 HES1 HES5 | CP -0.07615 0.01619 -0.02618 | GZ -0.03132 -0.02849 0.014161 | Ell markers Deep -0.03356 0.106875 NA | Upper -0.0347 -0.02001 -0.00804 | Deepyounger -0.035486649 0.110969832 NA | NA NA NA NA |
| | SOX2 HES1 | CP -0.07615 0.01619 -0.02618 -0.01897 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 | II markers Deep -0.03356 0.106875 NA -0.04313 | Upper -0.0347 -0.02001 -0.00804 -0.01155 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 | NA NA NA NA NA |
| | SOX2 HES1 HES5 | CP -0.07615 0.01619 -0.02618 -0.01897 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 | II markers Deep -0.03356 0.106875 NA -0.04313 NA | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA | NA NA NA NA NA NA |
| | SOX2 HES1 HES5 NKX2.1 | CP -0.07615 0.01619 -0.02618 -0.01897 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 | II markers Deep -0.03356 0.106875 NA -0.04313 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA | Deepyounger -0.035486649 0.110969832 NA -0.045383925 | NA NA NA NA NA NA |
| | SOX2 HES1 HES5 NKX2.1 GSX2 | CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04377 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA | Deepolder NA NA NA NA NA -0.26848178 |
| | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 | CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04377 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 | Deepyounger - 0.035486649 0.110969832 NA - 0.045383925 NA - 0.025786734 | Deepolder NA NA NA NA NA -0.26848178 |
| | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 | CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.03103 NA | GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04377 -0.04411 NA | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.025786734 -0.046597916 | Deepolder NA NA NA NA NA -0.26848178 NA NA |
| | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 | CP -0.07615 0.01619 -0.02618 -0.01269 0.026381 -0.03103 NA -0.07609 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04317 NA -0.04317 -0.04411 NA -0.04107 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.025786734 -0.046597916 NA | Deepolder NA NA NA NA -0.26848178 NA NA -0.18156184 |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED | CP -0.07615 0.01619 -0.02618 -0.01269 0.026381 -0.03103 NA -0.07609 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.058217 | Il markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04411 NA -0.04107 -0.0036 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.069176 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.025786734 -0.046597916 NA -0.03318227 0.021532431 | Deepolder NA NA NA NA -0.26848178 NA -0.18156184 -0.281554 |
| /ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 | em cell/pro CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.01269 0.026381 -0.07609 0.063111 -0.01909 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.058217 0.01342 | Il markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04375 0.106875 0.004313 0.006875 0.004313 0.006875 0.004313 0.006875 0.004313 0.006875 0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.0036 0.137325 0.137325 0.13755 0.137555 0.137555 0.1375555 0.13755555 0.1375555555 0.1375555555555 0.13 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.069176 0.019172 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.025786734 -0.046597916 NA -0.03318227 0.021532431 0.143222956 | Deepolder NA NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA |
| 1ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 | em cell/pro CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.01269 0.026381 -0.07609 0.063111 -0.01909 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.058217 0.01342 | Il markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04377 -0.04375 0.106875 0.004313 0.006875 0.004313 0.006875 0.004313 0.006875 0.004313 0.006875 0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.004377 -0.0036 0.137325 0.137325 0.13755 0.137555 0.137555 0.1375555 0.13755555 0.1375555555 0.1375555555555 0.13 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.069176 0.019172 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.025786734 -0.046597916 NA -0.033318227 0.021532431 | Deepolder NA NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA |
