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Dynamic and Cell-Specific DACH1 Expression in Human Neocortical and Striatal Development

Valentina Castiglioni^{1,†}, Andrea Faedo ^{1,8,†}, Marco Onorati^{1,2,3,†}, Vittoria Dickinson Bocchi^{1,†}, Zhen Li³, Raffaele Iennaco¹, Romina Vuono⁴, Gaetano P. Bulfamante⁵, Luca Muzio⁶, Gianvito Martino⁶, Nenad Sestan^{3,7}, Roger A. Barker⁴ and Elena Cattaneo¹

¹Department of Biosciences, Istituto Nazionale di Genetica Molecolare, University of Milan and INGM, 20122 Milan, Italy, ²Department of Biology, Unit of Cell and Developmental Biology, University of Pisa, 56124 Pisa, Italy, ³Department of Neuroscience and Kavli Institute for Neuroscience, Yale School of Medicine, New Haven, CT 06510, USA, ⁴Department of Clinical Neuroscience, John van Geest Centre for Brain Repair, University of Cambridge, CB2 0PY Cambridge, UK, ⁵Unit of Human Pathology and Developmental Pathology, Department of Health Sciences, Università degli Studi di Milano, San Paolo Hospital, 20142 Milan, Italy, ⁶Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute, 20132 Milan, Italy, ⁷Department of Genetics, of Psychiatry and of Comparative Medicine, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale Child Study Center, Yale School of Medicine, New Haven, CT 06510, USA and ⁸Cell Biology Unit, Axxam, 20091 Bresso-Milan, Italy

Address Correspondence to Elena Cattaneo, Department of Biosciences, University of Milan and INGM, (Istituto Nazionale di Genetica Molecolare) Padiglione Invernizzi, Via Francesco Sforza 35, 20122 Milan, Italy. Email: elena.cattaneo@unimi.it.

[†]Co-first authors.

Abstract

DACH1 is the human homolog of the *Drosophila dachshund* gene, which is involved in the development of the eye, nervous system, and limbs in the fly. Here, we systematically investigate *DACH1* expression patterns during human neurodevelopment, from 5 to 21 postconceptional weeks. By immunodetection analysis, we found that *DACH1* is highly expressed in the proliferating neuroprogenitors of the developing cortical ventricular and subventricular regions, while it is absent in the more differentiated cortical plate. Single-cell global transcriptional analysis revealed that *DACH1* is specifically enriched in neuroepithelial and ventricular radial glia cells of the developing human neocortex. Moreover, we describe a previously unreported *DACH1* expression in the human striatum, in particular in the striatal medium spiny neurons. This finding qualifies *DACH1* as a new striatal projection neuron marker, together with PPP1R1B, BCL11B, and EBF1. We finally compared *DACH1* expression profile in human and mouse forebrain, where we observed spatio-temporal similarities in its expression pattern thus providing a precise developmental description of *DACH1* in the 2 mammalian species.

Key words: *DACH1*, human neocortex, human striatum, medium spiny neurons, neuroepithelial cells

Introduction

During human development, a complex interplay of dynamic processes operates within a genetically organized context in the forebrain primordium, which ultimately defines area-specific molecular expression domains underlying brain functions (Monuki and Walsh 2001; Rakic 2009; Lui et al. 2011; Silbereis et al. 2016). Recent studies have described the human forebrain transcriptome organization, delineating the spatio-temporal regulation of gene expression that specifies early neocortical and striatal progenitors and their progeny (Kang et al. 2011; Miller et al. 2014; Onorati et al. 2014). These efforts produced a plethora of differentially expressed genes (DEGs) and gene modules that distinguish the developing human striatum from the neocortex. In our previous study, we were able to define a precise spatio-temporal map of developmental factors involved in striatal and neocortical neuron determination and differentiation in humans. Among these relevant genes, we identified Dachshund family transcription factor 1 (DACH1) as a neocortical enriched DEG and bimodal expressed gene (Onorati et al. 2014). Then, by looking at the Human Brain Transcriptome Atlas, we found that DACH1, later on, decreases its expression in the neocortex but shows a stable expression in the striatum until adulthood (Kang et al. 2011). Based on these expression data, and since no data were available in human brain on the protein expression level, we carried out a detailed expression analysis of DACH1 during human cortical and striatal development to characterize its cell type specificity in these 2 brain regions.

The DACH1 homolog in *Drosophila*, *dachshund*, plays a pivotal role in the differentiation of the eye imaginal disc, in leg morphogenesis and in controlling neural differentiation in the mushroom bodies, a neural structure required for learning and memory in flies. *Drosophila dachshund* mutant flies lack eyes, have truncated limbs and exhibit brain abnormalities (Mardon et al. 1994; Kurusu et al. 2000; Martini et al. 2000).

In mouse, *Dach1* encodes for a transcription factor expressed during development in restricted areas of the central nervous system (CNS), eye, neural crests, and limb buds (Mardon et al. 1994; Hammond et al. 1998; Caubit et al. 1999; Davis et al. 1999; Kozmik et al. 1999). Mouse *Dach1*-null homozygotes survive pregnancy but die within 24 h after birth (Davis et al. 2001; Backman et al. 2003). In the developing telencephalon, *Dach1* mRNA shows strong expression in the neural stem/progenitor cells of the ventricular and subventricular zone (VZ and SVZ, respectively) of the neocortex, in medial and lateral ganglionic eminences (MGE and LGE, respectively), and hippocampus (Machon et al. 2002).

Recently, DACH1 expression was detected in the early fetal human neocortex by PCR (Straccia et al. 2015). Moreover, DACH1 was reported to mark in vitro-derived rosette-like neuroepithelial stem cells (Elkabetz et al. 2008) and to act as a tumor suppressor gene (Chu et al. 2014). In particular, its expression is altered in several cancers, including glioblastoma (Watanabe et al. 2011).

