Published in final edited form as:

Int Rev Neurobiol. 2013 ; 113: 167–205. doi:10.1016/B978-0-12-418700-9.00006-X.

Transcriptional dysregulation of neocortical circuit assembly in ASD

Kenneth Y. Kwan

Molecular & Behavioral Neuroscience Institute (MBNI), Department of Human Genetics, University of Michigan, 5041 BSRB, 109 Zina Pitcher Place, Ann Arbor, MI, 48109-2200

Kenneth Y. Kwan: kykwan@umich.edu

Abstract

Autism spectrum disorders (ASD) impair social cognition and communication, key higher order functions centered in the human neocortex. The assembly of neocortical circuitry is a precisely regulated developmental process susceptible to genetic alterations that can ultimately affect cognitive abilities. Because ASD is an early onset neurodevelopmental disorder that disrupts functions executed by the neocortex, miswiring of neocortical circuits has been hypothesized to be an underlying mechanism of ASD. This possibility is supported by emerging genetic findings and data from imaging studies. Recent research on neocortical development has identified transcription factors (TFs) as key determinants of neocortical circuit assembly, mediating diverse processes including neuronal specification, migration, and wiring. Many of these TFs (TBR1, SOX5, FEZF2, and SATB2) have been implicated in ASD. Here, I will discuss the functional roles of these transcriptional programs in neocortical circuit development and their neurobiological implications for the emerging etiology of ASD.

Keywords

Transcriptional mechanisms; cerebral cortex; neurodevelopmental disorders; neurogenesis; neuronal migration; neural circuit wiring; axon projections; corpus callosum; corticospinal tract; corticothalamic tract

Introduction

Evolution of the neocortex is thought to underlie our species' remarkable cognitive, perceptive, and motor capabilities. It has been hypothesized that evolutionary advances in neocortical organization and circuitry, while enabling higher cognition, may have also increased our species' susceptibility to disorders that affect cognition. Autism spectrum disorders (ASD) impair higher cognitive functions executed by the human neocortex, including social reciprocity and communication. Although the mechanisms underlying ASD remain largely mysterious, emerging biological insights from genetic and imaging studies have implicated abnormal neocortical circuit assembly in ASD. The acquisition of neocortical organization and circuitry requires the coordinated execution of a series of developmental processes, including the specification of neuronal identity, neuronal migration, and wiring of neural circuits. In recent studies, transcription factors (TFs) have emerged as critical determinants of neocortical development (Kwan et al., 2012b; Leone et

al., 2008; MacDonald et al., 2012; Molyneaux et al., 2007; Rash and Grove, 2006; Rubenstein, 2011). Interestingly, many TFs that are required for the development of neocortical circuitry have been implicated in ASD. In this chapter, I will review the function of these ASD-implicated transcriptional mechanisms during neocortical development and discuss the insights they provide into the neurobiology underpinning ASD.

Although ASD is a strongly heritable disorder, phenotypic and genetic heterogeneity have impeded progress towards identifying loci that carry definitive risk. Reliable genetic findings, however, have begun to emerge from studies that utilized high-throughput methodologies to analyze well-characterized populations of patients and families (Iossifov et al., 2012; Jiang et al., 2013; Neale et al., 2012; O’Roak et al., 2012a; O’Roak et al., 2012b; Sanders et al., 2011; Sanders et al., 2012; Sebat et al., 2007; Talkowski et al., 2012; Weiss et al., 2003). From these data, it is now clear that no single locus accounts for more than 1% of ASD cases, with contributing loci likely numbering in the hundreds. Perhaps somewhat ironically, the genetic heterogeneity that has hindered progress in the previous decades may now provide an opportunity to illuminate the biological underpinnings of ASD, since the increasing number of genes makes possible analyses of convergent molecular pathways and cellular processes (State and Šestan, 2012). Indeed, this strategy has been used to intersect ASD-implicated genes with those that interact with FMRP (Iossifov et al., 2012), the RNA binding protein that is lost in fragile X syndrome, which is the leading monogenic cause of intellectual disability and syndromic autism (Chonchaiya et al., 2009). This analysis found that a significant number of ASD candidate genes are associated with FMRP, which is consistent with the possibility that common molecular pathways underlie autism and fragile X syndrome. In addition, weighted gene co-expression network analysis can reveal previously un-recognized connections between ASD risk genes. One such example of this unbiased approach was used to analyze gene expression in differentiating normal human neuronal progenitors, which revealed a significant overlap with ASD susceptibility genes annotated by the SFARI database (<http://gene.sfari.org>) (Konopka et al., 2012b). Future studies of the relationship between loci that confer ASD risks are likely to lead to additional insights about the neurobiological underpinnings of ASD.

With accumulating genetic data, it may now be possible to better pinpoint the timing and location of the biological events most relevant to the etiology of ASD. This possibility is facilitated by recent transcriptomic studies from multiple groups that have focused on spatio-temporal analyses of gene expression in the human brain (Colantuoni et al., 2011; Johnson et al., 2009; Kang et al., 2011). Available data from one of these resources (<http://www.humanbraintranscriptome.org>) revealed that many of the most reliable risk-carrying loci exhibit a sharp upregulation in the neocortex during the mid-fetal period (red arrowhead in Fig. 1) (State and Šestan, 2012), a key developmental window for the acquisition of neocortical organization and neural circuits. This distinct mid-gestation developmental pattern, which is not consistently observed in other brain regions (Fig. 1), suggests that ASD-associated genes may converge on pathways that function during the structural development and neural circuit wiring of the neocortex.

Additional evidence further implicate the mid-fetal period of neocortical development in ASD. Neuronal migration, the process by which new-born neurons are positioned away

from the germinal zones and towards their correct mantle layer destinations (Angevine and Sidman, 1961; Caviness, 1982; Lambert de Rouvroit and Goffinet, 1998; Rakic, 1974), occurs during early- to mid-gestation and has been shown to be disrupted in some ASD patients (Hutsler et al., 2007; Peñagarikano et al., 2011; Wegiel et al., 2010). Furthermore, there is evidence for abnormalities in the minicolumnar organization of neocortical neurons in cases of ASD and other neuropsychiatric disorders (Casanova et al., 2002). Neocortical minicolumns, which are composed of vertically arranged neurons connected into a local network, are thought to originate from developmental radial units and their formation is likely to be related to neuronal migration during the fetal period (Mountcastle, 1997; Peters, 2010; Rakic, 1988). Interestingly, even subtle alterations in the arrangement and spacing of minicolumns, which are basic units of neocortical neural circuitry, can significantly alter the architecture of inhibitory connectivity in the neocortex (Casanova et al., 2003). The alteration of mid-fetal processes required for the structural development of the neocortex, therefore, may have global consequences on the functioning of neocortical circuits that can contribute to ASD.

Indeed, functional disruption of neocortical connectivity has been hypothesized to underlie ASD and other neurodevelopmental disorders. Evidence supporting the dis- or under-connectivity theory of ASD has been reviewed elsewhere (Geschwind and Levitt, 2007; Just et al., 2012). In particular, data from some functional imaging studies support a reduction in the communication bandwidth between frontal and parietal areas, which leads to a redistribution of executive capability from the frontal areas to the posterior areas of the neocortex (Just et al., 2012). This decrease in fronto-posterior functional connectivity, as assessed by synchronized activity, is thought to lead to diverse consequences in neocortical function consistent with the widespread phenotypes of ASD.

Data from anatomical imaging studies have also implicated alterations in neocortical connectivity. Studies of axonal fiber tracts by diffusion tensor imaging (DTI) have consistently reported reductions in callosal projections in ASD, in both young and adult patients (Alexander et al., 2007; Keller et al., 2007; Kumar et al., 2010; Shukla et al., 2010; Weinstein et al., 2011). As the corpus callosum is the most prominent intracortical tract, alterations therein may indicate additional changes to other intracortical connections. Indeed, decreases in intrahemispheric connectivity have also been implicated in ASD (Ingallhalikar et al., 2011; Kumar et al., 2010; Lange et al., 2010; Lee et al., 2007; Nagae et al., 2012; Shukla et al., 2010; Sundaram et al., 2008; Weinstein et al., 2011). In addition to intracortical connectivity, defects in corticofugal tracts have been reported in ASD as well (Ingallhalikar et al., 2011; Wolff et al., 2012), suggesting more widespread changes to neocortical connections.

Interestingly, these changes do not simply reflect a general decrease in connectivity but may be the outcome of altered developmental trajectories during the formation of these axonal projections. In a recent longitudinal study of high risk infants (younger siblings of ASD children), axon tract development was prospectively studied from 6 to 24 months of age (Wolff et al., 2012). Comparison between infants who developed ASD and those who did not revealed altered developmental trajectories in 12 out of 15 white matter pathways examined, with a transient increase in fractional anisotropy at 6 months of age followed by a

sustained decrease likely lasting beyond 24 months. These data suggest that the alterations to neural connectivity in ASD have emerged by early postnatal development, preceding the onset of behavioral abnormalities, and are widespread in the neocortex, affecting both intracortical and corticofugal connections.

ASD is, fundamentally, a disorder of development. Consistent with the early childhood onset of the disorder, the aberrant neocortical circuits in ASD are likely to be developmental in origin, resulting from defective fetal and early postnatal mechanisms, a possibility supported by converging genetic and imaging data. Below, I will first provide an overview of the cellular processes that underlie the structural development of the neocortex and the assembly of its neural circuits. This will be followed by a discussion of the roles of four ASD-implicated transcriptional programs in multiple aspects of neocortical development and their potential contribution to the pathophysiology of ASD.

The organization of the cerebral cortex

The cerebral cortex, a thin sheet of gray matter at the most superficial part of the cerebral hemispheres, is involved in conscious sensory, cognitive, and motor processes. The emergence of the six-layered neocortex, phylogenetically the most recent division of the cerebral cortex, is thought to be a key advance in mammalian evolution and higher order brain function (Nieuwenhuys, 1994; Northcutt and Kaas, 1995). The neocortex is organized, cytoarchitectonically and functionally, into six horizontal laminae, layer (L) 1 to L6 (DeFelipe and Farinas, 1992; Jones, 1986; Mountcastle, 1997; O'Leary and Koester, 1993), and numerous tangential areas, broadly classified as sensory, motor, or association (O'Leary and Sahara, 2008; Rakic, 1988; Rash and Grove, 2006). Neocortical neuronal identity and connectivity exhibit considerable laminar and areal dependence that is well conserved among mammalian species, suggesting that the establishment of this complex neocortical organization is likely to be critical to neocortical function. Accordingly, the incorrect acquisition of organization and circuitry during neocortical development may contribute to cognitive impairments and increased susceptibility to psychiatric and neurological disorders (Liu, 2011; Manzini and Walsh, 2011; Rubenstein, 2011; Valiente and Marín, 2010).