| 1ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC3E | CP -0.07615 0.01619 -0.02638 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013056 | GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.058217 0.01555 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04313 NA -0.04317 -0.04411 NA -0.04107 -0.0036 0.137252 -0.00393 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.069176 0.019172 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.025786734 -0.046597916 NA -0.03318227 0.021532431 0.143222956 | Deepolder NA NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA |
| /ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC3E | CP -0.07615 0.01619 -0.02618 -0.01269 -0.01269 0.026381 -0.01269 0.063111 -0.01909 0.033656 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00648 -0.00648 0.00723 0.013109 NA -0.00839 0.058217 0.01342 0.051555 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04317 NA -0.04317 -0.04317 NA -0.04317 -0.04317 -0.04317 -0.0366 0.137325 -0.00393 ers | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.069176 0.019172 0.010116 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.0455786734 -0.046597916 NA -0.03318227 0.021532431 0.143222956 -0.007842848 | Deepolder NA NA NA -0.26848178 NA -0.28156184 -0.281554 NA 0.02524271 |
| IASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC38 | CP -0.07615 0.01619 -0.02618 -0.01269 0.026381 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.01909 0.03656 iate progen CP | GZ -0.03132 -0.02849 0.014161 -0.00648 -0.00646 0.00723 0.013109 NA -0.00839 0.058217 0.01342 0.051555 hitor market GZ | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.0437 -0.0437 -0.0437 -0.0437 -0.0437 -0.0437 -0.0437 -0.0437 -0.00393 -0.00393 -0.0499 -0.0499 -0.0497 -0.00393 -0.0039 - | Upper -0.0347 -0.02001 -0.03127 -0.03127 -0.02308 NA 0.000784 0.009176 0.019172 0.010116 Upper | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger | Deepolder NA NA NA NA -0.26848178 NA -0.21554 NA -0.21554 NA 0.02524271 |
| NASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC3E FOXO3 | -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013656 iate progen | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.05855 -0.0051555 -0.01542 0.051555 -0.008789 | Il markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04317 -0.04407 -0.04107 -0.0036 0.137225 -0.00393 ers Deep -0.06343 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.009172 0.019172 0.019172 0.01916 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.050903247 | Deepolder NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA 0.02524271 Deepolder -0.19263858 |
| //ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC38 | -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013656 iate progen | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.05855 -0.0051555 -0.01542 0.051555 -0.008789 | Il markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04317 -0.04407 -0.04107 -0.0036 0.137225 -0.00393 ers Deep -0.06343 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.009172 0.019172 0.019172 0.01916 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger | Deepolder NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA 0.02524271 Deepolder -0.19263858 |
| /ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC3I Intermedi FOXO3 PROX1 | -0.07615 0.01619 -0.02618 -0.012618 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.01909 0.033656 iate progen CP 0.016586 -0.0377 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00648 0.00723 0.013109 NA -0.00839 0.058217 0.01342 0.051555 hitor marke GZ 0.008789 0.017161 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04411 NA -0.04107 -0.0036 0.137225 -0.00393 ers Deep -0.06343 -0.06343 -0.0051 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.009176 0.019172 0.010116 Upper 0.026971 -0.01332 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.045597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.050903247 -0.005767163 | Deepolder NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA 0.02524271 Deepolder -0.19263858 |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 KK167 HIST3H3 EZH2 EED ASCL1 MAP1LC3F ED ASCL1 MAP1LC3F FOXO3 PROX1 | -0.07615 0.01619 -0.02618 -0.01807 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013056 iate progen CP 0.016586 -0.0377 co.01878 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00688 -0.00639 NA -0.00839 0.051555 hitor marke GZ 