COUP-TFII Expressing Interneurons in Human Fetal Forebrain

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Transcription factor COUP-TFII in rodents is important for migration of cortical interneurons from caudal ganglionic eminence (CGE) to the neocortex. Since in human, unlike in rodents, cortical interneurons have both ganglionic eminence (GE) and dorsal cortical origin, we studied the distribution of COUP-TFII in the human developing neocortex from 9 to 22 gestational weeks. COUP-TFII is expressed at all stages studied in the GE and in various cortical zones, from the proliferative ventricular/subventricular zone (VZ/SVZ) to layer I. Gradients of COUP-TFII expression are present in the GE, with peak expression in the CGE, and in the neocortex, from high expression in the temporal and occipital cortex to moderate in the frontal and dorsal cortex. Double immunofluorescence with γ-aminobutyric acid (GABA), calretinin, or calbindin, established that subpopulations of interneurons express COUP-TFII. A small fraction of COUP-TFII+ cells are progenitor cells that proliferate in the CGE (3.4 ± 0.3%) and in the cortical VZ/SVZ (1.7 ± 0.1%). In summary, COUP-TFII is expressed in the human fetal forebrain in GABAergic cells, according to its possible role in migration of cortical interneurons. The source of these cells seems to be the CGE and, to a smaller extent, the cortical VZ/SVZ.

Keywords: caudal ganglionic eminence, fetal cerebral cortex, human cortical progenitors, interneurons

Introduction

Cortical interneurons are inhibitory γ-aminobutyric acid positive (GABAergic) cells, further subdivided by the expression of calcium-binding proteins (e.g., parvalbumin—PV, calbindin—CB, and calretinin—CalR) or neuropeptides (somatostatin—Sst, neuropeptide Y—NPY, vasoactive intestinal polypeptide—VIP). They are critically involved in the formation of cortical circuitry, which is indispensable for normal brain function (Haydar et al. 2000; Owens and Kriegstein 2002; Rakic 2009; Zecevic et al. 2011). In rodents, cortical interneurons originate in the ganglionic eminence (GE) of the ventral pallium and tangentially migrate to the neocortex (Parnavelas 2000; Marin et al. 2003). The GE is a transitory embryonic structure, which consists of 3 subdivisions: lateral (LGE), medial (MGE), and caudal (CGE), based on their anatomical position and the expression of transcription factors. Two types of cells, cortical interneurons and oligodendrocytes, have been described to be generated in the GE both in rodents (Marshall and Goldman 2002) and in primates (Letinic et al. 2002; Rakic and Zecevic 2003a; Zecevic et al. 2005; Petanjek, Berger, et al. 2009; Petanjek, Kostović, et al. 2009; Jakovcevski et al. 2011).

In rodents, the 3 GE regions generate cortical interneurons from specific populations of progenitors, which differ in their molecular signature and their final destination (Xu et al. 2003; Butt et al. 2005; Fogarty et al. 2007). The LGE generates interneurons for the olfactory bulb and striatum. The MGE is the main source of the early-born parvalbumin-expressing (PV+) and Sst+ cortical interneurons that migrate tangentially into the deep cortical layers (Wichterle et al. 2001; Marin et al. 2003; Wonders and Anderson 2006). The CGE generates late-born interneurons that migrate to the caudal neocortex, hippocampus, and amygdala (Nery et al. 2002; Butt et al. 2005). The majority of GE-derived interneurons that migrate to the caudal neocortex, hippocampus, and amygdala (Nery et al. 2002; Butt et al. 2005) migrate through a caudal migratory stream either prenatally (Yozu et al. 2005; Kanatani et al. 2008) or after birth (Inta et al. 2008) under the regulation of the orphan nuclear receptor COUP-TF I/II (chicken ovalbumin upstream promoter transcription factors I and II) also known as Nr2f (Nuclear receptor subfamily 2 group F member) (Tripodi et al. 2004; Yozu et al. 2005; Kanatani et al. 2008; Miyoshi et al. 2010). In rodents, one-third of all cortical interneurons are estimated to be derived from the CGE (Nery et al. 2002; Butt et al. 2005; Miyoshi et al. 2010).

