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The Evolution of the Human Cerebral Cortex Development

A Genomic Perspective

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Abstract

The extraordinary cognitive abilities that humans possess, such as syntactical-grammatical language, abstract thinking, episodic memory, or complex reasoning, are largely dependent on the brain, and more specifically on its surface, the cerebral cortex, as was initially proposed by Thomas Willis in 1664. Since then, neuroscience has endeavored to decipher what makes the human brain so unique when compared with other species. Early studies were based on comparative brain anatomy between humans and other extant or extinct species, in the latter case based on data compiled from fossil records. More recently, comparison of the number of neurons and studies of cortical development have improved our understanding of the field. Nowadays, in the era of genomics, new possibilities have arisen for determining changes in gene expression or regulatory activity that underlie the observed differences in phenotypes. This chapter summarizes what is known about the human cerebral cortex. It focuses on the neocortex, which represents about 80% of the human brain mass, and places it into an evolutionary context by considering other hominins, nonhuman primates, and mammals. Finally, it explores the role of genomics in elucidating the shared and unique features of human nervous system development, organization, and function.

Organization and Development of the Human Cerebral Cortex

The cerebral cortex represents half of the volume of the human nervous system and consists of two main parts: the neocortex and the allocortex (i.e., olfactory system and hippocampus) (Krubitzer and Kaas 2005). The former, the largest section, is involved in higher-order brain functions. Recent studies estimate that the human cerebral cortex contains approximately 16.34 billion neurons that pass signals to each other via synaptic connections (Azevedo et al. 2009;

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Sigaard et al. 2016). It has been estimated that there are approximately 149,000 to 176,000 kilometers of myelinated axons connecting these neurons in the adult cerebral white matter (Marner et al. 2003). A total of approximately 164 trillion synapses have been estimated in an adult neocortex (Tang et al. 2001), while the published number of synapses received per neocortical neuron varies from 7,200 to 80,000 (Huttenlocher 1979; DeFelipe et al. 2002; Pakkenberg et al. 2003).

To support the complex circuitry and functions of the cortex, its component neurons are highly specialized and exhibit a variety of axonal projections, dendritic patterns, and electrophysiological properties. As a consequence, there may be thousands of distinct neuronal subtypes in the cerebral cortex, far more than in any other central nervous system structure. However, these subgroups can be broadly classified into two major groups: the glutamatergic excitatory projection neurons and GABAergic inhibitory interneurons (DeFelipe and Fariñas 1992; Petilla Interneuron Nomenclature Group et al. 2008). Accounting for about 80% of cortical neurons, the projection neurons occupy a central position in all cortical circuits. Extending long axonal projections to other structures, both within and beyond the cortex, projection neurons constitute the sole output system of the cortex. They also compose the largest input system of the cortex (i.e., corpus callosum) (Aboitiz and Montiel 2003; Chedotal and Richards 2010) and represent the major target of afferents from other structures of the brain. The great majority of projection neurons are pyramidal neurons, which exhibit a highly polarized morphology with a pyramid-shaped cell body, a long apical dendrite directed to the pia mater, and multiple basal dendrites extending laterally from the cell soma. These dendrites contain numerous spines—bulbous postsynaptic protrusions—along their lengths. The other major class of cortical neurons, the GABAergic inhibitory interneurons, do not have significant numbers of dendritic spines and tend to project locally rather than sending long axons across or beyond the cortex.

A hallmark of the mammalian cerebral neocortex is that its neurons are functionally organized at two levels into six somewhat arbitrary layers and roughly 200 cytoarchitectonically and functionally distinct areas (Krubitzer and Kaas 2005; Glasser et al. 2016). This immensely complex organization of the human cerebral cortex is the result of a prolonged and dynamic development, and will be summarized next (for a comprehensive review, see Geschwind and Rakic 2013; Silbereis et al. 2016). A critical and evolutionary conserved initial step in cortical development involves the establishment of dorsal-ventral, rostral-caudal, and medial-lateral axes within the forebrain anlage. Studies have uncovered a number of transcription factors, morphogens, and receptors involved in defining these axes (Grove and Fukuchi-Shimogori 2003; Sur and Rubenstein 2005; O'Leary et al. 2007). Neuroepithelial cells in the ventricular zone (VZ) serve as the stem or founder cells of the nervous system and divide predominately symmetrically to generate two progenitor daughter cells, thus exponentially expanding the pool of progenitor cells. After