0.008789 0.017161 tion/linea | II markers Deep -0.03356 0.106875 NA -0.04377 -0.04377 -0.04377 -0.04307 -0.0036 0.13725 -0.00393 ers Deep -0.06343 -0.0051 ge progres | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper 0.026971 -0.01332 sion market | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.025786734 -0.046597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.050903247 -0.055767163 | Deepolder NA NA NA NA NA NA NA NA NA 0.25548178 NA 0.02524271 Deepolder -0.19263858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 KK167 HIST3H3 EZH2 EED ASCL1 MAP1LC3F ED ASCL1 MAP1LC3F FOXO3 PROX1 | -0.07615 0.01619 -0.02618 -0.01807 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013056 iate progen CP 0.016586 -0.0377 co.01878 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00688 -0.00639 NA -0.00839 0.051555 hitor marke GZ 0.008789 0.017161 tion/linea | II markers Deep -0.03356 0.106875 NA -0.04377 -0.04377 -0.04377 -0.04307 -0.0036 0.13725 -0.00393 ers Deep -0.06343 -0.0051 ge progres | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper 0.026971 -0.01332 sion market | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.045597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.050903247 -0.005767163 | Deepolder NA NA NA NA NA NA NA NA NA 0.25548178 NA 0.02524271 Deepolder -0.19263858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 KK167 HIST3H3 EZH2 EED ASCL1 MAP1LC3F ED ASCL1 MAP1LC3F FOXO3 PROX1 | -0.07615 0.01619 -0.02618 -0.01897 -0.01897 -0.01897 -0.01269 0.026381 -0.030365 0.033161 -0.03365 0.03356 -0.03377 -0.016586 -0.0377 | GZ -0.03132 -0.02849 0.014161 -0.006846 -0.00646 0.00723 0.013109 NA -0.00839 0.053155 hitor markt GZ 0.008789 0.017161 tion/linea GZ | II markers Deep -0.03356 0.106875 NA -0.04377 -0.04377 -0.04377 -0.04307 -0.036 0.137325 -0.0036 Deep -0.06343 -0.0051 ge progress Deep | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.019172 0.010116 Upper 0.026971 -0.01332 sion market Upper | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.025786734 -0.046597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.050903247 -0.055767163 | Deepolder NA NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA 0.025242711 Deepolder -0.19263858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 MKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC3E FOXO3 PROX1 | CP -0.07615 0.01619 -0.02618 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.01309 0.03656 iate progen CP -0.016586 -0.0377 CP -0.02658 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00648 0.00723 0.013109 NA -0.00639 0.058217 0.01342 0.051555 nitor marka GZ 0.008789 0.017161 tion/lineag GZ 0.005552 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04377 -0.04411 NA -0.04107 -0.0036 0.137325 -0.00393 ers Deep -0.06343 -0.00511 ge progres Deep -0.01702 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.009716 0.019172 0.010116 Upper -0.026971 -0.01332 sion market Upper -0.0585 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.04597916 NA -0.033318227 0.021532431 0.14322956 -0.07842848 Deepyounger -0.050903247 -0.055767163 Deepyounger | Deepolder NA NA NA -0.26848178 NA -0.18156184 -0.281554 NA 0.02524271 Deepolder -0.19263858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 KK167 HIST3H3 EZH2 EED ASCL1 MAP1LC3E FOXG3 PROX1 Interneur | -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.033656 iate progen CP 0.016586 -0.0377 conal migra CP -0.02658 | genitor ce GZ -0.03132 -0.02849 0.0214161 -0.00688 -0.00646 0.00723 0.013109 NA -0.00839 0.013122 0.051555 itor market GZ 0.008789 0.017161 tior/linea GZ 0.005552 -0.00761 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04313 NA -0.04317 -0.04317 -0.0036 0.13725 -0.00393 ers Deep -0.06343 -0.0051 ge progres Deep -0.01702 -0.04313 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper -0.01332 sion market Upper -0.026971 -0.01332 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.025786734 -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.055093247 -0.005767163 The second sec | Deepolder NA NA NA NA NA -0.26848178 NA -0.25648178 NA -0.25648178 NA 0.02524271 Deepolder -0.19263858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 HIST3H3 EZH2 EED ASCL1 MAP1LC3F FOXO3 PROX1 Interneur FOXO3 PROX1 | CP -0.07615 0.01619 -0.02618 -0.01897 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.033656 iate progen