This work describes the dynamic expression profile of DACH1 in the human neocortex and striatum of samples aged 5–21 post-conceptual weeks (pcw). We found that DACH1 is expressed in the VZ/SVZ regions of the developing human cerebral cortex at all the stages analyzed. Furthermore, by analyzing single-cell RNA-seq dataset on the human developing neocortex (Onorati et al. 2016), we identified DACH1 as a specific marker of neuroepithelial and ventricular radial glial cells (vRGCs). Starting from 10 pcw, we found a DACH1 protein expression in the human striatum that was not previously reported, to the best of our knowledge, suggesting a different role for DACH1 in 2 distinct regions and times,

and qualifying DACH1 as a marker of striatal medium spiny projection neurons (MSNs).

Materials and Methods

Human Tissue

Deidentified postmortem human brain specimens were obtained from patients that requested pregnancy terminations and autopsy diagnostic procedures. All procedures were approved by the research ethical committees and research services division of the University of Cambridge and Addenbrooke's Hospital in Cambridge (protocol 96/85, approved by Health Research Authority, Committee East of England—Cambridge Central in 1996 and with subsequent amendments, with the latest approved November 2017) and by the Ethics Committee of San Paolo Hospital, Milano (protocol 11 186, approved on 19 July 2013). The ethics were fully reviewed and approved in the UK, in accordance with the Human Tissue Act 2006. Both documents were submitted to the Ethics Committee of the University of Milano and ethical approval was obtained on 27 March 2013. Appropriate informed consent was obtained and all available nonidentifying information were recorded for each specimen. Tissue was handled in accordance with ethical guidelines and regulations for the research use of human brain tissue set forth by the National Institute of Health (NIH) (<http://bioethics.od.nih.gov/humantissue.html>) and the World Medical Association Declaration of Helsinki (<http://www.wma.net/en/30publications/10policies/b3/index.html>).

Definition of Human Developmental Periods

We used pcw to indicate the developmental age of the human embryo and fetus (also termed postfertilization or postovulatory age). For embryonic stages (up to 8 pcw), we based age on multiple physical features (e.g., somites). For fetal stages, we calculated the age by subtracting 2 weeks to the gestational age (calculated on the mother's last menstruation), crown to rump length, anatomical landmarks, and by visual inspection, as previously described (Onorati et al. 2014).

According to Kang et al. (2011), we used the following developmental periods to describe the samples employed in this study:

- Embryonic development, up to 8 pcw
- Early fetal development, 8–13 pcw
- Early midfetal development, 13–19 pcw
- Late midfetal development, 19–24 pcw

Human Tissue Collection

This study was conducted with postmortem human brain specimens from tissues collected at the John van Geest Centre for Brain Repair, University of Cambridge, Cambridge, UK. Additional specimens were procured from the San Paolo Hospital, Milan, Italy. Details of the specimens analyzed are provided in Supplementary Table 1.

Brain Sampling for Immunostaining

As previously described (Onorati et al. 2014), specimens were chilled on ice during dissection and placed onto a chilled plate on ice. The entire brain (when possible) was removed and fixed for 2–4 days in 4% formaldehyde (FA) at 4°C. Samples were

then cryoprotected in 30% sucrose (in PBS), embedded in tissue-tek, stored at -80°C and then cryosectioned (12–30 μm).

Immunofluorescence

Brain sections were rehydrated for 10 min with PBS, then permeabilized with 0.5% Triton X-100, and blocked with 5% FBS for 1 h at room temperature. Brain sections were incubated overnight at 4°C with primary antibodies, listed in Supplementary Table 2. Alexa 488 or 568-conjugated secondary antibodies (Molecular Probes) were diluted 1:500 and mixed with DAPI (Molecular Probes, Invitrogen) to counterstain the nuclei. Sections were mounted with fluorescent mounting medium (Dako). Images were acquired with Leica DMI 6000B and DM4000B (Leica Microsystems) microscopes, analyzed with LAS-AF imaging software and then processed with Adobe Photoshop. Confocal images were acquired with a Leica TCS SP2 microscope (Leica Microsystems) and then processed with Adobe Photoshop.

Cell Counting

The positivity and specificity of each primary and secondary antibody used in this work was evaluated against a negative control intended as both nonexpressing human brain regions (e.g., spinal cord) and slices stained exclusively with secondary antibody. The majority of antibodies used were selected from the literature and previously tested on mouse and human brain. Automatic cell counting performed using Cell Profiler. Statistical tests were performed with PRISM software (GraphPad, ver 6). All results are expressed as mean \pm SD.

In situ Hybridization

In situ hybridization experiments were performed as previously described (Onorati et al. 2014), on 15–25 μm thick brain cryosections upon postfixation in 4% FA. Sections were then washed 3 times in PBS and incubated with 0.5 mg/mL of proteinase K in 100 mM Tris-HCl (pH 8) and 50 mM EDTA for 10 min at 30°C and then 15 min in 4% FA. Slices were washed 3 times in PBS before receiving H_2O washes. Sections were incubated in Triethanolamine 0.1 M (pH 8) for 5 min, then 400 μL of acetic anhydride was added 2 times for 5 min each. Finally, sections were rinsed in H_2O for 2 min and air-dried. Hybridization was performed overnight at 60°C , incubating either sense or antisense P^{33} labeled riboprobes at a concentration of 6×10^6 counts per minute (cpm/slide). The following day, sections were rinsed in SSC 5 \times for 5 min then washed in formamide 50% SSC 2 \times for 30 min at 60°C , before receiving Ribonuclease-A (Roche) treatment (20 mg/mL in 0.5 M NaCl, 10 mM Tris-HCl pH 8, and 5 mM EDTA 30 min at 37°C). Sections were further washed in formamide 50% SSC 2 \times for 30 min at 60°C and then rinsed 2 times in SSC 2 \times . Slides were dried by using ethanol series. NTB (Kodak) emulsion was applied in a dark room, according to manufacturer's instructions. Sections were developed after 1 week and mounted with DPX (BDH) mounting solution. Human DACH1 probe was cloned by PCR from nucleotide 1545 to nucleotide 2450 according to Genbank information (NM_080759.5). Mouse *Dach1* probe was cloned by PCR from nucleotide 1668 to nucleotide 2606, according to Genbank information (NM_007826.3). Bright field imaging was performed by Olympus BX52 and Leica Aperio AT2—digital scanner.