Present within each horizontal layer of the neocortex is a unique complement of glutamatergic excitatory projection (pyramidal) neurons and GABAergic inhibitory interneurons (DeFelipe and Farinas, 1992; Jones, 1986). The projection neurons, which account for approximately 80% of all neocortical neurons, extend long axonal projections. Intracortical projections connect proximal and distal regions of the neocortex, whereas corticofugal projections, which constitute the neocortical output system, connect the neocortex with subcortical brain structures. Projection neurons, which develop post-synaptic specializations known as dendritic spines, are also the largest input system, being the major target of afferents from other structures of the brain (DeFelipe and Farinas, 1992; O'Leary and Koester, 1993). Positioned within L2–L6, projection neurons exhibit layer- and subtype-dependent differences in molecular identity and axonal projections (Fig. 2) (DeFelipe and Farinas, 1992; Kwan et al., 2012b; Leone et al., 2008; MacDonald et al., 2012; Molyneaux et al., 2007; O'Leary and Koester, 1993). Corticofugal axonal projections originate strictly from the deep layers (L5 and L6) and the subplate (SP), a neocortical structure that contains

early-born neurons positioned between L6 and white matter (MW) (Allendoerfer and Shatz, 1994; Herrmann et al., 1994; Kostovic and Rakic, 1980, 1990; Molliver et al., 1973; Rakic, 1976). Axons that innervate the thalamus, which form the corticothalamic tract, originate largely from SP and L6, whereas axons that project subcerebrally, including the corticotectal, corticobulbar, and corticospinal tracts, arise exclusively from L5. Projection neurons positioned in the upper layers (L2–L4), in contrast, project only within the cortex, either intrahemispherically or contralaterally. The corpus callosum, which is formed by contralateral intracortical axons, enables communication between the two cerebral hemispheres. Neural imaging studies have implicated alternations to this layer-dependent neocortical connectivity in ASD. In particular, deficits in intracortical axon tracts, most notably the corpus callosum, have been strongly implicated (Alexander et al., 2007; Ingahalikar et al., 2011; Keller et al., 2007; Kumar et al., 2010; Lange et al., 2010; Lee et al., 2007; Nagae et al., 2012; Shukla et al., 2010; Sundaram et al., 2008; Weinstein et al., 2011). Changes in corticofugal connectivities, including the internal capsule, have been reported as well (Ingahalikar et al., 2011; Wolff et al., 2012).

The generation and migration of neocortical projection neurons

Diverse subtypes of neocortical projection neurons are generated from terminal divisions that take place within the germinal zones of the dorsal telencephalon, namely the ventricular and subventricular zones (VZ and SVZ) and undergo radial migration to their destination in the mantle layers of the neocortex (Fig. 2) (Angevine and Sidman, 1961; Rakic, 1974). Prior to the onset of neurogenesis, the dorsal telencephalic VZ is composed of proliferating neuroepithelial progenitors (NPs), which primarily undergo symmetric divisions, generating two daughter cells that would re-enter the cell cycle and thus exponentially expand the pool of NPs (Caviness et al., 1995; Rakic, 1995). At the onset of neurogenesis, around embryonic day (E) 10.5 in the mouse, some NPs assume radial glial morphology to become radial glial cells (RGCs) (Rakic, 1971). RGCs function as progenitors for projection neurons both directly, by undergoing neurogenic divisions in the VZ (Anthony et al., 2004; Malatesta et al., 2003; Miyata et al., 2004; Noctor et al., 2004), and indirectly, by giving rise to intermediate progenitors (IPs), which migrate away from the VZ and undergo further neurogenic divisions in the SVZ (Englund et al., 2005; Kowalczyk et al., 2009; Noctor et al., 2008; Sessa et al., 2010). In addition, RGCs provide a scaffold for the radial migration of their neuronal progeny into the mantle layers (Bentivoglio and Mazzarello, 1999; Rakic, 1971).

The earliest neocortical neurons form a band of cells, termed the preplate (PP), at the superficial part of the cerebral wall (Fig. 2) (De Carlos and O'Leary, 1992; Marin-Padilla, 1971; Marin-Padilla, 1978; The Boulder Committee, 1970). The first projection neurons, generated around E11.5 in the mouse, migrate radially away from the VZ and through the IZ before settling as a layer within the PP to form the nascent cortical plate (CP), from which L2–L6 of the neocortex would eventually emerge (Marin-Padilla, 1978). With the incoming CP neurons, the PP is split into two layers; the superficial marginal zone (MZ), which forms L1 of the postnatal cortex, and the deeper SP, which is positioned below L6. The early-born neurons of the MZ and SP are the first to mature morphologically and synaptically, form pioneering connections, and play a key role in the subsequent migration of CP neurons and

assembly of cortical input and output circuits (Allendoerfer and Shatz, 1994; Herrmann et al., 1994; Kostovic and Rakic, 1980, 1990; Molliver et al., 1973; Rakic, 1976). Interestingly, the SP is enriched in its expression of ASD-implicated genes (Hoerder-Suabedissen et al., 2013), which is consistent with the possibility that defects in SP pioneering connections may contribute to wider disruption of neocortical wiring relevant to ASD.

From E11.5 to E16.5 in the mouse, neurogenesis progresses to produce, in a sequential manner, diverse subtypes of neocortical projection neurons (Fig. 2). Because newly generated neurons migrate past older, post-migratory neurons to settle within the most superficial portion of the CP, projection neurons are added to the CP following an inside-first, outside-last (L6 to L2) sequence (Angevine and Sidman, 1961; Caviness, 1982; Lambert de Rouvroit and Goffinet, 1998; Rakic, 1974). At the end of radial migration, newly post-migratory neurons undergo molecular and morphological differentiation into diverse neuronal subtypes and form neural circuits appropriate of their layer location (Anton et al., 1999; D'Arcangelo and Curran, 1998; Lambert de Rouvroit and Goffinet, 1998; Yokota et al., 2007).

The sequential nature of neocortical neurogenesis from related progenitor lineages directly links neuronal birth-date and location of terminal division to neuronal position, identity, and axonal connectivity (Fig. 2). During early cortical neurogenesis (~E11.5–E13.5 in the mouse), the majority of neurons are generated directly via asymmetric neurogenic divisions in the VZ. These early-born neurons are mostly destined for the deep neocortical layers that comprise the corticofugal output of the neocortex. Later in neurogenesis (~E14.5–E16.5), many neurons are derived indirectly through divisions of IPs in the SVZ (Noctor et al., 2004; Sessa et al., 2010; Tabata et al., 2009; Tarabykin et al., 2001). These late-born neurons are mostly destined for the upper layers that form intracortical connections and extensively innervate the corpus callosum. IPs are thought to contribute a significant number of cortical projection neurons (Haubensak et al., 2004; Miyata et al., 2004) and may have played a role in the relatively recent evolutionary increases in upper-layer intracortical projections in the primates (Marín-Padilla, 1992). Consistent with this possibility, many primates, and a number of other mammals with large brains, have a specialized zone containing additional IPs known as the outer SVZ (oSVZ) (Lui et al., 2011). Because the SVZ contributes to a significant number of upper-layer neurons, perturbation in subventricular divisions may more severely alter intracortical connectivity, which has been implicated in ASD.

Defects in neurogenesis and neuronal migration can lead to severe brain malformations in humans (Kerjan and Gleeson, 2007; Liu, 2011; Manzini and Walsh, 2011; Thornton and Woods, 2009). Mutations affecting the cellular machinery required for VZ mitoses, most notably mechanisms involving the assembly of the centrosome, can lead to primary microcephaly, whereas asismigration can lead to lissencephaly and gyration disorders.

Perhaps the most widely studied neuronal migration phenotype is that of the *Reeler* mutant. In mice with the *Reeler* mutation, new-born projection neurons are able to migrate away from the germinal zones and through the IZ, but fail to migrate past older neurons within the CP, prematurely arresting immediately below (Rice and Curran, 2001; Tissir and Goffinet,

2003). As a result of this failure at the final step of neuronal migration, the partitioning of the PP is defective and the ordering of L2-SP becomes inverted. *Reeler* mice have a loss-of-function mutation in *Reln*, which encodes an extracellular matrix protein secreted by Cajal-Retzius (CR) neurons positioned in the MZ. Importantly, multiple studies have shown *RELN* to be a genetic risk factor in ASD (Holt et al., 2010; Kelemenova et al., 2010; Li et al., 2008; Persico et al., 2001; Serajee et al., 2006; Skaar et al., 2005), suggesting that altered neuronal positioning in the neocortex may underlie some ASD cases.

In addition to gross structural abnormalities, defective neuronal migration can lead to more subtle alterations, including changes to the columnar organization of the human neocortex (Kwan et al., 2012a; Mountcastle, 1997; Rakic, 1988). In addition to altered arrangement of neocortical minicolumns, which has been reported in the post-mortem brains of ASD and schizophrenic patients (Casanova et al., 2003; Casanova et al., 2002), other detailed studies of neuropathology have also revealed subtle neuronal migration defects in a proportion of examined autistic brains (Hutsler et al., 2007; Uppal and Hof, 2013; Wegiel et al., 2010). Furthermore, the absence of *contactin associated protein-like 2* (*CNTNAP2*, previously known as *CASPR2*), which has been implicated in ASD, leads to migration defects in both mice and humans (Peñagarikano et al., 2011; Strauss et al., 2006). Together, these data corroborate the possibility that mismigration of neocortical projection neurons, and potential associated changes in neural circuitry, can contribute to ASD and other neurodevelopmental disorders.

Generation and migration of neocortical inhibitory interneurons

Layer-dependent differences in the neocortex are further augmented by the remaining approximately 20% of neocortical neurons. The interneurons of distinct morphological, neurochemical, and electrophysiological subtype, also populate the neocortex with selective layer preferences (Markram et al., 2004; Miyoshi et al., 2007; Miyoshi and Fishell, 2011). Neocortical interneurons are generated from progenitors in the ventral forebrain, primarily within the medial and caudal ganglionic eminences (MGE and CGE) (Anderson et al., 1997; Marín and Rubenstein, 2003; Nadarajah and Parnavelas, 2002; Wonders and Anderson, 2006; Xu et al., 2004). In order to reach the neocortex, newborn interneurons undergo tangential migration, primarily via migratory corridors above or below the CP. Upon arrival to their destination cortical area, interneurons undergo radial migration to enter the CP (Ang et al., 2003; Nadarajah and Parnavelas, 2002; Yokota et al., 2007) before settling into cortex lamina based on their subtype, origin, and birthdate (Miyoshi and Fishell, 2011; Miyoshi et al., 2010).

Disruption of the balance between cortical excitation and inhibition has been hypothesized to be relevant to ASD (Rubenstein and Merzenich, 2003), as suggested by the high proportion of ASD patients that suffer from epilepsy and other observations. Alterations to the generation, migration, and wiring of interneurons, therefore, may contribute to this mechanism. Consistent with this possibility, genes that are critical to the early specification of interneuronal progenitors (genes of the *Dlx* family) have been associated with ASD (Liu et al., 2009; Nakashima et al., 2010). Furthermore, defective GABAergic signaling has been reported to underlie some ASD-related phenotypes in Rett syndrome (Chao et al., 2010).

Transcriptional regulation of ASD-related layer-dependent identity and connectivity

The molecular mechanisms regulating the layer-dependent identities and connectivities of distinct neocortical projection neurons are being unraveled (Fishell and Hanashima, 2008; Kwan et al., 2012b; Leone et al., 2008; MacDonald et al., 2012; Molyneaux et al., 2007; Polleux et al., 2007; Rash and Grove, 2006). Although much remains unknown, genes expressed in layer-selective or neuronal subtype-specific patterns are likely to be important. Transcription factors (TFs) are known to play pivotal roles in processes including the early patterning, sequential generation, arealization, dendritic morphology, and axonal connectivity of distinct neuronal cell types (Guillemot, 2007; Hevner et al., 2006; Hébert and Fishell, 2008; Jessell, 2000; Kwan et al., 2012b; MacDonald et al., 2012; Mallamaci and Stoykova, 2006; Monuki and Walsh, 2001; O'Leary and Sahara, 2008). In the neocortex, a number of layer- and neuronal subtype-specific TFs have been identified and characterized (Gray et al., 2004; Kwan et al., 2012b; MacDonald et al., 2012; Molyneaux et al., 2007).

