

# Genetic Changes Shaping the Human Brain

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The development and function of our brain are governed by a genetic blueprint, which reflects dynamic changes over the history of evolution. Recent progress in genetics and genomics, facilitated by next-generation sequencing and single-cell sorting, has identified numerous genomic loci that are associated with a neuroanatomical or neurobehavioral phenotype. Here, we review some of the genetic changes in both protein-coding and noncoding regions that affect brain development and evolution, as well as recent progress in brain transcriptomics. Understanding these genetic changes may provide novel insights into neurological and neuropsychiatric disorders, such as autism and schizophrenia.

All life forms develop, reproduce, and age based on their genetic blueprint. The human genetic blueprint is written in approximately three billion base pairs (bp) and contains protein-coding genes (estimated at 21,000 or fewer), RNA genes (e.g., microRNAs, promoter-associated short RNAs, small nucleolar RNAs, and long noncoding RNAs, which are estimated at 18,400), *cis*-regulatory elements (including promoters and transcription factor binding sites), long-range regulatory elements (namely, enhancers, repressors/silencers, and insulators), and transposable elements (ENCODE Project Consortium, 2011, 2012; Lander, 2011; Lander et al., 2001; Pennisi, 2012).

The human genome changes over both short and long timescales. Genetic changes that occurred as *Homo sapiens* diverged from the common ancestor of primates are identifiable as human-specific regions of the genome (Pollard et al., 2006). Subtler genetic changes, especially relating to susceptibility to infectious diseases, have occurred during human migration out of Africa and settlement in certain geographical and biological environments (Sabeti et al., 2007; Tishkoff et al., 2007). Furthermore, genetic changes continue to occur, and can cause disease, when inherited within a family as dominant (e.g., Huntington's disease) or recessive (e.g., microcephaly) alleles (Gilmore and Walsh, 2013; Ross and Tabrizi, 2011). Most recently, we have been discovering the extent to which genetic changes that occur somatically, during the development and lifetime of an individual, can affect the brain (Jamuar et al., 2014; Poduri et al., 2013).

One may wish that the development and evolution of the human brain could be explained simply by a discrete set of human-specific genetic changes within the context of conserved brain development among mammals, including mice. However, there are many qualitative and quantitative differences in the development of the cerebral cortex (neocortex) between humans and mice (Geschwind and Rakic, 2013; Hill and Walsh, 2005), and even our understanding of the development of the mouse brain is far from complete. Nonetheless, there has been recent progress in our understanding of human brain development and evolution due to new methods and tools available in genetics, genomics, and developmental biology (Table 1). Here, we will review the present state of research on the genetic

changes affecting the development and evolution of the human neocortex.

## Identification of Evolutionarily Important Genomic Regions by Comparisons between Species

One way to identify genetic changes that may have contributed to the evolution of the human brain is to compare the human genome to genomes of other species, both closely and distantly related, and determine which genetic changes may be relevant. Currently, genome sequences are available for archaic humans, such as the Neanderthal (Green et al., 2010) and the Denisovan (Reich et al., 2010), and for apes, such as the chimpanzee (Chimpanzee Sequencing and Analysis Consortium, 2005), the macaque (Gibbs et al., 2007), the orangutan (Locke et al., 2011), the gorilla (Scally et al., 2012), and the bonobo (Prüfer et al., 2012), as well as dozens of non-primate species.

However, mere comparison of the genomes from different species is not sufficient to identify functionally relevant genetic changes, as there are countless differences between the human genome and the genomes of other species. For example, there are nearly 20 million genomic loci that differ between humans and chimpanzees. One important caveat here is that some of the apparent differences between humans and non-humans may simply reflect technical effects such as low sequencing coverage and improper annotation of primate genomes compared to the human genome. The currently annotated human genome is a compilation of thousands of individual genomes. Thus, intra-species population variability is well documented in humans (Abecasis et al., 2012). In contrast, some of the archaic human and primate genomes are based on a single individual. Second, in most cases, the functional consequences of species differences in sequence are unknown. Any genetic change has the potential to elicit a robust phenotypic change, but it is nearly impossible to predict which genetic change is relevant. Then, out of 20 million or more candidates, where should we start?

One plausible starting point is to focus on the sequences that are uniquely present, absent, or variable in the human genome, as those sequences might contribute to unique features of the human brain. A search based on protein basic local alignment search tool (BLASTP) revealed a few *de novo* genes that are

**Table 1. Tools for Identification and Characterization of Genetic Changes Shaping the Human Brain**

Tool	Advantages	Disadvantages
Whole-exome sequencing	efficient sequencing of all the protein-coding genes (~180,000 exons) in the human genome	unable to identify structural and noncoding variants, although there are some tools to detect copy number variations from exome data
Whole-genome sequencing	sequencing of the entire genome of an individual	the function of the majority of the human genome is incompletely understood; thus, much sequencing data are often difficult to interpret; also, whole-genome sequencing is costly, at least for now
Single-cell sequencing	genome and transcriptome sequencing of individual cells detects cell-to-cell variability	requires amplification of the limited DNA and RNA in a single cell, which can introduce errors
RNA sequencing	reveals how each protein-coding gene or RNA gene is utilized in a given cellular context	detection of genes with low expression levels is difficult; multiple cell types can confound the interpretation
Chromosome conformation capture	reveals chromosomal interactions influencing gene expression, such as interaction between an enhancer and a promoter of a protein-coding gene	can be costly due to depth of sequencing needed, depending on method; requires a great number of cells; multiple cell types can generate noise and confound the interpretation, although single-cell chromosome conformation capture was recently developed
Clustered regularly interspaced short palindromic repeats (CRISPR)	genome editing by breaking the target DNA, which introduces frameshift mutations via non-homologous end joining or facilitates homologous recombination	off-target effects; efficiency in the brain awaits further optimization