Primers used for making human DACH1 probe:
Forward: GTCCGACTGGAACCTCTTTTATGATG
Reverse: GGGTCAGAGAGTCATTTAAGACCCTG

Probe length 904 from Genbank NM_080759.5
Primers used for making mouse *Dach1* probe:
Forward: GTTAGCCATCCTCTCAACCATCTGC
Reverse: TCATTTAAGACCCGGAGACTGTCCG
(Allen brain atlas probe).

Single-Cell RNA-seq Data Analysis

Single-cell RNA-seq datasets of the developing neocortex were retrieved from an earlier study (Onorati et al., 2016). Prenatal neocortical cells from 5 to 20 pcw were analyzed. Only cells that passed quality control according to Onorati et al. (2016) were used. Cell clustering was conducted by supervised hierarchical clustering with a solicited list of marker genes identifying major cell types in the neocortex. The R program, Monocle, was used to reconstruct cell lineage and pseudotime for the cells in the dataset using default parameters (Trapnell et al. 2014). Log_2 transformed RPKM values were used as input. Violin plots were generated using R package *ggplot2* and gene RPKM values were used. The analysis pipeline to identify genes correlated and anticorrelated with DACH1 was performed using the package described by Lun et al. (2016) on log_2 RPKM values. Briefly, genes that had Spearman's $\rho > 0$ and a FDR < 0.05 with DACH1 were considered as correlated with DACH1, whereas genes with a Spearman's $\rho < 0$ and a FDR < 0.05 with DACH1 were considered as anticorrelated with DACH1.

Hub Gene Identification

Identification of hub genes was performed using the TopGO function of the Functional Gene Networks (FGNet), an R/Bioconductor package (Aibar et al. 2015) on genes that highly correlate with DACH1. Node size was set to 20 and the P-value threshold < 0.05 . The gene universe was defined as all expressed genes that had 25 accumulative gene counts in all samples and RPKM > 1 in at least 5 of the samples and only GO "Biological processes" were considered. Filter threshold for metagroups was set to be > 40 .

Results

DACH1 Shows a Peculiar Pattern in the Human VZ and SVZ

In order to define the regional expression of DACH1 in the cerebral cortex during human fetal development, we carried out an immunofluorescence analysis, first focusing our attention to a 10-pcw specimen, corresponding to early fetal development. This period is characterized by the consolidation of the cortical plate (CP), while the developing striatum starts to be separated into the caudate and putamen by the internal capsule. By immunostaining on whole human coronal brain sections, we found that DACH1 clearly showed a rostral to caudal expression pattern, with a very weak expression rostrally (Fig. 1A–C). At the level of the paracentral section, DACH1 expression was present in the VZ of the pallium, with a sharp edge at the level of the pallial–subpallial boundary (PSB; Fig. 1B, arrow). We performed a colabeling of DACH1 with KI67, a static marker of proliferative activity (Gerdes et al. 1983). DACH1 was expressed in the proliferative regions (VZ, SVZ) of the developing human neocortex (Fig. 1A–C). Double immunofluorescence analysis at caudal levels clearly showed that many cells in the VZ/SVZ regions were proliferative as assessed by KI67 coexpression (Fig. 1D–F). In particular, we found that $48 \pm 12.2\%$ of total cells was DACH1 $^+$ /KI67 $^+$ (Supplementary Table 3A). Finally, we compared DACH1 expression with that of BCL11B (also known as