Here, I will discuss four transcriptional mechanisms critical to neocortical development that have been implicated in ASD. As revealed by recent genetic findings, there exists considerable overlap of risk-carrying genes in disorders that are phenotypically distinct (Marshall et al., 2008; Mefford et al., 2010; Smoller et al., 2013; State and Šestan, 2012). Genes that have been implicated in ASD have also been consistently implicated in epilepsy, intellectual disability, schizophrenia, and bipolar disorder, suggesting that shared genetic liabilities can lead to diverse clinical manifestations. Therefore, in my discussion of genetic findings below, studies of neuropsychiatric disorders in addition to ASD will also be included.

TBR1

T-box brain factor 1 (TBR1) encodes a T-box-containing transcription factor that controls multiple processes during the assembly of neocortical circuits. The spatiotemporal expression of TBR1 is consistent with its roles in regulating the laminar and regional identity and axonal pathfinding of early-born projection neurons (Fig. 3) (Bedogni et al., 2010; Han et al., 2011; Hevner et al., 2001; McKenna et al., 2011).

Genetic evidence implicating *TBR1* in ASD has been reported by several groups (Fig. 4). Recently, four 2q24 microdeletion cases in which disruption of *TBR1* is shared were reported (Traylor et al., 2012). These patients exhibited severe speech and language difficulties, autistic-like behavioral problems, and moderate to severe intellectual disability. Additionally, larger interval deletions that included *TBR1* have been reported (Krepischi et al., 2010; Magri et al., 2011; Palumbo et al., 2012; Takatsuki et al., 2010). Delayed or absent speech and language and intellectual disability were shared by all of the reported cases. In addition to copy number variants (CNVs), other *de novo* mutations have also been found in ASD cases. A recent, trio study using the Simon's collection of simplex families (Fischbach and Lord, 2010), reported a heterozygous *de novo* frameshift mutation (Ala136ProfsX80) that leads to a premature stop codon (O'Roak et al., 2012b). As the entire T-box domain critical for DNA binding is truncated as a result, the mutant allele is not expected to be able to express functional TBR1 protein. Subsequent analyses from the same group by targeted re-sequencing in additional ASD cases further revealed two novel single nucleotide variants

(SNVs) within the coding region (O’Roak et al., 2012a). The first (Lys228Glu) is a missense mutation that affects a highly conserved residue within the T-box domain. The second (Ser351X) is a nonsense mutation that truncates a significant portion of the T-box domain. Both of these variants are predicted to be highly deleterious to protein function.

Additional evidence of *TBR1* involvement in molecular pathways relevant to ASD includes its regulation of *Autism susceptibility candidate 2* (*AUTS2*) and *Reelin* (*RELN*) (Bedogni et al., 2010; Hevner et al., 2001). Rare mutations in *AUTS2* have been identified in ASD in studies from numerous groups (Ben-David et al., 2011; Huang et al., 2010; Kalscheuer et al., 2007; Prasad et al., 2012; Sultana et al., 2002; Talkowski et al., 2012). *AUTS2* was further identified as a hypermutable ASD-associated gene (Michaelson et al., 2012). The expression of *Auts2*, which is highly enriched in frontal neocortex, is dependent on *Tbr1* (Bedogni et al., 2010). In the *Tbr1* null mouse, *Auts2* expression is severely decreased, suggesting that loss of *AUTS2* may contribute mechanistically to *TBR1* dysfunction in ASD. *TBR1* further controls the expression of *Reln* (Hevner et al., 2001). Association studies have repeatedly implicated *RELN* in ASD (Holt et al., 2010; Kelemenova et al., 2010; Li et al., 2008; Persico et al., 2001; Serajee et al., 2006; Skaar et al., 2005). Furthermore, rare variants in *RELN* have also been identified (Neale et al., 2012). In the *Tbr1*-deficient neocortex, the early expression of *Reln* in Cajal-Retzius neurons of the MZ is greatly reduced (Hevner et al., 2001), suggesting an additional candidate mechanism by which *TBR1* may contribute to ASD.

The expression and function of *Tbr1* may provide key insights into the neurobiology of ASD. The expression of *TBR1* is highly enriched in the developing neocortex (Fig. 3) (Hevner et al., 2001), which is consistent with a contribution of neocortical dysfunction to ASD. Within the neocortex, *TBR1* expression is restricted to the corticothalamic projection neurons of L6 and the SP, and to the CR neurons of the MZ from an early embryonic age (~E12.5) (Han et al., 2011; Hevner et al., 2001; McKenna et al., 2011). *TBR1*, however, is absent from VZ and SVZ progenitor cells, and therefore functions post-mitotically in neurons after their terminal division and during their differentiation. In the neonatal mouse cortex, *TBR1* is absent from the upper-layer neurons that contribute to the majority of callosal axons. During the first post-natal week, however, a number of upper-layer neurons begin to upregulate *TBR1* expression (Han et al., 2011; Hevner et al., 2001; McKenna et al., 2011). This upper-layer expression of *TBR1* likely serves functions in addition to its roles in early-born neurons. Accordingly, *TBR1* expression in the upper layers is required for upper-layer *Auts2* expression (Bedogni et al., 2010; Srinivasan et al., 2012).

Consistent with its expression in a subset of early-born neocortical neurons, *TBR1* controls the formation of early corticofugal circuits. *Tbr1* is required for the normal formation of the corticothalamic tract, which originates mostly from L6 and SP neurons, the early-born cells in which *TBR1* is highly expressed (Han et al., 2011; Hevner et al., 2001; McKenna et al., 2011). Furthermore, a recent study showed, in number of mouse mutants, that the expression of *TBR1* consistently correlates with subcortical axons innervating the dorsal thalamus (Srinivasan et al., 2012), corroborating *Tbr1* as a key regulator of corticothalamic connectivity. *TBR1* executes this function, at least in part, by suppressing the expression of *Fez family zinc finger 2* (*Fezf2*, formerly *Fezl* and *Zfp312*) and *B cell leukemia/lymphoma*

11B (Bcl11b, formerly Ctip2), which are determinants of L5 subcerebral projection neurons (Arlotta et al., 2005; Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005), to low levels in L6 and SP corticothalamic neurons (Han et al., 2011; McKenna et al., 2011). In the *Tbr1* null neocortex, L6 and SP neurons, as a result of ectopic upregulation of high *Fezf2* expression, misroute their corticothalamic axons to aberrantly innervate the corticospinal tract (Han et al., 2011; McKenna et al., 2011). Conversely, when *Tbr1* is misexpressed in L5 corticospinal neurons, it represses L5 molecular identity and abolishes the formation of the corticospinal tract (Han et al., 2011; McKenna et al., 2011). Therefore, TBR1 deficiency disrupts two key aspects of corticofugal projections, nearly abolishing neocortical connectivity with the thalamus and leading to an ectopic tract that incorrectly innervates the spinal cord.

In addition to corticofugal connectivity, TBR1 is also required for normal intracortical connections. In the *Tbr1*-deficient cortex, callosal axons fail to cross the midline, and instead form Probst bundles (Hevner et al., 2001). As discussed above, alterations to the integrity of the corpus callosum are often reported in ASD (Ingalhalikar et al., 2011; Kumar et al., 2010; Lange et al., 2010; Lee et al., 2007; Nagae et al., 2012; Shukla et al., 2010; Sundaram et al., 2008; Weinstein et al., 2011; Wolff et al., 2012). This role of TBR1 in callosal connections, therefore, may be a contributory mechanism to TBR1 dysfunction in ASD in addition to TBR1 control of corticofugal connectivity. In the post-natal neocortex, upper-layer intracortical neurons up-regulate TBR1 expression (Han et al., 2011; Hevner et al., 2001; McKenna et al., 2011). Therefore, TBR1 may function cell-autonomously in callosal neurons. Consistent with this possibility, the expression of TBR1 in upper-layer neurons is sufficient to rescue the callosal defects observed in the *Satb2* mutant neocortex (Srinivasan et al., 2012), which is further discussed below. An additional explanation, however, may be found in TBR1 regulation of early SP circuits, which are known to orchestrate the development of both callosal and corticofugal connectivities (Allendoerfer and Shatz, 1994; Del Río et al., 2000; Herrmann et al., 1994; Kostovic and Rakic, 1980, 1990; Molliver et al., 1973; Rakic, 1976).

The SP is a transient zone in the developing neocortex in which some of the earliest synapses and pioneering circuits develop (Allendoerfer and Shatz, 1994; Del Río et al., 2000; Herrmann et al., 1994; Kostovic and Rakic, 1980, 1990; Molliver et al., 1973; Rakic, 1976). The SP, the thickest zone in the human mid-fetal cerebral wall, is thought to play critical roles in the migration and synaptogenesis of CP neurons, as well as in the assembly of proper neocortical efferent and afferent axonal projections. Examination of *Tbr1*-deficient mice revealed a severe disruption of SP formation, which is part of a complex mismigration phenotype that is distinct compared to the wholly inverted cortex of the *Reeler* mutant (Rice and Curran, 2001). In the *Tbr1* null neocortex, early-born SP neurons form an ectopic band in the center of the CP. This “mid-plate” exhibits a rostral-caudal gradient, being more deeply positioned in the rostral cortex (Han et al., 2011; Hevner et al., 2001). These areal-dependent migration defects are consistent with a role of *Tbr1* in the control of regional identity (Bedogni et al., 2010). In addition to mismigration of SP neurons, L5 neurons are also disrupted, being more widely distributed throughout the CP, whereas upper-layer neurons are distributed bimodally, with a majority positioned below the ectopic band of SP

neurons (Han et al., 2011; Hevner et al., 2001). These complex migration defects suggest that, other than regulation of *Reln* expression, *Tbr1* is likely to control migration via additional mechanisms.

Interestingly, consistent with the central role of SP in orchestrating cortical connectivities, recent data have implicated SP in ASD. Comprehensively profiling of gene expression in the mouse SP through multiple developmental ages was combined with network analysis, which unbiasedly revealed a significant enrichment of ASD- and schizophrenia-implicated genes being expressed in the SP (Hoerder-Suabedissen et al., 2013). These data suggest the possibility that disruption of early pioneering SP circuits can ultimately lead to wider alterations of neocortical organization and circuitry in disease and is further consistent with SP defects as an additional contributory mechanism of TBR1 deficiency in ASD.

SOX5

Sex determining region Y-box 5 (Sox5) encodes a member of the large family of SOX transcription factors that play key roles in diverse cellular functions, including sex determination, stem cell maintenance, and cell fate specification (Lefebvre et al., 2007). Although *Sox5* phenotypes exhibit key differences compared to *Tbr1*, *Sox5* is also required for multiple neocortical developmental processes, such as the molecular specification and positioning of early-born neurons, and development of corticofugal projections (Kwan et al., 2008; Lai et al., 2008).

Genetic evidence implicating *SOX5* contribution to ASD includes multiple studies of CNVs (Fig. 5). *SOX5* is affected by microdeletions in numerous cases of ASD and ID (Lamb et al., 2012; Rosenfeld et al., 2010; Schanze et al., 2013; Talkowski et al., 2012). The deletion intervals in the majority of the reported cases are small and restricted to *SOX5*. At least 10 reported intragenic microdeletions affect either the HMG box, which is required for DNA binding, or the coiled-coil domain, which is necessary for homo- and heterodimerization (Lefebvre et al., 2007), and are therefore predicted to be highly deleterious. Many of these patients suffer from speech and language delays, moderate to severe intellectual disability, and behavioral deficits that include autistic-like features and stereotypies (Lamb et al., 2012; Rosenfeld et al., 2010; Schanze et al., 2013; Talkowski et al., 2012). Furthermore, several cases of larger deletions that span beyond the entire *SOX5* gene have also been reported (Gläser et al., 2003; Lu et al., 2009; Nagai et al., 1995; Stumm et al., 2007). In addition to CNVs, *SOX5* interruption by chromosomal rearrangement has been reported in ASD (Talkowski et al., 2012). With a breakpoint located between the HMG box and coiled-coil domain, this t(11;12)(p13;p12.1) translocation is also predicted to lead to *SOX5* loss-of-function.

