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Single-Cell Transcriptome Profiling of Thyroid Hormone Effectors in the Human Fetal Neocortex: Expression of *SLCO1C1*, *DIO2*, and *THRB* in Specific Cell Types

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Background: Thyroid hormones are crucial for brain development, acting through the thyroid hormone nuclear receptors (TR)α1 and β to control gene expression. Triiodothyronine (T3), the receptor-ligand, is transported into the brain from the blood by the monocarboxylate transporter 8 (MCT8). Another source of brain T3 is from the local deiodination of thyroxine (T4) by type 2 deiodinase (DIO2). While these mechanisms are very similar in mice and humans, important species-specific differences confound our understanding of disease using mouse models. To fill this knowledge gap on thyroid hormone action in the human fetal brain, we analyzed the expression of transporters, DIO2, and TRs, which we call thyroid hormone effectors, at single-cell resolution.
Methods: We analyzed publicly available single-cell transcriptome data sets of isolated cerebral cortex neural cells from three different studies, with expression data from 393 to almost 40,000 cells. We generated Uniform Manifold Approximation and Projection scatterplots and cell clusters to identify differentially expressed genes between clusters, and correlated their gene signatures with the expression of thyroid effectors.
Results: The radial glia, mainly the outer radial glia, and astrocytes coexpress *SLCO1C1* and *DIO2,* indicating close cooperation between the T4 transporter OATP1C1 and DIO2 in local T3 formation. Strikingly, *THRB* was mainly present in two classes of interneurons: a majority expressing *CALB2*/calretinin, from the caudal ganglionic eminence, and in somatostatin-expressing interneurons from the medial ganglionic eminence. By contrast, many cell types express *SLC16A2* and *THRA*.
Conclusions: *SLCO1C1* and *DIO2* coexpression in the outer radial glia, the universal stem cell of the cerebral cortex, highlights the likely importance of brain-generated T3 in neurogenesis. The unique expression of *THRB* in discrete subsets of interneurons is a novel finding whose pathophysiological meaning deserves further investigation.

Keywords: fetus, interneurons, MCT8, nuclear receptors, OATP1C1, type 2 deiodinase

Introduction

Thyroid hormone signaling is crucial for mammalian brain development. Circulating thyroxine (T4) and triiodothyronine (T3) reach the brain parenchyma through transporter proteins in the blood–brain barrier (1). The primary thyroid hormone transporters are the *SLC16A2*-encoded monocarboxylate transporter 8 (MCT8), and the *SLCO1C1*-encoded organic anion transporter polypeptide (OATP1C1). MCT8 is a T4 and T3 transporter, whereas OATP1C1 transports T4, reverse T3, and T4 sulfate (2). *SLC16A2* mutations cause the X-linked Allan–Herndon–Dudley syndrome, with profound neurological impairment of prenatal origin (3,4).

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The authors confirm that the research described in this article meets the ethics guidelines and adheres to the legal requirements of Spain and Japan.

In the brain, 5′-deiodination of T4 in glia by type 2 deiodinase (DIO2) produces T3, the active thyroid hormone. Type 3 deiodinase (DIO3), expressed in neurons, inactivates T4 and T3 by 5-deiodination. T3 binds to the type II nuclear receptors TRα1 and TRβ, products of the *THRA* and *THRB* genes (5), and regulates the developmental timing of gene expression in most neural cell types.

Transporter-facilitated delivery of thyroid hormones and deiodinase action ensures that the proper amount of T3 reaches the nuclear receptors in a timely manner in response to cellular needs. These mechanisms are likely optimized to the brain cytoarchitecture, which differs greatly between human and mouse (6), making rodent disease models poorly applicable to humans. A relevant