In contrast to rodents, primate cortical interneurons have a prolonged generation period originating from both the cortical ventricular/subventricular zones (VZ/SVZ) and from the GE (Letinic et al. 2002; Rakic and Zecevic 2003a; Zecevic et al. 2005; Petanjek, Berger, et al. 2009; Petanjek, Kostović, et al. 2009; Jakovcevski et al. 2011; Yu and Zecevic 2011; Zecevic et al. 2011). Furthermore, bipolar and double-bouquet CalR+ cells are more numerous in human brains than in other species (Condé et al. 1994; Gabbott and Bacon 1996; Gabbott et al. 1997; Gonchar and Burkhalter 1997; Kawaguchi and Kubota 1997; DeFelipe et al. 2006, Bayatti et al. 2008). Their origin is of considerable interest for better understanding how cell circuitry is established in the human cerebral cortex. Impaired cortical circuitry and interneuron development has been implicated in numerous neurological and psychiatric disorders, from epilepsy to schizophrenia and autism (e.g., DeFelipe 1999; Lewis and Levitt 2002; Levitt 2003; Baraban and Tallent 2004).

In primates, the question of the CGE as a source of cortical interneurons has not been addressed. To better characterize GE-derived cortical interneurons, we studied the expression of COUP-TFII in the human fetal brain during the first half of the intrauterine period. This period of development in human brain coincides with interneuron genesis and their migration into emerging cortical layers (e.g., Letinic et al. 2002; Jakovcevski et al. 2011; Zecevic et al. 2011). Our results are based largely on immunohistochemistry on fixed brain tissue sections and represent the first study to demonstrate COUP-TFII distribution in the human fetal forebrain. COUP-TFII is expressed from the earliest stage studied here (9 gestational weeks [GW]) both in the ventral (CGE) and the dorsal forebrain regions, including the cerebral cortex. COUP-TFII has nuclear...
expression in a subpopulation of GABA+, CalR+, and CB+ cells, suggesting that some of these interneuron subtypes have CGE origin. A number of COUP-TFI+ cells are still dividing, as shown by their colabeling with proliferation markers, Ki67 and phosphorylated histone 3 (PH3). These progenitor cells are demonstrated more often in the CGE than in the cortical VZ/SVZ. Although it is hard to make firm conclusions on fixed brain tissue, gradients of COUP-TFI expression over the GE and cerebral cortex at developmental stages studied here suggest that tangential migration of CGE-derived interneurons to the neocortex in humans might be a conserved evolutionary mechanism, similar to that which has been described in rodents. A number of additional cortical COUP-TFI+ cells, however, have local neocortical origin, which may represent a human-specific feature.

Materials and Methods

**Human Fetal Brain Tissue**

Human fetal brain tissue, ranging in age from 9 to 22 GW (n = 11, Table 1), was obtained from the Brain Bank, Albert Einstein College of Medicine, Bronx, NY with the postmortem delay of approximately 15 min. Handling of the human material was done with special care following all necessary requirements and regulations set by the Institutional Ethics Committees. In all studied cases, ultrasonography and gross neuropathological examination confirmed that the brain tissue was normal. Brain tissue was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) overnight, cryoprotected in 30% sucrose, frozen in precooled 2-methylbutane, and stored at −70 °C until sectioned (15-μm-thick) in the frontal or sagittal plane and processed for immunohistochemistry.

**Immunohistochemistry**

Cryosections were incubated in blocking solution (1% bovine serum albumin [Sigma, St. Louis, MO], 5% normal goat serum [Vector Laboratories, Burlingame, CA], and 0.5% Tween 20 in phosphate buffered saline [PBS]) for 30 min at room temperature. Primary antibodies were applied overnight at 4 °C. We used the following antibodies: COUP-TFI (mouse, 1:1000, R&D Systems, Minneapolis, MN), calretinin (rabbit, 1:2000, Swant, Bellinzona, Switzerland), calbindin (rabbit, 1:2000, Sigma), GABA (mouse, clone GB-69, 1:1000 and rabbit, # A 2052, 1:2000, Sigma), proliferation markers Ki67 (rabbit, 1:100, Anaspec, San Jose, CA) and PH3 (mouse, 1:500, Immunex, San Diego, CA), NeuN (mouse, 1:100, Chemicon), as well as oligodendrocyte markers platelet-derived growth factor receptor alpha (PDGFRα, rabbit, 1:500, gift from Dr W. Stallcup, La Jolla, CA) and Olig2 (rabbit, 1:10,000, a gift from Dr C. D. Stiles, Harvard Medical School, Boston, MA).