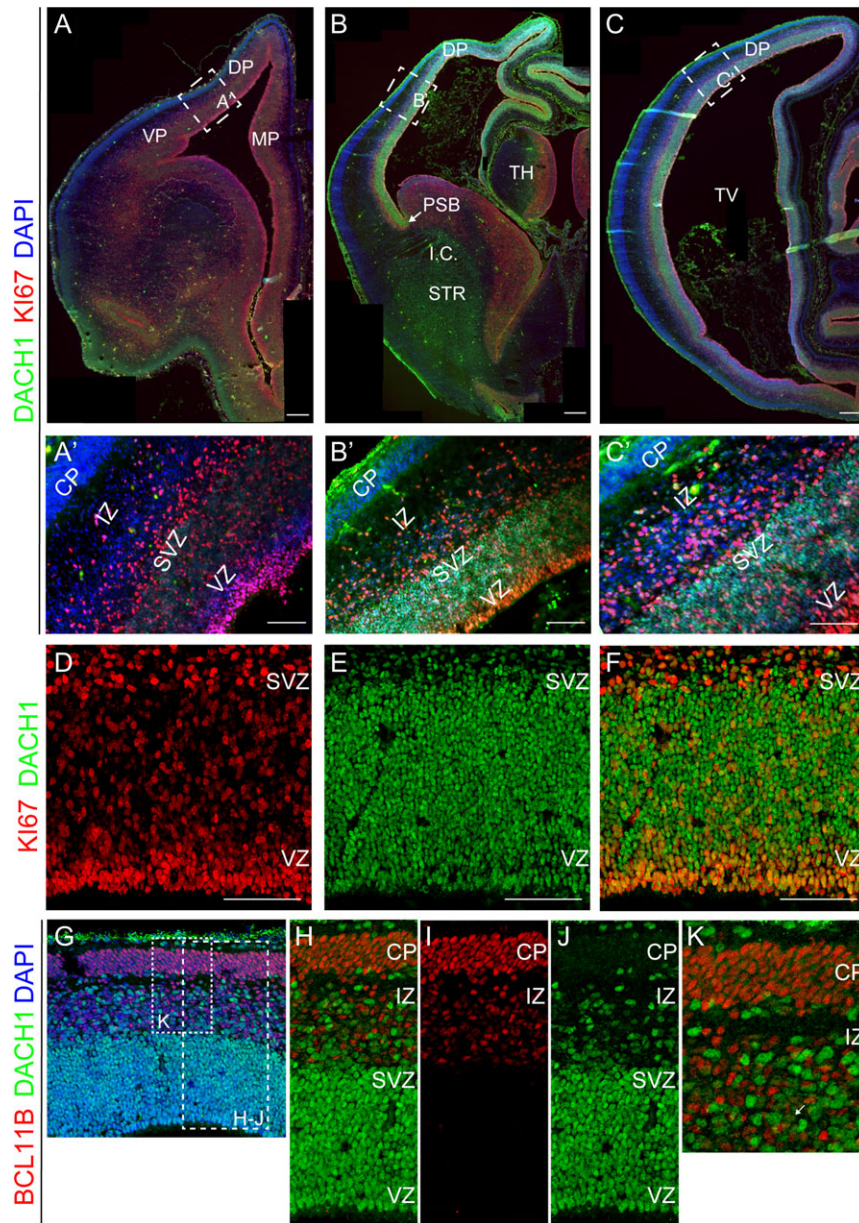


Figure 1. Expression pattern of DACH1 in the human fetal cerebral cortex at 10 pcw. (A–C) Rostro-caudal expression pattern for DACH1 compared with Ki67⁺ proliferative cells. DACH1 shows a very low expression in rostral sections (frontal cortex (A)), and a higher expression when moving towards the paracentral (B) and occipital cortex (C). (A'–C') Higher magnification of the boxes in A–C showing the strong DACH1 expression in more caudal sections. DACH1 is expressed in the ventricular/subventricular zone (VZ/SVZ) where Ki67⁺ proliferative cells reside. In the cortical plate (CP) there is no discernible expression. (D–F) High magnification of DACH1 and Ki67 expression in the dorsal pallium (DP) VZ/SVZ (caudal level) showing that they are coexpressed in these proliferative regions. The images are from the DP of a section adjacent to the one shown in (B). Some DACH1⁺ cells are Ki67⁺, probably migrating neuroblasts. (G–K) DACH1 expression in the DP compared with BCL11B (CTIP2), a layer 5 marker. The 2 proteins are expressed in a complementary fashion. (H–J) High magnification of the region highlighted in the dashed box in (G), showing DACH1 expression in the VZ/SVZ and scattered in the intermediate zone (IZ), compared with BCL11B expression in the CP and IZ. (K) High magnification of the region highlighted in the dashed box in (G), showing the complementary expression pattern of DACH1 and BCL11B. Arrows in K point to occasional single cells coexpressing both BCL11B and DACH1. VP, ventral pallium; MP, medial pallium; TV, telencephalic ventricle; PSB, pallium–subpallium boundary; TH, thalamus; STR, striatum; I.C., internal capsule. Scale bars: 100 μ m in A–C, 50 μ m in A'–F.

CTIP2), a transcription factor involved in the development of layer 5 projection neurons (Leid et al. 2004; Arlotta et al. 2005). DACH1 and BCL11B showed a mutually exclusive expression pattern, with DACH1 expressed mainly in the VZ/SVZ regions (Fig. 1G–K), and BCL11B mostly confined to the intermediate zone (IZ)/CP (Fig. 1G–K). We found only few cells coexpressing BCL11B and DACH1 (Fig. 1K, arrow).

DACH1 is Expressed in the VZ/SVZ Regions During Embryonic and Early Fetal Development Along the Medio-Lateral Axis

To determine whether DACH1 was also present at earlier stages of human development, we performed an immunodetection study on a 7-pcw + 2d embryonic specimen (Fig. 2A–C). We found

