Sylvian fissure development is linked to differential genetic expression in the pre-folded brain

Arka N. Mallela, Hansen Deng, Alyssa K. Brisbin, Alan Bush & Ezequiel Goldschmidt

The mechanisms by which the human cerebral cortex folds into its final form remain poorly understood. With most of the current models and evidence addressing secondary folds, we sought to focus on the global geometry of the mature brain by studying its most distinctive feature, the Sylvian fissure. A digital human fetal brain atlas was developed using previously obtained MRI imaging of 81 healthy fetuses between gestational ages 21 and 38 weeks. To account for the development of the Sylvian fissure, we compared the growth of the frontotemporal opercula over the insular cortex and compared the transcriptome of the developing cortices for both regions. Spatiotemporal mapping of the lateral hemispheric surface showed the highest rate of organized growth in regions bordering the Sylvian fissure of the frontal, parietal and temporal lobes. Volumetric changes were first observed in the posterior aspect of the fissure moving anteriorly to the frontal lobe and laterally in the direction of the temporal pole. The insular region, delineated by the limiting insular gyri, expanded to a much lesser degree. The gene expression profile, before folding begins in the maturing brain, was significantly different in the developing opercular cortex compared to the insula. The Sylvian fissure forms by the relative overgrowth of the frontal and temporal lobes over the insula, corresponding to domains of highly expressed transcription factors involved in neuroepithelial cell differentiation.

The human cerebral cortex at birth has completed the formation of all major gyral and sulcal folds. The cerebral surface grows from a largely smooth surface beginning at the sixth month of gestation to the characteristic patterns of gyrification. A number of hypotheses have been proposed on the mechanisms of preterm brain folding. These include mechanical instability that can arise from the outer gray matter expanding at a faster rate than the underlying white matter, the axonal tension hypothesis in which white matter axons draw together overlying cortical regions to form gyri, and genetic prepatterning of the cortex to form convolutions. Postnatally, cortical expansion continues through differential local growth that extend into adulthood, without altering the new born's brain folded outline. Observations of cortical development in preterm infants have shown that maximal directional growth occurs from the central sulcus toward the parietal lobe, then toward the frontal and temporal regions. The normal process of cortical development follows a predictable sequence. The Sylvian fissure, the deepest sulcus on the lateral hemispheric surface, can be identified as early as 12 weeks of gestation and serves as a major landmark for the dynamic changes of the brain surface. Abnormal morphologic features of the Sylvian fissure can be frequently associated with neuronal migration disorders. The development of this prominent fold defines the global shape of the brain and cannot be explained by current models, which render aleatory sulci and gyri, with no distinctive and reproducible large scale structure.

The Sylvian fissure forms by the convergence of the frontal and temporal lobes over the insula, which is a distinctive and unique mechanism not shared by any other sulcus. The characteristic radial migration pathway from the ventricular and subventricular zones, that forms most of the brain gyri, appears to be absent in the insula, which cells originate from the pallial/subpallial boundary and migrate in an oblique fashion around the basal ganglia.

1Department of Neurological Surgery, University of Pittsburgh Medical Center, UPMC Presbyterian Hospital, 200 Lothrop Street, Suite B-400, Pittsburgh, PA 15213, USA. 2University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. 3Department of Neurosurgery, Massachusetts General Hospital, Boston, MA, USA. *email: goldschmidt10@upmc.edu
In the present study, our goal is to evaluate the development of the Sylvian fissure as a result of asymmetric cortical growth between the frontoparietal and temporal opercula with respect to the insular cortex. Our hypothesis is that the convergence of the frontal and temporal opercula over the insula, driven by discrete regions of high growth, are responsible for the formation of the fissure and are associated with differential genetic expression patterns.

Results
Differential cortical expansion and convergence drives the formation of the Sylvian fissure. We calculated the local expansion (growth) of each area of the brain by registering each gestational week to the next one on a week by week basis. To determine local growth, we calculated the Jacobian determinant, which represents the local volumetric change (Fig. 1). This spatiotemporal mapping of the lateral hemispheres demonstrates early growth in the frontal, temporal, and parietal opercula, closing the Sylvian fissure from gestational weeks (GW) 23–25. Our analysis demonstrates that focal areas of cortical growth (volumetric expansion) and convergent growth of the opercula close the Sylvian fissure. In later weeks (GW 27–31) differential cortical expansion drives the formation of the major cortical sulci, progressing outward from the central sulcus in the frontal and parietal lobes and inferiorly in the temporal lobe. Although comparable, the left and right hemispheres exhibited asymmetric deformations and gained volume in similar places but at slightly different times, never exceeding 2 weeks of dyscoordination.

Using the same registration described previously, we created a vector map demonstrating the magnitude and direction of local tissue displacements (Fig. 2). This analysis demonstrates the direction of tissue growth in contrast to the Jacobian analysis which demonstrates the degree of overall tissue growth. Only cortical regions exhibited high magnitude vectors whereas the telencephalic white matter rendered neutral or small deformation levels. Both the temporal and frontotemporal opercula converged over the insula, coinciding with volumetric growth described previously. Convergence of the frontal and temporal lobes over the insula led to the formation of the Sylvian fissure. Notably, no posteroventral folding or bending was observed. This was evident at every gestational age but diminished in magnitude as the gestation progressed (Fig. 2).

Figure 1. Volumetric changes on the lateral hemispheric surface during gestation. Spatiotemporal mapping of the lateral hemispheres. Blue–red color code indicates the Jacobian determinant of the week-to-week co-registration warping, a measure of local volume growth. The Jacobian determinant is normalized for global volume growth, as described in the methods. Postconceptional weeks are indicated. As can be observed, the highest rate of organized growth localizes to the opercula of insula. The first of these “hot zones” is located to the supramarginal gyrus, with the frontal and temporal poles increasing their local expansion at later times. The scale represents the weekly proportional growth (i.e. 1.1 represents a 10% weekly relative expansion). Note that week labels are gestational week (GW).
Developmental gene expression. Cortical progenitors commonly form and migrate outward from the ventricular zone by using radial glial fibers as a scaffold. As a consequence, neurons born from the same progenitor area in the ventricular zone occupy neighboring positions in the mature cortex. This is, however, not true for the insular cortex, which neurons migrate obliquely from the pallial/subpallial boundary around the basal ganglia. We therefore analyzed the transcriptomes of the maturing cortices of the areas of high growth (defined by the opercula outside the insular limiting sulci, target structure) and the insula (limited by the limiting insular sulci, contrast structure). We report the top 0.1% most overexpressed genes relative to the insula, for each cellular layer of interest: subpial granular zone (SZ), marginal zone (MZ), cortical plate (CP) and subcortical plate (SP) (Table 1).