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the onset of neurogenesis, these progenitors transform into apical radial glial cells (aRGCs), which have the capacity to divide asymmetrically and generate two different cell types. An identical aRGC, a neuron, or a basal progenitor cell (i.e., intermediate progenitor, basal radial glial cell, bRGC, or others) are possible outcomes of mitosis occurring in an apical radial glial cell (Lui et al. 2011; Hevner and Haydar 2012; Taverna et al. 2014). Different subtypes of these basal progenitors lie in an incipient subventricular zone (SVZ) and are also capable of differentiating. In fact, most cortical neurons are generated from basal progenitors, which are abundant in the human cerebral cortex during cortical development. Once neurons are born, they migrate using radial glial cells as a guiding scaffold toward their final laminar destination, forming the subplate and layer 1, and then, consecutively, prospective layers 6 to 2 in the cortical plate (Molnár and Clowry 2012; Geschwind and Rakic 2013; Guo and Anton 2014; Johnson and Walsh 2017). After neurogenesis, gliogenesis starts as RGCs lose their apicobasal polarity and differentiate into different types of glial cells, followed by synaptogenesis, myelination, and the synaptic pruning that continues through adolescence (Silbereis et al. 2016). These neurons, which originate in the VZ/SVZ and radially migrate into the developing cortical plate, are primarily projection neurons (Leone et al. 2008; Greig et al. 2013; Han and Sestan 2013; Lodato and Arlotta 2015; Dwyer et al. 2016; Jabaudon 2017); in contrast, cortical interneurons, which are notoriously diverse in morphology and function, originate largely in the ganglionic eminences and migrate tangentially into the cortical plate (Wonders and Anderson 2006; Fertuzinhos et al. 2009; Bartolini et al. 2013; Hansen et al. 2013; Ma et al. 2013; Hu et al. 2017; Wamsley and Fishell 2017).

Evolution of the Human Cerebral Cortex

Comparison of brain features between humans and related species is essential to understand which traits are specific to humans, as well as to develop an understanding of the brains of common ancestors. Humans are hominins, a subgroup of primates which, in turn, are a subgroup of mammals. About 340 million years ago (mya) the early amniotes emerged from amphibians and divided into sauropsids, which evolved into extant reptiles, birds, and synapsids, leading to modern mammals. A basic brain structure of an external cortex and a few subcortical nuclear structures with olfactory, sensory, and memory functions are shared between reptiles, birds, and mammals (Krubitzer and Kaas 2005). However, whereas reptiles have a thin dorsal cortex with a single layer of pyramidal neurons and fewer inhibitory neurons, all extant mammals have a more complex six-layer neocortex (Krubitzer and Kaas 2005). In fact, although the time and manner in which the neocortex appeared remains unknown, this trait seems to be specific to mammals, as it has not been found in nonmammals. Over 4,600 present-day species of mammals, which are greatly diverse

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in terms of absolute and relative brain size, number of neurons and areas, and even gyrencephalization, have evolved from early mammals that inhabited the earth ~200 mya. These animals were probably small in size with a small neocortex organized into few areas, about 15 to 20 mostly sensory areas. Motor and premotor cortex emerged in placental mammals at most 125 mya. Early primates, in turn, were small, nocturnal, and arboreal animals that emerged about 80 mya with an already elaborated visual and motor cortex. Indeed, the visual cortex in all primates is remarkably complex. Finally, the number of specialized areas in primate neocortex is higher than in nonprimates.