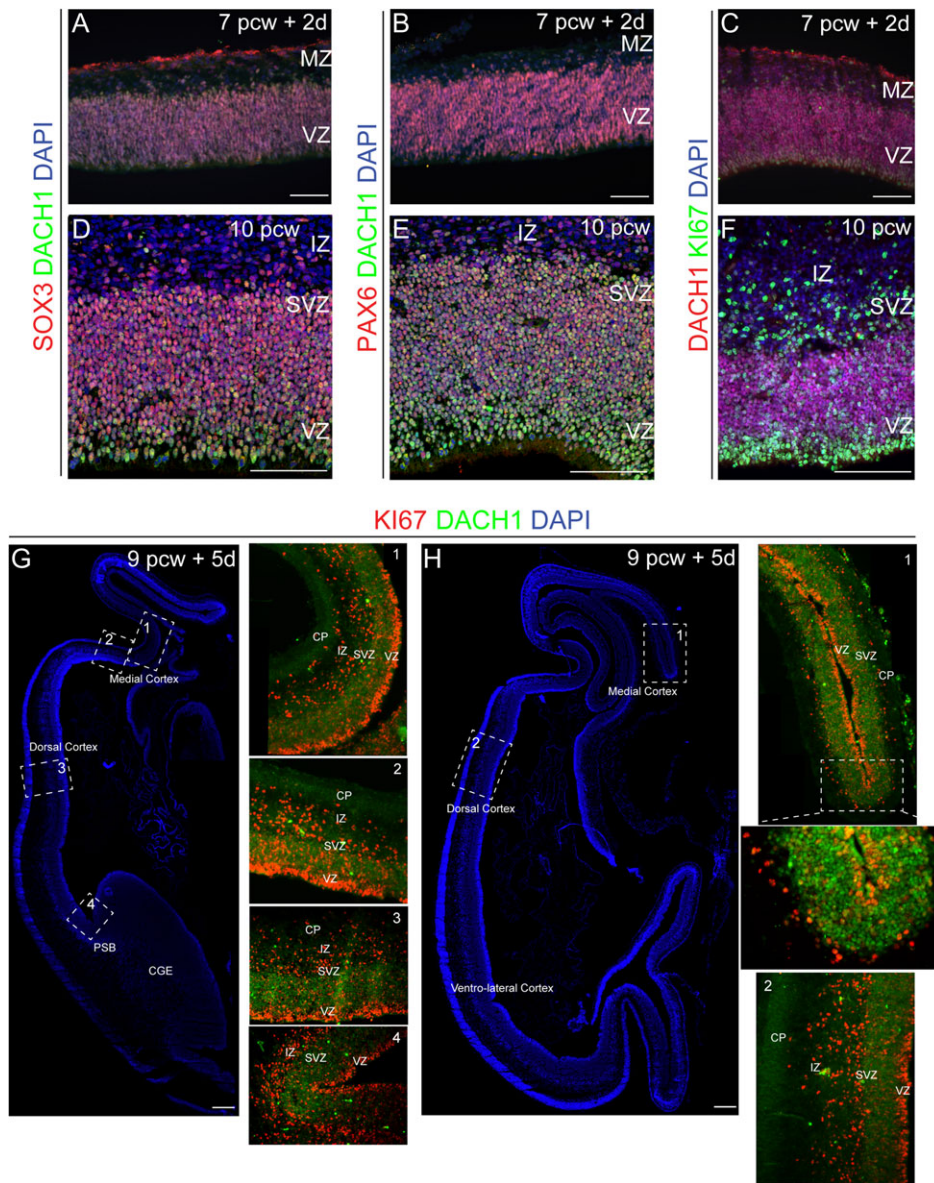


Figure 2. Spatial-temporal expression pattern of DACH1 in the developing human cerebral cortex. (A–C) Double immunodetection of DACH1 with SOX3 (A), PAX6 (B), and KI67 (C) at 7 pcw + 2d showing the coexpression of the 3 VZ markers with DACH1. (D–F) At 10 pcw DACH1 shows a similar coexpression pattern with SOX3, PAX6, and KI67. (G,H) DACH1 expression pattern in medio-lateral cortex at 9 pcw + 5d. The regions in the dashed box represent the area magnified in the boxes. DACH1 signal is localized in the VZ/SVZ, with no detectable signal in the CP. This pattern is conserved in more caudal sections (H). MZ, marginal zone; CP, cortical plate; IZ, intermediate zone; VZ/SVZ, ventricular/subventricular zone; PSB, pallium–subpallium boundary; CGE, caudal ganglionic eminence. Scale bars: 50 μm in A–F, 100 μm in G,H.

that DACH1 was coexpressed in the VZ together with SOX3 (Fig. 2A), PAX6 (Fig. 2B), well-known neural progenitor markers, and KI67 (Fig. 2C). This pattern of expression was then conserved at 10 pcw (Fig. 2D–F) where $77 \pm 4.1\%$ of the cells was DACH1/SOX3 double-positive and $76 \pm 4.0\%$ was DACH1/PAX6 double-positive in the VZ/SVZ regions (Supplementary Table 3A).

Next, we decided to investigate the medio-lateral expression pattern of DACH1 (Fig. 2G,H), using a (9 pcw + 5d) human fetal brain specimen in order to substantiate the expression analysis described above. We decided to focus our attention on the rostro-caudal levels analyzed in Figure 1B,C where DACH1 expression was stronger. DACH1 signal was localized with similar levels along the medio-lateral axis (Fig. 2G, and boxes 1–4) in the VZ/SVZ regions, with no detectable signal in the CP. This

pattern was conserved also in more caudal sections (Fig. 2H, and boxes 1–2).

Finally, we analyzed DACH1 expression at 21 pcw (Supplementary Fig. 1A–D). In this late midfetal specimen, DACH1 was localized in the cortical VZ, with fading expression in SVZ, IZ, subplate (SP), and CP (Supplementary Fig. 1A,A'). Supplementary Fig. 1A'' shows the reduced DACH1 expression moving from the VZ to the SVZ.

We then performed *in situ* hybridization (ISH) for DACH1 on adjacent sections to study its weak expression in the CP (Supplementary Fig. 1B,B'). By using this more sensitive assay, we could detect the DACH1 transcript signal in the CP, although at low level. In the VZ/SVZ regions, also at this stage DACH1 colocalized with SOX3 (Supplementary Fig. 1C) and the RGC marker TFAP2C (also known as AP2γ) (Supplementary Fig. 1D).

GRIA4 and the plexin receptor *PLXNA4* (Fig. 3C). Within the set of correlated genes, *DACH1* emerged as a hub gene (Fig. 13D) in functional annotations like “Nuclear DNA replication”, “Regulation of DNA biosynthetic process”, “Neuronal stem cell population maintenance”, and “Cell proliferation in forebrain” ($P < 0.05$, Supplementary Table 5). This picture is in line with the nature of dividing neuroepithelial cells and vRGCs and with immunodetection results that showed *DACH1* coexpressed with *PAX6* and *KI67* in the VZ (Fig. 1D–F).

DACH1 is Expressed in the Human Fetal Striatum

We further studied *DACH1* expression pattern during human brain development by taking into account the previously observed striatal expression (see Fig. 1B). First, we compared the *DACH1* profile during early fetal development with *FOXP1*, *FOXP2*, and *BCL11B*, 3 transcription factors highly expressed during human striatum development and in particular in MSNs (Onorati et al. 2014) (Supplementary Fig. 3A–L). We found that *DACH1* was similarly expressed in the developing striatum from 8 pcw to 10 pcw (Supplementary Fig. 3A–C).