The expression pattern of *SOX5* suggests that it plays a role in establishing early neocortical circuits, perturbations of which may contribute to ASD (Fig. 3). In the embryonic neocortex, *SOX5* expression is highly enriched in L6 and SP corticothalamic projection neurons and a subset of L5 subcerebral projection neurons, starting at around E14.5 (Kwan et al., 2008; Lai et al., 2008). The absence of *SOX5* from cortical VZ and SVZ progenitor cells throughout embryonic development indicates that it is likely to function post-mitotically (Kwan et al., 2008; Lai et al., 2008).

Consistent with this spatiotemporal expression, *Sox5* cell-autonomously controls the migration, differentiation, and axonal projections of these early-born neurons (Fig. 3) (Kwan et al., 2008; Lai et al., 2008). Analysis of *Sox5*-deficient mice revealed marked disruption of layer-dependent corticofugal connectivities. In the absence of *Sox5*, the vast majority of corticothalamic axons arising from SP and L6 neurons fail to reach the dorsal thalamus and are instead misrouted to the hypothalamus (Kwan et al., 2008). In addition, subcerebral axons originating from L5 neurons are greatly reduced, with projections to the pons and spinal cord, including the corticospinal tract, being nearly completely abolished (Kwan et al., 2008). Interestingly, although *Sox5* is required for the normal formation of the CST, its misexpression in upper layer neurons was not sufficient to respecify their projectional fate (Kwan et al., 2008), suggesting that the role of *Sox5* is not instructive. The corpus callosum, which is known to be altered in some cases of ASD, is grossly normal in the *Sox5* null neocortex. The remarkable defects in corticothalamic and corticospinal projections in the *Sox5* mutant mouse, however, are consistent with studies that show changes to the internal capsule in ASD, which indicate alterations to corticofugal connectivities (Ingahalikar et al., 2011; Wolff et al., 2012).

In addition to axonal projections, *Sox5*, like *Tbr1*, also regulates the molecular identities and positioning of early-born projection neurons. Consistent with its high expression in L6 and SP neurons, *Sox5* is selectively required for their migration (Kwan et al., 2008; Lai et al., 2008). In *Sox5*-deficient mice, L6 and SP neurons are unable to migrate past earlier-born neurons to settle more superficially, thus failing at this final step of neuronal migration. As a result, the deep cortical layers of the *Sox5*-null cortex exhibit a laminar inversion with some similarities to the *Reeler* phenotype (Rice and Curran, 2001). Interestingly, unlike *Reeler* and related mutants, the late-born neurons destined for the upper-layers are unaffected and migrate normally. Furthermore, in the *Sox5*-deficient neocortex, many SP neurons do not separate from the MZ, instead becoming ectopically positioned at the upper edge of L2 (Kwan et al., 2008; Lai et al., 2008). This failure in PP splitting disrupts the proper formation of the SP and may affect its early pioneering circuits (Kwan et al., 2008). Notably, in addition to wider migration defects in ASD (Wegiel et al., 2010), aberrant cell clustering and supernumerary cells in LI and SP have been reported (Hutsler et al., 2007). These findings suggest that defects in PP splitting may be present in rare ASD cases and that the migration defects that result from the absence of *SOX5* may have potential relevance to ASD.

In addition to positioning, *SOX5* also controls the molecular differentiation of L6 and SP neurons. *SOX5* is required not only for the expression of *connective tissue growth factor* (*Ctgf*), a reliable marker of SP neurons, but also for the progressive downregulation of L5 marker genes *Fezf2* and *Bcl11b* from L6 and SP neurons during late embryogenesis (Kwan et al., 2008). In the absence of *Sox5*, L6 and SP neurons aberrantly maintain L5 marker expression and therefore express a combination of L5 and L6/SP molecular identities (Kwan et al., 2008; Lai et al., 2008). Thus, changes to the morphological and molecular development of the SP, as well as its circuitry may further compound alterations of neocortical connectivities in *Sox5* deficiency in ASD. As discussed above, the pioneering neurons of the SP play key roles in the organization and wiring of CP neuronal circuits

(Allendoerfer and Shatz, 1994; Del Río et al., 2000; Herrmann et al., 1994; Kostovic and Rakic, 1980, 1990; Molliver et al., 1973; Rakic, 1976). The control of SP positioning and differentiation represents a potential point of convergence between *Tbr1* and *Sox5* mechanisms and is consistent with a putative role of SP circuits in ASD, as suggested by the enrichment of ASD-implicated gene expression in SP neurons (Hoerder-Suabedissen et al., 2013).

FEZF2

FEZ family zinc finger 2 (FEZF2), previously known as *FEZL* and *ZFP312/ZNF312* is one of the first transcription factors shown to be a necessary and sufficient determinant of corticofugal connectivity (Fig. 3) (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). It encodes a zinc finger-containing nuclear protein (Hashimoto et al., 2000; Hirata et al., 2004), that is functionally conserved at the protein level between fish and mammals (Shim et al., 2012).

Genetic association has been reported between *FEZF2* and ASD in two large cohorts of European ancestry and replicated in two additional cohorts (Fig. 6) (Wang et al., 2009). This SNP occurs within the highly conserved proximal promoter region of *FEZF2* that has been shown to be important to drive neocortical gene expression (Hirata et al., 2006). Interestingly, the major allele (T) seems to have a recent evolutionary origin, having emerged in primates, within which it has become highly conserved, likely after purifying selection (Fig. 6). Importantly, the allele associated with ASD (C) represents a reversion to the ancestral non-primate allele. Although it is quite possible for this basepair substitution itself to have a functional consequence on *FEZF2* gene expression, it may also represent a reversion to a more ancient haplotype that may have additional functional changes. Furthermore, a rare mutation in *FEZF2* has been identified in ASD (Sanders et al., 2012). This missense mutation alters an arginine residue that is highly conserved in mammalian and avian species and the mutated cysteine residue is predicted to be highly deleterious to the zinc finger domain critical for protein function.

Further evidence of *FEZF2* contribution to molecular pathways of ASD includes its regulation of the expression *TBR1* (Chen et al., 2005b), whose role in neocortical development and ASD was discussed above. In addition, *Fezf2* is required for the neocortical expression of *Forkhead box P2 (FOXP2)* (Chen et al., 2005a; Molyneaux et al., 2005), the disease-causing gene of a severe speech and language disorder (Lai et al., 2001) thought to be important to the emergence of human speech (Enard et al., 2002; Konopka et al., 2009). *FOXP2* and related *FOXP* genes have been implicated in ASD (Bowers and Konopka, 2012; Girirajan et al., 2013; Hamdan et al., 2010; O’Roak et al., 2011; Palumbo et al., 2013; Schaaf et al., 2011), and are discussed in depth in Chapter 4 of this volume.

As a key determinant of corticofugal connections, *FEZF2* may be important to cortical circuits relevant to ASD in several ways. Starting from an early developmental age (~E10.5) *Fezf2* is expressed exclusively in the corticofugal projection neurons of the deep cortical layers and the early (~E10.5–E13.5) embryonic progenitor cells that generate these neurons (Fig. 3) (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). It is absent from the intracortical projection neurons, in upper or deep layers, and in the late (>14.5) VZ

progenitors that give rise to upper-layer neurons. Within the deep layers, *Fezf2* is most highly expressed in L5 subcerebral neurons and less strongly expressed in L6 corticothalamic neurons (Chen et al., 2005b). The exclusivity of *Fezf2* expression in corticofugal projection neurons, and its graded expression levels in different corticofugal subtypes (i.e. corticospinal versus corticothalamic) suggests that *Fezf2* expression is under very precise gene regulation and that this regulation is likely to have a functional significance (Chen et al., 2005b; Kwan et al., 2012b).

Consistent with this highly specific expression pattern, *Fezf2* regulates the molecular specification and axonal projections of deep-layer subcortical projection neurons (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). *Fezf2* is required for the early specification of L5 molecular identity. In the absence of *Fezf2*, the expression of *Bcl11b*, *Etv1*, *Foxo1*, *Crym*, *Diap3*, *Clim1*, *Crim1*, and other L5 markers are lost or severely reduced (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). Accordingly, *Fezf2* is necessary for the formation of the corticospinal tract that originates from these neurons. When *Fezf2* is genetically removed (Chen et al., 2005a; Molyneaux et al., 2005) or knocked-down by RNAi (Chen et al., 2005b), corticospinal axons fail to enter the pons or spinal cord and some of these axons aberrantly invade the anterior commissure (Chen et al., 2005a; Molyneaux et al., 2005). *Fezf2*, however, is not only necessary for normal corticospinal tract formation, but also sufficient to induce it ectopically. When *Fezf2* is specifically mis-expressed in upper-layer neurons that normally project only intracortically, ectopic subcortical projections to the pons and spinal cord originate from these neurons (Chen et al., 2005b). Furthermore, *Fezf2* is sufficient to reprogram intracortical projection neurons to project subcortically in the early post-natal neocortex (De la Rossa et al., 2013; Rouaux and Arlotta, 2013). In addition to L5 identity and projections, *Fezf2* also controls the molecular development of L6 neurons and their corticothalamic projections (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). Furthermore, Probst bundles containing callosal axons that are unable to cross the midline have been reported in the *Fezf2* null cortex (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). Although this *Fezf2* phenotype may not be cell autonomous, changes in callosal connectivity can contribute to circuit alternations relevant to ASD. Furthermore, the loss of subcerebral connectivity in the absence of *Fezf2* is consistent with reduced internal capsule innervation reported in some ASD patients (Ingahalikar et al., 2011; Wolff et al., 2012).

These diverse roles of *Fezf2* on axon projections are mediated by its involvement in complex transcription networks that mediate multiple aspects of neocortical development (Chen et al., 2008; Han et al., 2011; Kwan et al., 2008; Leone et al., 2008; McKenna et al., 2011; Shim et al., 2012; Srinivasan et al., 2012). These networks include direct and indirect interactions between *FEZF2* and *SOX5*, *TBR1*, and *SATB2*, the other ASD-implicated TFs discussed in this chapter. This central role of *FEZF2* in neocortical transcriptional networks is consistent with its high intramodular connectivity in a gene co-expression network, the cortical development module, assembled unbiasedly from the developing human brain transcriptome (Kang et al., 2011).

Interestingly, *Fezf2* may further contribute to circuit assembly by regulating the morphological development of dendrites in projection neurons. When *Fezf2* is silenced, the

complexity of the basal dendritic arbors and the number of dendritic spines are both reduced (Chen et al., 2005b). Furthermore, the vertical orientation of the apical dendrites is also disrupted. These post-synaptic changes may further add to circuits alterations in the absence of *Fezf2*. Consistent with a potential contribution to ASD, dendritic and spine morphologies are often disrupted in ASD and animal models of ASD (Comery et al., 1997; Hutsler and Zhang, 2010; Irwin et al., 2000).