We found extensive differences in the transcriptome for every region, which may indicate a different origin of the cells forming these structures and may drive their differing growth rates. In the subpial granular zone, the 0.1% relative highest expressed genes had a 13.7-fold (opercula/insula) expression ratio (Fig. 3A). Amongst
| Layer | OMIM ID | Gene symbol | Full name | Chr | Fold change | Function | Pathological conditions | References |
|-------|----------|-------------|-----------|-----|-------------|----------|------------------------|------------|
| SG    | 617314   | SH3YL1      | SH3 domain containing, Ysc84-like 1 | 2   | 21.843      | Hair follicle development, meiosis, cell migration, and dorsal ruffle formation | Blessing 2016, Hasegawa 2011 |
| SG    | 605012   | SUPT16H     | Suppressor of Ty 16 homolog | 14  | 20.767      | Component of the FACT (facilitates chromatin transcription) complex, a chromatin-specific factor required for transcription elongation as well as for DNA replication and repair | Belotserkovskiyay 2003 |
| SG    | 608788   | SOCS7       | Suppressor of cytokine signaling 7 | 17  | 19.992      | Expressed at high levels in the nervous system at embryonic day 12.5 and in the cortical plate at embryonic day 15.5 | Hydrocephalus in mouse model | Krebs 2004 |
| SG    | 604522   | DEFA3       | Defensin, alpha 3, neutrophil-specific | 8   | 19.385      | Anti-HIV activity by directly inactivating HIV particles | Mackiewicz 2003 |
| SG    | 609842   | EDC3        | Enhancer of mRNA decapping 3 homolog | 15  | 19.23       | Removal of the 5-prime cap from mRNA prior to its degradation from the 5-prime end | Mental retardation, autosomal rec. 50 | Feng-Gron 2005, Ahmed 2015 |
| SG    | N/A      | ZNF563      | Zinc finger protein 563 | 19  | 18.768      | DNA Binding | https://www.uniprot.org/uniprot/Q8TA94 |
| SG    | 600981   | PGM5        | Phospho-glucomutase 5 | 9   | 16.804      | Phosphotransferase involved in interconversion of glucose-1-phosphate and glucose-6-phosphate | Edwards 1995 |
| SG    | 609518   | THAP7-AS1   | THAP7 antisense RNA 1 | 22  | 16.626      | Binds to N-terminal histone tails of histones H3 and H4. Promotes deacetylation (repression) | Macfarlan 2005 |
| SG    | N/A      | IQUB        | IQ motif and ubiquitin domain containing | 7   | 16.172      | Ubiquitin protein | https://www.genecards.org/cgi-bin/carddisp.pl?gene=IQUB |
| SG    | 610519   | LOC146880   | Rho GTPase activating protein 27 pseudogene | 17  | 15.708      | Involved in many cellular processes, inactive in the GDP-bound state and active in the GTP-bound state | Katoh and Kato 2004 |
| SG    | N/A      | VSG8        | Immunoglobulin domain | 1   | 15.628      | | https://www.genecards.org/cgi-bin/carddisp.pl?gene=VSG8 |
| SG    | 603560   | SBFI        | SET binding factor 1 | 22  | 14.732      | SBFI acts as a protective factor that prevents substrate dephosphorylation, modulates growth control | Charcot-Marie-Tooth disease, type 4B3 | Cui 1998, Nakhiro 2013 |
| SG    | 609207   | MREG        | Melanoregulin | 2   | 14.473      | Melanocyte regulation | O’Sullivan 2004 |
| SG    | 607753   | SMUG1       | Single-strand-selective monofunctional uracil-DNA glycosylase 1 | 12  | 14.45       | Base excision repair—glycosylase that removes uracil from single- and double-stranded DNA in nuclear chromatin | Boorstein 2001 |
| SG    | 614308   | FONG        | 2 | 14.128      | Unknown | Kou 2011 |
| SG    | 609209   | IVNS1ABP    | Influenza virus NS1A binding protein | 1   | 14.044      | Actin cytoskeletal stabilization | Sasagawa 2002 |
| SG    | 601534   | KCNJ3       | Potassium inwardly-rectifying channel, subfamily J, member 3 | 2   | 13.96       | Subunit of inward-rectifying potassium channel | Kennedy 1999 |
| SG    | 608214   | SCN3B       | Sodium channel, voltage-gated, type III, beta subunit | 11  | 13.792      | Subunit of voltage-sensitive sodium channel | Atrial fibrillation (familial, type 16), Brugada syndrome 7 | Morgan 2000, Wang 2010, Hu 2009 |
| MZ    | 601038   | DIO3        | Deiodinase, iodothyronine, type III | 14  | 39.355      | Deactivates T4/T3. Too high T4/T3 can be deleterious to CNS development | Salvatore 1995 |
| MZ    | 162660   | NTF3        | Neurotrophin 3 | 12  | 27.893      | Thalamocortical connection formation, promotes the survival of, and induces neurite outgrowth from, a subset of neural crest and placode-derived neurons | Kalcheim 1992, Ma 2002 |
| MZ    | N/A      | OR14C36     | Olfactory receptor, family 14, subf. C, member 36 | 1   | 22.236      | Olfactory receptor | https://www.genecards.org/cgi-bin/carddisp.pl?gene=OR14C36 |
| MZ    | 300255   | OGT         | O-linked N-acetylglucosamine (GlcNAc) transferase | X   | 22.162      | Single N-acetylglucosamine in O-glycosidic linkage to serine or threonine residues | Mental retardation, X-linked 106 | Shafi 2000, Vaidyanathan 2017, Willems 2017 |
| MZ    | N/A      | OR11A1      | Olfactory receptor, family 11, subf. A, member 1 | 6   | 18.384      | Olfactory receptor | https://www.genecards.org/cgi-bin/carddisp.pl?gene=OR11A1 |

