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Left–Right Asymmetry of Maturation Rates in Human Embryonic Neural Development

Carolien G.F. de Kovel, Steven Lisgo, Guy Karlebach, Jia Ju, Gang Cheng, Simon E. Fisher, and Clyde Francks

ABSTRACT

BACKGROUND: Left–right asymmetry is a fundamental organizing feature of the human brain, and neuropsychiatric disorders such as schizophrenia sometimes involve alterations of brain asymmetry. As early as 8 weeks postconception, the majority of human fetuses move their right arms more than their left arms, but because nerve fiber tracts are still descending from the forebrain at this stage, spinal–muscular asymmetries are likely to play an important developmental role.

METHODS: We used RNA sequencing to measure gene expression levels in the left and right spinal cords, and the left and right hindbrains, of 18 postmortem human embryos aged 4 to 8 weeks postconception. Genes showing embryonic lateralization were tested for an enrichment of signals in genome-wide association data for schizophrenia.

RESULTS: The left side of the embryonic spinal cord was found to mature faster than the right side. Both sides transitioned from transcriptional profiles associated with cell division and proliferation at earlier stages to neuronal differentiation and function at later stages, but the two sides were not in synchrony (p = 2.2 E-161). The hindbrain showed a left–right mirrored pattern compared with the spinal cord, consistent with the well-known crossing over of function between these two structures. Genes that showed lateralization in the embryonic spinal cord were enriched for association signals with schizophrenia (p = 4.3 E-05).

CONCLUSIONS: These are the earliest stage left–right differences of human neural development ever reported. Disruption of the lateralized developmental program may play a role in the genetic susceptibility to schizophrenia.

Keywords: Development, Gene expression, Hindbrain, Lateralization, Maturation rate, Schizophrenia, Spinal cord

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Many human cognitive processes are partially lateralized toward either the left or right side of the brain (1). Identifying genes involved in brain asymmetry is important because it can shed light on developmental processes that fine-tune neural circuits for their high-level functions (2,3) and because various psychiatric and cognitive disorders can involve alterations of brain asymmetries (4,5). At 8 weeks postconception, or roughly embryonic Carnegie stage (CS) 23, 85% of fetuses move their right arms more than their left arms, a proportion strikingly similar to the adult rate of right handedness (6). Furthermore, fetal hand motor asymmetry at 13 weeks postconception predicts handedness at age 12 years (7). Left–right anatomical differences of the cerebral hemispheres have been detected by ultrasound scanning as early as 18 weeks postconception (8). In infants, the right hemisphere has been reported to develop functionally and anatomically earlier than the left hemisphere in various respects, including that left hemisphere language-related brain regions develop more slowly than their contralateral counterparts (9–11). These observations indicate a broadly lateralized program of motor and cognitive development that begins in utero in our species and suggest that a general model can involve different rates of maturation on the two sides of the central nervous system (CNS).

Gene expression profiling from the left and right cerebral cortices in postmortem human fetuses has revealed little, or only tentative, evidence for laterality of messenger RNA (mRNA) expression in utero (12–15). One study, however, suggested that in 12- to 17-week-old fetuses the right cerebral cortex matures faster than the left one in terms of gene expression profiles (16). However, the corticospinal tract, which descends from the motor and somatosensory cerebral cortices to the spinal cord, only reaches the point of left–right crossover in the inferior hindbrain at 8 weeks postconception —the age of observed lateralization of arm movements—but does not yet extend into the spinal cord (17). Thus, lateralization of motor behavior at this stage is unlikely to reflect top-down asymmetry projected from the cerebral cortex. Instead, a bottom-up model is that molecular asymmetries already arise in the developing spinal cord prior to 8 weeks postconception. Developing vertebrate spinal cords show spontaneous electrophysiological activity that is affected by the formation of chemical synaptic networks (18), such that any left–right molecular differences might be reflected in patterns of spontaneous limb movements. In this study, we used RNA sequencing to measure gene expression profiles in the left and right spinal cords and hindbrains of human embryos aged

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4 to 8 weeks postconception to gain new insights into left–right differentiation of the human CNS within the earliest period of development yet analyzed in this way.

People with schizophrenia have an elevated rate of left handedness (19) and have shown alterations of various aspects of brain asymmetry in some, but not all, studies (20–26). Recent genome-wide association analysis in tens of thousands of participants has implicated individual genes and genetic networks involved in embryonic and fetal brain development in susceptibility to schizophrenia, bipolar disorder, and depression (27–29). Studies of patient-derived neural progenitor cells have also indicated disruptions of early developmental processes in schizophrenia (30). Therefore, we hypothesized that genes showing early developmental lateralization in the spinal cord or hindbrain might also show an enrichment of association signals within genome-wide association study (GWAS) results for schizophrenia. Although spinal cord abnormalities are not a known feature of schizophrenia, if subtle embryonic asymmetries within the spinal cord are important for patterning broader asymmetries of the brain later in development, then it is possible that disruptions of this early program might have consequences for disorder susceptibility.