Multiple types of genome and transcriptome sequencing tools are available for identification of genetic changes that may contribute to human brain development, evolution, and disease. Functional characterization of the genetic changes is expedited by CRISPR, a genome-editing tool.

unique to the human genome (Knowles and McLysaght, 2009; Wu et al., 2011). Interestingly, RNA sequencing (RNA-seq) data indicate that these genes have their highest expression levels in the neocortex and testes, although their functions have not been systematically examined. Conversely, a systematic search for human-specific deletions compared with other primate genomes identified 510 such deletions in humans that fall almost exclusively in noncoding regions (McLean et al., 2011). One such deletion removes a forebrain germinal zone enhancer near the tumor suppressor gene *growth arrest and DNA-damage-inducible, gamma* (*GADD45G*), suggesting a possible role in the expansion of specific brain regions in humans. However, a general consensus is that humans have a remarkably similar number of protein-coding genes to model organisms such as mice and worms and that most of the essential evolutionary changes did not happen by simple gene addition or subtraction.

Another evolutionary approach has been to focus on genomic loci that are well conserved throughout vertebrate evolution but are strikingly different in humans; these regions have been named “human accelerated regions (HARs)” (Bird et al., 2007; Bush and Lahn, 2008; Pollard et al., 2006; Prabhakar et al., 2008). So far, ~2,700 HARs have been identified, again most of them in noncoding regions: at least ~250 of these HARs seem to function as developmental enhancers in the brain (Capra et al., 2013). One of them (HAR1) encodes a long noncoding RNA (lncRNA) that is expressed specifically in Cajal-Retzius neurons in the developing human neocortex. Cajal-Retzius neurons are a heterogeneous population of cell types in the marginal zone and layer 1 of the developing neocortex that produces reelin, a large, secreted, extracellular matrix glycoprotein. Reelin controls processes of neuronal migration and positioning in the developing neocortex, so the expression pattern of this HAR suggests intriguing potential roles in regulating the shape or structure of the cerebral cortex.

The analysis of convergent evolution represents a third avenue of comparative genomics that has been very powerful of late but has not yet been explored in relation to the structure of the neocortex per se. A particular phenotype can evolve independently in species belonging to different lineages, creating analogous structures that have similar form or function. An evolutionarily convergent phenotype may be correlated with similar genomic differences occurring independently in the two lineages that evolved the same trait independently. Using this approach, the genetic basis of vitamin C deficiency in certain primates including humans has recently been uncovered (Hiller et al., 2012). Similarly, echolocation in bats and dolphins (Parker et al., 2013), the electric organs (Gallant et al., 2014), the origin of the nervous system (Moroz et al., 2014), and the domestication of wild rabbits (Carneiro et al., 2014) have been studied. This method may be very powerful in the future to apply to cerebral cortical evolution as well.

#### Identification of Evolutionarily Important Genomic Regions Using Human Genetics

The basic premise of human genetics is simple: where there is a phenotype, there is likely to be an associated genetic (or epigenetic) change. Identification of a genetic change in humans and validation in cell and animal models can explain the mechanistic cause of the phenotype. There are more than seven billion people manifesting diverse phenotypes, which are skillfully identified and characterized by physicians (Brenner, 2003). In conjunction with traditional human genetics approaches, next-generation, high-throughput, deep sequencing is a powerful tool to identify genetic changes that are compatible with life and affect development of the human brain.

It is relatively easy to detect and understand the functional consequences of changes in protein-coding sequences, compared to noncoding mutations. Mutations in a coding sequence

often cause more severe phenotypes than mutations in a regulatory element associated with the same coding sequence. Nonsense mutations, as well as missense mutations, especially in evolutionarily conserved amino acids (aa), can lead to gain or loss of function of a protein. A coding sequence mutation can be introduced into model organisms by overexpressing the mutated coding sequence or by knocking out, knocking down, or knocking in the orthologous gene. The tissues, cell types, and subcellular organelles in which a protein is expressed can suggest potential roles of the protein. Knowledge of any interacting proteins and of the aa sequence homology to other well-characterized proteins can also help identify the potential functions of a protein.

In this regard, *forkhead box protein p2* (*FOXP2*) provides a prime example of protein-coding sequence mutations that identify genes with potential evolutionary importance. A mutation in *FOXP2* was first identified in a British family with a severe language disorder (Lai et al., 2001). The affected individuals have a severe impairment in the selection and sequencing of fine oral and facial movements, the ability to break up words into their constituent phonemes, and the production and comprehension of word inflections and syntax. Their non-verbal abilities are relatively intact. *FOXP2* encodes a forkhead-winged helix family transcription factor that is highly expressed in the developing and mature neocortex (Ferland et al., 2003), and the mutation (R553H) disrupts the forkhead DNA-binding domain.