Unlike *Tbr1* and *Sox5*, *Fezf2* is not required for the migration of projection neurons. The layer-dependent arrangement and positioning of projection neurons exhibit no alternations in the absence of *Fezf2* (Chen et al., 2005a; Chen et al., 2005b; Molyneaux et al., 2005). Interestingly, *Fezf2* can indirectly control the layer distribution of specific subpopulations of cortical interneurons (Lodato et al., 2011). In the *Fezf2*-null cortex, the number of interneurons of certain subtypes is specifically reduced in L5, indicating that loss of *Fezf2*-dependent L5 identity alters the distribution of subclasses of interneurons (Lodato et al., 2011). As discussed above, interneurons are critical to the balance between excitation and inhibition in the neocortex (Rubenstein and Merzenich, 2003). Therefore, their disruption in the absence of *Fezf2* may further contribute to ASD.

SATB2

Special at-rich sequence-binding protein 2 (SATB2) is a DNA-binding protein that interacts with DNA matrix attachment regions (MARs) to alter gene expression by inducing local chromatin remodeling. In contrast to *TBR1*, *SOX5*, and *FEZF2*, *SATB2* controls the expression profiles, migration, and connectivity of intracortical projection neurons (Fig. 3) (Alcamo et al., 2008; Britanova et al., 2008).

Numerous mutations, including CNVs, SNVs, and chromosomal rearrangements that disrupt *SATB2* have been reported in ASD cases (Fig. 7). In one of the earlier studies of CNVs in ASD, a balanced translocation, t(2;6)(q32;p22), that interrupts *SATB2* was described (Marshall et al., 2008). A more recent study found an additional balanced translocation, t(2;6)(q33;q21), that disrupts *SATB2* in a case of ASD (Talkowski et al., 2012). Further study from the same group identified five intragenic deletions that affect the CUT domain critical for DNA binding in cases of ASD and other neurodevelopmental disorders (Talkowski et al., 2012). Three additional intragenic deletions have been found to underlie a 2q32q33 microdeletion syndrome characterized by severe intellectual disability (Rosenfeld et al., 2009). Furthermore, a nonsense mutation (Arg239X) positioned upstream of the CUT domain (Leoyklang et al., 2007) and a missense mutation (Val381Gly) that alters a well-conserved residue within the CUT domain (Rauch et al., 2012) have been found in cases of intellectual disability. A recent ASD study using whole genome sequencing further identified an inherited rare missense variant (Pro655Ser) (Jiang et al., 2013) that affects a residue that is conserved in mammalian and avian species. Together, these data support the possibility that loss of *SATB2* function can contribute to ASD.

There is additional evidence of *Satb2* involvement in ASD-related molecular pathways. *Satb2* controls the layer-dependent expression of the ASD-associated gene *Auts2* and may do so via its control of *Tbr1* (Srinivasan et al., 2012), itself an ASD-implicated gene as discussed above. Together with its interactions with several key transcriptional determinants

of neocortical development, including *Fezf2*, *Bcl11b*, and *basic helix-loop-helix family, member e22* (*Bhlhe22*, also *Bhlhb5*) (Srinivasan et al., 2012), which are discussed below, *Satb2* may occupy a central position in transcriptional networks relevant to ASD. Consistent with this possibility, *SATB2* is also a highly connected hub gene in a gene co-expression network that contains *FEZF2*, *SOX5*, and *TBR1* (Kang et al., 2011).

SATB2 controls multiple processes required for the formation of intracortical circuits that may be relevant to ASD (Fig. 3). The expression *SATB2* is greatly enriched in the intracortical projection neurons positioned in L2–L5 (Alcamo et al., 2008; Britanova et al., 2008). This expression starts in post-mitotic neurons at around E13.5 as they migrate away from VZ and SVZ enroute to their layer destination. Consistent with its expression, *Satb2* controls the molecular and axonal development of these projection neurons (Alcamo et al., 2008; Baranek et al., 2012; Britanova et al., 2008; Srinivasan et al., 2012). In the absence of *Satb2*, the molecular profiles of upper layer neurons are greatly altered, including the expression of layer-specific markers of intracortical projection neurons. *Cut-like homeobox 2* (*Cux2*), *caderhin 10* (*Cdh10*), and *RAR-related orphan receptor beta* (*Rorb*) are severely reduced in the *Satb2*-deficient neocortex (Alcamo et al., 2008). In addition, *Satb2* regulates, in a layer-dependent manner, the expression of *Bhlhe22* (Srinivasan et al., 2012), which encodes a transcription factor that post-mitotically controls neocortical areal identity (Joshi et al., 2008). Furthermore, *Satb2* suppresses the aberrant expression of other layer markers. In *Satb2*-null mice, the expression of L5 subcortical neuronal marker *Bcl11b* is de-repressed, and thus ectopically upregulated in the upper cortical layers (Alcamo et al., 2008; Britanova et al., 2008).

Accompanying these alterations in molecular identity, intracortical projection neurons aberrantly project their axons to subcortical brain structures in the absence of *Satb2*. This leads to the agenesis of the corpus callosum and a misrouting of callosal axons to the anterior commissure (Alcamo et al., 2008; Britanova et al., 2008). Unlike mutants in which callosal defects occur secondary to structural changes in the brain, such as the absence of a glial sling or other midline structures, callosal agenesis in the *Satb2*-null neocortex was not associated with Probst bundles, which suggests that *Satb2* is a cell-autonomous determinant of intracortical connectivity. Consistent with this possibility, this role of *Satb2* is mediated, likely cell-autonomously, by its control of axonal guidance molecules in upper-layer neurons. In the *Satb2* mutant neocortex, the upper-layer expression of *Eph receptor A4* (*EphA4*), *plexin A4* (*PlxnA4*), and *unc-5 homolog C* (*Unc5c*, also known as *Unc5H3*) is reduced (Srinivasan et al., 2012). Notably, the callosal defects that result from the absence of *Satb2* can be rescued by re-expression of *EphA4* or *Unc5c*, or *Tbr1*, the upper-layer expression of which may play a cell-autonomous role in specifying callosal connectivity (Srinivasan et al., 2012). As discussed above, defects in intracortical connectivity, including callosal projections, have been consistently implicated in ASD (Ingahalikar et al., 2011; Kumar et al., 2010; Lange et al., 2010; Lee et al., 2007; Nagae et al., 2012; Shukla et al., 2010; Sundaram et al., 2008; Weinstein et al., 2011; Wolff et al., 2012). These defects in the *Satb2*-deficient neocortex, therefore, are consistent with the potential contribution of *SATB2* to ASD-relevant neural circuits. In addition, *Satb2* controls the dendritic arborization and soma density of upper-layer neurons (Zhang et al., 2011), which may further contribute to

the overall development of input and output connections in intracortical circuits. This role of *Satb2* is also consistent with the disruption of dendritic spine morphology in ASD (Comery et al., 1997; Hutsler and Zhang, 2010; Irwin et al., 2000).

In addition to axonal projections, *Satb2* further regulates neuronal migration (Fig. 3). Similar to its control of molecular identity and connectivity, this role of *Satb2* is also layer-dependent. In *Satb2*-deficient mice, the migration of early-born neurons to the SP and deep cortical layers is unaffected. In contrast, a significant proportion of late-born neurons destined for the upper layers are stalled in the IZ, being unable to enter the CP (Alcamo et al., 2008; Britanova et al., 2008). This neuronal migration defect, however, was restricted to the perinatal ages, as these defects are corrected within the first post-natal week, with the majority of late-born neurons eventually arriving at their normal upper layer positions (Britanova et al., 2008; Zhang et al., 2011). These data suggest that, in the absence of *Satb2*, neuronal migration is not altogether defective, but rather, delayed. This delay is distinct from the neuronal migration defects described for *Tbr1* and *Sox5*, as well as those reported in post-mortem ASD brains (Wegiel et al., 2010). Whether delay of neuronal migration is present in ASD, however, might not be readily assessed by examination of post-mortem adult brains, as this defect is only transiently present during development. Although the presence of this defect in ASD remains to be fully examined, incorrect timing of the arrival of projection neurons to their CP destination can have functional consequences on neuronal circuits. The wiring of neocortical circuitry involves a highly-coordinated series of events. The early-postnatal ages in mouse neocortical development, which is approximately equivalent to mid-fetal development in the human neocortex (Clancy et al., 2007), represent a period of significant synaptogenesis and a time when both efferents and afferents tracts are being assembled. A delay in the arrival of neurons to the upper layers, therefore, can alter the formation of key neocortical circuits and contribute to neocortical miswiring in ASD.

Discussion

As the pace of genetic discoveries accelerates, functional studies are required to understand how alterations in candidate risk genes can contribute to ASD. Mechanistic studies of ASD neurobiology, however, are challenging not only because of the significant degree of phenotypic and genetic heterogeneity, but also because the complex cognitive and social deficits in ASD cannot be readily modeled in rodents.

Genetic studies in mice, however, do have the potential to illuminate mechanisms of neural circuitry assembly relevant to ASD. Many fundamental aspects of neocortical development are well conserved between rodent and primate species, including the establishment of primary neocortical areas and major axon tracts, and the genetic programs underlying their specification. Indeed, the layer-dependent expression patterns of the four transcriptional determinants discussed in this chapter (*Tbr1*, *Sox5*, *Fzf2*, and *Satb2*) are conserved between the mouse and human neocortices (Ip et al., 2011; Kwan et al., 2012a; Kwan et al., 2008; Saito et al., 2011). This suggests not only that their highly specific gene expression patterns are controlled by the same upstream transcriptional regulators in the two species, but also that their functions during neocortical development are shared. These common mechanisms should be amenable to being modeled in the mouse. Furthermore, although the mouse is an

imperfect model, given the technical, logistical, and ethical considerations, no better alternatives exist for experimental interrogation of intact neural circuits.

Genetic removal of *Tbr1*, *Sox5*, *Fezf2*, or *Satb2* from the mouse leads to profound defects in the neocortex, which are not observed in human ASD cases. It should be noted, however, that the mouse genetic studies discussed in this chapter involved the complete, homozygous removal of the genes of interest from the animal in order to reveal the most severe phenotypes. In human cases, these genes are likely to be affected less severely. Often, the reported ASD variants are SNVs or CNVs that do not remove the entire gene. Furthermore, all of the ASD cases discussed in this chapter are heterozygous at the locus in question, suggesting that one functional copy of the gene is present. Subtle alterations in gene dosage or functional capacity may more subtly affect neocortical circuit wiring in a manner consistent with ASD. In future experiments, recapitulating patient mutations in the mouse is likely to reveal mechanistic insights most relevant to human ASD.

Studies of mouse genetics have revealed diverse neocortical phenotypes in the *Tbr1*, *Sox5*, *Fezf2*, and *Satb2* mutants. There are, however, points of convergence that may illuminate the biology of ASD.

First, relationships in gene function can be inferred based on gene expression patterns if the spatiotemporal expression data are sufficiently comprehensive. The unbiased assembly of gene co-expression networks from large datasets represents a powerful tool to uncover previously unrecognized functional relationships between genes (Konopka et al., 2012a; Oldham et al., 2008). Network analysis of human brain transcriptome data revealed that *TBR1*, *FEZF2*, and *SATB2* are not only present within the same cortical development module, but also exhibit high intramodular connectivity, being amongst the top 10 hub genes within the module (Kang et al., 2011). These data indicate that during human brain development, *TBR1*, *FEZF2*, and *SATB2* exhibit considerable spatiotemporal overlap not only with each other, which indicates related function, but also with many other genes within the module. The latter suggests the possibility that these TFs regulate, and perhaps co-regulate, a large number of genes within the cortical development module, which is consistent with their roles as key determinants in numerous aspects of neocortical development.