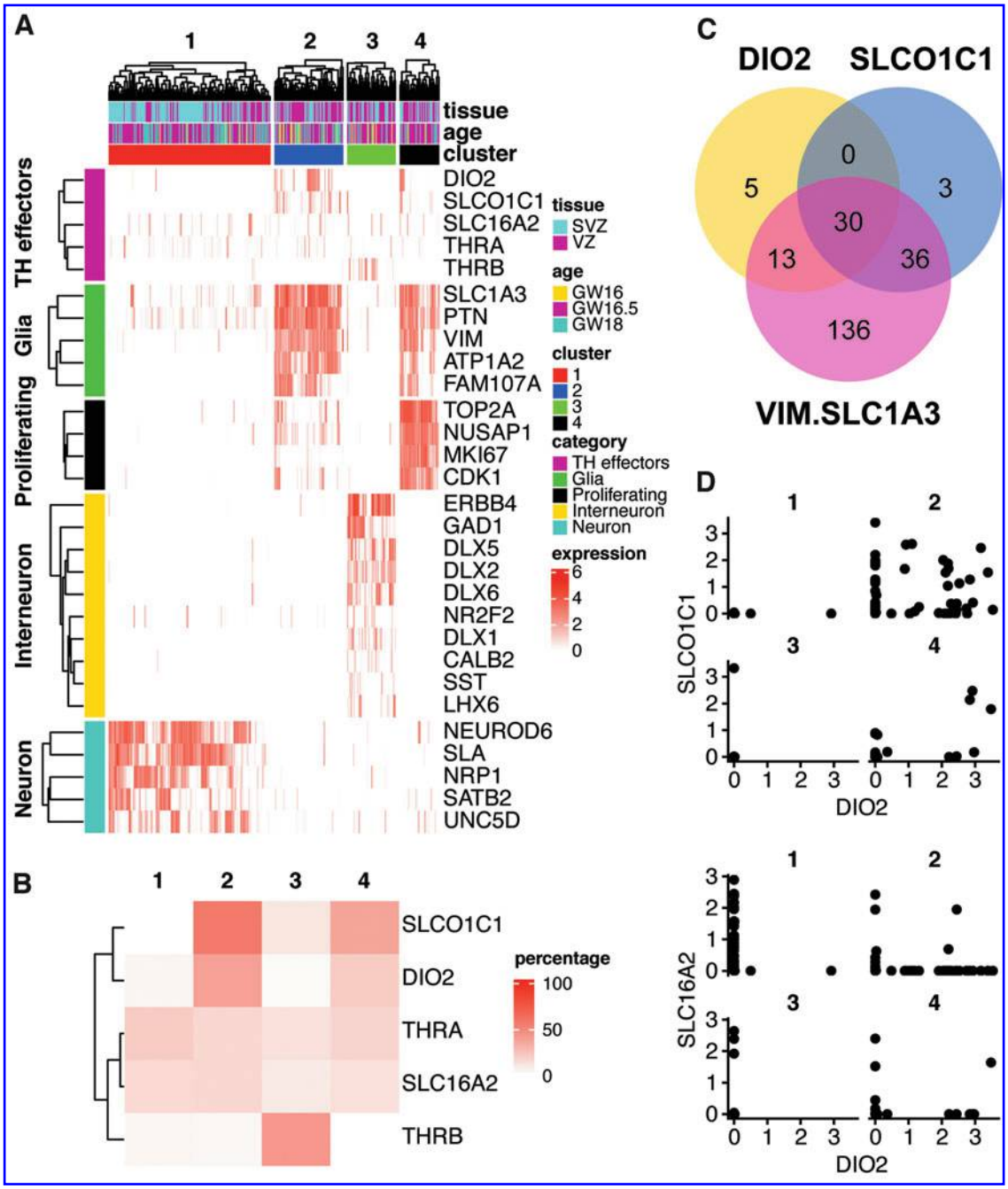

FIG. 1. Expression of thyroid hormone transporters, 5′-deiodinase, and receptors (thyroid hormone effectors) in radial glial cells. Data from a single-cell RNA sequencing data set of 393 individual radial glial cells isolated from human fetuses at gestational weeks 16–19 (11). (**A**) Heatmap constructed from the top differentially expressed genes grouped the cells into four clusters expressing markers of neurons (cluster #1), glia (cluster #2), GABAergic interneurons (cluster #3), and glia and proliferating cells (cluster #4). (**B**) Percentage of cells expressing the thyroid hormone effectors. (**C**) Venn diagram showing the number of cells expressing *DIO2*, *SLCO1C1*, and glia markers. (**D**) Correlation plots in each of the four clusters show coexpression of *DIO2* and *SLCO1C1* in radial glial cells and in some proliferating cells. *SLC16A2* did not correlate with *DIO2*. *DIO2*, type 2 deiodinase.

example is that disruption of these mechanisms in MCT8 deficiency leads to severe neurological impairment in humans but not in mice.

Understanding the mechanisms of disease resulting from altered function of transporters, deiodinases, and receptors requires detailed understanding of the precise timing and cellular expression of each of the components of this thyroid hormone effector triad, as well as their interaction. Despite the obvious difficulties in analyzing human fetal tissues, data on the expression of the T3 receptor, deiodinases, and transporters are available but with little or no insight at the cellular level (7–9).

Recent development of technologies to analyze gene expression in many individual cells simultaneously by single-cell RNA sequencing (scRNA-seq) (10) offers an opportunity to identify specific cells expressing genes of interest. In this study, we explored the expression of the thyroid hormone effectors in publicly available scRNA-seq data sets of the human fetal cortex at midgestation. We find that *DIO2* and *SLCO1C1* coexpress in glia, notably the outer radial glia, *THRB* is present mainly in GABAergic interneurons, and *SLC16A2* and *THRA* show widespread expression in most cell types. These observations provide a cytoarchitectural framework for a better understanding of thyroid hormone metabolism and action in the developing human brain.

Methods

The Ethics Committee of Consejo Superior de Investigaciones Cientificas of Spain (CSIC No. 042/2019) authorized this study. We analyzed three publicly available human fetal cortex scRNA-seq data sets: (i) 393 radial glial cells from gestational week (GW) 16–19 fetuses by Pollen *et al.* (11); (ii) 4261 neocortical cells from GW5.85–37, by Nowakowski *et al.* (12); and (iii) 33,976 neocortical cells from GW17–18, by Polioudakis *et al.* (13). Analysis of an additional data set of 2309 cells from GW8–26 prefrontal cortex (14) confirmed the results from the aforementioned three data sets and are not included in this report. The Pollen and Nowakowski studies used the Fluidigm C1 (www.fluidigm.com) platform, and the Polioudakis study used the Drop-seq workflow (15). We used raw counts for the Polioudakis study. For the Pollen and Nowakowski studies, only normalized transcripts per million reads were available. Data sets were processed and analyzed in R (16) using Seurat (17) and other packages. Genes not detected in any cells were filtered out. We removed from the Pollen data set a small cluster of 13 cells with low expression of ribosomal RNA, likely representing apoptotic cells. We normalized the data with NormalizedData, selected the top 2000 most variable features, and scaled them with ScaleData to calculate principal component analysis coordinates. The top 10 principal components were used to obtain Uniform Manifold Approximation and Projection (UMAP) scatterplots (18), and for clustering using the Leiden algorithm (19). We identified differentially expressed genes between clusters using the FindAllMarkers or FindMarkers functions from Seurat (17). To select differentially expressed genes, we used the Wilcoxon rank-sum test with an adjusted p-value cutoff of 0.05 and $\log_2$-fold change of 1.0 in absolute value (equivalent to a twofold change).