CP 0.016586 -0.0377 CP -0.02658 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00686 0.00723 0.00839 0.058217 0.01342 0.05555 -0.008789 0.017161 -0.008789 0.017161 -0.005552 -0.005552 -0.005552 -0.005552 -0.003512 | Il markers Deep -0.03356 0.106875 NA -0.04377 -0.04377 -0.04377 -0.04307 -0.0036 0.137325 -0.0038 ers Deep -0.06343 -0.00511 ge progres Deep -0.01702 -0.04313 -0.01702 -0.04313 -0.02841 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009176 0.019172 0.010116 Upper -0.01332 sion market Upper -0.00585 -0.01243 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.025786734 -0.04597916 NA -0.033318227 0.021532431 0.143222831 0.14322924 -0.0050903247 -0.005767163 Peepyounger -0.01817155 Deepyounger -0.01817155 -0.045383925 -0.045383925 -0.030696649 | Deepolder NA NA NA NA NA -0.26848178 NA -0.28154184 NA 0.02524271 Deepolder -0.19263858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 KKI67 HIST3H3 EZH2 EED ASCL1 MAP1LC3E Internedii FOXO3 PROX1 Interneur LHX6 FEZF1 SOX6 NR2F2 | CP -0.07615 0.01619 -0.02618 -0.01269 0.026381 -0.03303 NA -0.07609 0.033656 iate proger CP -0.018586 -0.0377 CP -0.02658 -0.01236 -0.024214 | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00648 -0.00638 -0.00639 0.05552 -0.005155 -0.00761 -0.000761 -0.00761 | Il markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04317 -0.04411 NA -0.04107 -0.0366 0.137325 -0.00363 -0.0051 ge progress Deep -0.01702 -0.04313 -0.02431 -0.0251 -0.0 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.000784 0.059176 0.019172 0.010116 Upper -0.026971 -0.01332 sion marks Upper -0.02585 -0.01283 -0.01283 -0.01284 -0.012 | Deepyounger -0.035486649 0.110969832 NA -0.045383925 NA -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.07842848 Deepyounger -0.050903247 -0.005767163 PS Deepyounger -0.01817155 -0.01817155 -0.01817155 -0.018174583925 | Deepolder NA NA NA -0.26848178 NA -0.26848178 NA -0.281554 NA -0.281554 NA -0.281554 NA -0.281554 NA -0.28254271 Deepolder NA -0.12963858 NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 EED ASC11 MK167 HIST3H3 EED ASC11 MAP1LC31 FOX03 FOX03 FOX03 FOX03 FOX03 FOX03 FOX03 FOX04 FOX04 FOX04 FOX04 FOX05 FO | CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.03103 NA -0.077609 0.063111 -0.013056 iate progen CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.01237 CP 0.016586 -0.01237 CP -0.01247 -0.024747 -0.02247 -0.0247 | genitor ce GZ -0.03132 -0.02849 0.0014161 -0.00688 -0.00688 -0.00839 0.013109 NA -0.00839 0.015155 itor marke GZ 0.008789 0.017161 tior/linea GZ 0.005552 -0.00761 -0.03051 0.063678 0.016194 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04313 NA -0.04317 -0.04317 -0.04107 -0.0036 0.13725 -0.00393 ers Deep -0.06343 -0.00511 ge progres Deep -0.06343 -0.00511 ge progres Deep -0.04313 -0.00511 0.038341 -0.04313 -0.02841 0.038341 -0.04313 -0.04313 -0.02841 0.038341 -0.04313 -0.04313 -0.02841 -0.04313 -0.02841 -0.038341 -0.04313 -0.04313 -0.02841 -0.038341 -0.04313 -0.04313 -0.02841 -0.038341 -0.04313 -0.04313 -0.02841 -0.04313 -0.02841 -0.04313 -0.02841 -0.04313 -0.038341 -0.04313 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04333 -0.04333 -0.04313 -0.04335 -0.04335 -0.04335 -0.04335 -0.04335 -0.04335 -0.04335 -0.0455 -0. | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper 0.026971 -0.01332 sion marks Upper -0.01283 -0.01283 -0.01283 -0.01143 -0.02194 -0.01143 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.045383925 NA -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.05767163 Peepyounger -0.045383925 -0.045383925 -0.045383925 -0.045383925 | Deepolder NA NA NA NA NA NA NA -0.26848178 NA -0.26848178 NA -0.25648178 NA -0.2524271 Deepolder -0.19263858 NA Deepolder NA NA NA NA NA NA NA NA NA NA NA NA NA |
| IASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 EED ASC11 MK167 HIST3H3 EED ASC11 MAP1LC31 FOX03 FOX03 FOX03 FOX03 FOX03 FOX03 FOX03 FOX04 FOX04 FOX04 FOX04 FOX05 FO | CP -0.07615 0.01619 -0.02618 -0.01897 -0.01269 0.026381 -0.03103 NA -0.077609 0.063111 -0.013056 iate progen CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.0377 CP 0.016586 -0.01237 CP 0.016586 -0.01237 CP -0.01247 -0.024747 -0.02247 -0.0247 | genitor ce GZ -0.03132 -0.02849 0.0014161 -0.00688 -0.00688 -0.00839 0.013109 NA -0.00839 0.015155 itor marke GZ 0.008789 0.017161 tior/linea GZ 0.005552 -0.00761 -0.03051 0.063678 0.016194 | II markers Deep -0.03356 0.106875 NA -0.04313 NA -0.04313 NA -0.04317 -0.04317 -0.04107 -0.0036 0.13725 -0.00393 ers Deep -0.06343 -0.00511 ge progres Deep -0.06343 -0.00511 ge progres Deep -0.04313 -0.00511 0.038341 -0.04313 -0.02841 0.038341 -0.04313 -0.04313 -0.02841 0.038341 -0.04313 -0.04313 -0.02841 -0.04313 -0.02841 -0.038341 -0.04313 -0.04313 -0.02841 -0.038341 -0.04313 -0.04313 -0.02841 -0.038341 -0.04313 -0.04313 -0.02841 -0.04313 -0.02841 -0.04313 -0.02841 -0.04313 -0.038341 -0.04313 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.03841 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04313 -0.04333 -0.04333 -0.04313 -0.04335 -0.04335 -0.04335 -0.04335 -0.04335 -0.04335 -0.04335 -0.0455 -0. | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper 0.026971 -0.01332 sion marks Upper -0.01283 -0.01283 -0.01283 -0.01143 -0.02194 -0.01143 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.045383925 NA -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.05767163 Peepyounger -0.045383925 -0.045383925 -0.045383925 -0.045383925 | Deepolder NA NA NA NA NA -0.26848176 NA -0.25648176 NA -0.2554271 Deepolder -0.21554 NA Deepolder -0.19263855 NA Deepolder NA NA NA NA NA NA NA NA NA NA NA NA NA |
| MASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 KK167 HIST3H3 EZH2 EED ASCL1 MAP1LC3F FOXO3 PROX1 Interneum FOXO3 PROX1 Interneum LHX6 FEZF1 SOX6 NR2F2 SP8 DLX1 | -0.07615 0.01619 -0.02618 -0.01897 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013056 -0.033656 -0.03376 -0.01256 -0.0377 -0.01256 -0.0377 -0.0258 -0.0377 -0.01259 -0.0258 -0.0377 -0.01259 -0.0377 -0.01259 -0.0258 -0.0377 -0.0258 -0.0377 -0.01259 -0.0258 -0.0377 -0.01259 -0.0258 -0.0377 -0.01258 -0.01258 -0.0377 -0.01258 -0.0258 -0.01258 -0.0258 - | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00688 -0.00682 0.013109 NA -0.00839 0.051555 -0.008789 0.017161 -0.005552 -0.005552 -0.005552 -0.005552 -0.005552 -0.005552 -0.005552 -0.00516 -0.005152 -0.00516 -0.005152 -0.0051555 -0.005152 -0.005152 -0.005152 -0.005152 -0 | Il markers Deep -0.03356 0.106875 NA -0.04377 -0.04411 NA -0.04107 -0.0036 0.13725 -0.00393 Peep -0.06343 -0.00518 Beep -0.06343 -0.00518 Beep -0.01702 -0.04313 -0.02841 0.038341 -0.02548 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper 0.026971 -0.01322 sion market Upper -0.02194 -0.01143 -0.02194 -0.021 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.025786734 -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.005767163 Deepyounger -0.05767163 Pepyounger -0.035767163 Pepyounger -0.01817155 -0.045383925 -0.030696649 0.047188293 -0.0458383925 -0.0458383925 -0.0458383925 -0.0458383925 -0.0459783575 -0.05978575 -0.05978575 | Deepolder NA NA NA NA NA NA NA -0.2684817 NA -0.252427 Deepolder O.1926385 NA Deepolder NA Deepolder NA NA NA NA NA |
| ASH1 | SOX2 HES1 HES5 NKX2.1 GSX2 FOXG1 EED ASC11 MK167 HIST3H3 EED ASC11 MAP1LC31 FOX03 FOX03 FOX03 FOX03 FOX03 FOX03 FOX03 FOX04 FOX04 FOX04 FOX04 FOX05 FO | -0.07615 0.01619 -0.02618 -0.01897 -0.01897 -0.01269 0.026381 -0.03103 NA -0.07609 0.063111 -0.013056 -0.033656 -0.03376 -0.01256 -0.0377 -0.01256 -0.0377 -0.0258 -0.0377 -0.01259 -0.0258 -0.0377 -0.01259 -0.0377 -0.01259 -0.0258 -0.0377 -0.0258 -0.0377 -0.01259 -0.0258 -0.0377 -0.01259 -0.0258 -0.0377 -0.01258 -0.01258 -0.0377 -0.01258 -0.0258 -0.01258 -0.0258 - | genitor ce GZ -0.03132 -0.02849 0.014161 -0.00688 -0.00688 -0.00682 0.013109 NA -0.00839 0.051555 -0.008789 0.017161 -0.005552 -0.005552 -0.005552 -0.005552 -0.005552 -0.005552 -0.005552 -0.00516 -0.005152 -0.00516 -0.005152 -0.0051555 -0.005152 -0.005152 -0.005152 -0.005152 -0 | Il markers Deep -0.03356 0.106875 NA -0.04377 -0.04411 NA -0.04377 -0.04107 -0.0036 0.13725 -0.00393 Peep -0.06343 -0.00518 ge progres Deep -0.06343 -0.00518 ge progres Deep -0.01702 -0.04313 -0.02841 0.038341 -0.02548 | Upper -0.0347 -0.02001 -0.00804 -0.01155 NA 0.03127 -0.02308 NA 0.009712 0.010116 Upper 0.026971 -0.01322 sion market Upper -0.02194 -0.01143 -0.02194 -0.021 | Deepyounger -0.035486649 0.110969832 NA -0.025786734 -0.045383925 NA -0.04597916 NA -0.033318227 0.021532431 0.143222956 -0.007842848 Deepyounger -0.05767163 Peepyounger -0.045383925 -0.045383925 -0.045383925 -0.045383925 | Deepolder NA NA NA NA NA NA NA -0.2684817 NA -0.2684817 NA -0.26155 NA Deepolder -0.28155 NA Deepolder NA Deepolder NA NA NA NA NA NA NA NA NA NA NA NA NA |