Second, recent evidence points to the presence of complex transcriptional networks in the specification of neocortical projection neuron identities and connectivities (Chen et al., 2008; Han et al., 2011; Kwan et al., 2008; Leone et al., 2008; McKenna et al., 2011; Shim et al., 2012; Srinivasan et al., 2012). The direct and indirect interactions between *TBR1*, *SOX5*, *FEZF2*, and *SATB2* are emerging as important cross-regulatory mechanisms central to the establishment of distinct neuronal subtypes and projections. Perturbations of key determinants within this transcriptional network may alter or switch certain neuronal fates and disturb the fine balance between intracortical, corticofugal, and more subtle neuronal and projectional subtypes. Such changes are likely to have wider functional consequences on neocortical circuitry.

The convergence of *TBR1*, *SOX5*, *FEZF2*, and *SATB2* in molecular pathways within the cortical development strongly implicates that they contribute to ASD by regulating the proper assembly of neocortical circuits. Future experiments aimed at the identification and characterization of effectors downstream of these transcriptional programs are expected to shed further light on pathways necessary for neocortical circuit assembly and relevant to ASD pathophysiology.

Acknowledgments

I thank Yuka Imamura Kawasaki and Mingfeng Li for help with the human brain transcriptome data in Fig. 1. This work was supported by the National Institutes of Health (MH096939).

References

- Alcamo E, Chirivella L, Dautzenberg M, Dobрева G, Fariñas I, Grosschedl R, McConnell S. *Satb2* regulates callosal projection neuron identity in the developing cerebral cortex. *Neuron*. 2008; 57:364–377. [PubMed: 18255030]
- Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, Miller JN, Lu J, Jeong EK, McMahon WM, et al. Diffusion tensor imaging of the corpus callosum in Autism. *Neuroimage*. 2007; 34:61–73. [PubMed: 17023185]
- Allendoerfer KL, Shatz CJ. The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annu Rev Neurosci*. 1994; 17:185–218. [PubMed: 8210173]
- Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes. *Science*. 1997; 278:474–476. [PubMed: 9334308]
- Ang ES, Haydar TF, Gluncic V, Rakic P. Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. *J Neurosci*. 2003; 23:5805–5815. [PubMed: 12843285]
- Angevine JB Jr, Sidman RL. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature*. 1961; 192:766–768. [PubMed: 17533671]
- Anthony TE, Klein C, Fishell G, Heintz N. Radial glia serve as neuronal progenitors in all regions of the central nervous system. *Neuron*. 2004; 41:881–890. [PubMed: 15046721]
- Anton ES, Kreidberg JA, Rakic P. Distinct functions of $\alpha3$ and $\alpha(v)$ integrin receptors in neuronal migration and laminar organization of the cerebral cortex. *Neuron*. 1999; 22:277–289. [PubMed: 10069334]
- Arlotta P, Molyneaux BJ, Chen J, Inoue J, Kominami R, Macklis JD. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. *Neuron*. 2005; 45:207–221. [PubMed: 15664173]
- Baranek C, Ditttrich M, Parthasarathy S, Bonnon CG, Britanova O, Lanshakov D, Boukhtouche F, Sommer JE, Colmenares C, Tarabykin V, et al. Protooncogene *Ski* cooperates with the chromatin-remodeling factor *Satb2* in specifying callosal neurons. *Proc Natl Acad Sci U S A*. 2012; 109:3546–3551. [PubMed: 22334647]
- Bedogni F, Hodge R, Elsen G, Nelson B, Daza R, Beyer R, Bammler T, Rubenstein J, Hevner R. *Tbr1* regulates regional and laminar identity of postmitotic neurons in developing neocortex. *Proc Natl Acad Sci U S A*. 2010; 107:13129–13134. [PubMed: 20615956]
- Ben-David E, Granot-HersHKovitz E, Monderer-Rothkoff G, Lerer E, Levi S, Yaari M, Ebshtein RP, Yirmiya N, Shifman S. Identification of a functional rare variant in autism using genome-wide screen for monoallelic expression. *Hum Mol Genet*. 2011; 20:3632–3641. [PubMed: 21680558]
- Bentivoglio M, Mazzarello P. The history of radial glia. *Brain Res Bull*. 1999; 49:305–315. [PubMed: 10452351]
- Bowers JM, Konopka G. The role of the *FOXP* family of transcription factors in ASD. *Dis Markers*. 2012; 33:251–260. [PubMed: 22960337]

- Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D, et al. Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. *Neuron*. 2008; 57:378–392. [PubMed: 18255031]
- Casanova MF, Buxhoeveden D, Gomez J. Disruption in the inhibitory architecture of the cell minicolumn: implications for autism. *Neuroscientist*. 2003; 9:496–507. [PubMed: 14678582]
- Casanova MF, Buxhoeveden DP, Switala AE, Roy E. Minicolumnar pathology in autism. *Neurology*. 2002; 58:428–432. [PubMed: 11839843]
- Caviness VS Jr. Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography. *Brain Res*. 1982; 256:293–302. [PubMed: 7104762]
- Caviness VS Jr, Takahashi T, Nowakowski RS. Numbers, time and neocortical neuronogenesis: a general developmental and evolutionary model. *Trends Neurosci*. 1995; 18:379–383. [PubMed: 7482802]
- Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, Neul JL, Gong S, Lu HC, Heintz N, et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*. 2010; 468:263–269. [PubMed: 21068835]
- Chen B, Schaevitz L, McConnell S. Fezl regulates the differentiation and axon targeting of layer 5 subcortical projection neurons in cerebral cortex. *Proc Natl Acad Sci U S A*. 2005a; 102:17184–17189. [PubMed: 16284245]
- Chen B, Wang S, Hattox A, Rayburn H, Nelson S, McConnell S. The Fezf2-Ctip2 genetic pathway regulates the fate choice of subcortical projection neurons in the developing cerebral cortex. *Proc Natl Acad Sci U S A*. 2008; 105:11382–11387. [PubMed: 18678899]
- Chen J, Rasin M, Kwan K, Sestan N. Zfp312 is required for subcortical axonal projections and dendritic morphology of deep-layer pyramidal neurons of the cerebral cortex. *Proc Natl Acad Sci U S A*. 2005b; 102:17792–17797. [PubMed: 16314561]
- Chonchaiya W, Schneider A, Hagerman RJ. Fragile X: a family of disorders. *Adv Pediatr*. 2009; 56:165–186. [PubMed: 19968948]
- Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL. Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics*. 2007; 5:79–94. [PubMed: 17426354]
- Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, Leek JT, Colantuoni EA, Elkahoul AG, Herman MM, Weinberger DR, et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature*. 2011; 478:519–523. [PubMed: 22031444]
- Comery TA, Harris JB, Willems PJ, Oostra BA, Irwin SA, Weiler IJ, Greenough WT. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc Natl Acad Sci U S A*. 1997; 94:5401–5404. [PubMed: 9144249]
- D’Arcangelo G, Curran T. Reeler: new tales on an old mutant mouse. *Bioessays*. 1998; 20:235–244. [PubMed: 9631651]
- De Carlos JA, O’Leary DD. Growth and targeting of subplate axons and establishment of major cortical pathways. *J Neurosci*. 1992; 12:1194–1211. [PubMed: 1556593]
- De la Rossa A, Bellone C, Golding B, Vitali I, Moss J, Toni N, Lüscher C, Jabaudon D. In vivo reprogramming of circuit connectivity in postmitotic neocortical neurons. *Nat Neurosci*. 2013; 16:193–200. [PubMed: 23292682]
- DeFelipe J, Farinas I. The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog Neurobiol*. 1992; 39:563–607. [PubMed: 1410442]
- Del Río J, Martínez A, Auladell C, Soriano E. Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages. *Cereb Cortex*. 2000; 10:784–801. [PubMed: 10920050]
- Enard W, Przeworski M, Fisher SE, Lai CS, Wiebe V, Kitano T, Monaco AP, Pääbo S. Molecular evolution of FOXP2, a gene involved in speech and language. *Nature*. 2002; 418:869–872. [PubMed: 12192408]
- Englund C, Fink A, Lau C, Pham D, Daza RA, Bulfone A, Kowalczyk T, Hevner RF. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. *J Neurosci*. 2005; 25:247–251. [PubMed: 15634788]

- Fischbach GD, Lord C. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. *Neuron*. 2010; 68:192–195. [PubMed: 20955926]
- Fishell G, Hanashima C. Pyramidal neurons grow up and change their mind. *Neuron*. 2008; 57:333–338. [PubMed: 18255026]
- Geschwind D, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol*. 2007; 17:103–111. [PubMed: 17275283]
- Girirajan S, Dennis MY, Baker C, Malig M, Coe BP, Campbell CD, Mark K, Vu TH, Alkan C, Cheng Z, et al. Refinement and discovery of new hotspots of copy-number variation associated with autism spectrum disorder. *Am J Hum Genet*. 2013; 92:221–237. [PubMed: 23375656]
- Gläser B, Rossier E, Barbi G, Chiaie LD, Blank C, Vogel W, Kehrer-Sawatzki H. Molecular cytogenetic analysis of a constitutional de novo interstitial deletion of chromosome 12p in a boy with developmental delay and congenital anomalies. *Am J Med Genet A*. 2003; 116A:66–70. [PubMed: 12476454]
- Gray PA, Fu H, Luo P, Zhao Q, Yu J, Ferrari A, Tenzen T, Yuk DI, Tsung EF, Cai Z, et al. Mouse brain organization revealed through direct genome-scale TF expression analysis. *Science*. 2004; 306:2255–2257. [PubMed: 15618518]
- Guillemot F. Cell fate specification in the mammalian telencephalon. *Prog Neurobiol*. 2007; 83:37–52. [PubMed: 17517461]
- Hamdan FF, Daoud H, Rochefort D, Piton A, Gauthier J, Langlois M, Foomani G, Dobrzyniecka S, Krebs MO, Joober R, et al. De novo mutations in FOXP1 in cases with intellectual disability, autism, and language impairment. *Am J Hum Genet*. 2010; 87:671–678. [PubMed: 20950788]
- Han W, Kwan KY, Shim S, Lam MM, Shin Y, Xu X, Zhu Y, Li M, Sestan N. TBR1 directly represses Fezf2 to control the laminar origin and development of the corticospinal tract. *Proc Natl Acad Sci U S A*. 2011; 108:3041–3046. [PubMed: 21285371]
- Hashimoto H, Yabe T, Hirata T, Shimizu T, Bae Y, Yamanaka Y, Hirano T, Hibi M. Expression of the zinc finger gene fez-like in zebrafish forebrain. *Mech Dev*. 2000; 97:191–195. [PubMed: 11025224]
- Haubensak W, Attardo A, Denk W, Huttner WB. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. *Proc Natl Acad Sci U S A*. 2004; 101:3196–3201. [PubMed: 14963232]
- Herrmann K, Antonini A, Shatz CJ. Ultrastructural evidence for synaptic interactions between thalamocortical axons and subplate neurons. *Eur J Neurosci*. 1994; 6:1729–1742. [PubMed: 7874312]
- Hevner R, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet A, Campagnoni A, Rubenstein J. Tbr1 regulates differentiation of the preplate and layer 6. *Neuron*. 2001; 29:353–366. [PubMed: 11239428]
- Hevner RF, Hodge RD, Daza RA, Englund C. Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. *Neurosci Res*. 2006; 55:223–233. [PubMed: 16621079]
- Hirata T, Nakazawa M, Muraoka O, Nakayama R, Suda Y, Hibi M. Zinc-finger genes Fez and Fez-like function in the establishment of diencephalon subdivisions. *Development*. 2006; 133:3993–4004. [PubMed: 16971467]
- Hirata T, Suda Y, Nakao K, Narimatsu M, Hirano T, Hibi M. Zinc finger gene fez-like functions in the formation of subplate neurons and thalamocortical axons. *Dev Dyn*. 2004; 230:546–556. [PubMed: 15188439]
- Hoerder-Suabedissen A, Oeschger FM, Krishnan ML, Belgard TG, Wang WZ, Lee S, Webber C, Petretto E, Edwards AD, Molnár Z. Expression profiling of mouse subplate reveals a dynamic gene network and disease association with autism and schizophrenia. *Proc Natl Acad Sci U S A*. 2013; 110:3555–3560. [PubMed: 23401504]
- Holt R, Barnby G, Maestrini E, Bacchelli E, Brocklebank D, Sousa I, Mulder EJ, Kantojärvi K, Järvelä I, Klauck SM, et al. Linkage and candidate gene studies of autism spectrum disorders in European populations. *Eur J Hum Genet*. 2010; 18:1013–1019. [PubMed: 20442744]

