Association between the thickness of transient fetal cortical compartments and gene expression

*Lana Vasung[1], *Chenying Zhao[2], Jennings Zhang[1], Hyuk Jin Yun[1], Caitlin Rollins[1], Clemente Velasco-Annis[1], Kiho Im[1], Simon K Warfield[1], P Ellen Grant[1], Ali Gholipour[1], Hao Huang[2]

***Co-first authors**

[1] Boston Children's Hospital/Harvard Medical School, Boston, United States, [2] Children's Hospital of Philadelphia, Philadelphia, PA

Introduction

- During prenatal development, the human cortex is composed of three cortical compartments (**cortical plate [CP], subplate [SP**], and marginal zone [MZ]) that undergo substantial reorganizational changes (Kostović et al. 2014). The changes of the CP and SP can be identified on both T1-weighted and T2-weighted (T2w) MRI by measuring their regional thickness and volume (Vasung et al. 2016, 2019).
- The **aim of our work** was to identify genes whose expression levels are associated with changes in CP and/or SP thickness.

Material and Methods

MRI acquisition and processing

In-vivo MRIs of 25 healthy fetuses (age range 20.71-32.14 gestational weeks [GW]) were acquired on a 3T Siemens Skyra MRI scanner using multiple T2w Half-Fourier Single Shot Turbo Spin Echo scans (TR=1400-2000ms, TE=100-120ms, voxel size=0.9x1.1x2mm3).
MRIs were preprocessed with an in-house built pipeline (Gholipour et al. 2017) composed of the following steps:

(i) motion correction and super-resolution 3D reconstruction,
(ii) brain masking,
(iii) N4 bias field correction with intensity normalization,
(iv) rigid registration, and
(v) initial segmentation.

We manually refined the segmented CP, SP, and cerebrospinal fluid (CSF) according to anatomical guidelines (Vasung et al. 2019). Next, the inner and outer surfaces of CP and SP were extracted, with minor modifications of the parameters, using the CLASP algorithm (Vasung et al. 2016).
The **thickness of the compartments** was measured by taking the absolute distance between the corresponding vertices of its inner and outer surface (Boucher et al. 2009). On the reconstructed surfaces, we manually annotated vertices [ROIs] that belonged to 12 cortical regions described in the Developmental Transcriptome repository ((Kang et al. 2011), see Figure 1).
The mean thickness of CP and SP of each annotated ROI was calculated for each brain.

Tissue mRNA sequencing and transcriptome level measuring

Tissue-level mRNA sequencing was conducted on high-quality tissue from three post-mortem human brain samples aged 23-25 GW (Kang et al. 2011). Normalized mRNA level, RPKM (Reads Per Kilobase of transcript per Million mapped reads) from a total of 60155 genes, was measured for twelve cortical regions and averaged across brains.

Correlation between transcriptome, CP, and SP thickness

For each gene, Pearson's correlation between regional CP or SP thickness [across all gestational weeks] and log of mRNA level (RPKM) was calculated.

Results

Spatio-temporal analysis of CP and SP thickness in 12 cortical regions revealed a significant increase in the thickness of CP and SP from 20.71 to 32.14 GW (**Figure 1**).
Next, for each region, we calculated the mean CP and SP thickness across gestational age (**Figure 2A**).
As expected, the regions with the thickest SP were primary sensory areas (**Figure 2A**). Finally, the mean CP and SP thickness of all regions across all ages (**Figure 2A**) were correlated with the gene expression levels. Six genes showed a significant positive correlation with SP thickness after Bonferroni correction (**Figure 2B**), while none of the genes were significantly related to the CP thickness (**Figure 2C**).