| | | CP | GZ | Deep | Upper | Deepyounger | Deepolder |
|---------------------------|--|--|---|--|---|---|--|
| | CALB1 | 0.13242 | -0.00645 | 0.023783 | 0.119282 | 0.02435309 | NA |
| | CALB2 | -0.00982 | 0.005753 | 0.061192 | -0.02206 | 0.067710201 | -0.1396070 |
| | PVALB | NA | NA | NA | NA | NA | NA |
| | NPY | 0.035586 | -0.01316 | 0.008697 | 0.034629 | 0.008337948 | NA |
| | VIP | 0.000677 | -0.0046 | NA | -0.00907 | NA | NA |
| | SST | -0.05238 | 0.032697 | NA | -0.02053 | NA | NA |
| | ССК | 0.087405 | -0.00525 | NA | 0.059807 | NA | NA |
| SERT | SLC6A4 | 0.118185 | -0.01143 | 0.066011 | 0.01044 | 0.068530306 | NA |
| | SCGN | -0.03366 | -0.02674 | 0.002719 | 0.052298 | 0.007559216 | -0.1396070 |
| VGAT | SLC32A1 | -0.02846 | 0.061928 | NA | 0.039818 | NA | NA |
| | RELN | 0.096125 | -0.02222 | 0.05679 | -0.00253 | 0.064613433 | -0.1396070 |
| | GAD1 | -0.06189 | 0.034091 | -0.07706 | 0.008261 | -0.078986887 | -0.1396070 |
| | GAD2 | -0.06255 | -0.02956 | -0.04313 | -0.02035 | -0.045383925 | NA |
| | | | | | | | |
| | Oligoden | Iroglia | | | | | |
| | ongouene | СР | GZ | Deep | Upper | Deepyounger | Deenolder |
| | OLIG1 | | -0.00815 | | -0.00956 | | NA |
| | OLIG2 | | -0.00888 | | -0.00814 | | NA |
| | PLP1 | | | | | -0.027897856 | |
| | MBP | | | 0.130232 | | 0.135810378 | |
| | CLDN11 | | | -0.06803 | | -0.071601869 | |
| | | | | | | | |
| | Microglia | | | | | | |
| | | СР | GZ | Deep | Upper | Deepyounger | Deepolder |
| | AIF1 | -0.03096 | -0.00617 | NA | -0.00994 | NA | NA |
| | CD68 | -0.01929 | -0.00615 | NA | -0.01008 | NA | NA |
| CD45 | PTPRC | -0.03117 | 0.115858 | -0.03439 | 0.035071 | -0.036599636 | NA |
| | | 0.01007 | -0.00755 | 0.095797 | 0 070437 | 0.099819422 | NA |
| | TMEM119 | -0.01007 | | | | | |
| | TMEM119 | -0.01807 | 0.00755 | | 0.070107 | 0.033013422 | |
| | | | | | 0.070107 | 0.035013422 | |
| | | populatio | | Deep | | | |
| | | populatio | ns | Deep NA | Upper -0.01792 | Deepyounger | |
| | Astrocytic | populatio CP -0.00637 | ns GZ -0.0389 | NA | Upper -0.01792 | Deepyounger NA | Deepolder NA |
| | Astrocytic GFAP | populatio CP -0.00637 -0.02593 | ns GZ -0.0389 -0.01933 | NA -0.04313 | Upper -0.01792 | Deepyounger NA -0.045383925 | Deepolder NA NA |
| | Astrocytic GFAP S100B | populatio CP -0.00637 -0.02593 -0.01197 | ns GZ -0.0389 -0.01933 | NA -0.04313 0.078988 | Upper -0.01792 -0.02029 | Deepyounger NA -0.045383925 | Deepolder NA NA NA |
| | Astrocytic GFAP S100B ALDH1L1 | populatio CP -0.00637 -0.02593 -0.01197 -0.0073 | ns GZ -0.0389 -0.01933 0.028255 0.036547 | NA -0.04313 0.078988 0.027346 | Upper -0.01792 -0.02029 -0.00633 0.012699 | Deepyounger NA -0.045383925 0.082144635 | Deepolder NA NA NA 0.4234630 |
| Cnxin 43 | Astrocytic GFAP S100B ALDH1L1 GLUL CD44 | population CP -0.00637 -0.02593 -0.01197 -0.0073 -0.00143 | ns GZ -0.0389 -0.01933 0.028255 0.036547 | NA -0.04313 0.078988 0.027346 -0.04313 | Upper -0.01792 -0.02029 -0.00633 0.012699 | Deepyounger NA -0.045383925 0.082144635 -0.0195301 -0.045383925 | Deepolder NA NA NA 0.4234630 |
| Cnxin 43 | Astrocytic GFAP S100B ALDH1L1 GLUL CD44 | population CP -0.00637 -0.02593 -0.01197 -0.0073 -0.00143 0.043886 | ns GZ -0.0389 -0.01933 0.028255 0.036547 -0.00049 | NA -0.04313 0.078988 0.027346 -0.04313 NA | Upper -0.01792 -0.02029 -0.00633 0.012699 0.173295 -0.00857 | Deepyounger NA -0.045383925 0.082144635 -0.0195301 -0.045383925 | Deepolder NA NA NA 0.4234630 NA NA |
| Cnxin 43 | Astrocytic GFAP S100B ALDH1L1 GLUL CD44 GJA1 | population CP -0.00637 -0.02593 -0.01197 -0.0073 -0.00143 0.043886 | GZ -0.0389 -0.01933 0.028255 0.036547 -0.00049 0.050774 | NA -0.04313 0.078988 0.027346 -0.04313 NA | Upper -0.01792 -0.02029 -0.00633 0.012699 0.173295 -0.00857 | Deepyounger NA -0.045383925 0.082144635 -0.0195301 -0.045383925 NA | Deepolder NA NA NA 0.4234630 NA NA |
| | Astrocytic GFAP S100B ALDH1L1 GLUL CD44 GJA1 AQP4 | Populatio CP -0.00637 -0.02593 -0.01197 -0.0073 -0.00143 0.043886 0.006953 NA | GZ -0.0389 -0.01933 0.028255 0.036547 -0.00049 0.050774 -0.01927 NA | NA -0.04313 0.078988 0.027346 -0.04313 NA -0.00129 NA | Upper -0.01792 -0.02029 -0.00633 0.012699 0.173295 -0.00857 -0.01295 NA | Deepyounger NA -0.045383925 0.082144635 -0.0195301 -0.045383925 NA -0.001727968 | Deepolder NA NA NA 0.4234630 NA NA NA NA |
| Cnxin 43 GLAST BLBP | Astrocytic GFAP S100B ALDH1L1 GLUL CD44 GJA1 AQP4 AQP9 | populatio CP -0.00637 -0.02593 -0.01197 -0.0073 -0.00143 0.043886 0.006953 NA -0.04007 | rs GZ -0.0389 -0.01933 0.028255 0.036547 -0.00049 0.050774 -0.01927 NA -0.02986 | NA -0.04313 0.078988 0.027346 -0.04313 NA -0.00129 NA -0.01135 | Upper -0.01792 -0.02029 -0.00633 0.012699 0.173295 -0.00857 -0.01295 NA -0.06293 | Deepyounger NA -0.045383925 -0.0195301 -0.0195301 -0.045383925 NA -0.001727968 NA | Deepolder NA NA 0.4234630 NA NA NA NA NA NA |
| GLAST | Astrocytic GFAP S100B ALDH1L1 GLUL CD44 GJA1 AQP4 AQP9 SLC1A3 | populatio CP -0.00637 -0.02593 -0.01197 -0.0073 -0.00143 0.043886 0.006953 NA -0.04007 | rs GZ -0.0389 -0.01933 0.028255 0.036547 -0.00049 0.050774 -0.01927 NA -0.02986 -0.05344 | NA -0.04313 0.078988 0.027346 -0.04313 NA -0.00129 NA -0.01135 0.082096 | Upper -0.01792 -0.02029 -0.00633 0.012699 0.173295 -0.00857 -0.01295 NA -0.06293 | Deepyounger NA -0.045383925 0.082144635 -0.0195301 -0.045383925 NA -0.001727968 NA -0.011877965 0.063326124 | Deepolder NA NA 0.4234630 NA NA NA NA -0.1396070 0.30357812 |