Comparison of the *FOXP2* cDNAs from multiple species indicates that the human *FOXP2* protein differs at only three aa residues from the mouse ortholog and at two residues from the chimpanzee, gorilla, and rhesus macaque orthologs (Enard et al., 2002). These aa changes are functionally critical as the human and chimpanzee *FOXP2* have strikingly different transcriptional targets, many of which are involved in CNS development (Konopka et al., 2009). Human-specific *FOXP2* targets also include genes involved in branchial arch formation and craniofacial development, which may be required for spoken language. Mice carrying humanized *FoxP2* show accelerated learning, qualitatively different ultrasonic vocalizations, and increased dendrite length and synaptic plasticity in the medium spiny neurons of the striatum. These findings suggest that the cortico-striatal circuitry mediates speech and language in humans (Enard et al., 2009; Fujita et al., 2008; Schreiweis et al., 2014; Shu et al., 2005). Although it is hard to know whether mouse ultrasonic vocalizations are analogous to human language, *FoxP2* and cortico-striatal circuitry seem to control vocal learning in songbirds, which communicate via vocalizations like humans (Wohlgemuth et al., 2014). Perhaps surprisingly, Neanderthals, Denisovans, and modern humans share an identical *FOXP2* protein (Meyer et al., 2012; Noonan et al., 2006). Thus, at least some neurobiological and physiological aspects of speech and language probably emerged after divergence from chimpanzees.

*FOXP2* is a part of a larger *FOXP* family that includes *FOXP1*, *FOXP3*, and *FOXP4*. Their expression patterns are different, but all *FOXP* transcription factors have a highly similar structure, suggesting that they emerged by duplication. Conserved forkhead domains are found in eukaryotic organisms from yeast to humans, and the human genome contains more than forty *FOX* genes that play diverse roles in development, metabolism, immunity, and cancer (Benayoun et al., 2011). Indeed, the majority

of human genes have been generated by gene duplication (Ohno, 1970; Zhang, 2003). Duplicated genes diverge functionally over time by accumulating changes in the coding sequence as well as in associated noncoding regulatory elements, which alter protein expression patterns (Carroll et al., 2005; Conant and Wolfe, 2008).

Analysis of the *Slit-Robo Rho GTPase-activating protein 2* (*SRGAP2*) genes shows the potential role of gene duplication in brain evolution (Charrier et al., 2012; Dennis et al., 2012). *SRGAP2* has been implicated in cerebral cortical development in mice, especially in neuronal migration and morphogenesis, and the F-BAR domain of *SRGAP2* is required for its function (Guerrier et al., 2009). Mice have only one form of the *Srgap2* gene, but humans have four forms: one ancestral form (*SRGAP2A*), which is orthologous to mouse *Srgap2*, and three human-specific paralogs (*SRGAP2B*, *SRGAP2C*, and *SRGAP2D*) that were produced by incomplete segmental duplication. Unlike *SRGAP2A*, which consists of 1,071 aa, the 459-aa *SRGAP2B* and *SRGAP2C* proteins have a truncated F-BAR domain. *SRGAP2D* mRNA is subject to nonsense-mediated decay. The three human-specific paralogs are located in the human-lineage-specific loci 1q21.1 and 1p12, both of which contain a number of genes implicated in neurodevelopment (O'Bleness et al., 2012). The human-specific paralogs are also present in the Denisovan and Neanderthal genomes, but not in the non-human primate genomes. Thus, the human-specific *SRGAP2* paralogs emerged when the genus *Homo* diverged from *Australopithecus* 2–3 million years ago (Dennis et al., 2012). *SRGAP2B* is expressed as a pseudogene. With its truncated F-BAR domain, *SRGAP2C* dimerizes with full-length *SRGAP2A* and inhibits *SRGAP2A* function in a dominant-negative manner. Overexpression of *SRGAP2C* in the mouse brain results in sustained radial migration of neurons as well as increased spine density and neoteny during spine maturation, which are human-specific features of neuronal development.

Genes controlling brain size or shape also exemplify evolutionarily dynamic genomic regions identified by human genetics. Primary microcephaly is an autosomal recessive neurodevelopmental disorder resulting in an abnormally small brain volume of >2–3 SDs below the mean, and some of the genes for this condition have also been implicated as potentially active in human brain evolution. Many of the known genetic mutations causing human primary microcephaly target one subcellular organelle—the centrosome (Gilmore and Walsh, 2013). Individuals with primary microcephaly show no obvious motor deficits but suffer from intellectual disability and language delay. Microcephaly is largely caused by mutations that disrupt genes encoding centrosomal proteins: *MCPH1*, *ASPM*, *CDK5RAP2*, *CENPJ*, *STIL*, *WDR62*, *CEP152*, and *CEP63* (Bilgüvar et al., 2010; Bond et al., 2002; Bond et al., 2005; Guernsey et al., 2010; Jackson et al., 2002; Kumar et al., 2009; Nicholas et al., 2010; Sir et al., 2011; Yu et al., 2010). Intriguingly, a few microcephaly-associated genes, notably *ASPM* and *CDK5RAP2*, show evidence for positive selection not only in primates but also across placental mammals. The evolution of *ASPM* and *CDK5RAP2* is strongly correlated with brain size, suggesting their roles in brain evolution (Montgomery and Mundy, 2014).

As microcephaly proteins are ubiquitously present in the centrosome of most animal cells (Nigg and Raff, 2009), it is

unclear why mutations in these genes affect the brain more than the rest of the body in most cases. The centrosome is the main microtubule-nucleating organelle during mitosis (M phase). It is composed of two centrioles: an old, fully mature mother centriole and a young, immature daughter centriole. The mother centriole forms the basal body of the primary cilium during G1 phase. After centriole duplication at G1/S, the cell contains two centrosomes, each consisting of a mother centriole and a daughter centriole. However, one of the centrosomes contains the oldest (“grandmother”) centriole in the cell, which was the mother centriole in the previous cell cycle. This inherent asymmetry between the mother and daughter centrioles and, by extension, between the two centrosomes in a dividing cell, seems to be critical to the maintenance of stem cell character (Wang et al., 2009; Yamashita et al., 2007). During cell division, the grandmother centriole and the ciliary membrane linked to this centriole are preferentially inherited by the daughter cell that is destined to remain a neural stem or apical radial glial cell (Paridaen et al., 2013; Taverna et al., 2014). Thus, in the absence of a microcephaly protein, human neural progenitors may fail to asymmetrically inherit the ciliary membrane or grandmother centriole, thereby losing stem cell character prematurely.

