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In this issue of Neuron, Johnson et al. employ a unique whole-genome exon-level analysis of the developing human brain showing that 76% of genes are expressed along with unexpectedly high levels of differential expression. These results have important consequences for understanding normal and pathological function and provide implications about the uniqueness of being human.

The human brain is beyond doubt the most sophisticated computational machine known to man, about whose self-construction or function we know tantalizingly little. The developmental study from Sestan and his colleagues makes a major contribution to our knowledge of the former area with far-reaching implications for the second (Johnson et al., 2009 [this issue of Neuron]). They report on the analysis of whole-genome exon-level expression of 13 regions in the midgestation human brain. This technique allows the identification of alternative splicing, which concerns 75% of the human multiexon genes. This is an important advance because alternative splicing is an established mechanism for gene diversification that can generate multiple proteins, and it is known to have important roles in normal and pathological brain function.

Rodents are the most widely used model for the investigation of brain development. However, alongside a number of core mechanisms that are conserved between rodents and primates, there are major differences in the nature and timing of ontogenetic processes characterizing primate corticogenesis (Dehay and Kennedy, 2007). Studies of human brain development, combined with interspecies comparisons, are therefore much needed in order to progress in understanding how the highly developed cortical areas in humans have acquired the capacity to support the rich repertoire of complex cognition and behaviors characteristic of our species. Because the Exon Array platform provides unparalleled resolution in its coverage of the genome (it reveals the prevalence and importance of alternative splicing and other fine transcriptional regulation), the work of Sestan and his colleagues opens the exciting possibility of better nailing down the evolutionary and developmental mechanisms that underlie unique human cognitive abilities such as language, abstract thinking, and creativity.

One key aim in the field of developmental neurobiology is to unravel the genetic mechanisms that underlie the specification of the identity of cortical areas. Because of the sheer resolution power of the Exon Array technology combined with an astute experimental design, this work represents a step forward in the search for the Holy Grail of cortical specification. The study compares gene expression in nine cortical regions. Four belong to the prefrontal cortex: orbito prefrontal cortex (OPFC), dorsolateral prefrontal cortex (DLPFC), medial prefrontal cortex (MPFC), and ventrolateral prefrontal cortex (VLPFC), and five regions are nonfrontal areas, including motor-somatosensory cortex, parietal association cortex, temporal association cortex, occipital visual cortex, and auditory cortex. The study is the first genome-wide-scale genomic approach of the human developing cortex. It reveals that over 76% of human genes are expressed in at least one brain region. Out of these 76%, 44% are differentially regulated and 28% are differentially alternatively spliced. Differentially expressed genes were more frequently associated with human-specific evolution of putative

cis-regulatory elements. By showing that hundreds of genes are differentially expressed or alternatively spliced within the fetal human frontal cortex, this work uncovers for the first time a large number of transcriptional differences between functionally distinct prefrontal areas. This study confirms previous findings concerning the regional enrichment of certain genes identified via other methods (PCDH17, CNTNAP2, EPHA3, EPHA7). More importantly, the vast majority of their data reveal so far unidentified complex expression patterns, which indicates a large number of candidate genes—regionally enriched or alternatively spliced genes not previously identified as such—to be explored.

The clustering results reveal a clear molecular distinction between the four frontal areas and the four non-frontal regions. It is noteworthy that the motor-sensory region shows a correlation with both frontal and nonfrontal regions, in accordance with its mixed frontal/parietal location. Besides showing genetic differences between functionally distinct prefrontal regions, this work also reveals two sets of data related to language. First, VLPFC (which includes presumptive Broca's area) was found to be more molecularly related to the motor-somatosensory area than to other prefrontal areas and FOXP2 is enriched in VLPFC and motor-somatosensory cortex. This observation would go along with the hypothesis that the FOXP2 phenotype is more related to the development and function of connections involved in sensory integration and vocal motor learning than to language per se (Varki et al., 2008). Second, a fascinating finding is that the perisylvian areas share molecular features, possibly correlated to their involvement in speech and language, in spite of being located across lobes (frontal, parietal, and temporal). This distributed network contains molecular signatures that include both known and unknown genes and does so where FoxP2 shows only a modest enrichment. Interestingly, a high proportion (20%) of the genes enriched in perisylvian regions were associated with human-specific accelerated evolution, suggesting that accelerated evolution of putative enhancers characterizes a fraction of genes showing specific expression patterns in the developing brain.

Of course, differential gene expression analysis alone fails to capture the functional role of changes in gene expression; for example, it does not tell you if regional changes in gene expression are neutral or adaptive. An alternative approach is to look at the position of a given gene in the context of the network in which it is embedded. Measurement of coexpression relationships reveals gene expression networks, where genes that cluster together define modules of functionally related genes. The topological properties of these networks are described as being scale free, which means that their degree distribution follows a power law. Scale-free networks are found in numerous complex systems ranging from the world wide web to some social networks, and have been fully characterized mathematically. The characteristic feature of scale-free gene networks is that the nodes (genes) can have widely different numbers of links to other genes. Genes that are highly connected constitute hubs and these genes ensure minimal path lengths in the network and therefore may play a particular role in the biological properties of the network. This approach revealed a number of region-specific modules, and indicated hub genes that could be of particular functional significance. For instance in the cortex modules hub genes included ZIC2 and ZIC4 (crucial in midline patterning of the dorsal forebrain), LRRC7 (a postsynaptic protein involved in dendritic morphology), and FOXG1 (linked to Ret syndrome).