Compared to nonhuman primates, the human brain is larger in size and in number of neurons; indeed, it is about three times the size and has about twice as many neurons as the chimpanzee brain (Collins et al. 2016). Compared to Neanderthals, human brain volume is similar, although the parietotemporal lobe is bigger in the modern human brain (Balzeau et al. 2012). Importantly, the human neocortex has dramatically increased in size as compared to more distantly related mammalian species—a 1,000-fold increase in comparison with mouse neocortex (Geschwind and Rakic 2013). That expansion, though, was not accompanied by a similar change at the whole brain level. Instead of augmenting in thickness, cortical expansion is the result of (a) an increase of the number of progenitors in the VZ and (b) indirect neurogenesis from aRGCs via basal progenitors, especially bRGCs, which are highly abundant and proliferative in humans. The latter is related to the expansion of the primate SVZ, particularly the outer SVZ (oSVZ), where bRGCs are located. While brain size and number of neurons in primates is a general predictor of cognitive abilities, these features are not fully sufficient to explain human cognitive capabilities; other animals (e.g., elephants, certain whales) surpass humans in each of these characteristics (Sousa et al. 2017a). Similarly, the expansion of the oSVZ and an abundance of bRGCs are common in species with a large neocortex (Reillo et al. 2011). In addition, there is no conclusive evidence of any brain area, cell type, or neural circuit that is completely new in humans (i.e., not present in other primates). However, there is increasing evidence for potential and confirmed changes in the developmental features, morphology, molecular profiles, and quantity of particular neural cell types in the human lineage (Elston et al. 2011; Kwan et al. 2012a; Bianchi et al. 2013; Sousa et al. 2017b). For instance, a subtype of pyramidal neurons, the von Economo cells posited to promote rapid neuronal communication, are found in several primates, elephants, and cetaceans but are particularly large and abundant in humans (Nimchinsky et al. 1999). Small changes like these in the connectome (neural circuits and networks formed by different neural cell types and their synaptic connections) can lead to functional changes. In fact, several studies suggest that neural circuits have undergone structural, molecular, and functional reorganization in the human lineage (Sousa et al. 2017b). Notably, the singular complexity of the human brain takes over two decades to be fully constructed, from neurogenesis to neural circuit assembly and maturation. This translates into a particularly

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long gestational time, infancy, childhood, and adolescence compared to other mammals (Zhu et al. 2018).

The Genomic Revolution

A key challenge is to understand the countless molecular and cellular processes as well as the precise system that regulates those processes, which are activated in humans over the long period of time in which the complete formation of the human cerebral cortex occurs. Over the last decades, with the advent of genetic and genomic technologies and the sequencing of large amounts of DNA, some progress has been made (Figure 3.1). The availability of genetic sequences from different species has had a direct impact on the improvement of our knowledge about phylogenetic relationships in mammals and, consequently, on the feasibility of effective comparative studies. The whole genome sequencing of the mouse and human genomes (Lander et al. 2001; Mouse Genome Sequencing Consortium 2002), followed a few years later by that of the chimpanzee (Waterson et al. 2005), enabled the first comparative genomic studies at a genome-wide level. As a result of a substantial reduction in the cost of genomic sequencing, massive sequencing has become feasible, and we quickly transitioned from one or a few individual whole genome sequences to thousands of human individuals sequenced. It is noteworthy that bioinformatics tools as well as increasingly complex methodology are being implemented in parallel to process these massive amounts of data.

Multiple sequence alignments among primates revealed a plethora of human-specific substitutions altering protein-coding sequences. The majority of those human-specific variants have not been functionally characterized. One example, *FOXP2*, has been intensively analyzed due to its relationship with language. It is a highly conserved gene during mammal evolution, but it presents amino acid substitutions in the lineage leading to humans that have been related to deficiencies in speech. These variants were introduced in mice promoting a change in neural-restricted phenotypes, including dendrite morphology and transient differences in ultrasonic vocalization (Enard et al. 2009). In contrast to genetic substitutions, gene duplication provides new genetic material for evolutionary forces to act upon, and thus represents a major source of evolutionary novelty, as Susumu Ohno (1970) established in his seminal book *Evolution by Gene Duplication*. To study gene families that have undergone recent gene duplication presents a further difficulty due to the similitude between paralogous copies. Nonetheless, there are at least three gene families with human-specific expansions that have been related with neurodevelopmental functions:

1. The *SRGAP2* gene has several duplications in humans that arose after the separation with chimpanzees (Dennis et al. 2012). One duplication

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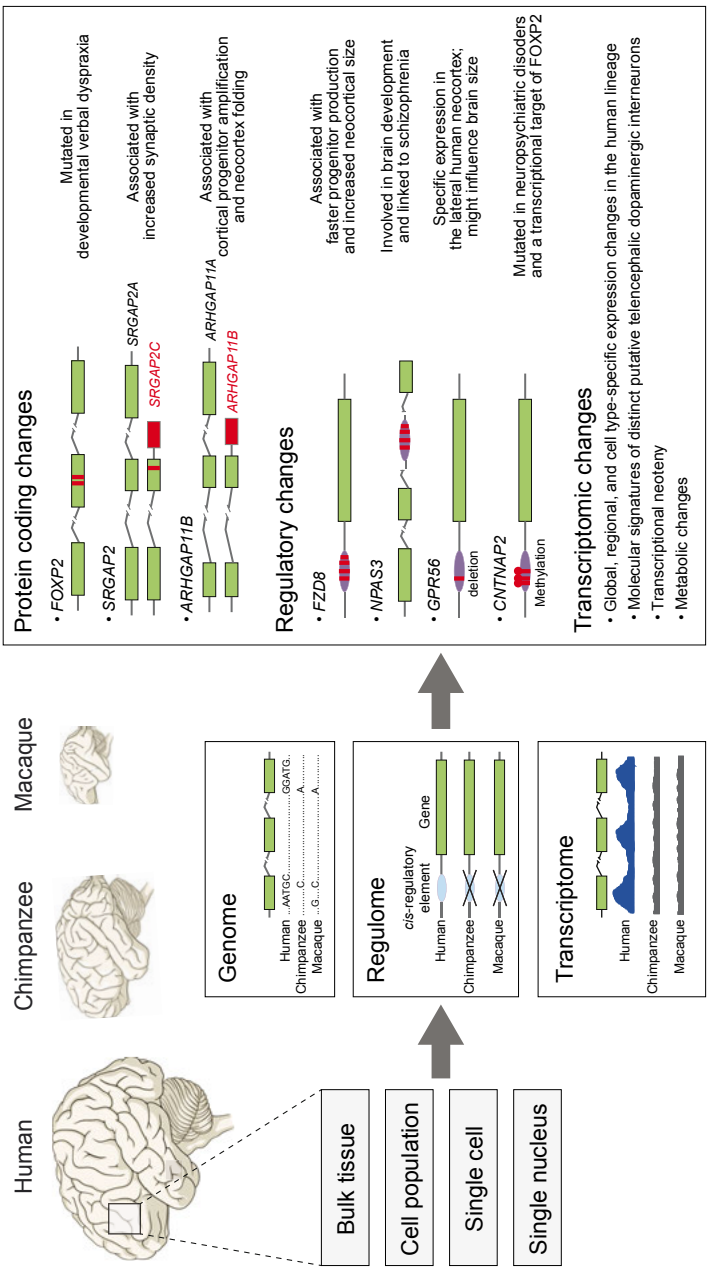

Figure 3.1 Evolutionary changes in the human cerebral cortex identified with genomics studies. Analysis at different levels can be done to unravel the genetic and molecular causes of brain phenotypic differences between species. General approaches are represented here as are examples of human-specific features (marked in red).

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is fixed in all human populations analyzed and interferes with the function of the ancestral copy of this gene, resulting in increased synaptic density in neocortical pyramidal neurons and prolonged spine maturation (Charrier et al. 2012; Fossati et al. 2016).