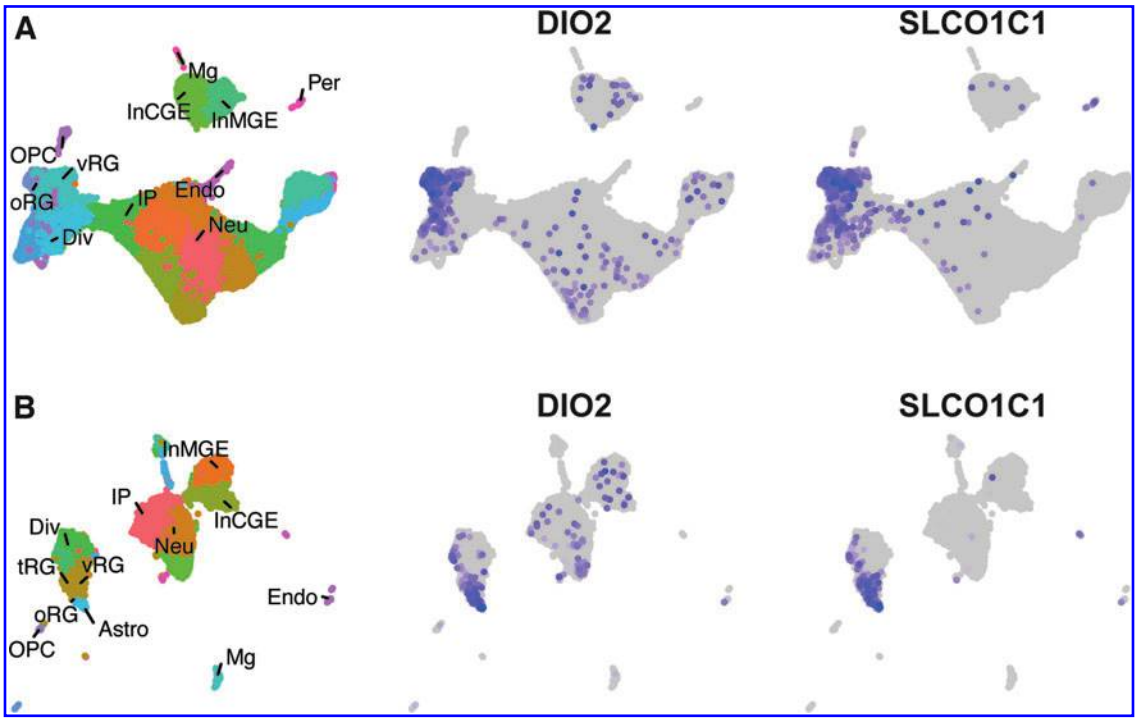

FIG. 2. *SLCO1C1* and *DIO2* are coexpressed in oRG and astrocytes in the developing human fetal neocortex. Shown are Uniform Manifold Approximation and Projection plots from the single-cell RNA sequencing data sets of human fetuses at gestational week 17–18 by Polioudakis *et al.* (**A**) (13) and gestational week 5.85–37 by Nowakowski *et al.* (**B**) (12). For clarity, only selected cell clusters are labeled. Astro, astrocytes; Div, dividing cells; Endo, endothelial cells; InCGE, interneurons from the caudal ganglionic eminence; InMGE, interneurons from the medial ganglionic eminence; IP, intermediate progenitors; Mg, microglia; Neu, excitatory neurons; OPC, oligodendrocyte precursor cells; oRG, outer radial glia; Per, pericytes; tRG, truncated radial glia; vRG, ventricular radial glia.

Results

We first analyzed the Pollen data set, with data from 393 radial glial cells from GW16–19 fetuses (11). This data set is of special interest as the radial glia is not only a guidance scaffold for neuronal migration, but is also the universal neural stem cell of the neocortex (20,21). In rodents, hypothyroidism affects radial glial cells (22). *THRA* mutations cause abnormal adhesion and proliferation of human cortical progenitors (23), suggesting alterations in the radial glia. Radial glial cells are immunoreactive for MCT8 and OATP1C1 in human fetal brain slices (9). These data indicate that the radial glia may be an important target for thyroid hormone at midgestation.

Using differential gene expression analysis, we identified four cell clusters (heatmap of Fig. 1A). Cluster #1 expressed markers of excitatory neurons. Cells in cluster #2 expressed the pan-glia markers *VIM* and *ATP1A2*, the radial glia markers *PTN* and *SLC1A3*, and the outer radial glia marker *FAM107A* (11). Cluster #3 showed specific expression of *ERBB4*, a tyrosine kinase and neuregulin receptor essential for early interneuron migration (24,25), GABAergic interneuron specification markers *DLX1/2* and *DLX5/6*, and the GABA synthesizing enzyme *GAD1*. *NR2F2* and *LHX6* transcription factors necessary for the maturation of interneurons from the caudal and medial ganglionic eminences (25), respectively, were also present. Finally, cells in cluster #4 expressed the glia markers of cluster #2 and proliferation markers such as *MKI67*, likely representing cycling progenitors. Figure 1B shows a semiquantitative assessment of these observations, with the percentage of cells expressing the thyroid hormone effectors in each cluster.

A clear pattern emerged: *SLCO1C1* and *DIO2* were associated with glial cells and cycling progenitors and *THRB* with GABAergic cells, in contrast to the broad expression of *SLC16A2* and *THRA*. After these initial findings, we performed more detailed analyses of the Pollen and other data sets comprising the whole cortex.

SLCO1C1 *and* DIO2

Further analysis of the Pollen data set (Fig. 1C) showed that of the 48 *DIO2*-expressing cells 43 (ca.90%) expressed the pan-glia marker *VIM* and/or the radial glia marker *SLC1A3*. Of these, 30 cells (61%) expressed *SLCO1C1*. *SLCO1C1* is highly enriched in the outer radial glia (26) and, indeed, 28 of the 30 cells expressing *DIO2* and *SLCO1C1* expressed the outer radial glia marker *FAM107A* (not shown). Of note, 43% of all cells expressing *SLCO1C1* also expressed *DIO2*. Correlation plots (Fig. 1D) revealed *DIO2* and *SLCO1C1* coexpression in glial cells (cluster #2). Some proliferating cells in cluster #4 also expressed *DIO2* and *SLCO1C1*. *DIO2* did not correlate with *SLC16A2*, indicating that OATP1C1 has a more prominent role than MCT8 in T4 transport to *DIO2*-expressing cells.

We confirmed the selective coexpression of *DIO2* and *SLCO1C1* in other data sets of the whole neocortex. Figure 2 shows the UMAP scatterplots from the Polioudakis *et al.* [GW17–18 (13); Figure 2A] and Nowakowski *et al.* [GW5.58–37 (12); Figure 2B] data sets. In Figure 2A, cells expressing *DIO2* and *SLCO1C1* correspond to the radial glia, particularly the outer radial glia (Fig. 3). Other *DIO2*-expressing cells were in neuronal areas, including interneurons from the caudal and medial subregions of the ganglionic eminence (labeled as InCGE and InMGE, respectively). *SLCO1C1* was most abundant in the radial glia, in close correspondence with *DIO2*. Figure 2B shows identical observations. As the temporal span of the Nowakowski data set extends to GW37, there was an additional set of cells labeled as astrocytes expressing both *DIO2* and *SLCO1C1*.