This study reports an order of magnitude increase in the numbers of differentially expressed genes, compared to studies in the developing rodent (Muhlfriedel et al., 2007). Alternative splicing is one potentially important mechanism for creation of new proteins during evolution. This strategy is common in the CNS where neurons are found to be rich in

regulated alternative splicing events (Grabowski, 1998). Much will probably be gained from a detailed comparison of alternative splicing between human and nonhuman primates, which has yet to be performed. Identifying human-specific alternative splice forms in the brain is important for understanding the mechanism of functional evolution and emergence of cognition in the human lineage. For instance, the work on neuropsin, involved in learning and memory, has revealed the existence of a longer spliced form that is only expressed in humans (Lu et al., 2007). Certain unique human cognitive traits, such as language, mathematical, and artistic capabilities, as well as planning, are assigned to specific areas of the cerebral cortex. Progress in the understanding of how genome evolution correlates with the human phenotype including the dimensions and the interconnections of these association areas is probably key to unraveling the mechanisms that underlie the unique repertoire of flexibility and adaptation that characterizes human cognition and behavior (Varki et al., 2008). The study by Sestan and colleagues reports evidence suggesting that transcriptional regulation in humans leads to increases in molecular specification of brain regions including cortical areas, in support of King and Wilson's suggestion of regulatory evolution (King and Wilson, 1975). The authors speculate that this could lead to the emergence of novel phenotypic traits. In this respect comparison of the developmental gene expression of a well-defined cortical area, such as the primary visual cortex of monkey and human, could be highly instructive given the in-depth understanding of the neurobiology of the visual cortex and the close similarity of the psychophysical visual function across primates. If it turns out that there are major differences between monkey and human in gene expression during the development of visual cortex, then this could indicate the human phenotype of a primary visual cortex more specialized in those attributes (e.g. perceptual learning, attention, visual imagery, memory, and associative memory) that go beyond the physiology underlying visual perception per se and could be hugely important in determining the cultural uniqueness of humans.

Noticeably, these results obtained at the midgestation stage, when key processes in the establishment of connections occur, stand in contrast with molecular studies in adult human brain, which reveal only modest differences in gene expression between cortical areas (Khaitovich et al., 2004). Furthermore, studies on adult brains point to a high conservation of general expression patterns, between for instance mouse and human (Strand et al., 2007). Taken together with the present results, this suggests that it is the gene expression during development that largely determines higher brain functions by specifying the complexity of neural connections. Numerically, the most important genes relating to cognitive differences between species may be genes that specify how the machinery is put together. In support of this hypothesis, many of the identified differentially expressed genes in this study are related to processes involved in connection formation, such as axonal guidance and cell adhesion.

Because cortical networks and microcircuits provide the computational architecture that mediates information processing and cognitive functions, these findings speak to ideas on the structure but also those on the function of the cortex in the adult. A widely held view of the organization of the cerebral cortex is of a lattice-like structure with a repeated canonical microcircuitry, suggesting that it is the differences in the subcortical input to different cortical regions that largely determines their functionality (Douglas and Martin, 2004). However, the extensive differential regional expression of genes shown in the present study would seem to be at odds with a strong theory of homogeneous cortical structure. Further, a number of studies point to the possibility of fine-grain structural differences between cortical areas. For instance at the single-neuron level, connections have been shown to be very specific, and local cortical circuits show highly nonrandom features that form a skeleton of strong connections in a sea of weak connections (Song et al., 2005). The activity of the strong

connections, embedded in the network of weak connections, determines the area-specific information processing properties of the cortex. Because the highly influential, strong synaptic connections are numerically few, it is hypothesized that their exact connectivity pattern and properties might therefore be of crucial importance in specifying the functional properties of the circuits. It would be reasonable to think that these structural features of the local microcircuitry, determined with single-cell precision, will be fine-tuned by the genetic properties of precursor cells. This is supported by recent results showing that the functional columnar microarchitecture in the mature neocortex is made of specific microcircuits linking preferentially excitatory neurons within ontogenetic radial clones (Yu et al., 2009).

In the current context of paucity of published genome-wide expression data from developing human, this data set represents an invaluable resource and unrivalled goldmine of information. Accessibility of this important and unique resource by other investigators will be a key factor in progressing in the endeavor to understand the molecular and evolutionary mechanisms underlying human brain development and the emergence of our most highly advanced cognitive abilities. Future efforts should be directed toward the generation of comparable data sets from additional developmental stages of the human brain, and importantly from other species.

Complementary to interspecies investigations, an interesting extension of the approach of Sestan and colleagues would be to examine the gene expression between the different compartments of the embryonic brain so as to better understand developmental mechanisms underlying the production, migration, and differentiation of neurons. We shall illustrate this with respect to proliferation. The hallmark of human and nonhuman primate cortex is the selective enlargement of the supragranular layer compartment that is considered to underlie the highly developed computational abilities of the human brain. The supragranular layer neurons of the primate are produced by a specialized germinal zone (the outer subventricular zone) that does not have a counterpart in the rodent brain (Lukaszewicz et al., 2005). Remarkably, this primate-specific specialized germinal zone expresses a transcription factor Pax6 (Fish et al., 2008) that in the rodent is restricted to the primary germinal zone (the ventricular zone). The unique cell cycle kinetics of the outer subventricular zone are known to underlie the cytoarchitecture of the cortex, so bringing the approach developed by Sestan and colleagues to the different germinal zones will elucidate the gene networks underlying the cellular mechanisms that generate a unique feature of primate corticogenesis (Rakic et al., 2009).

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