Next, we performed costainings with a panel of striatal markers at 10 pcw (Fig. 4A). Strong *DACH1* signal was present in the striatum at this stage, coexpressed with *EBF1* (Fig. 4B–C'), *ISL1* (Fig. 4D,D'), and *BCL11B* (Fig. 4E,E'). We investigated coexpression

patterns with different striatal markers at 10pcw where *DACH1* was expressed in $30.6 \pm 7.6\%$ of total striatal cells (Fig. 4F). We quantified the number double-positive cells, finding $24 \pm 7\%$ of cells coexpressing *DACH1* and *EBF1*, $25 \pm 6.6\%$ *DACH1* and *ISL1*, and $43.1 \pm 11.1\%$ *DACH1* and *BCL11B*. When the number of double-positive cells was normalized to the total number of *DACH1*⁺ cells, we found that $77.8 \pm 4.6\%$ of *DACH1* expressed *EBF1*, $78.9 \pm 6.7\%$ expressed *ISL1*, and 100% expressed *BCL11B* (Fig. 4F and Supplementary Table 3B). *DACH1* resulted also coexpressed with *PPP1R1B* (also known as *DARPP-32*), a mature striatal MSN marker (Onorati et al. 2014) (Fig. 4G).

We also evaluated *DACH1* signal in the 21 pcw human striatum. *DACH1* was localized in the mantle region together with *EBF1* (Supplementary Fig. 4A,A'), *ISL1* (Supplementary Fig. 4B, B'), *BCL11B* (Supplementary Fig. 4C,C'), and *PPP1R1B* (Supplementary Fig. 4D,D') with similar coexpression patterns found at 10 pcw (Supplementary Table 3C). Double immunostaining analysis revealed that *DACH1* was then expressed by *SST*⁺ interneurons but not by the *NR2F2*⁺ (also known as *COUPTFII*) or *CALB2*⁺ (also known as *calretinin*) interneurons (Supplementary Fig. 4E–G). Our finding of *DACH1* being strongly expressed in the human striatum is further supported by the Human Brain Transcriptome Atlas (Kang et al. 2011) where *DACH1* shows a stable expression until adulthood (Supplementary Fig. 5A).

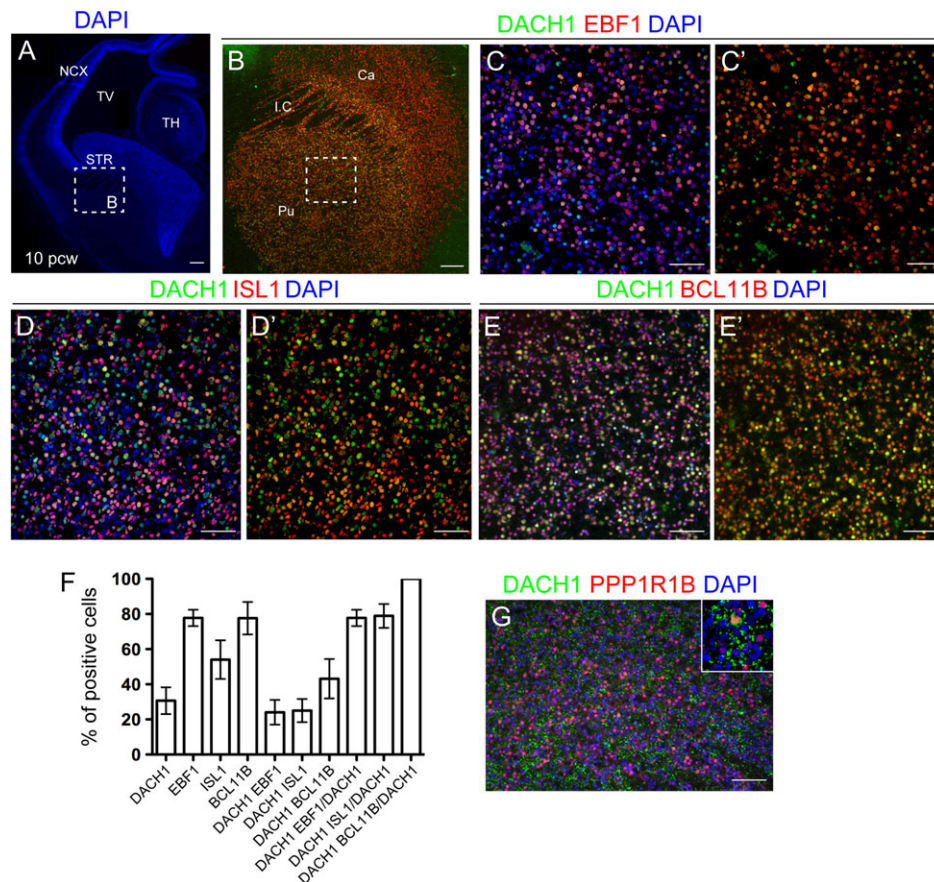


Figure 4. *DACH1* expression in the developing human striatum. (A) 10 pcw human brain coronal hemisection. The region highlighted in the dashed box represents the striatal area analyzed in B. (B–C') Coexpression analysis of *DACH1* and *EBF1*, a striatal marker. The region in the dashed box in B represents the area magnified in C–E'. (D,D') Coexpression analysis of *DACH1* with the striatal marker *ISL1*. (E,E') Coexpression analysis of *DACH1* and the MSN marker *BCL11B*. (F) Histogram showing the percentage of cells coexpressing *DACH1* and the indicated markers. All results are expressed as mean \pm SD. (G) *DACH1* coexpression with the MSN marker *PPP1R1B* (see arrows in the inset). NCX, neocortex; TV, telencephalic ventricle; TH, thalamus; STR, striatum; I.C., internal capsule; Ca, caudate; Pu, putamen. Scale bars: 100 μ m in A,B; 50 μ m in C–E', G.

We also suggest that *DACH1* is the main player in human neurodevelopmental processes since its paralog *DACH2* is not significantly expressed both at population and single-cell level in the human neocortex and striatum (Supplementary Fig. 6A,B).