Corresponding Alexa 488 or Alexa 555-conjugated secondary antibodies (1:500, Molecular Probes, Eugene, OR) were subsequently applied for 2 h at room temperature. For double staining, the primary antibodies were mixed at optimal dilutions and subsequently detected using mixtures of appropriate secondary antibodies. Nuclei were counterstained with a short (5 min) incubation in 1% bisbenzamide (Polysciences, Warrington, PA). For each antigen, specificity of staining was tested by omitting the primary antibody or replacing it with corresponding isotype controls (mouse IgG1, IgG2a or IgG2b, or rabbit serum), whereas the specificity of secondary antibodies was tested by omitting the primary antibodies from the protocol. Both tests resulted in a lack of immune reaction. Additionally, analysis was performed only on those sections in which staining patterns were consistent with the subcellular distribution of the antigen (e.g., COUP-TFI as a transcription factor within the cell nucleus).

**Cell Quantification**

The countings were performed on an Axioscope microscope (Carl Zeiss, Oberkochen, Germany) equipped with a motorized stage and Neurolucida software-controlled computer system (MicroBrightField, Williston, VT). Sections were observed and delineated under low-power magnification (10× objective) with a 365/420 nm excitation/emission filter set. The nuclear staining allowed delineation of areas of interest (e.g., GE, cortical plate etc.). A grid (60 × 60 μm) was placed over each field area of interest. Two-dimensional counts of the labeled cells/nuclear profiles were performed under 40× objective in 4-6 random square fields. Results are presented as a percentage of immunolabeled cells of the overall cell number (number of nuclear profiles labeled with nuclear counterstain). At least 3 sections per case were counted, and mean values along with standard deviations (SDs) were calculated. The data are expressed as mean ± SD and statistically

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**Table 1**

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<td>M</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>F</td>
</tr>
</tbody>
</table>

Note: F, Female; M, Male. Na, not available.

**Figure 1.** Scanned sections of fetal forebrains during the first half of gestation. (A) Sagittal section at 15 GW. (B) Coronal section at 15 GW. (C) Coronal section through the level of the thalamus at 22 GW. Abbreviations: Cer—cerebellum, Hip—hippocampus, Th—thalamus, iGE—inferior ganglionic eminence, IC—internal capsule, LV—lateral ventricle, CSB—corticostriate boundary.
compared using Student’s t-tests. The criterion for significance was set at \( P \leq 0.05 \).

**Western Blot**

Tissue samples from 3 fetuses were lysed in PBS containing a mixture of protease inhibitors and detergents (1 mM phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, 2 μg/mL pepstatin, 0.5 mM ethylenediaminetetraacetic acid, 1% Triton X-100) and diluted in sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate, 10% glycerol, 5% β-mercaptoethanol, 10% bromophenol blue). Equal amounts of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 4–15% polyacrylamide gels and transferred electrophoretically onto polyvinylidene difluoride membranes (Millipore). After blocking the membranes with 5% nonfat milk in Tris buffered saline (Tris 10 mM, NaCl 150 mM, Tween-20 0.05%, pH 7.4), they were incubated overnight with a monoclonal primary antibody to COUP-TFII (1:1000, R&D Systems) or a monoclonal antibody to actin (1:500, Sigma), followed by the incubation with antimouse secondary antibody conjugated with horseradish peroxidase (goat anti-mouse IgG, 1:5000, Thermo Fisher Scientific, Rockford, IL) and developed using ECL plus western blotting detection system and Hyperfilm (Amersham, Little Chalfont, UK). The optical density of the bands from 3 independent experiments was measured using NIH Image J program.

**Reverse Transcriptase Polymerase Chain Reaction**

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to manufacturer’s instructions. The amount of purified RNA was estimated by measuring the absorbance at 260 nm. Its purity was assessed by a 260/280 nm ratio. The RNA (1 μg) of each sample was reverse transcribed using the SuperScript First-Strand Synthesis System for reverse transcriptase polymerase chain reaction (RT-PCR) kit (Invitrogen). The complementary DNA was subsequently amplified by the polymerase chain reaction (PCR). The sequences of the primers used were as follows: **COUP-TFII** (sense, 5'-ACAAAACAGAAGGGCCT-3'; antisense, 5'-TGTTCTTACGGCACAGTATTGC-3'), **GAPDH** (sense, 5'-GTACTGACGGCAGACATCG-3'; antisense, 5'-AGCCACATCGCCTAGACACC-3').

**Image Acquisition and Processing**

Photomicrographs were taken on an Axioplan fluorescent microscope (Carl Zeiss). Image processing was done by Adobe Photoshop CS5 software (Adobe Systems Inc, San Jose, CA) and included only adjustments of brightness and contrast.