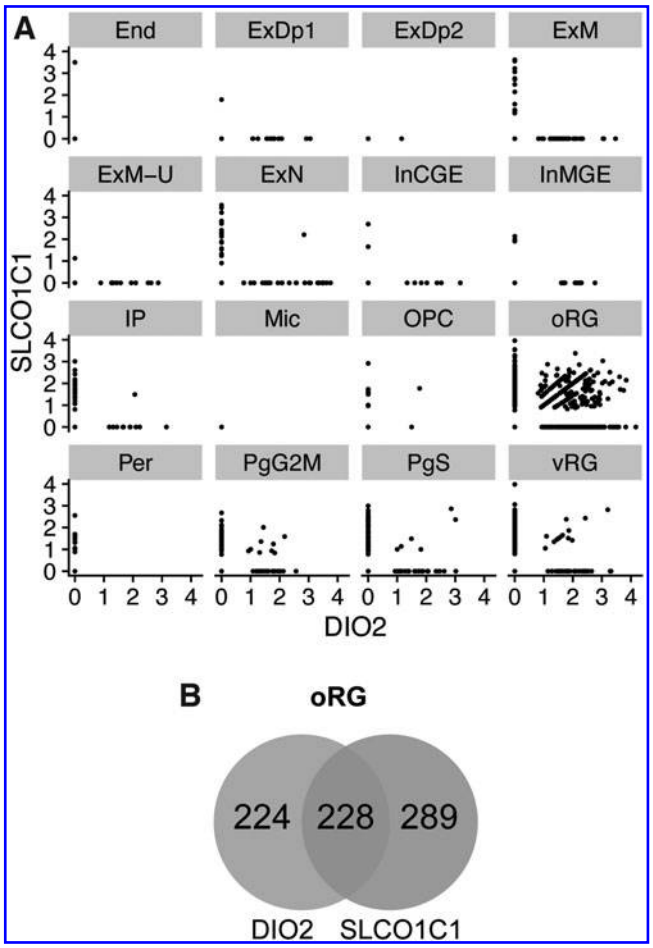

FIG. 3. Correlation plots of *SLCO1C1* and *DIO2* expression in different cellular clusters. The data were from the neocortex data set of human fetuses at gestational week 17–18 by Polioudakis *et al.* (13). (**A**) The strongest coexpression was in the outer radial glial cell cluster. Cluster labels were obtained from the original publication. (**B**) Venn diagram showing coexpression of *SLCO1C1* and *DIO2* in outer radial glial cells. ExDp1 and ExDp2, excitatory neurons of deep layers 1 and 2 (corresponding to layers 5–6 of the mature cortex); ExM, maturing excitatory neurons; ExM-U, maturing excitatory neurons upper enriched (corresponding to layers 2–4 of the mature cortex); ExN, migrating excitatory neurons; Mic, microglia; OPC, oligodendrocyte progenitor cells; PgG2M, cycling progenitors, G2/M phase; PgS, cycling progenitors S phase.

Figure 3 shows correlation plots of *DIO2* and *SLCO1C1* expression in different cell clusters of the Polioudakis data set. The highest correlation was present in the outer radial

glia and less in the ventricular radial glia and in progenitor cells in the G2M and S phases of the cell cycle (PgG2M and PgS, respectively). The Venn diagram of Figure 3 shows that among the outer radial glia (1293 cells), 35% (452 cells) expressed *DIO2* and 40% (517 cells) expressed *SLCO1C1*, with an overlap of 228 cells (31%) expressing both. Other cell clusters such as maturing excitatory neurons and migrating excitatory neurons showed *DIO2* and *SLCO1C1* expression without coexpression.

Overall, these observations indicate that during human neocortical development several cell types have the potential to produce local T3 through *DIO2* expression. The correlated

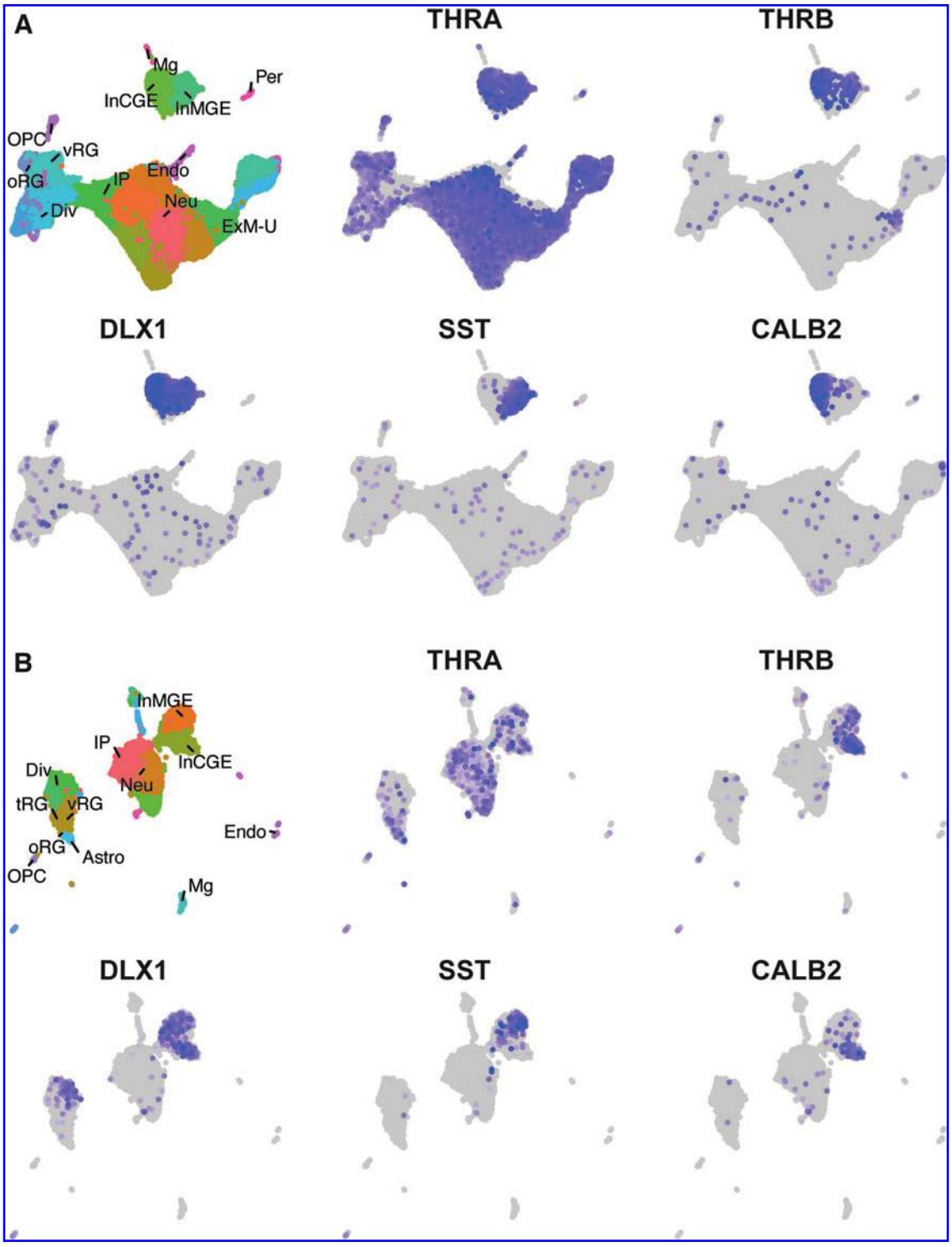

FIG. 4. Expression of *THRA* and *THRB* in the developing human fetal neocortex. *THRA* is expressed in most cells, and *THRB* shows specific expression in GABAergic interneurons of the caudal ganglionic eminence and the medial ganglionic eminence, which express the specification marker *DLX1* and either *CALB2* or *SST*.