2. Studies in mice demonstrate a second human-specific gene, *ARHGAP11B*. Highly expressed in radial glial cells, *ARHGAP11B* promotes aRGCs to undergo symmetric divisions with two basal progenitors as outcome, and is capable of producing neocortical folding in otherwise lissencephalic mouse brains (Florio et al. 2015).
3. *DUF1220* is a domain that has expanded in the human genome extraordinarily, mainly in the *NBPF* gene family (Popesco et al. 2006; O'Bleness et al. 2012). Deletions and duplications of this domain have been associated with micro and macrocephaly (Brunetti-Pierri et al. 2008) as well as correlated with brain size (Zimmer and Montgomery 2015), and it is putatively expressed in the VZ early during cortical neurogenesis, promoting proliferation in neural stem cells (Keeney et al. 2015).

As early as 1975, Mary-Claire King and Allan Wilson (1975) hypothesized that protein sequence-altering mutations could not account for the full range of phenotypic differences observed between humans and nonhuman primates. Since then, many advances have been made in the study of the impact of gene regulation on human brain evolution. Discussed in depth below, we first need to understand the relevance of gene expression changes during cortical development. Microarrays, RNA sequencing (RNA-seq), and other technologies allow for the transcriptomic profiling of particular tissues or specific regions of a given tissue. Some early comparison studies of gene expression among tissues reported that genes expressed in the brain have evolved at higher rates than those expressed in other tissues, but other studies have argued the contrary (Bustamante et al. 2005; Nielsen et al. 2005; Wang et al. 2007a). Recent comprehensive analyses of the human brain and particular neocortical areas across time have revealed that an immense number of coding and noncoding RNAs are involved in neuronal functions. In other words, the human brain is dynamically regulated across regions and time, more pronouncedly during early and mid-fetal development (Johnson et al. 2009; Kang et al. 2011; Miller et al. 2014; Li et al. 2018). Other genomic studies have been able to identify human genes displaying species-specific expression patterns that may contribute to evolutionary changes in cortical development, structure, and function (see reviews by Somel et al. 2013; Silbereis et al. 2016; Sousa et al. 2017a). Recent studies of gene expression profiles between adult brain regions of human, chimpanzee, and macaque monkey revealed extensive transcriptional differences between homologous brain regions and cell types in the human lineage (Sousa et al. 2017b; Zhu et al. 2018). Many of the genes that exhibit human-specific global, regional, and cell type-specific expression patterns

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encoded transcription factors, ion channels, and neurotransmitter enzymes and receptors. Changes in the expression of these proteins can affect function of neural circuits by altering transcription, electrophysiological properties, or neurotransmission. Furthermore, the same study showed that humans may have evolved a distinct dopaminergic neuron system in the cerebral cortex and striatum. Specifically, Andre Sousa, Ying Zhu, and colleagues characterized a rare and unusual population of subpallium-derived putative dopaminergic interneurons enriched in the human striatum and present in the human neocortex but not in the neocortices of African great apes (i.e., common chimpanzee, bonobo, and gorilla), which are our closest living relatives. Moreover, they uncovered evidence that these interneurons likely switch their transmitter system from somatostatin to dopamine, a phenomenon that has never been reported in the cerebral cortex.

Because bulk RNA-seq experiments aggregate data across the diverse range of cell types found in the brain, it is essential to analyze gene expression within individual cell populations. Thus, the ability to determine the gene expression pattern of a small number of cells or single cells is of great importance for resolving a variety of problems in the context of human brain evolution. In fact, the last decade has witnessed a rapid advance in single-cell RNA-seq technologies (Johnson and Walsh 2017; Lein et al. 2017), which represents an unbiased approach to explore evolution and development within the context of specific and defined cell types. An increasing number of cell types and subtypes in the developing and adult human cerebral cortex have been characterized in a systematic manner at an unprecedented pace using single-cell or nucleus RNA-seq (Florio et al. 2015; Pollen et al. 2015; Lake et al. 2016; Onorati et al. 2016; Nowakowski et al. 2017; Li et al. 2018; Zhong et al. 2018). These studies not only confirmed the transcriptomic and developmental signatures of well-established cell types, they discovered previously unknown gene expression patterns in individual human neural cell types. With increasing reproducibility and scalability, single-cell RNA-seq technology has undoubtedly become, and will remain, an important experimental platform for cell type discovery and is poised for cross-species comparison at cellular resolution.