**Results**

**The Position of CGE in Human Fetal Brain**

There is not a single gene that characterizes the CGE in a way that Nkx2.1 expression delineates the MGE in rodent brain (Sussel et al. 1999; Butt et al. 2005; Xu et al. 2008). In rodents, however, COUP-TFII is enriched in the CGE, and it is only sparsely expressed in the LGE (Kanatani et al. 2008; Willi-Monnerat et al. 2008; Miyoshi et al. 2010).

To determine the position of CGE in human fetal brains, we relied on its relative position in respect to other forebrain structures and on published data (e.g., Ulfig 2002). In sagittally cut human fetal brains, the CGE can be seen in the most caudal position protruding into the lateral ventricle, close to the hippocampus (Fig. 1A). Rostrally to, and in continuation with the CGE, one can observe the LGE in lateral sections and the MGE in medial parasagittal sections. In the coronally cut brain at the level through the thalamus, the CGE appears as superior and inferior subdivisions, separated by the thalamus and internal capsule (Fig. 1C). In coronal sections cut rostrally to the thalamus, the LGE and MGE are seen but not the CGE (Fig. 1B).

**COUP-TFII is Expressed in the Second Trimester Human Fetal Brain**

COUP-TFII mRNA levels and COUP-TFII protein expression were determined in fresh prepared tissue from 2 brains at 21 GW. To determine the expression level of COUP-TFII in human fetal brains, we used immunofluorescence for COUP-TFII in the cerebral wall at 9 GW; inset presents higher magnification of layer I. (A) COUP-TFII expression in the cerebral wall and (C) GE at 10 GW. (D–F) Immunofluorescence for COUP-TFII at 13 GW in (D) Cortical VZ/SVZ, (E) CGE, and (F) Cerebral cortex. Inset shows a scanned section with boxed areas from which the photographs in D and E were taken. Abbreviations: CP—cortical plate. Scale bars: 100 μm (A, C), 50 mm (B, D–F).
midterm (20, 21 GW). Both semiquantitative RT-PCR (Fig. 2A) and western blot (Fig. 2B) analyses confirmed the COUP-TFII expression in the fetal human GE and cortical VZ/SVZ at midterm. We next analyzed COUP-TFII expression at different developmental stages using immunohistochemistry on cryo-sections. At the earliest stage studied, 9 GW, COUP-TFII is expressed in cell nuclei distributed throughout the forebrain (Fig. 3A). In the cerebral wall, COUP-TFII is mainly seen in the cortical layer I, in the intermediate/subplate (SP) zone, and only occasionally in the cortical plate. Notably, at this stage of development, COUP-TFII is not present in the cortical VZ/SVZ (Fig. 3A). During subsequent weeks of intrauterine development (10, 13 GW), immunoreactivity for COUP-TFII becomes more prominent (Fig. 3B,C). At 13 GW, a dense cell population is demonstrated in the proliferative zone of the CGE, where COUP-TFII+ cells account for 12.4 ± 1.3% of all cells (Fig. 3C). By 13 GW, the number of COUP-TFII+ cells in the cortical plate also increases (Fig. 3F). Notably, at this age for the first time, COUP-TFII+ cells are present in the cortical VZ/SVZ although far less frequent (2.3 ± 0.5%) than in the CGE (Fig. 3D,E).

We next analyzed the subdivisions of the GE (MGE, LGE, and CGE) with respect to COUP-TFII expression. By studying both coronal and sagittal sections of a 15 GW brain, a gradient of COUP-TFII expression in the GE was observed (Fig. 4). A decreasing percentage of COUP-TFII+ cells is seen from the CGE (16 ± 2.1%) to the LGE (1.8 ± 0.3%), and very few immunolabeled cells were observed in the MGE (0.3 ± 0.1%). This distribution pattern suggests that, similar to rodents, early COUP-TFII+ cells originate in the CGE and migrate to the cerebral cortex through the LGE (Tripodi et al. 2004; Kanatani et al. 2008).