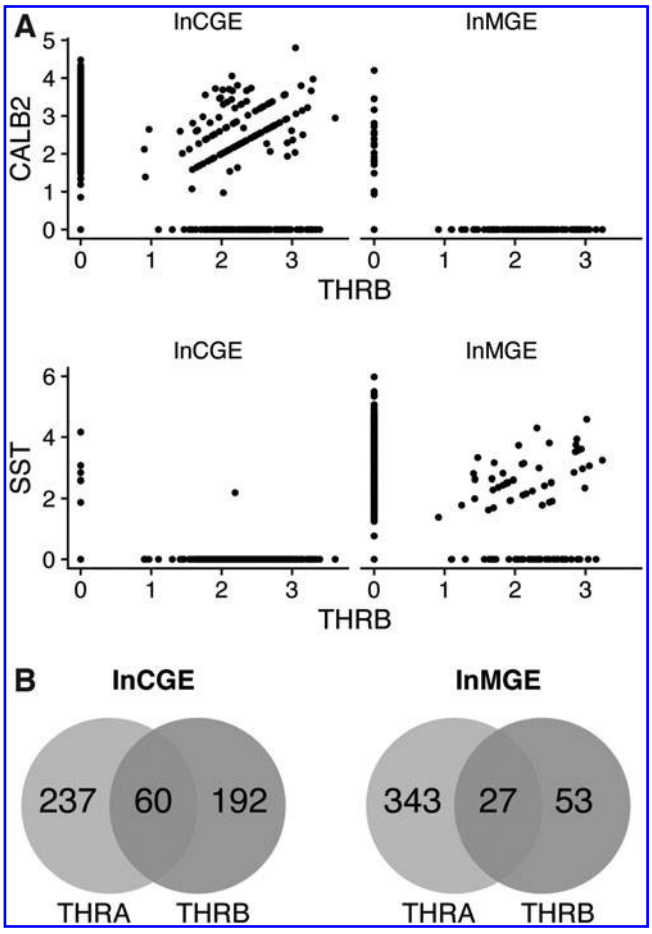

FIG. 5. Thyroid hormone receptors in interneurons. (**A**) Correlation plots between *CALB2* or *SST* and *THRB* in interneurons from the caudal (InCGE) or medial (InMGE) ganglionic eminence. (**B**) Venn diagrams of *THRA* and *THRB* expression in InCGE or InMGE, showing only partial overlap.

coexpression of *DIO2* and *SLCO1C1* makes the outer radial glia the putative main site of T3 production in the developing cortex at midgestation until astrocytes appear later in development. *DIO3* was present in a few scattered cells in all data sets (not shown), in agreement with its very low activity in the fetal cortex (8).

THRA *and* THRB

In the UMAP scatterplots of the Polioudakis (Fig. 4A) and Nowakowski (Fig. 4B) data set *THRA* was present in most cell clusters. *THRB* showed predominant expression in cell clusters from the caudal and medial subregions of ganglionic eminence. These clusters also showed prominent expression of the interneuron-specification factor *DLX1* and correspond to GABAergic interneurons (24,25,27).

Of the interneurons originating in the human ganglionic eminence, about half originate in the caudal subregion and express *CALB2* (calretinin) (28). The other half originates in the medial subregion and includes the parvalbumin and most somatostatin (SST) interneurons. *THRB* expression corresponded to the *CALB2* and *SST* clusters (Figs. 4A, B, and 5A). The thyroid hormone-dependent parvalbumin interneurons (4,29–31) were not present in these data sets, likely due to their late appearance during the postnatal period (32,33).

Cells derived from the ganglionic eminence also expressed *THRA*. In the Polioudakis data set (Fig. 5B), of 1434 interneurons from the caudal ganglionic eminence 237 (16.5%) expressed *THRA,* 192 (13.4%) *THRB*, and 60 (4.2%) expressed both TRs. In the medial ganglionic eminence, of 1705 cells, 343 (20.1%) expressed *THRA*, 53 (3.1%) *THRB*, and 27 (1.6%) both TRs. Thus, *THRA* and *THRB* might exert actions on different interneurons with a subset expressing both receptors. In the latter case, the effects of *THRA* and *THRB* might be additive, as their expression was positively correlated (Fig. 6).

A cluster of maturing excitatory neurons (Fig. 6) also showed correlated *THRA* and *THRB* coexpression. This cluster corresponds to neurons destined to cortex layers 2–4 and was defined by the combinatorial expression of upper layer-enriched genes, including the callosal projection neuron gene *LMO4* (13,34).

We also surveyed the expression of T3 receptor-interacting, transcriptional coregulators (5). Supplementary Figure S1 shows that the *NCOR1–2* corepressors, *NCOA1–3* coactivators, and the *MED30* subunit of the mediator complex have a wide distribution in the UMAP scatterplots.

SLC16A2

It was of obvious interest to examine *SLC16A2*/MCT8 expression and compare it with *SLCO1C1*. Most cells expressed *SLC16A2* (Fig. 7A, B). We compared *SLC16A2* and *SLCO1C1* expression across cell types using violin plots (Fig. 7C, D). In both data sets, *SLC16A2* expression occurred mainly in neurons, but was present in most cell clusters, including endothelial cells. By contrast, *SLCO1C1* was expressed in radial glia and astrocytes, and was practically absent from endothelial cells.

We also analyzed the expression of the secondary thyroid hormone transporters (35) *SLC7A5*/LAT1 and *SLC7A8*/LAT2, which are primarily L-type amino acid transporters (Supplementary Fig. S2). Endothelial cells, neurons, and medial ganglionic eminence interneurons expressed *SLC7A5*, and endothelial cells, neurons, and microglia expressed *SLC7A8*.

A summary of the percentage expression of each effector in every cell cluster is shown in Figure 8. Differences between the data sets reflect the different temporal spans. The main features are (i) predominant expression of *DIO2* and *SLCO1C1* in the outer radial glia and astrocytes; (ii) neurons, outer radial glia, and astrocytes express *THRA*; (iii) *THRB* is mainly expressed in interneurons from the caudal ganglionic eminence; (iv) endothelial cells express *SLC16A2* and not *SLCO1C1*; and (v) low expression of the thyroid hormone effectors in dividing cells.