Fig. 7 Correlation levels of *Serpini1* to different transcripts during development visualised by heatmap (based on Nowakowski et al. 2017). Transcripts are grouped in specific cell types. CP, cortical plate; GZ, granular zone; Deep, deep cortical; Upper, upper cortical; Deep younger, deep cortical <15 gestational week; Deep older, deep cortical > 15th gw.

single cell RNA sequencing data. This period coincides with the first major wave of neurons migrating from the germinal zones towards the cortical plate. As the telencephalic wall increased in thickness during the late second trimester, neuroserpin-ip cells gradually localised to deeper layers of the developing cortical plate and the subplate. This matched with single cell RNA sequencing data that characterised a novel neuroserpin-ip subplate cell type co-expressing *Cplx2*, *Bcl11b* (*Ctip2*) and *Map1b*. *Map1b* has already been predicted as a potential subplate marker in rat (Teng et al. 2001).

The selective expression of neuroserpin by deep cortical plate/subplate cells during the third trimester and early postnatal period (between the 25th gw and 1st postnatal month) suggests dynamically changing functions of this molecule, perhaps adapting to the need of the lamination sequence, thalamocortical fibre ingrowth into the cortical plate and maturation of cortical plate circuits. Our observations imply that neuroserpin expression is down-regulated in migrating neurons after passing the subplate/ deep cortical plate. A question arises why this process may be necessary because such a down-regulation may cause vulnerability to hypoxic stress. However, increased vulnerability of early cortical plate neurons may be within the scope of the developmental programme, as this phenomenon may assist the selection of the fittest neurons within the cortical plate. Those neurons which do not fit properly into the neural circuitry, i.e. those with impaired firing patterns/metabolic activity, do not survive and may undergo apoptosis and be phagocytosed by microglia