DACH1 Expression is Conserved in the Mouse

Finally, we performed a human versus mouse comparison by performing ISH and immunofluorescence analyses at different developmental time points in the mouse. We first found that at E13.5 (a stage comparable to the 10 pcw analyzed in Fig. 1) *Dach1* was strongly expressed in the VZ/SVZ of the developing pallium (Supplementary Fig. 7A). At the end of the neurogenesis (E18.5), *Dach1* was confined to the VZ/SVZ regions and virtually absent from the CP (Supplementary Fig. 7B–D). At E16.5 *DACH1* showed a transitional expression pattern compared with E13.5 and E18.5, with high expression in the neocortical VZ/SVZ regions, together with PAX6, TFAP2C, and SOX3 (Supplementary Fig. 7E–G'). We also observed a strong expression in the striatum (Fig. 5A–H, Supplementary Fig. 7B) where $88 \pm 1.63\%$ of *DACH1* cells coexpressed *BCL11B* and $82 \pm 1.34\%$ of the *DACH1* cells were *EBF1*⁺ at E16.5 (Fig. 5A–D, Supplementary Table 3D). Then at P8 *DACH1* marked MSNs together with *BCL11B* ($94.98 \pm 1.62\%$) and *PPP1R1B* ($89 \pm 1.10\%$) (Fig. 5D–H, Supplementary Table 3D).

Discussion

In this study, we demonstrate that *DACH1* is expressed in neuroepithelial cells and vRGCs of the human forebrain from the earliest stage analyzed (5 pcw) and continues to be expressed until midfetal stages (21 pcw). Moreover, we describe a peculiar *DACH1* expression in striatal MSNs, which is conserved in mouse. We show that *DACH1* expression is temporally and spatially dynamic, suggesting multiple roles at different times and regions.

DACH1 is Expressed in Neocortical Neuroepithelial Cells and vRGCs

Single-cell transcriptional analysis showed that *DACH1* expression significantly decreases between 5 and 20 pcw in proliferating neural progenitor populations in the neocortex. This was confirmed at protein level, as we observed a strong *DACH1* signal in the embryonic neocortical VZ/SVZ, coexpressed with PAX6. This expression persists in the proliferative regions of the neocortex at least until 21 pcw, the latest developmental stage analyzed in this study. Single-cell transcriptomics revealed that *DACH1* is found predominantly in neuroepithelial cells and vRGCs starting from 5 pcw, corroborating the expression in 15–18 gestational week-old vRGCs shown by Pollen et al. (2015). Genes with high correlation with *DACH1* show an enrichment in terms related to cell cycle and neural stem cell population maintenance, with most of the gene terms correlating to mechanisms involved in mitosis. Moreover, *DACH1* results as a hub gene in these processes suggesting a potential role in regulating the cell cycle within dividing progenitors. Further strengthening this hypothesis is the fact that we also observed a strong *DACH1* expression in the VZ of mice at E16.5. In line with these results, *Dach1* expression is detected from E8.25 in the anterior neural ridge (Caubit et al. 1999) and persists at least until P21 in the ependymal and subependymal zones (Machon et al. 2002). *DACH1* is considered one of the markers of neural rosette progenitors in vitro (Elkabetz et al. 2008). These observations, taken together, advocate for *DACH1* having a fundamental role in neural progenitors during early stages of development, both in mouse and human.

A previous study examined *Dach1* mutant mice with respect to proliferation defects at E17.5 (Backman et al. 2003), finding no impairments. However, they performed only a 2-h BrdU pulse at a single stage, thus probably missing more subtle defects that can be revealed by cumulative studies at different