Discussion

Our observations constitute an approximation to the cytoarchitecture of thyroid hormone transport and action in the developing human neocortex. The data allow several

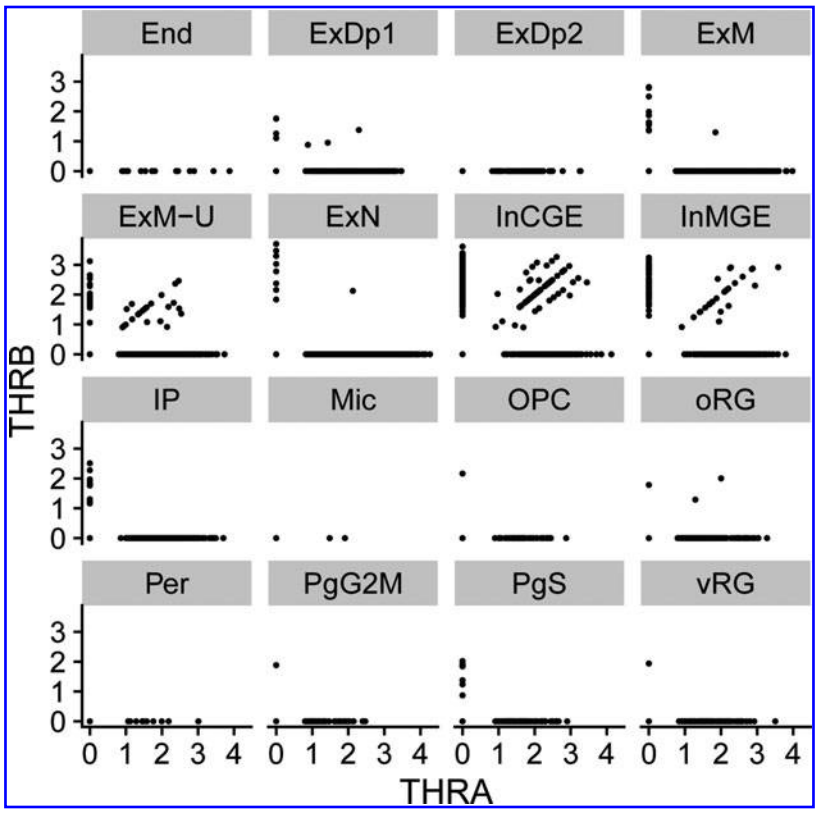

FIG. 6. Correlation plots of *THRA* and *THRB* expression in different cellular clusters. Data were obtained from the data set by Polioudakis *et al.* (13). Expression of *THRB* was limited to interneurons from the caudal or medial ganglionic eminence, and to a particular cluster of excitatory neurons (ExM-U). Cluster labels: ExDp1 and ExDp2.

conclusions to be drawn (illustrated in Fig. 9). During the second trimester of human fetal brain development, the T4 transporter gene *SLCO1C1*, and the 5′-deiodinase gene *DIO2* are coexpressed in radial glial cells, the universal stem cells of the cortex, whose appearance marks the onset of neurogenesis at about embryonic day 33 (6,21). Within the radial glia cell clusters, *SLCO1C1* and *DIO2* are present mainly in the outer radial glia. It is conceivable that OATP1C1 in these cells facilitates the influx of T4 and its DIO2-mediated conversion to T3. The importance of this observation cannot be underestimated, as the outer radial glial cells are the precursors of most human cortical projection neurons (36). These cells reside in an enlarged outer subventricular zone, typical of primates, and their neurogenic activity largely accounts for human cortical expansion (37). The outer radial glia gives rise to intermediate progenitors that differentiate first into neurons, and later in development into oligodendrocytes and astrocytes. The latter retain *DIO2* expression. Most cells expressing *DIO2* are glia, except for some interneurons, as previously observed (38).

The local formation of T3 by radial glial cells, which also form the scaffold for neuron migration, supports a role for T3 in the migration and/or differentiation of neural cells at midgestation. Given the widespread expression of *THRA* and coregulators, T3 formed in the radial glia might act on these cells or diffuse and act on nearby cells, such as migrating and/or differentiating neurons. At least in mice, TRα1 is present in neurons of the cortical plate as soon as the neuroblasts become postmitotic (39).

An intriguing novel finding was the almost restricted expression of *THRB* in subsets of GABAergic interneurons, mainly *CALB2* and *SST* interneurons. During neocortical evolution (40) there is a large expansion of GABAergic interneurons, which is largely due to an increase in the number of *CALB2*/calretinin interneurons. These cells, which in rodents account for 16–18% of total interneurons, increase by 35–40% in primates (41), especially in association areas, and appear early in the human dorsal telencephalon from about GW8 (20). It is tempting to speculate that *THRB* might be facilitating the evolution of the neocortex by actions on *CALB2*/calretinin interneurons.

THRA is present in the same interneuron cell types as *THRB*, but with limited coexpression. This might indicate different functions of TRα and TRβ on interneurons. In mice, thyroid hormones influence the differentiation of GABAergic cells presumably through TRα1 (4,29,30,39,42–44). The expression pattern points to a unique role of TRβ in humans. Comparisons with rodents are difficult, as the major transcriptomic study on mouse GABAergic interneuron subclasses is on adult brain (45).

The interpretation of *THRA* expression patterns needs to be cautious. The transcriptomic analysis includes the alternative splicing product, nonreceptor isoform TRα2, which is more abundant than TRα1 and present in most cell types, especially in the brain (46). Long reads sequencing would be needed to distinguish between the TRα1 and TRα2 splicing products, which is a limitation of our study. A second limitation, inherent to transcriptomic studies, is the lack of information on the abundance of the protein products. This could be critical to understand the relative roles played by TRα1 and TRβ, as there is poor correlation between TR mRNAs and their respective receptor proteins (46).