METHODS AND MATERIALS

Differential Expression Analysis

RNA sequencing was performed separately for left and right spinal cords, and for left and right hindbrains, dissected from 18 postmortem human embryos aged 4 to 8 weeks postconception at the times of their terminations (CS13 to CS23; Supplemental Table S1), which had been donated to the United Kingdom's Human Developmental Biology Resource following voluntary medical abortions. Details of sample collection, dissection, preparation, RNA sequencing, and data quality control can be found in the Supplement. In RStudio (version 99.9.9; Boston, MA), gene mRNA expression data were normalized and transformed into log2 (counts per million [cpm]). Genes were filtered to retain only those for which at least three libraries had at least five reads per gene, separately for each brain structure. Linear models with observational-level weights were fitted to obtain average expression values for each gene on each side (left or right), separately for spinal cord and hindbrain, and moderated *t* statistics were used to assess differential expression between sides using the Bioconductor limma package (version 3.22.7; Bioconductor, Roswell Park Cancer Institute, Buffalo, NY; limma package, Walter & Eliza Hall Institute for Medical Research, Melbourne, Australia) with *voom* (31). This design allowed paired analysis of the left and right samples from the same embryo for each tissue. In multidimensional scaling analysis of the gene expression data, no dimensions were found to relate to collection batch or time from clinic until freezing, and the number of pairs of samples was relatively low to support the inclusion of covariate effects (beyond the individual-level effect that was already accounted for by paired-sample testing). Therefore, we did not consider these factors in the differential expression analysis.

Many genes showed a pattern of increasing or decreasing expression with age, as assessed across the different embryos of varying ages. A linear slope was fitted through bilateral expression as log2 (cpm) on CS for each gene. The slope coefficient of each gene was plotted against the left–right differential expression *t* value, and the Pearson correlation between the slope coefficients and *t* values was computed (SPSS version 20.0.0.1; IBM Corp., Armonk, NY).

Gene Ontology Enrichment

Gene Ontology (GO) assigns genes to approximately 4000 gene sets representing biological processes in a loosely hierarchical structure (32). To search for biological process gene sets that were enriched on the left or right side of each structure, we used the Gene Set Enrichment Analysis (GSEA) tool from the Broad Institute (build 00044, January 2016; Cambridge, MA) combined with Molecular Signatures Database gene sets (c5.bp.5.1; http://www.go2msig.org/cgi-bin/prebuilt.cgi), curated genes updated April 2015 (33,34). Analysis was performed using the preranked mode provided in the software. For this analysis, genes were ranked by lateralization *t* value from positive (higher expression on the right) to negative (higher expression on the left) *t* values. This way, positive enrichment in the GSEA results would indicate that members of a particular gene set, on average, show relatively higher expression on the right side of the tissue, and negative enrichment would indicate relatively higher expression on the left side. GSEA reports a familywise error rate, which is a conservative measure to correct for multiple testing (35). To identify gene sets that were lateralized in both spinal cord and hindbrain, we selected gene sets that had an absolute normalized enrichment score (as calculated by GSEA software) exceeding 2.0 in both tissues. To cluster the GO gene sets into larger units in order to indicate the broad types of processes implicated, REVIGO (http://revigo.irb.hr/) was used (36). See Supplement for more details.

Transcription Factor Target Enrichment

To identify transcription factors (TFs) that play important roles in lateralization of spinal cord and hindbrain, we performed enrichment analysis for TF targets in our left–right differential expression data. Gene sets, provided by Molecular Signatures Database, were characterized by having a particular target motif near their transcription start site. Gene set collection c3.tft.5.0 from Molecular Signatures Database, updated June 2015, contained 615 gene sets. Enrichment analysis was performed as described for GO gene sets above. See Supplement for more details.

Overlap With Schizophrenia GWAS Results

We downloaded the publicly available association statistics from the Psychiatric Genomics Consortium schizophrenia genome-wide association study of up to 36,989 cases and 113,075 controls (https://www.med.unc.edu/pgc/results-and-downloads; SCZ2) (29). We tested the hypothesis that genes that showed lateralized expression in the embryonic spinal cord or hindbrain were enriched for association signals with schizophrenia by running the PASCAL software (http://www2.unil.ch/cbg/index.php?title=Pascal) (35). For this, we defined one gene set comprising the 681 genes that showed lateralization (toward either the right or the left) in the embryonic