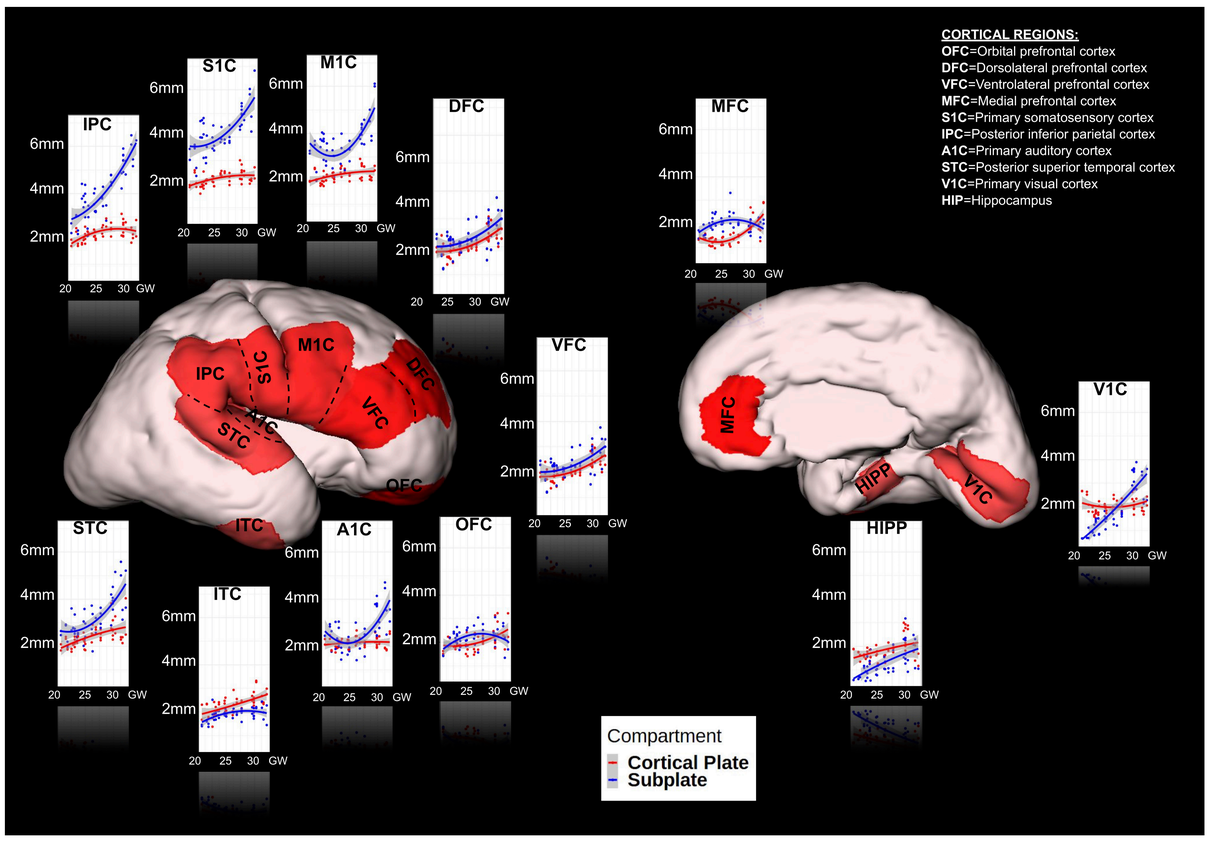

Figure 1. Mean CP (in red) and SP thickness (in blue) in12 cortical regions.

The majority of the genes that were significantly associated with SP thickness were pseudogenes: ATPase H+ transporting V0 subunit e1 (ATP6V0E1P2), RNA U6 Small Nuclear 1301 (RNU6-1301P), ribosomal protein L21 (RP11-690D19.1 and RPL21P9), and zinc finger protein 562 (CTD-3116E22.6).
Pseudogenes have been suggested to play a role in the regulation of gene expression and the generation of genetic diversity (Balakirev and Ayala 2003).