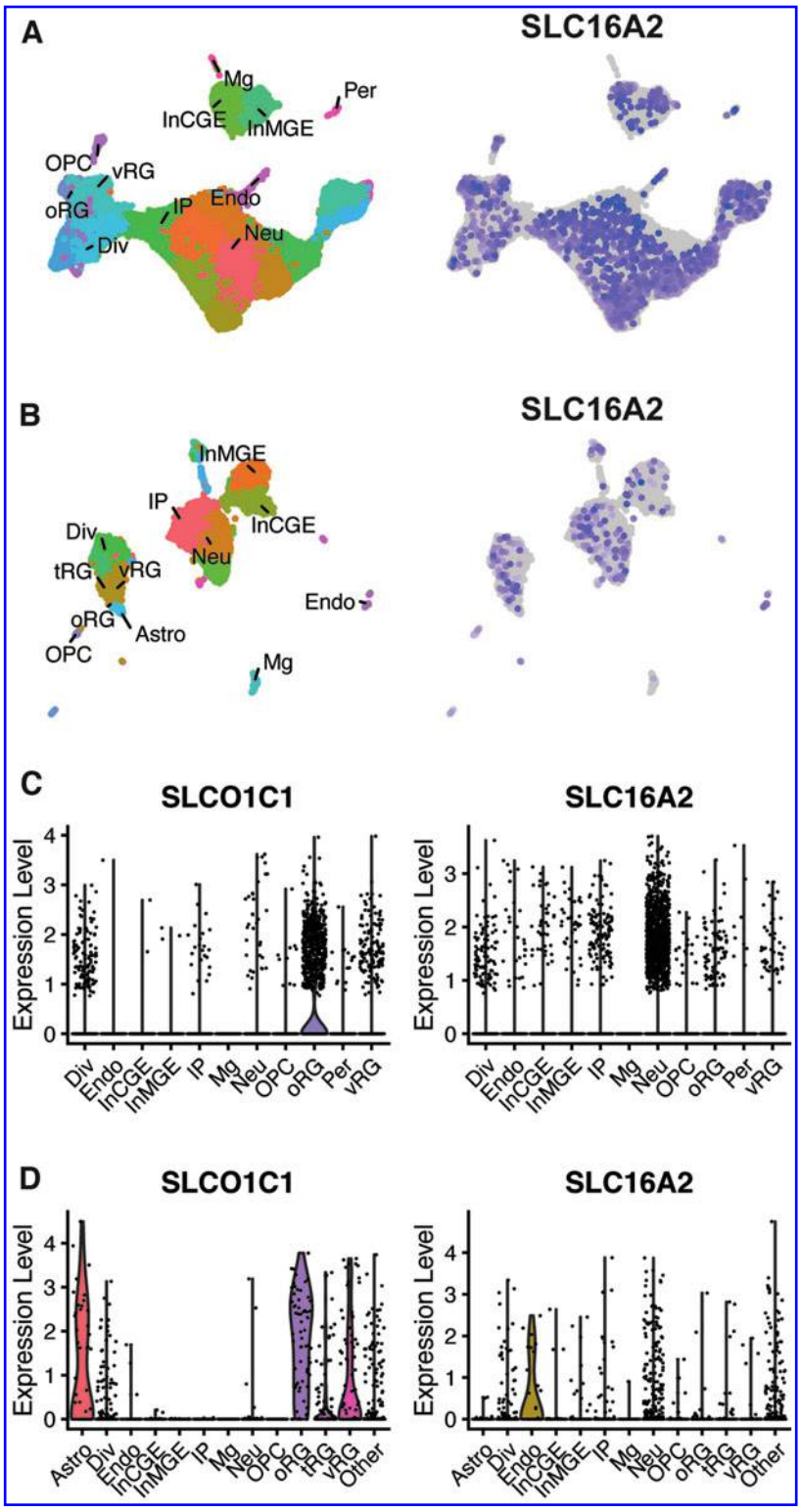

FIG. 7. Distribution of transporter expression in cell clusters. **(A, B)** Uniform manifold approximation and projection scatterplots of *SLC16A2* expression in the Polioudakis *et al.* (**A**) (13) and Nowakowski *et al.* (**B**) (12) data sets. (**C, D**) Violin plots of the comparison between *SLCO1C1* and *SLC16A2* expression from the Polioudakis (**C**) and Nowakowski (**D**) data sets. For clarity, only selected cell clusters are labeled. Neu, neurons; Other, undefined.

Despite these limitations, thyroid hormone effectors are present in the human fetal brain at midgestation. MCT8 and OATP1C1 immunoreactivity is present from at least GW14 (9). DIO2 activity increases in the cortex from GW12 to GW14, with very low DIO3 activity, in parallel to cortical T3 concentration (8). From GW10 to GW18 the T3 receptor protein, measured by T3 binding, also increases in the fetal brain up to 2000 molecules per nucleus (47). Notably, the receptor protein had analog binding affinities typical of TRβ. Therefore, it might well be that TRβ is a major TR subtype in the human fetal brain at midgestation, predominantly expressed in interneurons. In support of a role of TRβ, a recent study on the temporal pole cortex of adult individuals (48) showed that *THRB* correlated with an index of T3 action based on variations of the expression of genes sensitive to T3. There was no correlation with *THRA*.

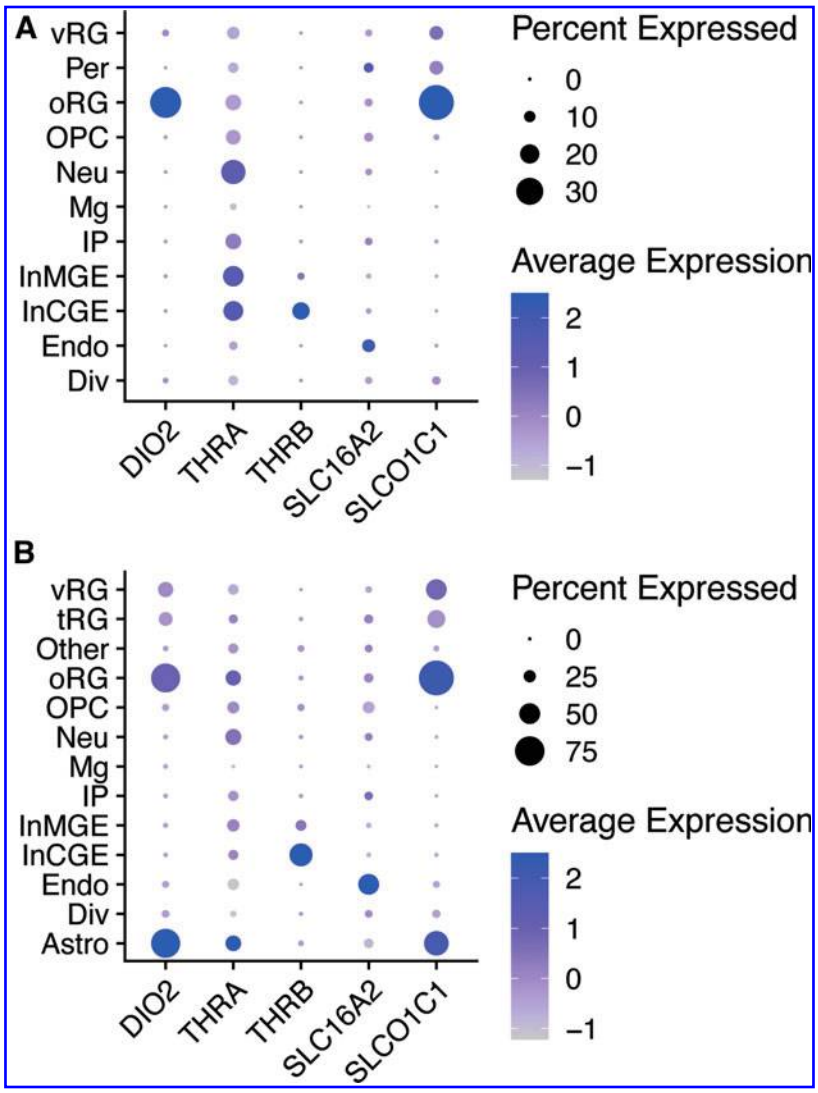

FIG. 8. Quantitative and percentual expression of thyroid hormone effectors in cell clusters. **(A)** Polioudakis (13) dataset; **(B)** Nowakowski (12) dataset.

