A Transcriptomic Atlas of Mouse Neocortical Layers

http://genserv.anat.ox.ac.uk/layers

Cell type-specific expression: Results

489 and 120 genes were predicted to be preferentially expressed in specific layers' neurons or in astrocytes, respectively. These predictions arose from the application of two additional sets of classifiers that were trained using sets of neuron- or astrocyte-specific genes (Cahoy et al., 2008). Their classification was only possible because they showed differing patterns of expression across samples (Online Supplementary Figure 6). By contrast, oligodendrocyte-specific genes could not be predicted as they were not generally found outside lower layers and there was no expression evidence of laminar subtypes of oligodendrocytes (Online Supplementary Figure 6). This confirms earlier observations that oligodendrocytes are rare in the neocortex, except in the deepest layers (Tan et al., 2009). We provide lists of layer-enriched neuron- and astrocyte-specific genes in Online Supplementary Tables 7 and 8 respectively.

Cell type-specific expression: Methods

All probe set IDs that were found (Cahoy et al., 2008) to be at least 4-fold enriched in neurons (932) were mapped onto mouse Ensembl IDs using the MGI batch query (April 26, 2010), yielding 745 unique Ensembl gene IDs (640 classifiable). All probe set IDs that were similarly at least 4-fold enriched in astrocytes (630) were mapped onto mouse Ensembl IDs using the MGI batch query (April 26, 2010), yielding 551 unique Ensembl gene IDs (356 classifiable). Classifiers were trained and tested

separately for each of these sets as was previously done for the complete set of classifiable genes.

Imprinting: Results

Imprinted genes, expressed in a controlled parent-of-origin manner, frequently act in the brain and influence behavior (Dulac). Approximately half of 34 maternally imprinted and 88 paternally imprinted protein-coding genes identified by a previous study (Gregg et al.) were patterned across layers (Online Supplementary Table 13). For example, expression of *Gabra5*, a paternally imprinted gene in mouse known also to be paternally imprinted among individuals with Prader-Willi and Angelman syndromes (Hogart et al., 2007) was enriched in layers 5 and 6b, which was confirmed by *in situ* hybridization curations (Lein et al., 2007).

We identified 80 lincRNAs expressed from genomic regions with parentally biased expression in adult mouse medial prefrontal cortex. We found direct evidence for imprinting (allele-specific expression within the locus) in cortex for 37 of these (16 maternal, 21 paternal) and in embryonic day 15 brain for 52 transcripts (28 maternal, 24 paternal) (Online Supplementary Table 14). These overlapped known imprinted loci such as *Rian*, *Miat* and *BC070450*. Only more highly expressed genes could be called as imprinted, enriching for previously known genes. Unpatterned or patterned lincRNAs across the layers were more likely to be imprinted in the mouse medial prefrontal cortex than in the embryonic brain. Identification of these 37 cortically imprinted lincRNAs, and 43 further candidate imprinted lincRNAs, now opens for investigation their functional roles in the cortex.

Imprinting: Methods

Genes having consensus maternally- or paternally- biased expression in medial prefrontal cortex (mPFC) were extracted from Supplementary Table 2 of Gregg et al. and translated with the MGI batch query followed by manual curation, resulting in 38 maternally- and 96 paternally-imprinted expressed known genes in our set. Of the imprinted genes, 34 maternally biased and 88 paternally biased genes were classifiable across layers. We did not find a significant bias favoring either parent in any layer.

To find novel imprinted lincRNAs to expand upon the ncRNA set previously reported (Gregg et al.), we first identified lincRNA transcripts overlapping imprinted clusters reported in Supplementary Table 5 of Gregg et al. We then extracted the counts of RNA sequence reads supporting each allele when mapped to mm9 (using only the autosomal chromosomes) from adult mPFC of the initial and reciprocal crosses given in GEO accession number GSE22131. Where there were at least ten reads per SNP and there was either a paternal or a maternal bias >50% in both crosses, significant differences in allelic expression were assessed using Fisher's exact test and a cutoff *p*-value of 0.05, which for these data yields a false discovery rate of 0.1 in mPFC (Gregg et al.). LincRNA transcripts that overlapped (from end-to-end, to include hnRNA) these significantly imprinted maternally- or paternally-imprinted SNPs were classified as being imprinted.

References

Cahoy, J.D., Emery, B., Kaushal, A., Foo, L.C., Zamanian, J.L., Christopherson, K.S., Xing, Y., Lubischer, J.L., Krieg, P.A., Krupenko, S.A., *et al.* (2008). A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci *28*, 264-278.
Dulac, C. Brain function and chromatin plasticity. Nature *465*, 728-735.
Gregg, C., Zhang, J., Weissbourd, B., Luo, S., Schroth, G.P., Haig, D., and Dulac, C. High-resolution analysis of parent-of-origin allelic expression in the mouse brain. Science *329*, 643-648.

Hogart, A., Nagarajan, R.P., Patzel, K.A., Yasui, D.H., and Lasalle, J.M. (2007). 15q11-13 GABAA receptor genes are normally biallelically expressed in brain yet are subject to epigenetic dysregulation in autism-spectrum disorders. Hum Mol Genet *16*, 691-703.
Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., *et al.* (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature *445*, 168-176.
Tan, S.S., Kalloniatis, M., Truong, H.T., Binder, M.D., Cate, H.S., Kilpatrick, T.J., and Hammond, V.E. (2009). Oligodendrocyte positioning in cerebral cortex is independent of projection neuron layering. Glia *57*, 1024-1030.