In the cerebral cortex, different cortical areas have a distinct distribution of COUP-TFII+ cells. Posterior (caudal) cortical areas have a more abundant COUP-TFII+ cell population than anterior (rostral) areas. This trend is seen in sagittally cut brains starting at 10 and 15 GW. We quantified COUP-TFII+ cells at 15 GW brain in layer I, which is a well-defined region suitable for evaluation of the percent of immunolabeled cells (Fig. 5). We found almost 4 times as many COUP-TFII+ cells in layer I of the caudal/temporal cortex (23.5 ± 6.3%) compared with the rostral/dorsal cortex (8.6 ± 3.3%). This indicates that COUP-TFII+ cells migrate first to caudal and then rostral cortical regions, but at midterm, this regional difference was more variable. The difference in COUP-TFII expression was especially apparent in the SP layer as seen in Fig. 6 (72.3% of cells in the temporal SP were COUP-TFII+ vs. only 4.3% in dorsal SP). However, when we pooled together 4 cases (20–21 GW), we found that 34.2 ± 4.2% of total cell number in layer I were

Figure 4. COUP-TFII expression in the subdivisions of GE at 15 GW. (A–D) Representative images of immunoflorescence for COUP-TFII in (A) MGE, (B) LGE, (C) CGE. (E) Scan of sagittally cut 15 GW brain, boxed area is presented in (D) a border (dashed line) of the high expression in the CGE to the low expression in the MGE. (F) Diagram represents relative densities of COUP-TFII+ cells in the different GE sub regions. Scale bars: 50 μm (A–C), 100 μm (D).
COUP-TFI⁺ cells in the caudal and temporal cortex versus 35.7 ± 3% in the rostral and dorsal cortex. In the cortical plate, percentage of COUP-TFI⁺ cells from the total cells was 3.7 ± 0.3% in the caudal versus 3.6 ± 1.4% in rostral brain regions.

When we grouped early cases (10, 11, 13, and 15 GW, \( n = 4 \)) and compared the percentage of COUP-TFI⁺ cells in layer I with a group of midterm cases (20–21 GW, \( n = 4 \)), we obtained similar numbers, 25.5 ± 4.5% versus 34.9 ± 2.1%, respectively, which are not significantly different (\( P > 0.05, t \)-test). Although the number of COUP-TFI⁺ cells per surface area increases, the newly formed highly cellular subpial granular layer, “dilutes” the percentage of COUP-TFI⁺ cells from all the cells at this location.

Considerable individual variations between human tissue specimens were noticed. For example, the percentage of COUP-TFI⁺ cells from total cells ranged from 15.4 to 37.1 in the early group (\( n = 4 \)) and 26.7 to 42.2 in midterm group (\( n = 4 \)).

**COUP-TFI is Expressed in a Subpopulation of Cortical Interneurons**

In rodents, COUP-TFs I and II are expressed in a subpopulation of migrating cortical interneurons (Tripodi et al. 2004; Kanatani et al. 2008; Miyoshi et al. 2010). Here, we performed double immunohistochemistry in order to characterize the types of cells that express COUP-TFI in the human cerebral cortex. At all ages studied, cells that express COUP-TFI often coexpress interneuron markers, GABA, calretinin, and calbindin (Figs 6 and 7).

We first tested colocalization of COUP-TFI and GABA, as a universal marker of interneurons (Fig. 6). We calculated the percentage of GABAergic cells that express COUP-TFI in various cerebral cortex zones at 15 GW. Similar percentages of GABA⁺/COUP-TFI⁺ cells were seen in the cortical plate (18.5 ± 5%) and 5P layer (19.3 ± 6.6%), but a much higher percentage was obtained in layer I, where half of GABAergic cells (50 ± 3.2%) coexpress COUP-TFI (Table 2). On the other hand, we
calculated that 27.2 ± 2.8% of COUP-TFI⁺ cells express GABA in layer I at this age (Table 3). A subfraction of COUP-TFI⁺ cells can also be labeled with oligodendrocyte progenitor markers Olig2 and PDGFRα (Supplementary Fig. 1).

Comparable results for GABA⁺ cells that express COUP-TFI were obtained at later ages (20--22 GW), whereas 22.5 ± 8.8% in the cortical plate, 17.3 ± 0.5% in the SP, one-fourth of GABAergic cells (24.2 ± 4.9%) in layer I coexpress COUP-TFI (Table 2). In this context, it is interesting that our preliminary results demonstrate that GABAergic cells represent up to 20.4 ± 1.2% of NeuN⁺ neurons in layer I, 9 ± 0.7% in the cortical plate, and 1.5 ± 0.7% in the SP layer at midterm. However, it should be noted that NeuN is not yet expressed by all cortical plate cells at this developmental stage, as previously reported (Sarnat et al. 1998). The percentage of COUP-TFI⁺ cells that expressed GABA is listed in Table 3.