While the critical T4 and T3 transport occurs in the blood–brain barrier (49,50), widespread *SLC16A2* expression supports a role for MCT8 in neuronal T3 transport. In the presence of a relatively constant maternal T4 supply, fetal thyroid secretion, cortex DIO2 activity, T3 concentrations, and T3 receptors increase steadily during the second trimester (7). The lack or reduced *DIO3* expression and activity should facilitate reaching a high T3 saturation of the receptor. In this context, MCT8 may serve to optimize neuronal T3 transport in and out of the cells. In contrast, the amino acid transporters LAT1 and LAT2 are present, but their role is uncertain (51).

The clinical phenotype of resistance to thyroid hormones due to *THRB* mutations (52) frequently includes varying degrees of intellectual disability and hyperactivity (53–55). Predominant expression of *THRB* in interneurons suggests a link with interneuron dysfunction. To the best of our knowledge, there are no brain structural analyses addressing this issue in beta-type resistance to thyroid hormone. Disruption of inhibitory circuits caused by interneuron dysfunction is linked to disorders such as schizophrenia, autism, intellectual disabilities, and epileptic encephalopathies (56,57), affecting mainly parvalbumin interneurons. Calretinin interneurons are reduced in number in lissencephaly (56), and in preterm infants with diffuse white matter damage (58). In most cases, the pathogenic role of specific interneurons is uncertain. In mice, expression of a dominant-negative TRα1 decreases parvalbumin and increases calretinin interneurons (59). Increased calretinin might be due to the elevated brain T3 in the mutant mice acting through TRβ.

This study describes the basic elements of the cytoarchitectural organization of thyroid hormone effectors—transporters, DIO2, and T3 receptors—in the developing human fetal neocortex. Our findings stress the relevance of the radial glia, the universal stem cell of the cortex, as a source of local T3, the widespread expression of *SLC16A2* (MCT8) and *THRA* genes across many cell types, and the predominant expression of *THRB* in interneurons. The latter finding suggests a novel specific role of TRβ in interneuron biology hitherto unsuspected.

Authors' Contributions

J.B. and B.M. conceived this study. D.D. performed bioinformatics analysis. J.B. wrote the article. All authors analyzed the data and their significance, and were involved in the final editing and approval of the submitted article.

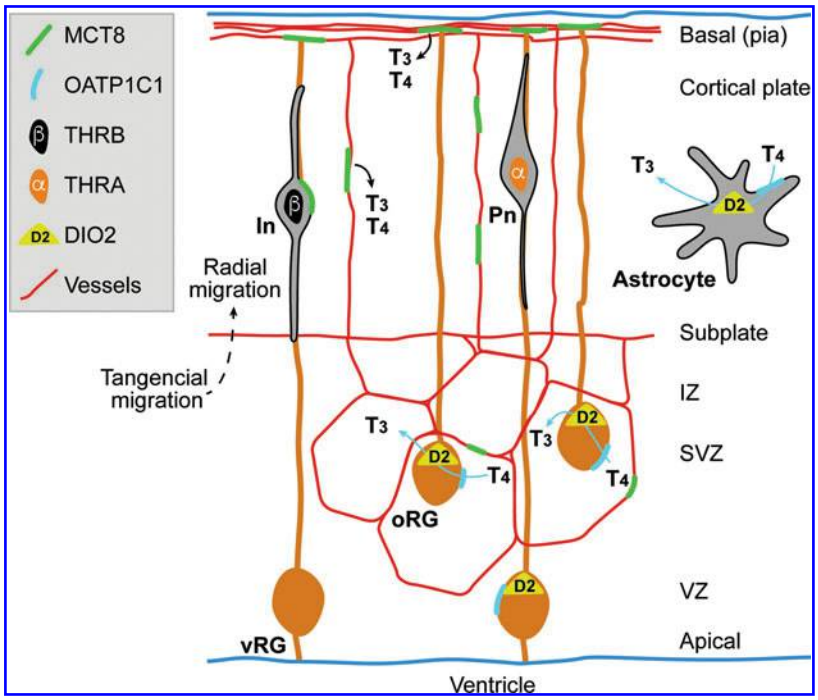

FIG. 9. Cartoon representing a highly simplified view on the cytoarchitecture of thyroid hormone effectors in the developing human neocortex. CP, SP, IZ, SVZ, VZ, In, Pn, vRG, with the apical process in contact with the ventricular surface and the basal process reaching the pial layer; oRG lacking the apical process. The RG cells are the universal stem cells of the cerebral cortex and in primates are located in two niches: the vRG with apical and basal processes, in the VZ, and the oRG with only a basal process, in the outer SVZ. The neurogenic potential of the oRG is responsible for the enlargement of the neocortex in primates. Shown is a *THRB*-expressing interneuron originating in the caudal ganglionic eminence, reaching the cortex by tangential migration through the IZ/SVZ and then by radial migration to the specific cortical position. Also shown is a radially migrating *THRA*-expressing excitatory neuron. The oRG and some vRG express *SLCO1C1* (OATP1C1) and *DIO2*. Neurons express *SLC16A2* (MCT8). T4 enters the cortex through MCT8 located in the vascular endothelial cells and reaches glial cells expressing OATP1C1 and DIO2, with the potential to generate local T3. The vessels run vertically through the cortical plate and form a honeycomb structure in the IZ and SVZ, according to Komabayashi-Suzuki *et al.* (60). CP, cortical plate; In, interneuron; IZ, intermediate zone; MCT8, monocarboxylate transporter 8; Pn, projection neuron; RG, radial glial; SP, subplate; SVZ, subventricular zone; T3, triiodothyronine; T4, thyroxine; VZ, ventricular zone.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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