Continued
| Layer | OMIM ID | Gene symbol | Full name | Chr | Fold change | Function | Pathological conditions | References |
|-------|---------|-------------|-----------|-----|-------------|----------|------------------------|------------|
| MZ 612176 | MYSM1 | Myb-like, SWIRM and MPN domains 1 | 1 | 17.024 | Metalloprotease that targets monoubiquitinated histone H2A, a mark for epigenetic transcriptional repression and chromatin inaccessibility | Bone marrow failure syndrome 4 | Panda 2015, Al Sultan 2013 |
| MZ 606198 | IRX2 | Iroquois homeobox 2 | 5 | 16.821 | Pattern formation of vertebrate embryos | Bosse 1997 |
| MZ 606446 | SLAMF6 | SLAM family member 6 | 1 | 16.416 | Expressed on NK cells and cooperates in the induction of NK cell activity | Bottino 2011 |
| MZ 602372 | ZAN | Zonadhesin | 7 | 15.498 | Localizes to the anterior part of the sperm head and acts as a receptor to the zona pellucida matrix of the egg | Gasper and Swanson 2006 |
| MZ 162660 | NTF3 | Neurotrophin 3 | 12 | 14.293 | Thalamocortical connection formation, promotes the survival of, and induces neurite outgrowth from, a subset of neural crest and placode-derived neurons | Kalcheim 1992, Ma 2002 |
| MZ 605799 | AMN | Amnionless homolog | 14 | 13.684 | Encodes a type I transmembrane protein that is expressed exclusively in the extracellular visceral endoderm layer during gastrulation | Megaloblastic anemia 1, Norwegian type | Kalantry 2001 |
| MZ N/A | CYB561D1 | Cytochrome b-561 domain containing 1 | 1 | 12.995 | Cytochrome | https://www.genecards.org/cgi-bin/carddisp.pl?gene=CYB561D1 |
| MZ 609082 | FBXL16 | F-box and leucine-rich repeat protein 16 | 16 | 12.441 | Acts as protein-ubiquitin ligase. F-box proteins interact with SKP1 through the F box, and they interact with ubiquitination targets | Jin 2004 |
| MZ 131550 | EGFR | Epidermal growth factor receptor | 7 | 11.919 | Involved in diverse cellular functions, including cell proliferation, differentiation, mobility, and survival, and in tissue development | Wang 2004 |
| MZ 114761 | CA5A | Carbonic anhydrase VA, mitochondrial | 16 | 11.394 | Encodes an intramitochondrial carbonic anhydrase, which is pivotal for providing bicarbonate (HCO3-) for multiple mitochondrial enzymes | Hyperammonemia due to carbonic anhydrase VA deficiency | van Karnebeek 2014 |
| MZ 612185 | CASKIN2 | CASK interacting protein 2 | 17 | 11.27 | Binds to CASK protein, neurexins | Tabuchi 2002 |
| MZ N/A | TRIM4 | Tripartite motif containing 4 | 7 | 10.999 | Tripartite motif, localizes to cytoplasm | https://www.genecards.org/cgi-bin/carddisp.pl?gene=TRIM4 |
| MZ N/A | OR10J3 | Olfactory receptor, family 10, subf. J, member 3 | 1 | 10.3 | Olfactory receptor | https://www.genecards.org/cgi-bin/carddisp.pl?gene=OR10J3 |
| MZ N/A | CSAG2 | CSAG family, member 2 | X | 10.177 | Associated with chondrosarcoma | https://www.genecards.org/cgi-bin/carddisp.pl?gene=CSAG2 |
| MZ N/A | GRIP2 | Glutamate receptor interacting protein 2 | 3 | 9.362 | Interacts with Glutamate receptor | https://www.genecards.org/cgi-bin/carddisp.pl?gene=GRIP2 |
| MZ 609626 | MDGA1 | MAM domain containing glycosyl phosphatidylinositol anchor 1 | 6 | 9.129 | GPI-anchored membrane protein | De Juan 2002 |
| MZ N/A | OR5B17 | Olfactory receptor, family 5, subf. B, member 17 | 11 | 8.702 | Olfactory receptor | https://www.genecards.org/cgi-bin/carddisp.pl?gene=OR5B17 |
| MZ 607419 | GEMIN7 | Gem (nuclear organelle) associated protein 7 | 19 | 8.537 | Assembly of small nuclear ribonucleoproteins (snRNPs). Component of Survival of Motor Neuron (SMN) Complex | Baccon 2002 |
| MZ 607971 | SLC6A15 | Solute carrier family 6 (neutral amino acid transporter), member 15 | 12 | 8.475 | Sodium-coupled amino acid (neurotransmitter) transporter | Takanaga 2005 |
| MZ 607407 | EBF3 | Early B-cell factor 3 | 10 | 8.26 | Transcription factor | Chao 2017, Harms 2017 |
| MZ 602767 | KRT85 | Keratin 85 | 12 | 8.245 | Keratin, component of hair follicle | Ectodermal dysplasia, Type 4 | Naeem 2006 |
| MZ N/A | ZNF677 | Zinc finger protein 677 | 19 | 8.192 | Zinc finger protein (hypotheical) | https://www.genecards.org/cgi-bin/carddisp.pl?gene=ZNF677 |