In the proliferative zones at midterm, similar percentages for coexpression of GABA and COUP-TFI were obtained in the CGE (27 ± 8%) and in the cortical VZ/SVZ (22.5 ± 4.5%). Considering that in rodents the CGE gives rise to CalR⁺ and CB⁺ interneurons (Kanatani et al. 2008, Miyoshi et al. 2010), we studied these subpopulations of cortical interneurons for COUP-TFI expression (Fig. 7). We estimated double-labeled COUP-TFI⁺/CalR⁺ cells in the proliferative zones of the CGE and cortical VZ/SVZ at 15 and 20 GW. A particularly dense population of double-labeled COUP-TFI⁺/CalR⁺ cells was demonstrated in the corticostriate boundary (CSB) of a 15 GW brain, where the LGE meets the cortical VZ/SVZ (Fig. 7A). It is possible that the population of double-labeled cells in the CSB represent migrating neurons, as we reported previously for Lhx6⁺ cells (Jakovcevski et al. 2011), but this needs to be verified on more cases. Another location where COUP-TFI⁺/CalR⁺ cells at 15 GW fetal brain were prominent is the hippocampal area (Fig. 7B).

In the CGE at 15 GW, 13.6 ± 1.7% of all COUP-TFI⁺ cells were CalR⁺ (Fig. 7A–C). On the other hand, from all CalR⁺ cells, one-third expressed COUP-TFI (31.3 ± 3.1%), and a similar percentage was obtained at 20 GW (26 ± 3%). In the cortical VZ/SVZ, CalR⁺ cells that expressed COUP-TFI increased from 18.5 ± 8% to 27.3 ± 4.8% from 15 to 20 GW (Table 4).

Similarly, the percentage of double-labeled COUP-TFI⁺/CalR⁺ cells increases in the cortical plate and layer I between 15 and 20 GW (Fig. 7 and Table 2). Cajal–Retzius cells, close to the pia, express both markers (Fig. 7F inset). In the expanding SP layer, this percentage is very high, around 60%, both at 15 and 20 GW (Table 2).

Calthbndin⁺ cells are less frequent in the cerebral cortex at midterm (Zecevic et al. 2011), but a subpopulation of these cells coexpress COUP-TFI, both in the layer I and in the cortical plate (Fig. 7G,H). Other interneuron populations, such as Sst⁺, PV⁺, VIP⁺, and NPY⁺ cells are sparse in the cerebral cortex at this stage.

**COUP-TFI in Progenitor Cells**

To investigate the origin of cells that express COUP-TFI, we used double immunofluorescence for proliferation markers (Ki67 and PH3), combined with the antibody to COUP-TFI. At 15 GW, similar percentages (between 2% and 4%) of COUP-TFI⁺/Ki67⁺ cells proliferate in the CGE and in the cortical VZ/SVZ (Fig. 8 A,B). At midterm (20--21 GW, n = 2), more COUP-TFI⁺/Ki67⁺ cells proliferate in the CGE (3.45 ± 0.3) than in the cortical VZ/SVZ (1.8 ± 0.1%), which is statistically significant (P < 0.05, t-test) (Fig. 8C–E). Similar results were obtained with PH3, another proliferation marker (not shown).

**Discussion**

In this study, we demonstrate that COUP-TFI is expressed in the human forebrain from the earliest age examined (9 GW)
and continues to be expressed throughout the midterm (20-22 GW). Although the total number of COUP-TFII+ cells increases, their density remains similar between 15 and 20 GW.

Table 2

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<th>Gestation weeks</th>
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<td>15 GW</td>
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<td>19.3 ± 6.8%</td>
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<td>60 ± 12.4%</td>
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<tr>
<td>20-22 GW</td>
<td>22.5 ± 8.8%</td>
<td>74.3 ± 1.1%</td>
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<td>57 ± 11.7%</td>
<td>24.2 ± 4.9%</td>
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Note: CP, Cortical plate.
Comparison of the Distribution of COUP-TFI\(^{II}\) Cells between Human and Rodent Forebrains