Gene Regulation and Human Accelerated Regions

The hypothesis of Mary-Claire King and Allan Wilson has been supported by many studies utilizing whole genome sequencing and chromatin immunoprecipitation with sequencing (ChIP-seq). In fact, a large-scale study of the impact of positive selection on coding and noncoding regions in the human genome emphasized not only the importance of noncoding regions in the evolution of human-specific traits, but also showed that noncoding changes have played a particularly large role in the evolution of the human nervous system (Haygood et al. 2010). This idea has led the way for a plethora of research delving into

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the role of noncoding DNA and gene regulation in the evolution of the human cortex. These studies are very diverse, given that gene regulation is a highly complex process that occurs at several levels. Underlying sequence changes (including substitutions, deletions, and duplications) can influence the regulatory activity of proximal regulatory elements, or promoters, as well as distal elements such as enhancers. However, gene regulation is also dictated by epigenetic factors (e.g., histone modifications and DNA methylation). Here we focus on examples of evolutionary changes in gene regulation at each of these levels, and their potential impact on the development of unique aspects of the human brain.

Sequencing-based comparative genomics studies have allowed for the identification of regulatory regions that have acquired an excess of substitutions in the human lineage, termed human accelerated regions (HARs). Scanning the genome for regions that are highly conserved in other species, while containing an enrichment of human-specific substitutions, has resulted in the identification of over 2,000 HARs. It is hypothesized that HARs are responsible for producing human-specific gene expression patterns and, thus, human-specific phenotypes and traits (Pollard et al. 2006a; Prabhakar et al. 2006; Bird et al. 2007). Further analysis of HARs regions utilized additional genomic data, including chromatin state and transcription factor binding sites, and found that at least 30% of HARs are predicted to act as developmental enhancers (Capra et al. 2013). Several subsequent studies have shown via transgenic mouse enhancer assays that HARs drive distinct reporter gene expression patterns in the developing mouse brain as compared to orthologous sequences from other species; this indicates that HARs may play a specific role in the evolution of the human brain (Capra et al. 2013; Kamm et al. 2013a; Boyd et al. 2015). Finally, the functional relevance of HARs to brain development is further indicated by associations between mutations in HARs and human-specific diseases, such as schizophrenia and autism spectrum disorder (ASD).

Several lines of evidence have indicated that noncoding HARs may be particularly relevant to the development of the human nervous system. For example, HARs are often located near genes that have been implicated in neural development, or genes that show differential expression between developing brain regions (Haygood et al. 2010). The first HAR that was identified, termed HAR1, is located within two overlapping noncoding RNA genes and has been implicated in cortical development (Pollard et al. 2006b). *HAR1F*, one of the noncoding RNAs spanning HAR1, is highly specifically expressed in Cajal-Retzius neurons during neocortical development. Its expression in the adult brain, however, is more diffuse, implying that HAR1 plays a role in regulating a developmentally dynamic gene. In 2015, Boyd et al. (2015) identified that the HARE5 region acts to enhance *FZD8*, a gene that encodes a receptor involved in the Wnt signaling pathway. Interestingly, HARE5 displays species-specific enhancer activity, acting earlier and more robustly in the human neocortex than that of the chimpanzee. Transgenic mouse assays demonstrated that the human

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HARE5 sequence directs faster progenitor production and larger cortical size as compared to the chimpanzee HARE5 sequence. Despite this compelling evidence for the role of HARs in human brain development, their function and the consequence of these human-specific sequences changes is still unknown.