The centrosome-associated cell division machinery contains at least three more proteins that carry modern human-specific aa substitutions. Cancer susceptibility candidate 5 (CASC5), kinesin family member 18A (KIF18A), and sperm-associated antigen 5 (SPAG5) localize to the mitotic spindle, which is organized by the centrosome, or to the kinetochore, which attaches chromosomes to the spindle microtubules (Pääbo, 2014; Prüfer et al., 2014). The three proteins are expressed in the germinal zones during mid-fetal brain development. These data suggest that the centrosome-associated cell division machinery may be critical to neocortical evolution.

Another crucial neurodevelopmental gene, *AHI1*, has undergone evolutionary changes along the human lineage (Ferland et al., 2004). Mutations in *AHI1* cause Joubert syndrome, a congenital brain malformation of the cerebellar vermis and brainstem. Individuals with Joubert syndrome have motor and behavioral abnormalities, including an inability to walk due to severe clumsiness and “mirror” movements, and cognitive and behavioral disturbances. In particular, the human and non-human primate aa sequences of the N-terminal coiled-coil domain of *AHI1* are highly divergent—and this domain is totally missing in mice and rats—suggesting that *AHI1* may play a crucial, human-specific role during neurodevelopment. Overall, the studies of changes in protein-coding sequences have highlighted some interesting leads.

### Noncoding DNA and Human Brain Evolution

Even before the completion of the human genome project, it was anticipated that protein-coding sequences alone could not fully explain the human-specific aspects of our anatomy and physiology. Human and chimpanzee proteins were already known to be so similar that other regulatory mechanisms would presumably be required to account for the biological differences between these species (King and Wilson, 1975). Indeed, the human genome project has made it clear that nearly 99% of the human genome does not encode proteins and that the approximately 21,000 human protein-coding genes are in general surprisingly

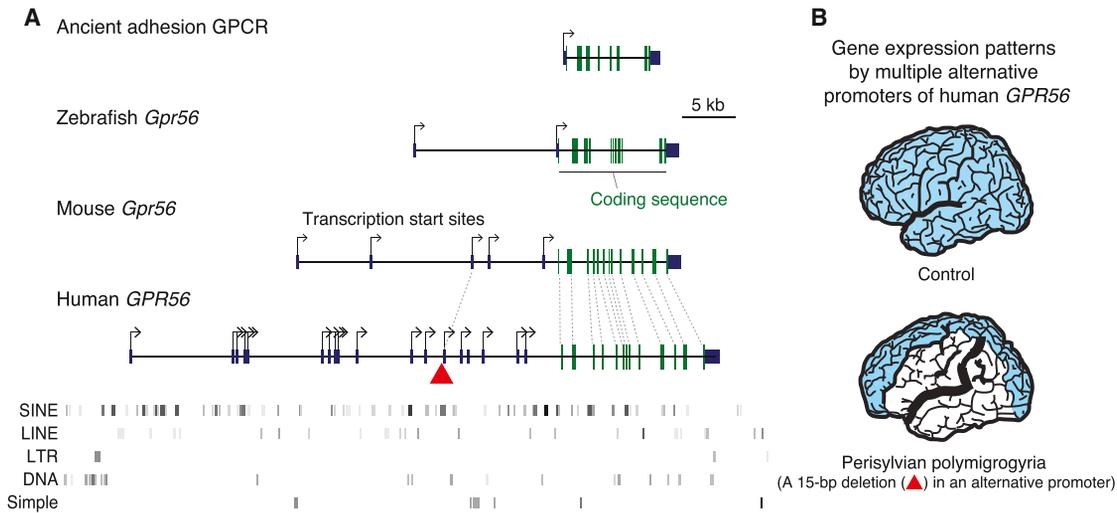
similar across placental mammals (Lander, 2011; Lander et al., 2001; Venter et al., 2001).

Although the actual number of protein-coding genes in humans is much smaller than initial estimates of approximately 100,000 genes, alternative splicing has been recognized as an important source of variation, potentially producing multiple different functional mRNAs and proteins with diverse expression patterns from the same gene (Keren et al., 2010; Nilsen and Graveley, 2010). The synapse provides ample examples of alternative splicing events that diversify its components. Clustered protocadherins are perhaps the most complex synapse-specificity and circuit-assembly molecules that are alternatively spliced (Zipursky and Sanes, 2010). With numerous alternative exons for multiple protein domains, there are approximately 350,000 possible, combinatorial protocadherin proteins from about 60 *Pcdh* loci.