- Huang XL, Zou YS, Maher TA, Newton S, Milunsky JM. A de novo balanced translocation breakpoint truncating the autism susceptibility candidate 2 (AUTS2) gene in a patient with autism. *Am J Med Genet A*. 2010; 152A:2112–2114. [PubMed: 20635338]
- Hutsler JJ, Love T, Zhang H. Histological and magnetic resonance imaging assessment of cortical layering and thickness in autism spectrum disorders. *Biol Psychiatry*. 2007; 61:449–457. [PubMed: 16580643]
- Hutsler JJ, Zhang H. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res*. 2010; 1309:83–94. [PubMed: 19896929]
- Hébert JM, Fishell G. The genetics of early telencephalon patterning: some assembly required. *Nat Rev Neurosci*. 2008; 9:678–685. [PubMed: 19143049]
- Ingalhalikar M, Parker D, Bloy L, Roberts TP, Verma R. Diffusion based abnormality markers of pathology: toward learned diagnostic prediction of ASD. *Neuroimage*. 2011; 57:918–927. [PubMed: 21609768]
- Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, et al. De novo gene disruptions in children on the autistic spectrum. *Neuron*. 2012; 74:285–299. [PubMed: 22542183]
- Ip BK, Bayatti N, Howard NJ, Lindsay S, Clowry GJ. The corticofugal neuron-associated genes ROBO1, SRGAP1, and CTIP2 exhibit an anterior to posterior gradient of expression in early fetal human neocortex development. *Cereb Cortex*. 2011; 21:1395–1407. [PubMed: 21060114]
- Irwin SA, Galvez R, Greenough WT. Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb Cortex*. 2000; 10:1038–1044. [PubMed: 11007554]
- Jessell T. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat Rev Genet*. 2000; 1:20–29. [PubMed: 11262869]
- Jiang YH, Yuen RK, Jin X, Wang M, Chen N, Wu X, Ju J, Mei J, Shi Y, He M, et al. Detection of Clinically Relevant Genetic Variants in Autism Spectrum Disorder by Whole-Genome Sequencing. *Am J Hum Genet*. 2013
- Johnson MB, Kawasaki YI, Mason CE, Krsnik Z, Coppola G, Bogdanovic D, Geschwind DH, Mane SM, State MW, Sestan N. Functional and evolutionary insights into human brain development through global transcriptome analysis. *Neuron*. 2009; 62:494–509. [PubMed: 19477152]
- Jones EG. Neurotransmitters in the cerebral cortex. *J Neurosurg*. 1986; 65:135–153. [PubMed: 2873211]
- Joshi PS, Molyneaux BJ, Feng L, Xie X, Macklis JD, Gan L. Bhlhb5 regulates the postmitotic acquisition of area identities in layers II–V of the developing neocortex. *Neuron*. 2008; 60:258–272. [PubMed: 18957218]
- Just MA, Keller TA, Malave VL, Kana RK, Varma S. Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. *Neurosci Biobehav Rev*. 2012; 36:1292–1313. [PubMed: 22353426]
- Kalscheuer VM, FitzPatrick D, Tommerup N, Bugge M, Niebuhr E, Neumann LM, Tzschach A, Shoichet SA, Menzel C, Erdogan F, et al. Mutations in autism susceptibility candidate 2 (AUTS2) in patients with mental retardation. *Hum Genet*. 2007; 121:501–509. [PubMed: 17211639]
- Kang HJ, Kawasaki YI, Cheng F, Zhu Y, Xu X, Li M, Sousa AM, Pletikos M, Meyer KA, Sedmak G, et al. Spatio-temporal transcriptome of the human brain. *Nature*. 2011; 478:483–489. [PubMed: 22031440]
- Kelemenova S, Schmidtova E, Ficek A, Celec P, Kubranska A, Ostatnikova D. Polymorphisms of candidate genes in Slovak autistic patients. *Psychiatr Genet*. 2010; 20:137–139. [PubMed: 20436377]
- Keller TA, Kana RK, Just MA. A developmental study of the structural integrity of white matter in autism. *Neuroreport*. 2007; 18:23–27. [PubMed: 17259855]
- Kerjan G, Gleeson JG. Genetic mechanisms underlying abnormal neuronal migration in classical lissencephaly. *Trends Genet*. 2007; 23:623–630. [PubMed: 17997185]
- Konopka G, Bomar JM, Winden K, Coppola G, Jonsson ZO, Gao F, Peng S, Preuss TM, Wohlschlegel JA, Geschwind DH. Human-specific transcriptional regulation of CNS development genes by FOXP2. *Nature*. 2009; 462:213–217. [PubMed: 19907493]

- Konopka G, Friedrich T, Davis-Turak J, Winden K, Oldham MC, Gao F, Chen L, Wang GZ, Luo R, Preuss TM, et al. Human-specific transcriptional networks in the brain. *Neuron*. 2012a; 75:601–617. [PubMed: 22920253]
- Konopka G, Wexler E, Rosen E, Mukamel Z, Osborn GE, Chen L, Lu D, Gao F, Gao K, Lowe JK, et al. Modeling the functional genomics of autism using human neurons. *Mol Psychiatry*. 2012b; 17:202–214. [PubMed: 21647150]
- Kostovic I, Rakic P. Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J Neurocytol*. 1980; 9:219–242. [PubMed: 7441294]
- Kostovic I, Rakic P. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J Comp Neurol*. 1990; 297:441–470. [PubMed: 2398142]
- Kowalczyk T, Pontious A, Englund C, Daza RA, Bedogni F, Hodge R, Attardo A, Bell C, Huttner WB, Hevner RF. Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb Cortex*. 2009; 19:2439–2450. [PubMed: 19168665]
- Krepisch AC, Knijnenburg J, Bertola DR, Kim CA, Pearson PL, Bijlsma E, Szuhai K, Kok F, Vianna-Morgante AM, Rosenberg C. Two distinct regions in 2q24.2–q24.3 associated with idiopathic epilepsy. *Epilepsia*. 2010; 51:2457–2460. [PubMed: 21204806]
- Kumar A, Sundaram SK, Sivaswamy L, Behen ME, Makki MI, Ager J, Janisse J, Chugani HT, Chugani DC. Alterations in frontal lobe tracts and corpus callosum in young children with autism spectrum disorder. *Cereb Cortex*. 2010; 20:2103–2113. [PubMed: 20019145]
- Kwan KY, Lam MM, Johnson MB, Dube U, Shim S, Rašin MR, Sousa AM, Fertuzinhos S, Chen JG, Arellano JI, et al. Species-dependent posttranscriptional regulation of NOS1 by FMRP in the developing cerebral cortex. *Cell*. 2012a; 149:899–911. [PubMed: 22579290]
- Kwan KY, Lam MM, Krsnik Z, Kawasawa YI, Lefebvre V, Sestan N. SOX5 postmitotically regulates migration, postmigratory differentiation, and projections of subplate and deep-layer neocortical neurons. *Proc Natl Acad Sci U S A*. 2008; 105:16021–16026. [PubMed: 18840685]
- Kwan KY, Sestan N, Anton ES. Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. *Development*. 2012b; 139:1535–1546. [PubMed: 22492350]
- Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature*. 2001; 413:519–523. [PubMed: 11586359]
- Lai T, Jabaudon D, Molyneaux B, Azim E, Arlotta P, Menezes J, Macklis J. SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. *Neuron*. 2008; 57:232–247. [PubMed: 18215621]
- Lamb AN, Rosenfeld JA, Neill NJ, Talkowski ME, Blumenthal I, Girirajan S, Keelean-Fuller D, Fan Z, Pouncey J, Stevens C, et al. Haploinsufficiency of SOX5 at 12p12.1 is associated with developmental delays with prominent language delay, behavior problems, and mild dysmorphic features. *Hum Mutat*. 2012; 33:728–740. [PubMed: 22290657]
- Lambert de Rouvroit C, Goffinet AM. A new view of early cortical development. *Biochem Pharmacol*. 1998; 56:1403–1409. [PubMed: 9827572]
- Lange N, Dubray MB, Lee JE, Froimowitz MP, Froehlich A, Adluru N, Wright B, Ravichandran C, Fletcher PT, Bigler ED, et al. Atypical diffusion tensor hemispheric asymmetry in autism. *Autism Res*. 2010; 3:350–358. [PubMed: 21182212]
- Lee JE, Bigler ED, Alexander AL, Lazar M, DuBray MB, Chung MK, Johnson M, Morgan J, Miller JN, McMahon WM, et al. Diffusion tensor imaging of white matter in the superior temporal gyrus and temporal stem in autism. *Neurosci Lett*. 2007; 424:127–132. [PubMed: 17714869]
- Lefebvre V, Dumitriu B, Penzo-Méndez A, Han Y, Pallavi B. Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. *Int J Biochem Cell Biol*. 2007; 39:2195–2214. [PubMed: 17625949]
- Leone DP, Srinivasan K, Chen B, Alcamo E, McConnell SK. The determination of projection neuron identity in the developing cerebral cortex. *Curr Opin Neurobiol*. 2008; 18:28–35. [PubMed: 18508260]