HARs are being increasingly implicated in human-specific diseases. For example, the highest density of HARs occurs within the gene *NPAS3*, a transcription factor involved in brain development that has been implicated in schizophrenia (Kamm et al. 2013b). Fourteen HARs occur within the introns of *NPAS3*, 11 of which were shown to drive reporter gene expression in the central nervous system in a zebrafish transgenic assay. Kamm et al. (2013a) further explored one of those regions and showed that orthologous mouse and chimpanzee sequences drove similar LacZ expression patterns, while the human sequence drove a more extensive pattern of expression that included the developing anterior telencephalon, lending evidence for human-specific regulatory activity of this region. Recent studies have been able to pinpoint specific mutations within HARs that might have an influence on human-specific disease phenotypes. For example, a rare homozygous mutation was identified in some unrelated individuals with ASD. This mutation falls within HAR246, a noncoding region upstream of *CUX1*, a gene involved in the morphology of cortical neurons and the formation of synaptic spines (Doan et al. 2016). It was shown that the mutated version of HAR246 displays increased enhancer activity compared to wild type, indicating that this mutation could disrupt cortical neuron morphology and signaling and contribute to the human-specific ASD-related phenotypes observed in individuals with this mutation. Together, these links between HARs and human-specific diseases suggest a role for HARs in the evolution of human-specific cognitive and social traits.

Duplications and Deletions in Regulatory Regions

In addition to identifying noncoding regions with human-specific substitutions, whole genome sequencing has led to the identification of regulatory regions with human-specific deletions or duplications. Over 500 noncoding regions that are highly conserved in other species, but deleted in humans, have been identified (McLean et al. 2011). As with HARs, transgenic assays have provided evidence for the role of these deleted regions in brain development. For example, there is a human-specific deletion of an enhancer near *GADD45g*, a tumor suppressor gene that is thought to repress proliferation and is expressed in the developing mouse neocortex. Further characterization of the function of this locus is needed, but it is possible that loss of this enhancer could increase proliferation in the developing brain and therefore contribute to the expansion of the human neocortex.

In addition, duplications or insertions in regulatory elements, as seen at the gene *GPR56*, have been implicated in brain development (Bae et al. 2014).

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Mouse transgenic assays showed that the human version of a cis-regulatory element of *GPR56*, containing several insertions and duplications, drives specific expression in the lateral neocortex, whereas the mouse element drives a broader expression pattern across the whole neocortex. Given that *GPR56* has been shown to play a role in progenitor proliferation and cortical patterning, and the fact that a deletion within the 5′ promoter of *GPR56* causes cortical polymicrogyria, it is speculated that alterations of the regulation of *GPR56* could also influence human brain size. These examples illustrate the utility of functional characterization of human-specific sequence changes in regulatory regions and provide a framework for similar future studies that will doubtlessly continue to improve our understanding of evolution and cortical development.

Evolutionary Changes in Regulatory Activity

As demonstrated above, sequencing-based studies have added substantially to our understanding of the role of noncoding regions and gene regulation in the evolution of the human brain. Contemporary techniques, such as ChIP-seq, have allowed us to delve into this field even further by performing comparative studies of the regulatory activity of noncoding DNA between species. Several groups have performed global comparisons of regulatory activity in humans and closely related species, revealing complicated evolutionary dynamics. By profiling active regulatory regions, marked by H3K27ac and H3K4me3, during early cortical neurogenesis in humans, macaques, and mice, Reilly et al. (2015) showed that embryonic enhancers with human-specific gains or losses of activity regulate genes that are enriched in co-expression modules associated with neuronal proliferation and differentiation. A subsequent study compared active regulatory regions in a variety of brain regions in the human, chimpanzee, and macaque (Vermunt et al. 2016). This comparison emphasized the importance of including closely related species in this kind of analysis; while about 1,400 enhancers and 90 promoters were specifically enriched in the adult human brain compared to macaque, only 193 enhancers and 17 promoters continued to show human-specific gain of activity when compared to chimpanzee. Thus, a similar comparison of prenatal regulatory activity would be necessary to identify high-confidence human-specific developmental regulatory programs, but due to the unavailability of prenatal chimpanzee data, this comparison has been heretofore impossible.