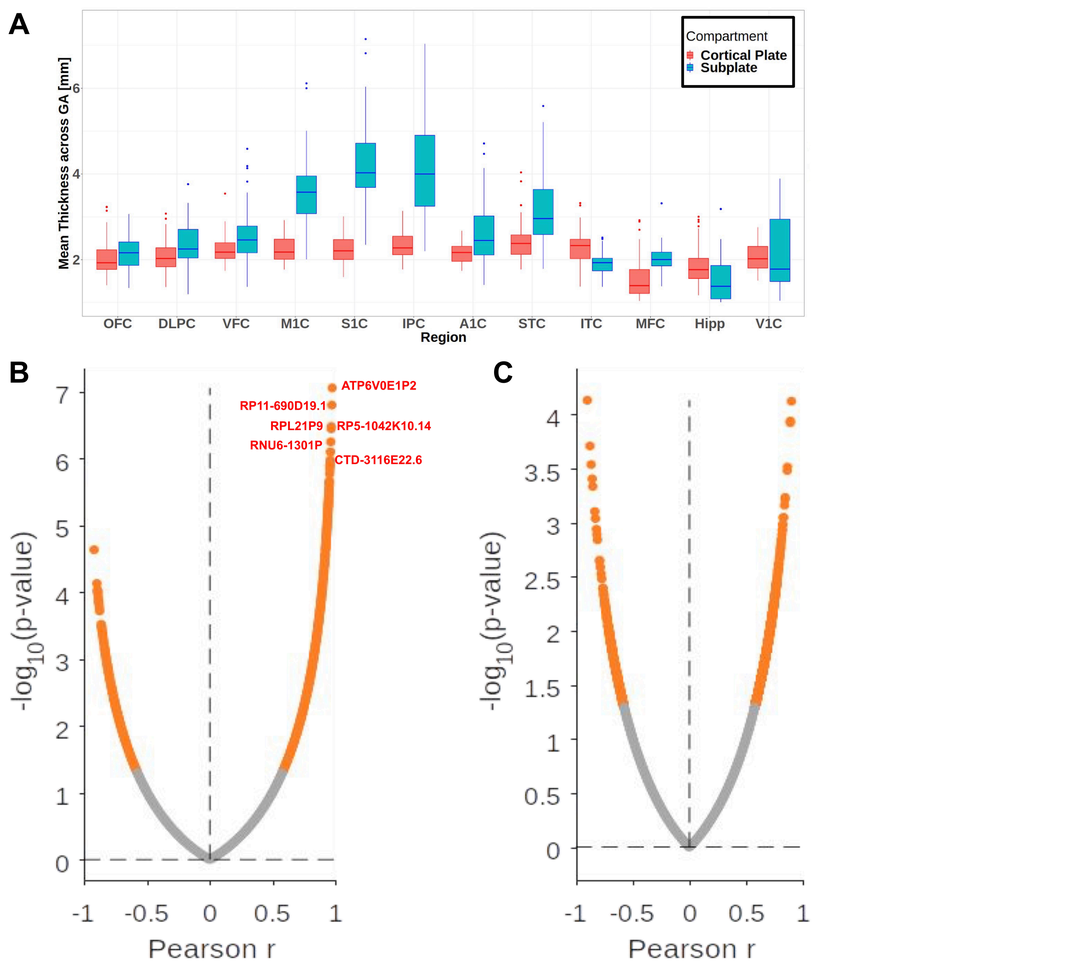

Figure 2A. Mean CP (in red) and SP thickness (in blue) across 12 cortical regions.
Figure 2B and 2C. Association between thickness of transient cones (SP left, CP right) and regional transcription level (significant genes in red).

Conclusion

In conclusion, our results suggest that there might be a link between the level of expression of certain pseudogenes and the thickness of the SP. Whether the SP regulation of cortical development and plasticity (Kanold 2009) is achieved via these pseudogenes remains to be determined in animal models.

Balakirev ES, Ayala FJ (2003) Pseudogenes: Are They "Junk" or Functional DNA? Annu Rev Genet 37:123–151 Boucher M, Whitesides S, Evans A (2009) Depth potential function for folding pattern representation, registration and analysis. Med Image Anal 13:203–214 Gholipour A, Rollins CK, Velasco-Annis C, et al (2017) A normative spatiotemporal MRI atlas of the fetal brain for automatic segmentation and analysis of early brain growth. Sci Rep 7:476 Kang HJ, Kawasawa YI, Cheng F, et al (2011) Spatio-temporal transcriptome of the human brain. Nature 478:483–489 Kanold PO (2009) Subplate neurons: crucial regulators of cortical development and plasticity. Front Neuroanat 3:16 Kostović I, Jovanov-Milošević N, Radoš M, et al (2014) Perinatal and early postnatal reorganization of the subplate and related cellular compartments in the human cerebral wall as revealed by histological and MRI approaches. Brain Struct Funct 219:231–253 Vasung L, Lepage C, Radoš M, et al (2016) Quantitative and Qualitative Analysis of Transient Fetal Compartments during Prenatal Human Brain Development. Front Neuroanat 10:11 Vasung L, Rollins CK, Yun HJ, et al (2019) Quantitative In vivo MRI Assessment of Structural Asymmetries and Sexual Dimorphism of Transient Fetal Compartments in the Human Brain. Cereb Cortex. https://doi.org/10.1093/cercor/bhz200