Continued
| Layer | OMIM ID | Gene symbol | Full name | Chr | Fold change | Function | Pathological conditions | References |
|-------|----------|-------------|-----------|-----|-------------|----------|-------------------------|------------|
| CP    | 609730   | PDZRN4      | PDZ domain containing ring finger 4 | 12  | 5.887       | Unknown  |                          | Katoh 2004 |
| CP    | 602150   | SNAI2       | Snail homolog 2 | 8   | 5.137       | SNAI2 triggers epithelial-mesenchymal transitions and plays an important role in developmental processes, evolutionarily conserved | Waardenburg syndrome type II; Perez-Mancaera 2007; Sanchez-Martin 2002 |
| CP    | 607047   | ATXN3       | Ataxin 3  | 14  | 4.983       | Exhibit deubiquitinate activity and appears to be a component of the ubiquitin proteasome system. It may also have roles in transcriptional regulation and neuroprotection | Machado-Joseph disease (spinocerebellar ataxia-3) | Haacke 2006, Kawaguchi 1994 |
| CP    | 608896   | SGCG        | Sarcoglycan, gamma (35 kDa dystrophin-associated glycoprotein) | 13  | 4.871       | The dystrophin-glycoprotein complex (DGC) comprises a group of proteins that span the sarcolema and bind actin to the extracellular matrix of muscle cells | Muscular dystrophy, limb-girdle, autosomal recessive 5 | Noguchi 1995, Piccolo 1996 |
| CP    | 603054   | GREM1       | Gremlin 1 | 15  | 4.7         | Proposed to control diverse processes in growth and development by selectively antagonizing the activities of different subsets of the transforming growth factor (TGF)-beta ligand | Hsu 1998 |
| CP    | 162660   | NTF3        | Neurotrophin 3 | 12  | 4.604       | Thalamocortical connection formation, promotes the survival of, and induces neurite outgrowth from, a subset of neural crest and placode-derived neurons | Kalcheim 1992, Ma 2002 |
| CP    | 137141   | GABRA4      | Gamma-aminobutyric acid (GABA) A receptor, alpha 4 | 4   | 4.453       | Posttranslational regulatory role of protein receptor GABRA4 subunit involved in GABAergic neurotransmission | Mu 2002 |
| CP    | 123900   | EZR         | Ezrin     | 6   | 4.069       | Scaffold between the actin cytoskeleton and transmembrane proteins facilitating cell–cell interactions and receptor retention | Roumier 2001 |
| CP    | 164860   | MET         | Met proto-oncogene (hepatocyte growth factor receptor) | 7   | 3.883       | Cell-surface receptor for hepatocyte growth factor | Deafness, autosomal recessive 97; Hepatocellular carcinoma | Bottaro 1991 |
| CP    | 615730   | DOCK7       | Dedicator of cytokinesis 7 | 1   | 3.86        | DOCK7 plays a role in priming 1 neurite to become the axon | Epileptic encephalopathy, early infantile, 23 | Watabe-Uchida 2006, Perrault 2014 |
| CP    | 608789   | NCKAP5      | NCK-associated protein 5 | 2   | 3.858       | The NAP5 protein contains pro-rich sequences and a putative nuclear localization signal. NAP5 expression was detected in fetal and adult brain, leukocytes, and fetal fibroblasts | Matsuoka 1997 |
| CP    | 142622   | HPCA        | Hippocalcin | 1   | 3.74        | Neuron-specific Ca(2+)-binding protein found in the retina and brain | Takamatsu 1994 |
| CP    | 605790   | STK31       | Serine/threonine kinase 31 | 7   | 3.707       | Encodes a putative protein kinase with a tudor domain, found in RNA-interacting proteins, and a coiled-coil domain | Wang 2001 |
| CP    | 610851   | APIAR       | Adaptor-related protein complex 1 associated regulatory protein | 4   | 3.637       | Membrane protein, unknown function | Simpson 2000 |
| CP    | 612891   | LRRC8E      | Leucine rich repeat containing eight family, member E | 19  | 3.635       | Unknown | Kubota 2004 |
| CP    | 601642   | IL12RB2     | Interleukin 12 receptor, beta 2 | 1   | 3.521       | Expressed on Th1 and Th2 lymphocytes | Kim 2001 |
| CP    | N/A      | OR4D6       | Olfactory receptor, family 4, subf. D, member 6 | 11  | 3.48        | Olfactory receptor | https://www.genecards.org/cgi-bin/carddisp.pl?gene=OR4D6 |
| CP    | 606899   | CACNG7      | Calcium channel, voltage-dependent, gamma subunit 7 | 19  | 3.359       | Component of voltage-gated calcium channel | Burgess 2001 |
| SP    | 606198   | IRX2        | Iroquois homeobox 2 | 5   | 3.02        | Pattern formation of vertebrate embryos | Bost 1997 |
| SP    | 615388   | ADAT2       | Adenosine deaminase, tRNA-specific 2 | 6   | 12.961      | Converts adenosine to inosine by hydrolytic deamination of genomically encoded adenosine on tRNAs | Gerber 1999 |

Continued
Table 1. Genetic expression analysis. Genes differentially expressed in the progenitor layers of the frontal, parietal, and temporal lobes when compared to the insula at 15 weeks post-conception in the BrainSpan atlas (https://atlas.brain-map.org/atlas/atlas=138322603) at the 99.9th percentile. Ref: 2010 Allen Institute for Brain Science. BrainSpan Atlas of the Developing Human Brain51. 5G subpial granular Zone, MZ marginal Zone, CP cortic plate, SP subplate.

| Layer | OMIM ID | Gene symbol | Full name | Chr | Fold change | Function | Pathological conditions | References |
|-------|---------|-------------|-----------|-----|-------------|----------|------------------------|------------|
| SP    | 617922  | GYP A       | Glycophorin A | 4   | 11.829      | One of the most abundant red cell proteins, with about 1 million copies of GYP A per red cell. | Sialomucin | Cooling 2015 |
| SP    | 607667  | CTNNA3      | Catenin (cadherin-associated protein), alpha 3 | 10  | 11.647      | Cell adhesion molecule. In intercalated discs of the heart, CTNNA3 is a component of a unique hybrid adhering junction, or area composita | Arrhythmogenic right ventricular dysplasia, familial, 13 | Li 2012, van Hengel 2013 |
| SP    | 601724  | NEUROD1     | Neuronal differentiation 1 | 2   | 10.769      | Generate functional neurons from human pluripotent stem cells as early as 6 days after transgene activation | Maturity-onset diabetes of the young 6 | Naya 1995, Pang 2011, Malecki 1999 |
| SP    | 602830  | HIST1H4E    | Histone cluster 1, H4e | 6   | 8.564       | | H4 Histone Family | Marzluff 2002 |
| SP    | 601567  | LMAN1       | Lectin, mannose-binding, 1 | 18  | 7.921       | May function as a molecular chaperone for the transport from ER to Golgi of a specific subset of secreted proteins, including coagulation factors V and VIII | Combined factor V and VIII deficiency | Nichols 1998 |
| SP    | 160740  | MYH2        | Myosin, heavy chain 2, skeletal muscle, adult | 17  | 7.796       | Encodes the myosin heavy chain isoform that is expressed in fast type 2A muscle fibers | Proximal myopathy and ophthalmoplegia | Tajikarhi 2014 |
| SP    | 118493  | CHRM2       | Cholinergic receptor, muscarinic 2 | 7   | 7.455       | Shares structural features with other muscarinic receptors, including 7 transmembrane domains, an extracellular N terminus, and an intracellular C terminus | | Peralta 1987 |
| SP    | 600618  | ETV6        | Ets variant 6 | 12  | 7.441       | May act as a tumor suppressor gene | Leukemia, acute myeloid, somatic | Stegmaier 1995 |
| SP    | 608255  | TRAF3IP3     | TRAF3 interacting prot. 3 | 1   | 7.262       | Interacted with the isoleucine zipper domain of Trf3 and activated JNK | | Dadgostar 2003 |
| SP    | 607937  | NANO G      | Nanog homebox | 12  | 7.101       | Nanog is a critical factor underlying pluripotency in both ICM and ES cells | | Mitsui 2003 |
| SP    | 615717  | PLK1S1      | Polo-like kinase 1 substrate 1 | 20  | 7.027       | Mediates mitotic chromosome stabilization | Retinitis Pigmentosa 69 | Ohshinori 2006, El Shamieh 2014 |
| SP    | 615680  | CARD16      | Caspase recruitment domain fam., member 16 | 11  | 7.001       | Caspase recruitment, apoptosis | | Lee 2001 |
| SP    | N/A     | LRRC70      | Leucine rich repeat containing 70 | 5   | 7.001       | Unknown | | https://www.genecards.org/cgi-bin/carddisp.pl?gene=LRRC70 |
| SP    | 607512  | ADAMTS18    | ADAM metalloproteinase with thrombospondin type 1 motif, 18 | 16  | 6.925       | Zinc-dependent protease | Microcornea, myopic chorioretinal atrophy, and telecanthus | Aldahmesh 2013 |