Our findings on COUP-TFI\(^{II}\) expression in fetal human brain, while showing some similarities, point to significant differences regarding data derived from rodent studies. In rodents, COUP-TFI\(^{II}\) is expressed primarily in the CGE and at the LGE/MGE border (Tripodi et al. 2004; Youz et al. 2005; Kanatani et al. 2008; Miyoshi et al. 2010). The majority (approximately 85%) of CGE-derived and only 10% of MGE-derived interneurons express COUP-TFI\(^{II}\), whereas LGE-derived cells do not express COUP-TFI\(^{II}\) (Miyoshi et al. 2010). It has been demonstrated that COUP-TFI\(^{II}\) is important for late-born cortical interneurons that migrate through the caudal migratory stream from the CGE to upper cortical layers of the caudal cortex (Youz et al. 2005; Kanatani et al. 2008; Miyoshi et al. 2010). However, both COUP-TFI\(^{II}\) and COUP-TFI\(^{II}\) were also reported in the early embryonic VZ in rodents, where they regulate transition from neural stem cells to neurons and glia (Naka et al. 2008). Using genetic fate mapping of CGE-generated cortical interneurons, with the Cre-Mash1 promoter, it has been described that in rodents, 75% of CGE-derived interneurons migrate to the upper cortical layers (I–III). Surprisingly, this distribution is not related to the time of their origin, as is the case for projection neurons that follow an inside-out pattern of migration (Miyoshi et al. 2010). COUP-TFI\(^{II}\), a close relative of COUP-TFI\(^{II}\), regulates the balance between MGE- and CGE-derived cortical interneurons (Lodato et al. 2011). Conditional loss of COUP-TFI\(^{II}\) results in a reduction of late-born CalR\(^{+}\) and VIP\(^{+}\) interneurons generated from the CGE and an increase in early-born PV\(^{+}\) cells derived from the MGE (Lodato et al. 2011).

In our study, the expression of COUP-TFI\(^{II}\) in the human cerebral cortex was detected fairly early (9 GW). COUP-TFI\(^{II}\) cells at 9 GW were located above the still thin emerging cortical plate. Some of CalR/COUP-TFI\(^{II}\) double-labeled cells are Cajal-Retzius cells as judged by their size and position close to the pia (Meyer et al. 2000, 2002, Zecevic et al. 2011). Cajal-Retzius cells at this and later stages could be colabeled with reelin and CalR, but reelin and COUP-TFI\(^{II}\) double labeling could not be done since both antibodies are produced in mouse. Notably, we did not observe proliferation of COUP-TFI\(^{II}\) cells in the cortical VZ/SVZ at this age (9 GW), suggesting that early COUP-TFI\(^{II}\) cells are generated either in the GE or in the cortical hem. The cortical hem in human is a site of origin of Cajal-Retzius cells (Meyer 2010). At later stages (15–22 GW), we found a gradient of COUP-TFI\(^{II}\) expression in the cerebral cortex, from abundant in the caudal (temporal and occipital) cortex to moderate expression in the rostral (frontal) cortex. This finding is in congruence with the results in mice, where COUP-TFI\(^{II}\) is necessary for migration through the caudal migratory stream from the CGE to the occipital cortex (Kanatani et al. 2008).

Several features regarding the distribution of COUP-TFI\(^{II}\) cells in the cerebral cortex, however, might be human specific. First, COUP-TFI\(^{II}\) cells are present quite early during human cortical development, whereas in rodents, they are reported as late generated cells in the CGE. Second, the extensive SP layer in humans (Kostovic and Rakic 1990) harbors a large number of COUP-TFI\(^{II}\) cells particularly in the caudally located temporal/occipital cortex. Third, a subpopulation of COUP-TFI\(^{II}\) cells proliferates in the cortical VZ/SVZ, which adds to the variety of cortical progenitors reported in primates. Finally, some COUP-TFI\(^{II}\) progenitors in the GE coexpress Olig2 and might be common neuron/oligodendrocyte progenitor cells. Olig2, a basic-helix-loop-helix transcription factor, has been described in neural progenitors in the ventral telencephalon and particularly in the MGE both in rodents (Miyoshi et al. 2007) and humans (Jakovevski and Zecevic 2005a). Only, occasionally, we found cells labeled with COUP-TFI\(^{II}\) and PDGFR\(x\), the early oligodendrocyte progenitor marker. At the same fetal stages studied here, we have previously demonstrated another ventral transcription factor expressed by interneuron progenitors, Mash1, in PDGFR\(x\) oligodendrocyte progenitors (Jakovevski et al. 2011).