Interestingly, these studies of the epigenetic landscape of the human brain all demonstrate that the positions of promoters and enhancers are largely conserved between humans and nonhuman primates, despite underlying sequence divergence (Cotney et al. 2013; Prescott et al. 2015; Vermunt et al. 2016). In addition, a subset of these positionally conserved regulatory regions exhibit tissue- and/or species-specificity, as they show evidence of activity in disparate tissues or across different brain regions in different species (Cotney et al. 2013;

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Vermunt et al. 2016). Interestingly, these regulatory regions can also show cell type-specific patterns of conservation. For example, Shulha et al. (2012) showed that in the prefrontal cortex, neuronal epigenomes are more similar between humans, macaques, and chimpanzees than they are across cell types (compared to non-neuronal cell types) within the same species. Overall, these studies have identified sets of putative regulatory regions that likely contain a plethora of information about the evolution of the human brain. The human-specific enhancers identified in these studies did show some overlap with the HARs discussed above, but an overall enrichment was not observed. Thus, these data sets of putatively human-specific regulatory regions displaying specific activity in various brain regions, developmental stages, or cell types provide a novel framework for future studies linking genes to human-specific traits and developmental phenotypes.

Gene Regulation via DNA Methylation

DNA methylation provides another layer of epigenetic gene regulation. Methylation can occur at any of the ~1 billion cytosines in the human genome but is most often found at the ~28 million CpG sites. Changes in methylation are driven by alteration of the expression of DNA methyltransferases and can direct species-, tissue-, or cell type-specific patterns of expression (Hernando-Herraez et al. 2015). Specifically, promoter methylation is associated with reduced gene expression and is thought to play a large role in the regulation of gene expression. For example, a comparison of liver, heart, and kidney between humans and macaques showed that 12–18% of the differential expression between the species could be explained by differences in promoter methylation (Pai et al. 2011). Studies of methylation levels in the brain have failed to produce large-scale patterns but have identified specific examples of important methylation changes.

In a study of putative regulatory regions of 36 genes, it was shown that CpG sites tend to be more methylated in human brain than in chimpanzee (Enard et al. 2004). A similar study showed, however, that promoter regions of several genes were significantly less methylated in human brain than chimpanzee brain (Zeng et al. 2012). In studies more specific to brain development, methylation differences in genes involved in neuronal function have been reported both at the level of DNA (Farcas et al. 2009; Schneider et al. 2012) and histones (Shulha et al. 2012). An example of a gene with species-specific methylation patterns is *CNTNAP2*, a transcriptional target of *FOXP2*. *CNTNAP2* has been implicated in neurological and psychiatric disorders, including language impairment (Rodenas-Cuadrado et al. 2014). *CNTNAP2* was shown to be differentially expressed in humans compared to chimpanzees, which could be attributed to changes in *CNTNAP2* isoforms and widespread gene methylation differences (Schneider et al. 2014). Thus, it has been hypothesized that

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differential methylation of *CNTNAP2* could ultimately be responsible for differential expression of the gene in humans, which is thought to be related to the development of human-specific language abilities. Finally, Gokhman et al. (2014) have shown that DNA methylation varies between Neanderthals and Denosivans, via an analysis that uses C→T ratio as an indicator of the amount of methylated cytosines that have decayed in a sample over time. This variance indicates that alterations in regulatory landscapes have occurred recently within human evolution and leaves room for novel studies of the evolution of higher cognitive functions within the recent human lineage.

Conclusions

Over the last several centuries, humans have devoted extensive time and effort to the study of the cerebral cortex, the source of our extraordinary cognitive abilities. Through these studies, we have developed a rich understanding of the function and development of the cerebral cortex and observed the vital role that genetics and gene regulation play in these processes. The link between genes (both mutations and expression) and phenotypes, however, is still very poorly understood and difficult to study. The examples that we have described here demonstrate how genomics can be used to begin to bridge this gap in understanding, in an effort to ultimately elucidate the evolutionary mechanisms underlying human brain development. To further our understanding of the fascinating human cortex, future genomic studies need to prioritize the functional characterization of genomic elements, especially noncoding regulatory regions.

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