 these, the SH3YL1 gene (related to cellular migration) and the SOCS7 gene (related to hydrocephalus in mice) are involved in CNS development. In the marginal zone the cutoff was 8.2-fold (Fig. 3B). Among the genes overexpressed in the opercular regions related to brain growth, we found Dio3, which deactivates T4 to T3 conversion; NTF3, involved in the formation of thalamocortical connections and neurite growth and survival and IRX2, involved in developmental pattern formation. For the cortical plate the fold difference to reach the 0.1 percentile was 3.4 (Fig. 3C). Several of the most overexpressed genes in this region are involved in neuronal growth including SNAI2, related to epithelial mesenchymal transitions; ATXN3, related to transcriptional regulation and neuroprotection; GREM1, proposed to control development by selectively antagonizing the activities of the transforming growth factor (TGF)-beta ligand; NTF3; GABRA4, that regulates GABAergic activity and; EZR4, that acts as a scaffold between the actin cytoskeleton and transmembrane proteins facilitating cell–cell interactions and receptor retention and DOCK7, that plays a role in priming neurites to become the axons. For the subcortical plate the threshold for reporting was 6.9 (Fig. 3D) including IRX2 and NEUROD1, that generates functional neurons from human pluripotent stem cells.

**Discussion**

During the last two-thirds of gestation, the cerebral cortex expands and folds into a conserved arrangement of sulci and gyri. The physical mechanisms by which the distinctive convoluted cortical pattern develops have been addressed in numerous publications. Nevertheless, these models fail to account for the global geometry
Figure 3. Comparison of the genetic expression in the maturing cortex of the opercula and the insula at 21 post-conceptional weeks (GW 23). A marked difference between the transcriptome of cortical areas (frontal parietal, temporal opercula) and the insular cortex. This may drive the higher degree of expansion that was observed at every level of the maturing cortex (A) subpial granular zone; (B) marginal zone; (C) cortical plate and (D) subplate). M1 primary motor cortex, SI primary somatosensory cortex, Lateral T/O lateral temporoparietal. Ref: 2010 Allen Institute for Brain Science. BrainSpan Atlas of the Developing Human Brain51.
of the brain and do not explain the development of the Sylvian fissure\textsuperscript{18–20}. Here, we found that the anterior and posterior poles of the telencephalic vesicle converge over the central region forming the frontal and temporal lobes, and the Insula, respectively. This process is triggered by the differential expansion of the fronto-temporal opercula very early during GW 23–25, preceding any bending process\textsuperscript{22}. These two different regions exhibited a consistent difference in their transcriptomes in the pre-folded brain, with the temporal, frontal and parietal opercular cortices having consistent expression patterns that were different from that of the insula.

We interrogated the BrainSpan Atlas to determine which genes are preferentially expressed in the developing cortices of the frontal, parietal and temporal lobes compared to the insular at 21 PCW, corresponding to gestation week (GW) 23. Given the lack of longitudinal data in the BrainSpan dataset, we were not able to determine the temporal sequence of transcriptional changes that lead to folding nor the specific pathways by which these transcriptional changes effect changes in growth. Per our analysis of the Gholipour atlas, the Sylvian fissure largely closes from 23 to 25 GW, suggesting that genetic expression immediately preceding this may drive this process. Further, while MRI changes may lag anatomic changes, the closure of the Sylvian fissure is a large-scale process that is clearly visible on fetal MRI (Fig. 1). Our aim was to identify transcriptional differences at the beginning of this period (week 21) that may drive this difference in growth pattern.

Many of the overexpressed genes in the opercula have a well-defined or plausible role in neuronal migration or survival during development. SOCS7 is a protein coding gene that regulates signaling cascades and its involved in terminating neuronal migration\textsuperscript{35}. Although the process is complex and incompletely understood, it is known that the complex SOCS7-Cul5-Rbx2 regulates termination of migration and disruption of that system results in abnormally position neurons in the CP\textsuperscript{36}.

Similarly, Neurotrophin 3 (NTF3), IRX2, and DOCK7, have roles in neuronal differentiation and survival. NTF3 controls the survival and differentiation of neurons and is thought to promote neuronal survival in the developing brain\textsuperscript{26,37}. In contrast, while IRX2 does not have a defined role in telencephalic development, it is known to participate in the rostro caudal differentiation of the hindbrain\textsuperscript{29}. DOCK7 codes for a guanine nucleotide exchange factor, which in turn activates Rac 1 and 3 and Rho amongst others. DOCK7 has a direct role in brain development by regulating the fate of the radial glia\textsuperscript{30,31}. Mutations on this gene cause epileptic encephalopathy and cortical blindness\textsuperscript{32}.

Other proteins we identified do not have a clearly identified role (as of yet) in brain development. Ataxin 3 (ATX3) is involved in protein ubiquitination, a trinucleotide expansion of this gene (CAG) causes Machado-Joseph disease or spinocerebellar ataxia, although this mutation is associated with neuronal death, so far ATX3 does not have a well-defined role in brain development\textsuperscript{33,34}. GREM1 codes for a bone morphogenetic protein (BMP) antagonist and can modulate organogenesis. In mouse it conveys the Sonic Hedgehog polarizing signal and is involved in limb development. A role in cortical folding has not been described\textsuperscript{35}.

Apart from the physical principles involved in brain folding, numerous reports evidenced that this process is under heavy genetic regulation\textsuperscript{5,30–40}. Recently de Juan Romero et al. described a genetic patterning in the ferret’s VZ and outer ventricular zone (OVZ), matching the folded geometry of the brain and pre-folded before gyrication started. These differentially expressed genes (DEG) are composed of thousands of sequences that are expressed in an on and off fashion alternating between sulci and gyri, including Trnp1, Ccnd1, EOMES, Notch, Shh, MAPK and Wnt\textsuperscript{7,29,40}. This pattern conforms a blueprint for individual folds but do not explain the large-scale arrangement of the brain lobes.