Neurexins, a highly polymorphic family of synaptic receptors, exhibit distinct isoform-specific biochemical interactions and synapse assembly functions. The KH-domain RNA-binding protein SAM68 alternatively splices the *Nrxn1* gene in an activity-dependent manner, thereby dynamically controlling *Nrxn1* molecular diversity in the CNS (Iijima et al., 2011). In fact, a few critical splicing factors in the nervous system have been reported, such as NOVA, FMRP, RBFOX, and most recently, nSR100/SRRM4 (Brown et al., 2001; Gehman et al., 2011; Irimia et al., 2014; Jensen et al., 2000). nSR100 controls alternative splicing of 3–15 nucleotide “microexons,” which in turn alters protein-protein interactions, during neurogenesis. Reduced levels of nSR100 and dysregulated neuronal microexons are implicated in some cases of autism (Irimia et al., 2014). RBFOX targets are also frequently dysregulated in autism, highlighting the critical role of alternative splicing in brain development and function (De Rubeis et al., 2014; Voineagu et al., 2011; Weyn-Vanhenryck et al., 2014).

In addition to alternative splicing, almost half of the known protein-coding genes in humans utilize alternative promoters. Each promoter is regulated by a distinct set of transcription factors, which drive diverse spatial and temporal expression patterns (Davuluri et al., 2008). For example, the human *brain-derived neurotrophic factor* (*BDNF*) gene has nine promoters that are used in specific tissues and brain regions (Pruunsild et al., 2007). Aberrant use of a promoter is associated with various diseases. A polymorphism in the promoter region of the human serotonin transporter gene *SLC6A4* has also been associated with several dimensions of neurosis and psychopathology (Hariri et al., 2002).

*G protein-coupled receptor 56* (*GPR56*) demonstrates how noncoding elements and alternative promoters can control key features of cerebral cortical development. *GPR56* encodes a G protein-coupled receptor that binds extracellular matrix ligands and regulates normal neocortical development (Jeong et al., 2013; Singer et al., 2013). Mutations in the coding sequences cause polymicrogyria (“too many small gyri”) in most of the neocortex (Piao et al., 2004). Recently, polymicrogyria restricted to the neocortical areas surrounding the Sylvian fissure including Broca’s area, the primary language area, was identified (Bae et al., 2014). The causative mutation is found in the *GPR56* locus but disrupts a noncoding element, which constitutes a strong neural promoter during development, in the 5′-upstream



**Figure 1. Emergence and Evolution of a Gene**

(A) *GPR56* exemplifies how a novel gene arises and evolves. *GPR56* is critical to neocortical development, especially gyral patterning. Its protein-coding sequence arose when vertebrates and invertebrates diverged, probably by gene duplication of a preexisting adhesion G protein-coupled receptor (GPCR) with a long N-terminal extracellular domain. When placental and non-placental mammals diverged, the number of noncoding elements was dramatically increased. The majority of the novel noncoding elements were derived from transposable elements. The placental *Gpr56* gene obtained a critical noncoding element (red triangle) that constitutes a robust neural promoter, which drives regional expression in the neocortex. *GPR56* continued to obtain new noncoding elements, alternative promoters, and untranslated exons in the primate lineage. Numerous transposon-derived noncoding elements are all over the human *GPR56* gene. Most of them are likely to be evolutionarily recent, since the older insertions become mutated and unrecognizable. SINE, short interspersed nuclear elements; LINE, long interspersed nuclear elements; LTR, long terminal repeat elements; DNA, DNA repeat elements; Simple, simple repeats or micro-satellites.

(B) Multiple alternative promoters of human *GPR56* collectively drive gene expression in the entire neocortex (colored in blue). Loss of a specific noncoding element, which corresponds to the red triangle in (A), and thus loss of the associated promoter, ablate *GPR56* expression and cause neocortical malformation in the areas surrounding the Sylvian fissure bilaterally (i.e., perisylvian polymicrogyria). The affected areas include Broca's area, the primary language area for speech. Presumably, the novel noncoding elements enabled more precise and complex neocortical patterning mediated by *GPR56*. Adapted from Bae et al., 2014.

regulatory region of *GPR56*. It turned out that the human *GPR56* has at least 17 alternative promoters, whereas mouse *Gpr56* has only a handful (Figure 1). Intriguingly, the human noncoding element directs gene expression in a highly restricted pattern, whereas the mouse element directs more diffuse expression. These data suggest that diversification of noncoding elements and promoters results in region-specific gene expression in the cortex, thus allowing independent development and functional specialization of distinct neocortical regions.

Noncoding regions in the human genome have numerous transposable elements, highly repetitive DNA, introns, and pseudogenes, as well as RNA genes. What proportion of noncoding DNA is functional is an open question, which deserves a substantial amount of careful study (Doolittle, 2013; ENCODE Project Consortium, 2012; Palazzo and Gregory, 2014). Obviously, not all DNA in the human genome can be functional. The human genome (comprising three billion bp) contains roughly eight times as much DNA as that of the pufferfish *Fugu rubripes* (0.4 billion bp) but is about one-fortieth the size of the genome of the lungfish *Protopterus aethiopicus* (140 billion bp) (<http://www.genomesize.com>) (Palazzo and Gregory, 2014). Therefore, the relationship between genome size and the complexity of the organism is not a straightforward one.

Transposable elements are the most prevalent type of noncoding DNA, accounting for approximately 45% of the human genome. Long interspersed nuclear element-1 (LINE1) retrotransposons are autonomous transposable elements that can retrotranspose a copy of their own RNA, as well as other

RNAs, such as short interspersed nuclear elements (SINEs), and cellular mRNAs. Most transposable elements are inactive due to mutations, but LINE1 is still active and has been implicated in dozens of diseases (Hancks and Kazazian, 2012). Transposable elements clearly have been active over evolutionary time to introduce innovations to the genome during evolution. Many conserved noncoding elements were derived from transposable elements. Comparison of placental and marsupial genomes demonstrates that the number of conserved noncoding elements is sharply increased in the genome of placental mammals (Lindblad-Toh et al., 2011; Mikkelsen et al., 2007). Over the course of evolution, transposable elements provide novel binding sites for the host transcriptional machinery and thus help create novel regulatory networks (Davidson, 2010; Wray, 2007). For example, many of the complex alternative promoters and exons of human *GPR56* were derived from LINES and SINEs (Bae et al., 2014).