(Harry & Kraft, 2012). The continuous expression of neuroserpin by deep cortical/subplate neurons and hence their selective resistance to hypoxic stress may be necessary because during the inside–out lamination sequence these neurons provide the directing cues for incoming waves of migrating neurons. Therefore it is important to have these neurons in place and not replaced by new-comers.

It is important to note that other factors may also contribute to the vulnerability of cortical plate/migrating neurons to hypoxia in the early stage of development (which coincides with the first propagation of neuroserpin expression). For example, the absence of glutamatergic input on these early cortical plate/migrating neurons can also lead to hypoxic vulnerability, as explained by the lack of NMDA receptor-mediated neuroprotection (Marini et al. 2001; Marini et al. 1998).

The propagation of neuroserpin expression after the 3rd postnatal month signals the end of the time-window when cortical plate neurons may be particularly vulnerable to hypoxic stress. Those neurons which were properly fitted into the neural circuitry and survived this selection period, may acquire the neuroprotective protease inhibitor, neuroserpin. The role of neuroserpin in adult cortex is underscored by its ubiquitous expression within almost all neuronal subtypes shown in this study and others (Teesalu et al. 2004). Because of the short half-life of this molecule (~ 10 min, Lee et al. 2017) its constitutive expression may be vital in blocking the activation of the proteolytic cascade in the CNS.

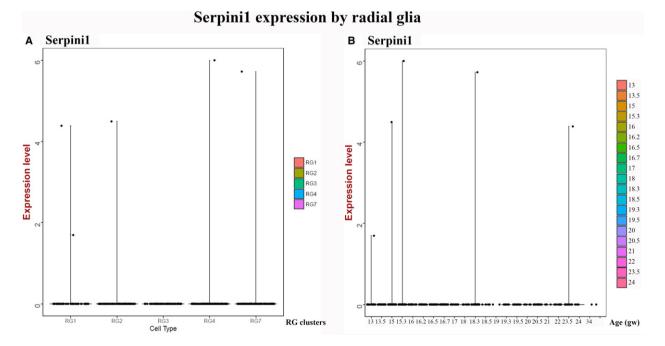


Fig. 8 Expression of neuroserpin in developing human cortex described by single cell transcriptomics analysis. (A) Violin plot for neuroserpin expression in different radial glial subtypes (counts per million). (B) Violin plot for neuroserpin expression by radial glial clusters plotted along the age (gestational week) (Nowakowski et al. 2017).

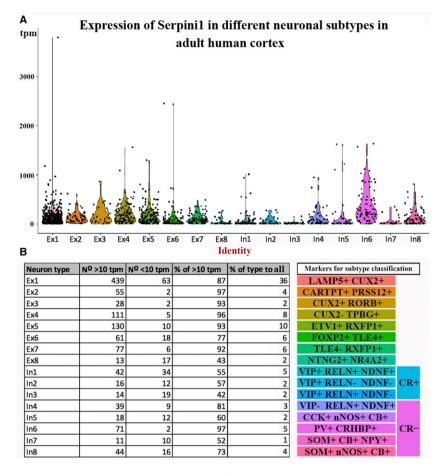


Fig. 9 Expression of neuroserpin in adult human temporal cortex as identified by single cell transcriptomics analysis. (A) Violin plot for neuroserpin expression in each subtype of excitatory and inhibitory neurons from dataset of single cell RNA sequencing data in Lake et al. (2016). (B) Table summarizing the number and percentage of neurons for each subtype that expressed neuroserpin above the threshold (tpm > 10). Subtype-markers are shown on the right hand side.

Results from developmental animal models reported the subplate specific expression of neuroserpin (mouse: Kondo et al. 2015). Nonetheless, caution is warranted when extrapolating data from rodent models to human because the deep cortical expression pattern observed in adult mouse (Wu et al. 2010; Kondo et al. 2015) is markedly different from that found in adult human. The almost panneuronal neuroserpin expression in adult human cortex (Teesalu et al. 2004) is confirmed by our study, which suggests different roles of this molecule or increased anti-proteolytic demand by neurons in human. Future studies should reveal whether the pan-neuronal cortical expression of neuroserpin is a unique feature in human or other primate taxa, and should decipher the possibility of changing functions of neuroserpin between different mammalian taxonomic groups.

Our work provides cues for the possible proteolytic mechanism involved in cortical plate development, especially for a selection period for cortical plate neurons which may coincide with the regression of neuroserpin from the cortical plate starting from the 18th gw. This period extends until the propagation of neuroserpin within the cortex starting from the 3rd postnatal month. The endogenous low level of neuroserpin expression observed in cortical plate neurons during the perinatal period may be promising for therapeutic approaches which aim to improve neuronal survival by increasing the baseline level of neuroserpin following perinatal hypoxia-ischaemia. Future studies are warranted to reveal the role of neuroserpin in human perinatal hypoxia and other relevant models (neuroserpin KO mouse, Rice-Vannucci) focusing on molecular interplays with hypoxia/proteolysis and synaptic assembly networks.

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Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

I.A. and Z.M. conceived the study. I.A., T.T. and K.M. did the immunohistochemical staining. I.A., T.T. and C.F. scanned sections and measured immunoreactivity. B.M. performed Nissl and PAS-Alcian Blue staining. S.M. and T.T. carried out the heatmap analysis. B.A., T.J.N. and A.R.K. provided single cell RNA sequencing data on developing human brain. K.K. and S.D. carried out single cell RNA sequencing data analysis on adult human dataset. All authors interpreted the data, and contributed to and approved the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Heatmaps showing the distribution of neuroserpin immunoreactivity during stage 2 (13th gw) from Fig. 6.