A comparison between our findings and the DEGs described by de Juan Romero would be inaccurate; on one side they analyzed the ventricular and subventricular zones and not the cortices themselves; on the other, the mechanism involved in forming cortical convolutions in ferrets and humans are different and may be governed by different mechanisms\textsuperscript{39,41,42}. The genetic expression at the pallial/subpallial boundary was described by Carney et al.\textsuperscript{43}. This region is highly heterogeneous and expresses a mixed array of genes from the pallium (Pax6) and subpallium (Gsh2).

Our findings suggest that the insular progenitor cells have a distinct transcriptional signature than the populations that develop into the frontal/temporal/parietal operculae. The latter expand at a much higher rate than the former, thus closing the Sylvian fissure on the lateral hemispheric surface. The work of de Juan Romero et al.\textsuperscript{40} and Carney et al.\textsuperscript{43} suggests that the insular progenitors may follow an oblique migration pathway in contrast to the direct radial migration pathway leading to the lateral hemispheric cortical surface (Fig. 4). The developing basal ganglia may block a direct pathway for progenitor cells in the ventricular zone to the insula. The distinct gyral configuration of the insula (5 relatively linear gyri that converge to the limen insulae) in contrast to the convoluted gyri of the lateral hemispheric surface may also be related to this\textsuperscript{44}. Future studies must investigate the genetic makeup of these progenitor populations and how this drives the connectivity and morphology of the insula and operculae.

The atlas developed by Gholipour et al. is derived from 81 healthy, normally-developing fetuses\textsuperscript{45}, while the gene expression data is derived from two 21 PCW (23 GW) fetuses as described in the BrainSpan documentation. It is important to note that the Gholipour atlas uses gestational week as a measure of time, which is approximately 2 weeks ahead of post-conceptional week. Thus, the genetic information from the BrainSpan Atlas corresponds to the very beginning of the Gholipour atlas. Although different individuals were used to construct both datasets, we argue that the genetic drivers of Sylvian fissure formation are highly conserved across individuals given the universal formation of the fissure in the healthy human brain.

Our findings are limited by the small number of analyzed specimens, the use of bulk mRNA instead of single cell expression, and the difficulty of matching the time and spatial dimensions between a dataset generated from MRI and one constructed directly from fetal brain tissue. Given the ethical constraints in obtaining fetal brain tissue, the number of specimens available for genetic analysis is inherently limited. Further, given these same ethical limits, longitudinal imaging and genetic analysis is not possible in the same individual. Despite
these constraints, this study deepens our understanding of the geometry and the transcriptome of the normal development of the Sylvian fissure.

Conclusion

The Sylvian fissure forms by the relative overgrowth of the frontal and temporal lobes over the insula, with the developing cortices exhibiting sharply different transcriptomes. It is interesting to note that some of these genes are related to neuronal proliferation and differentiation functions and can be part of the landscape of the genes responsible for the general geometry of the brain.

Methods

Guidelines statement. All data used in this analysis were obtained from publicly available databases that were obtained in accordance with all relevant guidelines and regulations. Specifically, for the fetal MRI atlas by Gholipour et al.\textsuperscript{45} the study was approved by the Boston Children’s Hospital Institutional Review Board and the Committee on Clinical Investigation and written informed consent was obtained from all participants. For the BrainSpan atlas, all work was performed following guidelines for the research use of human brain tissue of the University of Washington and Advanced Bioscience Resources (Alameda, California) with approval by the Human Investigation Committees and Institutional Ethics Committees of each institute.

Developmental MRI assessment. Atlas. We analyzed the fetal brain atlas described by Gholipour et al.\textsuperscript{45,46}. This atlas was reconstructed from weekly fetal T2-weighted MRI images of 81 healthy fetuses from gestational week 21 to 38 segmented to identify multiple key subcortical and cortical regions. A description of the Atlas and its full content can be accessed at https://crl.med.harvard.edu/research/fetal_brain_atlas/.

Registration. To analyze fetal brain development over time, we utilized the SyN algorithm in the ANTs (Advanced Normalization Tools)\textsuperscript{47} package to register the atlas fetal MRI on a week by week basis. This is a nonlinear, diffeomorphic registration paradigm. We performed two types of registration. First (part A), we registered images on a week-by-week basis (week 38 to week 37, week 37 to week 36, etc.) using the full SyN algorithm in the reverse direction.

Second (Part B), in order identify nonlinear changes such as gyral growth or folding and ignore gross volumetric changes, we registered all weeks to week 38 using an affine transformation (performed using ANTs). Subsequently we performed nonlinear registration using SyN to identify these nonlinear changes.

Registration validation. In order to validate the accuracy of our registration, we warped the week 38 segmentation to the desired week using our registration method\textsuperscript{45}. We then compared the overlap of the atlas segmentation for the desired week to the warped segmentation using the multiclass Dice similarity coefficient, as implemented in the Scikit-learn package in Python.
Jacobian analysis. To determine volumetric changes on a week by week basis, we calculated the Jacobian determinant of Part A registration described above (week-to-week) using ANTS. The Jacobian determinant is the determinant of the Jacobian matrix, a matrix of the first order partial derivatives of the transformation, and represents the local volumetric change associated with the transformation. A determinant greater than 1 represents expansion and vice versa. We used this to generate Jacobian maps for each week representing the relative volume expansion/contraction for each voxel for each week (after correcting for global scaling). Note that the Jacobian determinants are normalized for global volume change—e.g. if overall global volume growth was 9%, an area with Jacobian determinant of 0.95 still grew 3.5%. We rendered these maps in 2D and 3D using Nibabel, matplotlib, and nilearn, all publicly available Python packages.

Displacement maps. To visualize the direction and magnitude of local displacements, we rendered the normalized (part B), registration using arrow plots, which allow for visualization of local deformation. In addition, we overlaid these plots with a heat map of the magnitude (total displacement) of each area. This was performed using Nibabel and matplotlib.

Developmental gene expression. Atlas. In the current study, the BrainSpan atlas was utilized to profile gene transcription at different stages of brain maturation. The BrainSpan atlas is an ARRA-funded grant through the NIH to a consortium consisting of the Allen Institute for Brain Science; Yale University; the University of Southern California; Massachusetts General Hospital, Harvard-MIT Health Sciences and Technology, Athinoula A. Martinos Center for Biomedical Imaging; the University of California, Los Angeles; and the University of Texas Southwestern Medical Center. All data are publicly accessible. The methods and processes used to generate gene expression data have been previously published and can be accessed via https://www.brainspan.org.