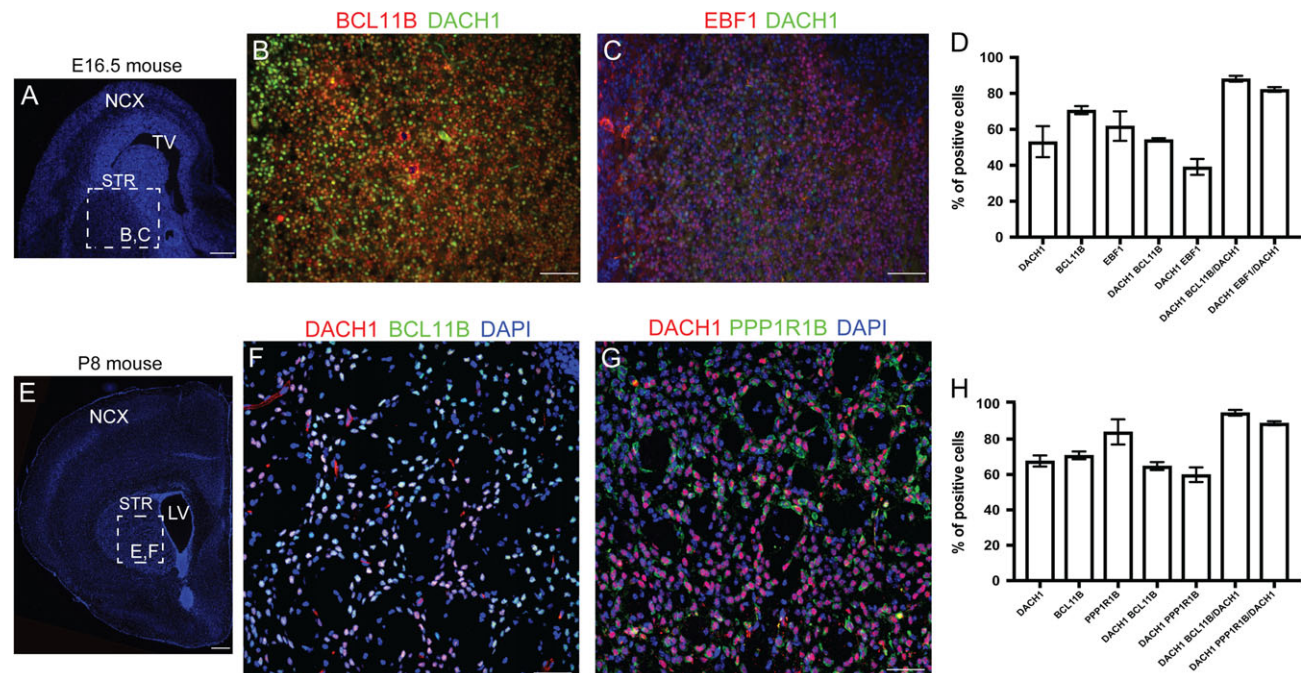


Figure 5. *DACH1* expression pattern in the mouse striatum. (A) A representative E16.5 mouse brain coronal section. The region highlighted in the dashed box represents the striatal area analyzed in B,C. (B) Coimmunodetection analysis of *DACH1* and *BCL11B*. (C) Coimmunodetection analysis of *DACH1* and *EBF1*. (D) Histogram showing the percentage of cells coexpressing *DACH1* together with *BCL11B* and *EBF1*. (E) A representative P8 mouse coronal brain section. The region highlighted in the dashed box represents the striatal area analyzed in E,F. (F) Coimmunodetection analysis showing *DACH1* and *BCL11B* coexpression. (G) Coimmunodetection analysis of *DACH1* and *PPP1R1B*. (H) Histogram showing the percentage of cells coexpressing *DACH1* together with *BCL11B* and *PPP1R1B*. NCX, neocortex; TV, telencephalic ventricle; STR, striatum; LV, lateral ventricle. Scale bars: 100 μ m in A,D; 50 μ m in B,C,E,F.

time points. DACH2 redundancy is unlikely because of its flat and lower expression in cerebral cortex proliferating zones (Supplementary Fig. 6A; Kang et al. 2011).

DACH1 has a Rostro-Caudal Expression Pattern in the Human Neocortex

Neocortical parcellation is an important developmental process that gives rise to 4 major areas: the primary visual (V1), somatosensory (S1), auditory (A1), and motor (M1) areas.

These areas are subdivisions that differ from one another by differences in cell composition, connections, and patterns of gene expression. The suggestion that intrinsic genetic mechanisms may control arealization was based on the observation of transcription factors with gradients of expression along the medio-lateral and rostro-caudal axes (Sur and Rubenstein 2005). In this study, we show that DACH1 at 10 pcw has a rostral-low to caudal-high expression pattern of gene expression, and thus it may have a role in correct cerebral cortical arealization similar to the transcription factors PAX6, EMX2, COUPTFI, and SP8 (Sur and Rubenstein 2005). Alternatively, DACH1 may have a dynamic expression pattern in different areas at different developmental ages.

A Secondary Site of DACH1 Expression, the Striatum

A few transcription factors, for example BCL11B and SP8, are expressed both in the neocortex and in the basal ganglia. In particular BCL11B is expressed, both in mouse (Arlotta et al. 2005, 2008) and in human (Onorati et al. 2014) in layer 5 projection neurons and in the MSNs of the striatum. In this study, we report that DACH1 has a similar dual expression. Its striatal expression was found both in mouse tissue (E13.5, E16.5, and E18.5) and in human specimens (10 pcw and 21 pcw). Interestingly, this striatal expression was not extensively reported before even in mouse. In Davis et al. (1999) they reported a generic ganglionic eminence expression at E12.5, while Caubit et al. (1999) reported an exclusive neocortical expression, even if their analysis was confined to the E12.5 stage. A more recent study examined *Dach1* expression by ISH at E12.5, P0, and P21, but did not describe any expression in the striatum, except for faint LGE staining (Machon et al. 2002). We now show, using both ISH and immunostaining, that DACH1 is strongly expressed in the striatum in mouse and human, and that DACH1 expression is stable until adulthood (Supplementary Fig. 5A; Kang et al. 2011). Moreover, at 10 pcw, DACH1 was coexpressed with important mature striatal markers such as EBF1 (Garel et al. 1999; Onorati et al. 2014), ISL1 (Toresson et al. 2000; Onorati et al. 2014), BCL11B, and PPP1R1B.

Together, our findings suggest that DACH1 can have a dual role in the developing human telencephalon. The first one is in human cortical progenitors, at early developmental stages, as a consequence of its expression in neuroepithelial cells and vRGCs and not in cortical projection neurons or interneurons. Of interest, at 21 pcw it was also expressed in the LGE VZ, probably extending its role to other progenitor types. The second role is in the striatum, mostly in postmitotic striatal MSNs where it is coexpressed with well-known MSN markers. DACH1 is not the only gene that acts in 2 different contexts within the nervous system. Also, PAX6 and OTX2, known neural progenitor markers for the ventricular neuroepithelium of the developing CNS, are found in certain neuronal subpopulations in the adult brain (Stoykova et al. 2000, Omodei et al. 2008). In particular, PAX6 characterizes dopaminergic neurons in the glomerular layer of the olfactory bulb, granule neurons in the cerebellum

and amacrine and ganglion cells of the retina (Stoykova et al. 2000). Instead, OTX2 is expressed in a neuronal subset within the ventral tegmental area (Di Salvio et al. 2010). In line, the dual role of DACH1 has been described in the literature with regards to promoting differentiation of the *Drosophila* eye and limb in postmitotic cells (Mardon et al. 1994; Hammond et al. 1998; Caubit et al. 1999; Davis et al. 1999; Kozmik et al. 1999) and in regulating proliferation and growth in cancer cells (Watanabe et al. 2011). In conclusion, here, we mirror this binary action in 2 different areas of the telencephalon: the neocortex and striatum. We describe its enrichment in neocortical progenitors in combination with genes involved in the cell cycle, together with its typifying role in defining MSNs in the developing striatum.

Supplementary Material

Supplementary material is available at *Cerebral Cortex* online.

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Notes

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