The concept that changes in noncoding sequence generated evolutionary changes has been strongly supported by increasingly systematic studies of regulatory elements that control nervous system expression. Active regulatory elements are usually associated with p300/CBP and epigenetically marked by H3K27ac. Thus, p300/CBP chromatin immunoprecipitation sequencing followed by *lacZ* reporter mouse transgenesis can systematically reveal enhancers active in the telencephalon (<http://enhancer.lbl.gov>). This approach identified numerous potential embryonic forebrain enhancers that are evolutionarily conserved or divergent between mice and humans. At least

**Table 2. Resources for Neurogenetics**

Name	Contents	Link
SFARI Gene	a database of genes implicated in autism	<a href="http://sfari.org/resources/sfari-gene">http://sfari.org/resources/sfari-gene</a>
SchizophreniaGene	a database of genes implicated in schizophrenia	<a href="http://www.szgene.org">http://www.szgene.org</a>
Allen Brain Atlas	a comprehensive database of gene expression patterns and connectivity maps in the brains of humans, non-human primates, and mice during development and in adulthood	<a href="http://www.brain-map.org">http://www.brain-map.org</a> , <a href="http://www.brainspan.org">http://www.brainspan.org</a>
Human Brain Transcriptome	a database of spatio-temporal gene expression patterns in the developing and adult human brain	<a href="http://hbatlas.org">http://hbatlas.org</a>
VISTA Enhancer Browser	a resource for experimentally validated human and mouse noncoding fragments with gene enhancer activity as assessed in transgenic mice	<a href="http://enhancer.lbl.gov">http://enhancer.lbl.gov</a>
ENCODE	a comprehensive database of potentially functional elements in the human genome: unannotated exons, regulatory elements, RNA genes, etc.	<a href="https://www.encodeproject.org">https://www.encodeproject.org</a>
UCSC Genome Browser	a user-friendly gateway to a large collection of genomes, gene expression, regulation, variation, and evolution	<a href="http://genome.ucsc.edu">http://genome.ucsc.edu</a>
Ensembl	a user-friendly gateway to a large collection of genomes, gene expression, regulation, variation, and evolution	<a href="http://www.ensembl.org">http://www.ensembl.org</a>
CEEHRC Platform	a database of epigenome	<a href="http://www.epigenomes.ca">http://www.epigenomes.ca</a>

Multiple online resources provide information on genomes, epigenomes, brain transcriptomes, brain connectivity maps, and genes implicated in autism and schizophrenia.

one-third of human brain candidate enhancers are unique to humans and not functionally conserved in mice, suggesting that a significant portion of human brain enhancers emerged after the divergence of primates and rodents (Visel et al., 2013) and that changes in enhancer elements represent a dynamic evolutionary mechanism.

Recently, the importance of noncoding RNAs in brain development, evolution, and disease has been gradually appreciated (Esteller, 2011; Qureshi and Mehler, 2012; Sauvageau et al., 2013). For example, there are more than 100 primate-specific and 20 human-specific microRNAs that are expressed in the developing brain (Berezikov, 2011). The primate-specific microRNAs target cell-cycle and neurogenesis regulators, thereby increasing proliferation of neocortical progenitors. Primate-specific co-evolution of microRNAs and their targets in the germinal zones may have contributed to neocortical expansion in primates (Arcila et al., 2014).

### Transcriptomic Analyses Reveal How the Human Brain Develops and Evolves

Changes in the noncoding genome are reflected in differences in levels or patterns of transcription, and our understanding of the architecture of gene transcription has advanced considerably in the last few years. RNA-seq of prenatal and postnatal brains in health and disease has provided an enormous wealth of data on gene expression (Table 2) (Hawrylycz et al., 2012; Johnson et al., 2009; Kang et al., 2011; Lein and Hawrylycz, 2014; Miller et al., 2014; Pletikos et al., 2014; Zeng et al., 2012). The human brain expresses numerous genes; approximately 80%–95% of protein-coding genes are expressed in at least one brain region during at least one period of development or adulthood. Co-expressed genes suggest anatomical structures, cell types, and molecular pathways that are potentially critical to brain development and function. Comparison of brain RNA-seq from different species reveals evolutionarily conserved and divergent gene expression patterns.

The adult human neocortex has multiple distinct functional areas, such as the visual area in the occipital lobe, the auditory area in the temporal lobe, and Broca's language area in the left frontal lobe. Surprisingly, it turned out that transcription profiles in the adult neocortex are relatively homogeneous throughout the cortex, although a certain level of heterogeneity still does exist (Hawrylycz et al., 2012; Kang et al., 2011; Pletikos et al., 2014), suggesting that the distinct function of each neocortical area becomes apparent only at a higher level of complexity beyond gene expression. Comparison of the adult human and non-human primate brain transcriptomes shows that primates share common, closely matched gene expression patterns in the neocortex, while the human frontal lobe is transcriptionally more complex (Bernard et al., 2012; Konopka et al., 2012; Pletikos et al., 2014). In contrast, the mouse neocortex transcriptome is markedly different from the primate transcriptome. For example, the *synaptic vesicle protein 2c* (*SV2C*) gene is expressed preferentially in layer 3 pyramidal neurons in primates but is expressed in layer 5 pyramidal neurons in mice. The *prodynorphin* (*PDYN*) gene, which encodes dynorphin and other kappa opioid receptor peptide agonists, is expressed in layers 4 and 5 in primary visual cortex in primates but is expressed only in scattered GABAergic interneurons in mice (Bernard et al., 2012).