Postmortem human brain specimens were from the following: the Department of Neurobiology at Yale School of Medicine and the National Institute of Mental Health; the Human Fetal Tissue Repository at the Albert Einstein College of Medicine; the Brain and Tissue Bank for Developmental Disorders at the University of Maryland; the Birth Defects Research Laboratory at the University of Washington; the MRC-Wellcome Trust.
The BrainSpan Atlas sampled over 90 substructures in the developing brain, listed at https://help.brain-map.org/download/attachment/3506181/Prenatal_LMD_Microarray.pdf, building on the previous work by Kang et al. This includes the granular and dysgranular insular cortices (listed under "Neocortex" in Table 2 in the white paper at the link), and the surrounding opercular areas.

Because the BrainSpan Atlas transcriptome is not referenced in the stereotactic space (it is provided in coronal slices), we defined the insula and operculae with anatomical landmarks visible both in the MRI dataset and the BrainSpan Atlas. The insula was defined as the cortical region within the limiting insular sulci, which corresponded to the dysgranular and granular insular cortices in the BrainSpan Atlas. We then identified substructures in the BrainSpan atlas that correspond to the superior (frontal and parietal lobe), and inferior (temporal) operculae. Note that not all substructures had transcriptomic data for all layers (subpial granular zone, marginal zone, cortical plate, subplate).

Human Developmental Biology Resource at the Institute of Human Genetics, University of Newcastle, U.K. The protocol was approved by the respective IRBs. Informed consent was obtained.

The BrainSpan Atlas sampled over 90 substructures in the developing brain, listed at https://help.brain-map.org/download/attachment/3506181/Prenatal_LMD_Microarray.pdf, building on the previous work by Kang et al. This includes the granular and dysgranular insular cortices (listed under "Neocortex" in Table 2 in the white paper at the link), and the surrounding opercular areas.

Because the BrainSpan Atlas transcriptome is not referenced in the stereotactic space (it is provided in coronal slices), we defined the insula and operculae with anatomical landmarks visible both in the MRI dataset and the BrainSpan Atlas. The insula was defined as the cortical region within the limiting insular sulci, which corresponded to the dysgranular and granular insular cortices in the BrainSpan Atlas. We then identified substructures in the BrainSpan atlas that correspond to the superior (frontal and parietal lobe), and inferior (temporal) operculae. Note that not all substructures had transcriptomic data for all layers (subpial granular zone, marginal zone, cortical plate, subplate). The included opercular structures from the BrainSpan atlas were—frontopolar cortex, ventrolateral prefrontal cortex, orbital frontal cortex, primary motor cortex, primary somatosensory cortex, inferior parietal cortex, parainsular temporal cortex (e.g. auditory cortex), lateral temporoooccipital cortex, and superior temporal cortex. We acknowledge that several of these structures span areas beyond the conventional definition of the operculae but given the parcellation from the BrainSpan atlas, we were limited to this. This is illustrated in Figs. 5 and 6.
Comparative whole transcriptome analysis. We analyzed the transcriptional profile at 21 postconceptional weeks (PCW) available for a total of two specimens. We interrogated the atlas based on the result of the fetal MR analysis described above. We aimed to compare the expression profile of the developing cortex in areas of high growth with cortical regions exhibiting minimal relative expansion. The subpial granular zone (SZ), marginal zone (MZ), cortical plate (CP) and subcortical plate (SP) corresponding to the frontoparietal and temporal opercula were compared to the insula.

A comparative search of genes consistently over expressed in the target regions (opercular cortex) compared to the contrast region (insular cortex) was performed. We report on the 99.9 highest expressed percentile of around 35,000 genes analyzed for each region, which selects the top 35 overexpressed genes in each area. The cut off (in folds) was 13.7 for the SG, 8.2 for the MZ, 3.4 for the CP and 6.9 for the SP.

Received: 1 November 2019; Accepted: 18 August 2020
Published online: 02 September 2020