A Subpopulation of Cortical Interneurons in Humans Might be Derived from the CGE

In rodents, distinct subtypes of cortical interneurons originate in each of the GEs. PV\(^{+}\) and Sst\(^{+}\) cells originate in the MGE, whereas the VIP\(^{+}\), CalR\(^{+}\), and CB\(^{+}\) cells are mainly derived from the CGE (Nery et al. 2003; Xu et al. 2004; Butt et al. 2005; Cobos et al. 2006; Kanatani et al. 2008; Miyoshi et al. 2010). Furthermore, in Nkx2.1 null mutants, genesis of PV\(^{+}\) and Sst\(^{+}\) interneurons is decreased but generation of CalR\(^{+}\) and VIP\(^{+}\) interneurons is increased. With a nonfunctional Nkx2.2 gene, the MGE progenitors switch fate and are restricted to generate more CalR\(^{+}\) and VIP\(^{+}\) interneurons, characteristic for the CGE (Butt et al. 2008). Notably, COUP-TFI\(^{II}\) becomes ectopically expressed in the MGE after turning off the Nkx2.2 gene at E10.5 (Butt et al. 2008).
In the human fetal brain tissue, we demonstrated a gradient of COUP-TFI⁺ cells in different parts of the GE with peak values in the CGE. In addition, in the cerebral cortex, we found the coexpression of GABA, CalR, and CB with COUP-TFI. A considerable number of these double-labeled cells are present in the cerebral cortex at midterm. In layer I, our preliminary results show that around 20% of NeuN⁺ neurons are GABAergic. Of these GABAergic cells, approximately 25% coexpress COUP-TFI.

Additionally, we demonstrated, by colabeling with the proliferation marker Ki67, that COUP-TFI⁺ cells proliferate more commonly in the CGE than in other forebrain regions at midterm (see below). Combined, these results suggest that a fraction of cortical interneurons may be generated in the CGE in human, similar to what has been reported in rodents.

**COUP-TFI in Progenitor Cells**

To investigate the origin of cells that express COUP-TFI, we colabeled our sections with a battery of proliferation markers, combined with an antibody to COUP-TFI. The most reliable results were obtained with anti-Ki67 rabbit polyclonal antibody. Indeed, a small fraction of COUP-TFI⁺ cells were proliferating in the GE and even fewer cells in the cortical VZ/SVZ. In sections where we could identify the CGE by its caudal position, this was a predilection site of COUP-TFI⁺ cells proliferating in vivo, and a variety of proliferation markers label various proportions of cells in the human fetal forebrain, it is possible that the extent of neurogenesis in the CGE at midterm is larger than we estimate.

Previous work, based on the expression of Dlx and Mash1 transcription factors, demonstrated that human GABAergic interneurons originate in both the GE and in the cortical VZ/SVZ (Letinic et al. 2002). Similarly, in nonhuman primates, late interneuron progenitors in the cortical VZ/SVZ were Mash1⁺/GAD65⁺ double-labeled cells (Petanjek, Berger, et al. 2009; Petanjek, Kostovic, et al. 2009). Our laboratory has recently identified several different subpopulations of potential interneuron progenitors in the cortical VZ/SVZ, including CalR⁺, Mash1⁺, and Nkx2.1⁺ proliferating cells (Jakovcevski et al. 2011; Zecevic et al. 2011). Thus, it was not surprising to find that a fraction of COUP-TFI⁺ cells proliferate in the cortical VZ/SVZ. Whether these cells represent genuine cortical VZ-derived progenitors, or intermediate progenitors that perform transit amplification, as suggested for the cortical principal neurons (Lui et al. 2011), remains to be studied.

In conclusion, our study provides the first insight into COUP-TFI⁺ cells in the human cerebral cortex. We confirmed that, similar to rodents, COUP-TFI⁺ cells are present in both the cerebral cortex and in the CGE and that a subpopulation of them coexpresses interneuron markers, including calretinin, calbindin, and GABA. Several results, however, have not been described in rodents and thus may present human-specific or primate-specific developmental events. Our findings further underline the need to study human cortical development with its complex progenitor pool unparalleled in any other species. The human neocortex has a long developmental period, which provides unique insights into neurodevelopmental disorders as well as into evolutionary adaptations that have created the intricate organization of the human brain.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/
Funding
This work was supported by National Institutes of Health (grant 2R01NS041489-10A1) and National Society for Multiple Sclerosis (grant RG 3083-D-5).

Notes
Midterm human fetal tissue was obtained from B. Poulos at the Albert Einstein College of Medicine, Tissue Repository. Conflict of Interest: None declared.

References


Sussel L, Marin O, Kimura S, Rubenstein JL. 1999. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular
respecification within the basal telencephalon: evidence for a
transformation of the pallidum into the striatum. Development. 15:3359-3370.