The transcriptome of the developing, prenatal human neocortex, however, is quite heterogeneous across different cortical areas (Johnson et al., 2009; Kang et al., 2011; Miller et al., 2014). First, transcriptional differences are found between different neocortical areas (e.g., orbital/dorsolateral/ventrolateral/medial prefrontal, motor-somatosensory, parietal association, temporal auditory, temporal association, and occipital visual neocortex). Each area expresses a large number of specific gene expression and alternative splicing patterns, often with a rostrocaudal, mediolateral, or frontotemporal gradient. For example, *cerebellin 2 precursor* (*CBLN2*) is enriched in the prefrontal cortex, *neuropeptide Y* (*NPY*) in non-frontal areas, and *FOXP2* in the perisylvian cortex; the full-length isoform *ROBO1a* is enriched in the

temporal lobe, whereas the alternative short isoform *ROBO1b* is slightly enriched in the prefrontal cortex. Regionally enriched transcriptomes form a co-expression network; for example, the developing prefrontal cortex has a distinct gene network that contains a number of schizophrenia genes (Gulsuner et al., 2013). Differentially expressed genes are more frequently associated with human-specific evolution of putative *cis*-regulatory elements (Johnson et al., 2009).

Transcriptional differences in the prenatal human are also pronounced in different germinal and postmitotic layers. Laminar transcriptomic signatures are likely to reflect the cellular diversity and developmental processes of the prenatal human neocortex. Excitatory neurons, which constitute the majority of neocortical neurons, are derived from neocortical progenitors called radial glial (RG) cells in a germinal zone called the ventricular zone (VZ) (Ayoub et al., 2011; Fietz et al., 2012). RG cells and the VZ are common to all mammals and express cell proliferation genes (e.g., *SOX2*, *PAX6*, *ASPM*), with human RG cells showing a number of species-specific signaling pathways such as platelet derive growth factor D (PDGFD) signaling (Lui et al., 2014). On the other hand, differentiated, postmitotic neurons express distinct neuronal genes (e.g., *LMO4*, *FOXP1*, *CUX1*). Remarkably, humans, non-human primates, and placental mammals with an enlarged brain have an additional, expanded germinal zone called the outer subventricular zone (OSVZ), where a unique population of neocortical progenitors called outer or basal RG cells are abundant (Betizeau et al., 2013; Fietz et al., 2010; Hansen et al., 2010; Lui et al., 2011; Reillo et al., 2011; Smart et al., 2002). However, the OSVZ shows minimal transcriptional differences from the inner subventricular zone (Miller et al., 2014), which awaits more sophisticated investigations, preferably based on single-cell transcriptomes, because individual differences of neocortical progenitors may be obscured by pooled population means.

In this regard, there are a couple of debates on progenitors and the OSVZ in the neocortical development field. The first debate is on how neocortical progenitors generate postmitotic neurons diverse in shape, size, connectivity, and electrophysiological and molecular properties. One theory is that a common kind of progenitor sequentially generates all subtypes of neurons, with progressive restriction of progenitor fate potential (Guo et al., 2013). The other theory is that lineage-restricted or distinct subtypes of progenitors co-exist and are pre-specified to generate different subtypes of neurons (Franco et al., 2012). As both theories were mainly explored in mice, other animals with an enlarged and gyrencephalic neocortex should be examined rigorously and carefully to resolve this debate. The second debate is on what roles outer RG cells in the OSVZ have played for neocortical expansion in carnivores and primates during evolution. Outer RG cells show unique morphology and cellular movements and are abundant in primates and carnivores, while they are virtually absent in mice. As neocortical expansion strongly correlates with gyrification (Tallinen et al., 2014), potential roles of outer RG cells in gyrification and expansion were explored. Examination of multiple mammals revealed that outer RG cells are also present in the Amazonian agouti, a gyrencephalic rodent, and the marmoset, a lissencephalic primate (García-Moreno et al., 2012; Kelava et al., 2012). Thus, whereas the mere presence of outer RG cells alone cannot explain gyrifica-

tion, they are nonetheless in a key context to potentially regulate gyral patterns. Perhaps specific ablation of outer RG cells in animal models other than mice or examination of outer RG cells in microcephalic brains during development may resolve this debate.

Human-specific transcriptomic analyses and the above-mentioned debates raise questions about the widespread use of mice as proxies for humans in research on neocortical development and function and in psychiatric drug development. The features that are conserved between mice and humans are, no doubt, of paramount importance, and thus will continue to be studied. However, there are a few evolutionarily novel features unique to the developing human or primate neocortex, such as precursor neurons migrating into the cortical primordium from the subpallium even before local neurogenesis in the VZ, von Economo neurons located in layer 5 of the anterior cingulate and fronto-insular cortex, and so on (Clowry et al., 2010; Geschwind and Rakic, 2013; Radonjić et al., 2014). Hence, the precise introduction of mutations that cause severe human neocortical phenotypes into the mouse genome often leads to negligible phenotypes in the mouse. Alternatively, larger animal models, such as the ferret, a gyrencephalic carnivore, and the marmoset (Fietz et al., 2010; García-Moreno et al., 2012; Kelava et al., 2012), or 3D culture of human neural stem cell-derived cerebral organoids or mini-brains (Bae and Walsh, 2013; Lancaster et al., 2013; Stein et al., 2014) may provide new insights into human neocortical development and evolution. Regardless, caution must be taken against quick extrapolation of results from ferrets, inbred mice, or cell culture systems to human neocortical development per se. Both human fetal brain tissues and animal/cell models need to be studied in parallel.