References
1. Chi, J. G., Dooling, E. C. & Gilles, F. H. Gyral development of the human brain. Ann. Neurol. 1, 86–93 (1977).
2. Bayly, P. V., Taber, L. A. & Kroenke, C. D. Mechanical forces in cerebral cortical folding: A review of measurements and models. J. Mech. Behav. Biomed. Mater. 29, 568–581 (2014).
3. Tallinen, T. et al. On the growth and form of cortical convolutions. Nat. Phys. 12, 588–593 (2016).
4. Garel, C. et al. Fetal cerebral cortex: Normal gestational landmarks identified using prenatal MR imaging. Am. J. Neuroradiol. 22, 184–189 (2001).
5. Fernández, V., Llinares-Benadero, C. & Borrell, V. Cerebral cortex expansion and folding: What have we learned?. EMBO J. 35, 1021–1044 (2016).
6. Bystron, I., Blakemore, C. & Rakic, P. Development of the human cerebral cortex: Boulder Committee revisited. Nat. Rev. Neurosci. 9, 110–122 (2008).
7. Ang, H. J. et al. Spatio-temporal transcriptome of the human brain. Nature 478, 483–489 (2011).
8. Paredes, M. F. et al. Extensive migration of young neurons into the infant human frontal lobe. Science 354, 6308 (2016).
9. Zilles, K., Armstrong, E., Schleicher, A. & Kretschmann, H. J. The human pattern of gyrification in the cerebral cortex. Anat. Embryol. (Berl.) 179, 173–179 (1988).
10. Van Essen, D. C. A tension-based theory of morphogenesis and compact wiring in the central nervous system. Nature 385, 313–318 (1997).
11. Tallinen, T. & Biggins, J. S. Mechanics of invagination and folding: Hybridized instabilities when one soft tissue grows on another. Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 92, 2 (2015).
12. Zilles, K. et al. Quantitative analysis of sulci in the human cerebral cortex: Development, regional heterogeneity, gender difference, asymmetry, intersubject variability and cortical architecture. Hum. Brain Mapp. 5, 218–221 (1997).
13. Tallinen, T. & Biggins, J. S. Mechanics of invagination and folding: Hybridized instabilities when one soft tissue grows on another. Phys. Rev. E Stat. Nonlinear Soft Matter Phys. 92, 2 (2015).
14. Vasung, L. et al. Quantitative and qualitative analysis of transient fetal compartments during prenatal human brain development. Front. Neuroanat. 10, 11 (2016).
15. Van Essen, C. A. Tension-based theory of morphogenesis and compact wiring in the central nervous system. Nature 385, 313–318 (1997).
16. Sarnat, H. B. & Flores-Sarnat, L. Telencephalic flexure and malformations of the lateral cerebral (Sylvian) fissure. Pediatr. Neurol. 63, 23–38 (2016).
17. Bush, A., Nuñez, M., Bribbin, A. K., Friedlander, R. M. & Goldschmidt, E. Spatial convergence of distant cortical regions during folding explains why arteries do not cross the sylvian fissure. J. Neurol. 1–10 (2019). (online ahead of print)
18. Gonzalez-Arnay, E., Gonzalez-Gómez, M. & Meyer, G. A radial glia fascicle leads principal neurons from the pallial-subpallial boundary into the developing human insula. Front. Neuroanat. 11, 111 (2017). https://doi.org/10.3389/fnana.2017.00111.
19. Hikone, K., Kudo, K. L. & Nakajima, K. How does Reelin control neuronal migration and layer formation in the developing mammalian neocortex?. Neurosci. Res. 86, 50–58 (2014).
20. Anderson, K. D. et al. Differential distribution of exogenous BDNF, NGF, and NT-3 in the brain corresponds to the relative abundance and distribution of high-affinity and low-affinity neurotrophin receptors. J. Comp. Neurosci. 357, 296–317 (1995).
21. Rodriguez-Tebã, A., Dechant, G. & Barde, Y. A. Neurotrophins: Structural relatedness and receptor interactions. Philos. Trans. R. Society Lond. Series B Biol. Sci. 311, 235–258 (1991).
22. Nakamura, H., Katakura, T., Matsunaga, E. & Sato, T. Thalamus organizer for midbrain and hindbrain development. Brain Res. Rev. 49, 120–126 (2005).
23. Yamauchi, M., Miyamoto, Y., Chan, J. R. & Tanoue, A. ErbB2 directly activates the exchange factor Dock7 to promote Schwann cell migration. J. Cell Biol. 181, 351–365 (2008).
24. Gadea, G. & Blangy, A. Dock-family exchange factors in cell migration and disease. Eur. J. Cell Biol. 93, 466–477 (2014).
25. Perrault, I. et al. Mutations in DOCK7 in individuals with epileptic encephalopathy and cortical blindness. Am. J. Hum. Genet. 94, 891–897 (2014).
26. Johnson, S. L. et al. Differential toxicity of ataxin-3 isoforms in drosophila models of spinocerebellar ataxia type 3. Neurobiol. Dis. 132, 104535 (2019).
27. Nóbrega, C. et al. Molecular mechanisms and cellular pathways implicated in Machado-Joseph disease pathogenesis. Adv. Exp. Med. Biol. 1049, 349–367 (2018).
28. Johnson, E. J., Neely, D. M., Dunn, J. C. & Davey, M. G. Direct functional consequences of ZRS enhancer mutation combine with secondary long range SHH signalling effects to cause preaxial polydactyly. Dev. Biol. 392, 209–220 (2014).
36. Lohmann, G., Von Cramon, D. Y. & Colchester, A. C. F. Deep sulcal landmarks provide an organizing framework for human cortical folding. *Cereb. Cortex* **18**, 1415–1420 (2008).
37. Walsh, C. A. Genetic malformations of the human cerebral cortex. *Neuron* **23**, 19–29 (1999).
38. Lohmann, G., von Cramon, D. Y. & Steinmetz, H. Sulcal variability of twins. *Cereb. Cortex* **9**, 754–763 (1999).
39. Reillo, I. & Borrell, V. Germinal zones in the developing cerebral cortex of ferret: Ontogeny, cell cycle kinetics, and diversity of progenitors. *Cereb. Cortex* **22**, 2039–2054 (2012).
40. de Juan Romero, C., Bruder, C., Tomasello, U., Sanz-Anquela, J. M. & Borrell, V. Discrete domains of gene expression in germinal layers distinguish the development of gyrencephaly. *EMBO J.* **34**, 1859–1874 (2015).
41. Elsen, G. E. *et al.* The protomap is propagated to cortical plate neurons through an Eomes-dependent intermediate map. *Proc. Natl. Acad. Sci. USA* **110**, 4081–4086 (2013).
42. Baala, L. *et al.* Homozygous silencing of T-box transcription factor EOMES leads to microcephaly with polymicrogyria and corpus callosum agenesis. *Nat. Genet.* **39**, 454–456 (2007).
43. Carney, R. S. E., Cocos, L. A., Hirata, T., Mansfield, K. & Corbin, J. G. Differential regulation of telencephalic pallial-subpallial boundary patterning by Pax6 and Gsh2. *Cereb. Cortex* **19**, 745–759 (2009).
44. Türe, U., Yaşargil, D. C. H., Al-Mefty, O. & Yaşargil, M. G. Topographic anatomy of the insular region. *J. Neurosurg.* **90**, 720–733 (1999).
45. Ghofpour, A. *et al.* A normative spatiotemporal MRI atlas of the fetal brain for automatic segmentation and analysis of early brain growth. *Sci. Rep.* **7**, 476 (2017).
46. Marami, B. *et al.* Temporal slice registration and robust diffusion-tensor reconstruction for improved fetal brain structural connectivity analysis. *Neuroimage* **156**, 475–488 (2017).
47. Avants, B. B. *et al.* A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* **54**, 2033–2044 (2011).
48. Tusnán, N. J. & Avants, B. B. Explicit B-spline regularization in diffeomorphic image registration. *Front. Neuroinform.* **7**, 39 (2013).
49. Avants, B. B., Epstein, C. L., Grossman, M. & Gee, J. C. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med. Image Anal.* **12**, 26–41 (2008).
50. Ventur, B., Dahne, S., Höhne, J., Heller, H. & Blankertz, B. Wyrm: A brain–computer interface toolbox in python. *Neuroinformatics* **13**, 471–486 (2015).
51. Miller, J. A. *et al.* Transcriptional landscape of the prenatal human brain. *Nature* **508**, 199–206 (2014).

**Author contributions**

E.G., A.B. and A.N.M. were involved in the design and conception of this manuscript. E.G., H.D. and A.M. performed the literature search. E.G., A.B., H.D. and A.N.M. compiled the primary manuscript. E.G., A.B., A.K.B. and A.M. compiled the figures. E.G., A.M. and A.B. critically revised the manuscript. All authors have approved the manuscript as it is written. E.G., A.M., A.K.B., H.D. all contributed to figures and tables. All figures/tables were created or assembled by the co-authors.

**Funding**

This research was partially supported by the American Association of Neurological Surgeons via the Van Wagenen Fellowship.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Correspondence** and requests for materials should be addressed to E.G.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit [http://creativecommons.org/licenses/by/4.0/](http://creativecommons.org/licenses/by/4.0/).

© The Author(s) 2020