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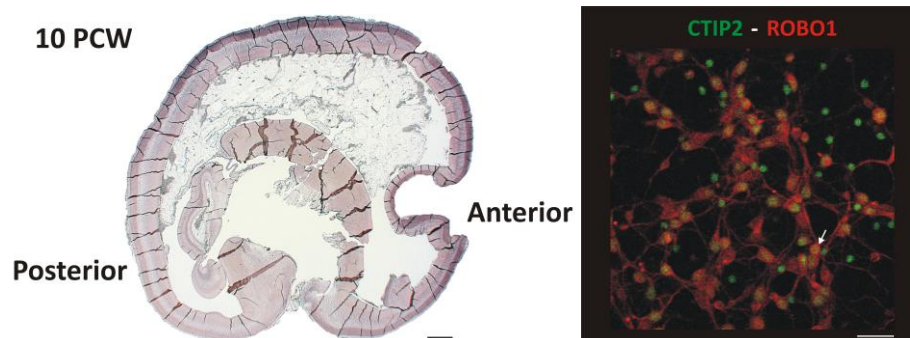
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## **Development of Functional Regionalisation in the Human Neocortex**

There is a stereotyped division of the cerebral cortex into functional domains. As there is both graded and regionalized gene expression from the earliest stages of rodent cortical development, we have used Affymetrix gene chip analysis of human foetal brain tissue from 8-12.5 post-conceptual weeks (PCW), obtained from the MRC-Wellcome Trust Human Developmental Biology Resource (<http://www.hdbr.org>), to discover genes expressed in gradients along the anterior-posterior axis of the human neocortex, and confirmed these findings using real-time PCR and in situ hybridisation for a subset of these genes.

We have investigated further Layer V corticofugal neuron-associated and anteriorly upregulated genes (the transcription factor CTIP2 and the axon guidance molecule ROBO1 and its downstream signaling molecule SRGAP1) and compared their expression patterns, using tissue in-situ hybridization and immunohistochemistry, with other laminar specific transcription factors (ER81, FEZF2, SATB2: Layer V; NURR1, SOX5: Layer VI/subplate) with no or opposite expression gradients. It was shown Layer V forms as early as 12 PCW in humans. Furthermore, prominent medullary pyramids were strongly immunoreactive for both ROBO1/SRGAP1 by 14-17 PCW, as corticospinal fibres extended to the decussation and beyond, around the time when a distinct ROBO1/SRGAP1-positive Layer V emerges in the neocortex. We propose that co-expression of these three genes in the anterior neocortex marks the early location of the human motor cortex, allowing further study of its early differentiation.

In order to study regulation of regionalization genes, we have established dissociated human cell cultures from anteriorly or posteriorly derived fetal neocortex that exhibits similar gradients of gene expression. We are stimulating FGF signaling pathways and examining changes in expression levels of the anteriorly upregulated corticofugal neuron-associated genes.



Left - CTIP2 gradients in sections from the developing human neocortex. Tissue in situ hybridization of sagittal section of human fetal brain for CTIP2 revealed that it was expressed at high levels anteriorly in comparison to the posterior region of the neocortex at 10 PCW. Scale bar represent 500  $\mu\text{m}$ .

Right - Co-localization of CTIP2 and ROBO1 in cells derived from human fetal brain culture. Immunocytochemistry of cells isolated and cultured from the anterior region of the human fetal neocortex. Image was captured by confocal microscopy and showed that cells expressing CTIP2 were in green, ROBO1 in red and both in yellow (arrow). Scale bar represents 200  $\mu\text{m}$ .

## ***Publications and Presentations resulting from this studentship:***

### *Journal Publications*

Ip BK, Wappler I, Peters H, Lindsay S, Clowry GJ, Bayatti N. (2010) Investigating gradients of gene expression involved in early human cortical development. *Journal of Anatomy* 217; 4: 300–311.

Ip BK, Bayatti N, Howard NJ, Lindsay S, Clowry GJ. (2010) The Corticofugal Neuron-Associated Genes ROBO1, SRGAP1, and CTIP2 Exhibit an Anterior to Posterior Gradient of Expression in Early Fetal Human Neocortex Development. *Cerebral Cortex*, in press.

Wang WZ, Hoerder-Suabedissen A, Oeschger FM, Bayatti N, Ip BK, Lindsay S, Supramaniam V, Srinivasan L, Rutherford M, Møllgård K, Clowry GJ, Molnár Z. (2010) Subplate in the developing cortex of mouse and human. *Journal of Anatomy* 217; 4: 368–380.

### *Published Abstract*

Clowry GJ, Bayatti N, Ip BK, Lindsay S. (2008) Gene expression studies of the development of functional regionalization in the human neocortex. Program No. 718.14 Abstract Viewer and Itinerary Planner. Washington, DC: Society for Neuroscience. Online

Ip BK, Bayatti N, Lindsay S, Clowry GJ. (2009) Development of functional regionalization in the human neocortex: preliminary findings of SLIT/ROBO/srGAP differential expression. *J Anatomy* 214:794-795.

Ip BK, Bayatti N, Howard N, Lindsay S, Clowry GJ. (2009) Expression of ROBO1/SRGAP1 and CTIP2 suggests that the motor cortex initially forms at the anterior pole during development of the human foetal neocortex, *Mechanisms of Development* 126:S82-S83.

Ip BK, Bayatti N, Howard NJ, Clowry GJ, Lindsay S. (2009) Localization of Expression of Motor Cortex Markers ROBO1/SRGAP1 and CTIP2 in the Early Foetal Human Neocortex. *British Neurosci. Assoc. Abstr.*, Vol 20, P52, Abstract 3.02.

Ip BK, Bayatti N, Howard NJ, Lindsay S, Clowry GJ. (2010) Graded expression patterns of proteins ROBO1, SRGAP1 and CTIP2 show the corticospinal tract arises from the anterior pole of the neocortex during early fetal human development. *FENS Abstr.* 127.14

### *Meetings Attended*

08-09/04/2008 North East Neuroscience and Chris Thompson Memorial Symposium

02-04/07/2008 The ASGBI 2008 Summer Meeting

19-22/04/2009 British Neuroscience Association 20<sup>th</sup> National Meeting

09-12/06/2009 16<sup>th</sup> MGC PhD Workshop

05-07/01/2010 The ASGBI 2010 Winter Meeting

03-07/07/2010 The 7<sup>th</sup> FENS Forum of European Neuroscience Meeting