### The Dynamic Genome of the Single Neuron

Somatic mutations, which arise during the cell divisions that generate the embryo, have recently been implicated in several important neurological diseases. Somatic mutations that were identified in human hemimegalencephaly (HMG) revealed the dysregulated phosphoinositide 3-kinase (PI3K)-AKT3-mTOR pathway as the causative mechanism (Lee et al., 2012; Poduri et al., 2012; Rivière et al., 2012). HMG patients suffer from intractable epilepsy, which can be controlled only by surgical resection of the affected brain tissue. Prior to the discovery of somatic mutations in the PI3K-AKT3-mTOR pathway in HMG, the genetic etiology of HMG was poorly understood, although there had been rare cases of HMG associated with the overgrowth syndromes tuberous sclerosis complex (TSC) (Cartwright et al., 2005) and Proteus syndrome (Griffiths et al., 1994). Direct study of resected tissue led to the identification of a recurrent AKT3 mutation (E17K) in HMG. AKT3 is a RAC-gamma serine/threonine protein kinase that is highly expressed in the developing neocortex, and the E17K mutation renders AKT3 constitutively active (Lee et al., 2012; Poduri et al., 2012). Additional somatic mutations causing HMG have been identified that implicate key upstream and downstream components of this pathway, namely PI3K and mTOR (Lee et al., 2012; Rivière et al., 2012). These results are consistent with previous reports that HMG is occasionally seen in patients with TSC. TSC is caused by mutations in *TSC1* and *TSC2*, which encode members of the mTOR pathway. Surprisingly, levels of mosaicism as low as

8% are sufficient to disrupt the normal architecture and function of the neocortex in HMG (Evrony et al., 2012; Lee et al., 2012; Poduri et al., 2012; Rivière et al., 2012).

Given the difficulty of detecting somatic mosaic mutations by standard methods such as Sanger sequencing or even whole-exome sequencing, it is not known how common a cause of complex neurogenetic disease they are. A recent study of brain malformations used “deep sequencing” of candidate genes to an average read depth of more than 200 to identify mutations that might be present in a small proportion of cells. Analysis of leukocyte-derived DNA samples from 158 persons with brain malformations identified somatic mosaicism that had been difficult to detect previously. This study revealed that such somatic mutations represented more than a quarter of all identified mutations and were often missed both by Sanger sequencing and by some whole-exome sequencing with typical calling algorithms. Substantial disability in the form of epilepsy and intellectual disability could be caused by mutations present in as few as 10% of brain cells. Presumably, these mutations occurred in the early post-zygotic stages before neurogenesis. This report suggests that high-coverage sequencing panels provide an important complement to whole-exome and genome sequencing in the evaluation of somatic mutations in the human brain (Jamuar et al., 2014).

While low levels of somatic mosaicism are clinically important, they are also difficult to detect. Typical whole-genome sequencing or Sanger sequencing of pooled DNA from many cells covers each base in the genome 50–60 times; at this level of coverage, it is possible to dismiss low levels of true somatic mosaicism as errors (false-negative results). One way to solve this problem is to amplify and sequence the genomes of individual cells. Single-cell sequencing has already been used to evaluate the clonal evolution of tumors (Hou et al., 2012; Navin et al., 2011; Xu et al., 2012) and the spontaneous mutation of sperm cells (Lu et al., 2012; Wang et al., 2012). In the human brain, single-cell sequencing has been successfully used to detect somatic mosaicism in HMG and to evaluate the rate of somatic LINE1 retrotransposition (Evrony et al., 2012). Single-cell sequencing has also been used to measure the prevalence of large copy number variants in the brain (McConnell et al., 2013; Cai et al., 2014). Less than one unique insertion was detected per neuron, suggesting that LINE1 retrotransposition is not a major generator of neuronal diversity in the brain. Interestingly, single-cell sequencing can repurpose the LINE1 retrotransposition events for cell lineage analysis in the developing human brain (Evrony et al., 2015).

One caveat of single-cell sequencing is that somatic mutations that occurred during or after neurogenesis can be identified only in surgically resected or postmortem brain tissues. Noninvasive identification of somatic mosaicism limited to the brain is impossible. Nonetheless, the optimization of single-cell and high depth sequencing will allow us to address the role of somatic mosaicism in brain disorders, in cases where mosaic mutations are detectable in a small proportion of accessible cells, such as leukocytes and skin fibroblasts (Poduri et al., 2013).

### Neurogenetics is Just the Beginning

Though the human brain is extremely complex, the proportion of genes that govern its development, and for which we have tenta-

tively assigned functions, grows inexorably. Functional studies of human neurogenetic mutations are obviously challenging, due to the fact that the human brain has evolved dramatically in the last 2–3 million years, gaining novel circuits, cell types, and signaling pathways that are rare or absent in animal models (Geschwind and Rakic, 2013; Molnár et al., 2014; Pääbo, 2014). Nonetheless, by identifying critical neural genes and studying their functions in diverse model organisms using innovative tools, *Homo sapiens* keeps marching on in the endeavor to understand its brain. Perhaps the process rather than the product of this endeavor may help define who we are.

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