- Leoyklang P, Suphapeetiporn K, Siriwan P, Desudchit T, Chaowanapanja P, Gahl WA, Shotelersuk V. Heterozygous nonsense mutation SATB2 associated with cleft palate, osteoporosis, and cognitive defects. *Hum Mutat.* 2007; 28:732–738. [PubMed: 17377962]
- Li H, Li Y, Shao J, Li R, Qin Y, Xie C, Zhao Z. The association analysis of RELN and GRM8 genes with autistic spectrum disorder in Chinese Han population. *Am J Med Genet B Neuropsychiatr Genet.* 2008; 147B:194–200. [PubMed: 17955477]
- Liu JS. Molecular genetics of neuronal migration disorders. *Curr Neurol Neurosci Rep.* 2011; 11:171–178. [PubMed: 21222180]
- Liu X, Novosedlik N, Wang A, Hudson ML, Cohen IL, Chudley AE, Forster-Gibson CJ, Lewis SM, Holden JJ. The DLX1 and DLX2 genes and susceptibility to autism spectrum disorders. *Eur J Hum Genet.* 2009; 17:228–235. [PubMed: 18728693]
- Lodato S, Rouaux C, Quast KB, Jantrachotechatchawan C, Studer M, Hensch TK, Arlotta P. Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex. *Neuron.* 2011; 69:763–779. [PubMed: 21338885]
- Lu HY, Cui YX, Shi YC, Xia XY, Liang Q, Yao B, Ge YF, Li XJ, Huang YF. A girl with distinctive features of borderline high blood pressure, short stature, characteristic brachydactyly, and 11.47 Mb deletion in 12p11.21–12p12.2 by oligonucleotide array CGH. *Am J Med Genet A.* 2009; 149A:2321–2323. [PubMed: 19764034]
- Lui JH, Hansen DV, Kriegstein AR. Development and evolution of the human neocortex. *Cell.* 2011; 146:18–36. [PubMed: 21729779]
- MacDonald JL, Fame RM, Azim E, Shnyder SJ, Molyneaux BJ, Arlotta P, Macklis JD. Specification of cortical projection neurons: transcriptional mechanisms. *Developmental Neuroscience: A Comprehensive Reference.* 2012:1.
- Magri C, Piovani G, Pilotta A, Michele T, Buzi F, Barlati S. De novo deletion of chromosome 2q24.2 region in a mentally retarded boy with muscular hypotonia. *Eur J Med Genet.* 2011; 54:361–364. [PubMed: 21211576]
- Malatesta P, Hack MA, Hartfuss E, Kettenmann H, Klinkert W, Kirchhoff F, Gotz M. Neuronal or glial progeny: regional differences in radial glia fate. *Neuron.* 2003; 37:751–764. [PubMed: 12628166]
- Mallamaci A, Stoykova A. Gene networks controlling early cerebral cortex arealization. *Eur J Neurosci.* 2006; 23:847–856. [PubMed: 16519650]
- Manzini MC, Walsh CA. What disorders of cortical development tell us about the cortex: one plus one does not always make two. *Curr Opin Genet Dev.* 2011; 21:333–339. [PubMed: 21288712]
- Marin-Padilla M. Early prenatal ontogenesis of the cerebral cortex (neocortex) of the cat (*Felis domestica*). A Golgi study. I. The primordial neocortical organization. *Z Anat Entwicklungsgesch.* 1971; 134:117–145. [PubMed: 4932608]
- Marin-Padilla M. Dual origin of the mammalian neocortex and evolution of the cortical plate. *Anat Embryol (Berl).* 1978; 152:109–126. [PubMed: 637312]
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci.* 2004; 5:793–807. [PubMed: 15378039]
- Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet.* 2008; 82:477–488. [PubMed: 18252227]
- Marín O, Rubenstein JL. Cell migration in the forebrain. *Annu Rev Neurosci.* 2003; 26:441–483. [PubMed: 12626695]
- Marín-Padilla M. Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: a unifying theory. *J Comp Neurol.* 1992; 321:223–240. [PubMed: 1500541]
- McKenna WL, Betancourt J, Larkin KA, Abrams B, Guo C, Rubenstein JL, Chen B. Tbr1 and Fezf2 regulate alternate corticofugal neuronal identities during neocortical development. *J Neurosci.* 2011; 31:549–564. [PubMed: 21228164]
- Mefford HC, Muhle H, Ostertag P, von Spiczak S, Buysse K, Baker C, Franke A, Malafosse A, Genton P, Thomas P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet.* 2010; 6:e1000962. [PubMed: 20502679]

- Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, Jian M, Liu G, Greer D, Bhandari A, et al. Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. *Cell*. 2012; 151:1431–1442. [PubMed: 23260136]
- Miyata T, Kawaguchi A, Saito K, Kawano M, Muto T, Ogawa M. Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. *Development*. 2004; 131:3133–3145. [PubMed: 15175243]
- Miyoshi G, Butt SJ, Takebayashi H, Fishell G. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. *J Neurosci*. 2007; 27:7786–7798. [PubMed: 17634372]
- Miyoshi G, Fishell G. GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. *Cereb Cortex*. 2011; 21:845–852. [PubMed: 20732898]
- Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJ, Battiste J, Johnson JE, Machold RP, Fishell G. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. *J Neurosci*. 2010; 30:1582–1594. [PubMed: 20130169]
- Molliver ME, Kostovic I, van der Loos H. The development of synapses in cerebral cortex of the human fetus. *Brain Res*. 1973; 50:403–407. [PubMed: 4705508]
- Molyneaux B, Arlotta P, Hirata T, Hibi M, Macklis J. Fezl is required for the birth and specification of corticospinal motor neurons. *Neuron*. 2005; 47:817–831. [PubMed: 16157277]
- Molyneaux B, Arlotta P, Menezes J, Macklis J. Neuronal subtype specification in the cerebral cortex. *Nat Rev Neurosci*. 2007; 8:427–437. [PubMed: 17514196]
- Monuki ES, Walsh CA. Mechanisms of cerebral cortical patterning in mice and humans. *Nat Neurosci*. 2001; (4 Suppl):1199–1206. [PubMed: 11687830]
- Mountcastle VB. The columnar organization of the neocortex. *Brain*. 1997; 120(Pt 4):701–722. [PubMed: 9153131]
- Nadarajah B, Parnavelas JG. Modes of neuronal migration in the developing cerebral cortex. *Nat Rev Neurosci*. 2002; 3:423–432. [PubMed: 12042877]
- Nagae LM, Zarnow DM, Blaskey L, Dell J, Khan SY, Qasmieh S, Levy SE, Roberts TP. Elevated mean diffusivity in the left hemisphere superior longitudinal fasciculus in autism spectrum disorders increases with more profound language impairment. *AJNR Am J Neuroradiol*. 2012; 33:1720–1725. [PubMed: 22492573]
- Nagai T, Nishimura G, Kato R, Hasegawa T, Ohashi H, Fukushima Y. Del(12)(p11.21p12.2) associated with an asphyxiating thoracic dystrophy or chondroectodermal dysplasia-like syndrome. *Am J Med Genet*. 1995; 55:16–18. [PubMed: 7702088]
- Nakashima N, Yamagata T, Mori M, Kuwajima M, Suwa K, Momoi MY. Expression analysis and mutation detection of DLX5 and DLX6 in autism. *Brain Dev*. 2010; 32:98–104. [PubMed: 19195802]
- Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, Lin CF, Stevens C, Wang LS, Makarov V, et al. Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature*. 2012; 485:242–245. [PubMed: 22495311]
- Nieuwenhuys R. The neocortex. An overview of its evolutionary development, structural organization and synaptology. *Anat Embryol (Berl)*. 1994; 190:307–337. [PubMed: 7840420]
- Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat Neurosci*. 2004; 7:136–144. [PubMed: 14703572]
- Noctor SC, Martínez-Cerdeño V, Kriegstein AR. Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. *J Comp Neurol*. 2008; 508:28–44. [PubMed: 18288691]
- Northcutt RG, Kaas JH. The emergence and evolution of mammalian neocortex. *Trends Neurosci*. 1995; 18:373–379. [PubMed: 7482801]
- O'Leary DD, Koester SE. Development of projection neuron types, axon pathways, and patterned connections of the mammalian cortex. *Neuron*. 1993; 10:991–1006. [PubMed: 8318235]
- O'Leary DD, Sahara S. Genetic regulation of arealization of the neocortex. *Curr Opin Neurobiol*. 2008; 18:90–100. [PubMed: 18524571]

- O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, Karakoc E, Mackenzie AP, Ng SB, Baker C, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat Genet.* 2011; 43:585–589. [PubMed: 21572417]
- O'Roak BJ, Vives L, Fu W, Egerton JD, Stanaway IB, Phelps IG, Carvill G, Kumar A, Lee C, Ankenman K, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science.* 2012a; 338:1619–1622. [PubMed: 23160955]
- O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature.* 2012b; 485:246–250. [PubMed: 22495309]
- Oldham MC, Konopka G, Iwamoto K, Langfelder P, Kato T, Horvath S, Geschwind DH. Functional organization of the transcriptome in human brain. *Nat Neurosci.* 2008; 11:1271–1282. [PubMed: 18849986]
- Palumbo O, D'Agruma L, Minenna AF, Palumbo P, Stallone R, Palladino T, Zelante L, Carella M. 3p14.1 de novo microdeletion involving the FOXP1 gene in an adult patient with autism, severe speech delay and deficit of motor coordination. *Gene.* 2013; 516:107–113. [PubMed: 23287644]
- Palumbo O, Palumbo P, Palladino T, Stallone R, Zelante L, Carella M. A novel deletion in 2q24.1q24.2 in a girl with mental retardation and generalized hypotonia: a case report. *Mol Cytogenet.* 2012; 5:1. [PubMed: 22214275]
- Persico AM, D'Agruma L, Maiorano N, Totaro A, Militeri R, Bravaccio C, Wassink TH, Schneider C, Melmed R, Trillo S, et al. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol Psychiatry.* 2001; 6:150–159. [PubMed: 11317216]
- Peters, A. *The Neurochemical Basis of Autism*. Springer; 2010. The morphology of minicolumns; p. 45–68.
- Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Sonnenblick LI, Gruver R, Almajano J, Bragin A, et al. Absence of CNTNAP2 Leads to Epilepsy, Neuronal Migration Abnormalities, and Core Autism-Related Deficits. *Cell.* 2011; 147:235–246. [PubMed: 21962519]
- Polleux F, Ince-Dunn G, Ghosh A. Transcriptional regulation of vertebrate axon guidance and synapse formation. *Nat Rev Neurosci.* 2007; 8:331–340. [PubMed: 17453014]
- Prasad A, Merico D, Thiruvahindrapuram B, Wei J, Lionel AC, Sato D, Rickaby J, Lu C, Szatmari P, Roberts W, et al. A discovery resource of rare copy number variations in individuals with autism spectrum disorder. *G3 (Bethesda).* 2012; 2:1665–1685. [PubMed: 23275889]
- Rakic P. Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res.* 1971; 33:471–476. [PubMed: 5002632]
- Rakic P. Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science.* 1974; 183:425–427. [PubMed: 4203022]
- Rakic P. Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. *Nature.* 1976; 261:467–471. [PubMed: 819835]
- Rakic P. Specification of cerebral cortical areas. *Science.* 1988; 241:170–176. [PubMed: 3291116]
- Rakic P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci.* 1995; 18:383–388. [PubMed: 7482803]
- Rash B, Grove E. Area and layer patterning in the developing cerebral cortex. *Curr Opin Neurobiol.* 2006; 16:25–34. [PubMed: 16426837]
- Rauch A, Wieczorek D, Graf E, Wieland T, Ende S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet.* 2012; 380:1674–1682. [PubMed: 23020937]
- Rice D, Curran T. Role of the reelin signaling pathway in central nervous system development. *Annu Rev Neurosci.* 2001; 24:1005–1039. [PubMed: 11520926]
- Rosenfeld JA, Ballif BC, Lucas A, Spence EJ, Powell C, Aylsworth AS, Torchia BA, Shaffer LG. Small deletions of SATB2 cause some of the clinical features of the 2q33.1 microdeletion syndrome. *PLoS One.* 2009; 4:e6568. [PubMed: 19668335]

- Rosenfeld JA, Ballif BC, Torchia BS, Sahoo T, Ravnan JB, Schultz R, Lamb A, Bejjani BA, Shaffer LG. Copy number variations associated with autism spectrum disorders contribute to a spectrum of neurodevelopmental disorders. *Genet Med*. 2010; 12:694–702. [PubMed: 20808228]
- Rouaux C, Arlotta P. Direct lineage reprogramming of post-mitotic callosal neurons into corticofugal neurons in vivo. *Nat Cell Biol*. 2013; 15:214–221. [PubMed: 23334497]
- Rubenstein JL. Annual Research Review: Development of the cerebral cortex: implications for neurodevelopmental disorders. *J Child Psychol Psychiatry*. 2011; 52:339–355. [PubMed: 20735793]
- Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. 2003; 2:255–267. [PubMed: 14606691]
- Saito T, Hanai S, Takashima S, Nakagawa E, Okazaki S, Inoue T, Miyata R, Hoshino K, Akashi T, Sasaki M, et al. Neocortical layer formation of human developing brains and lissencephalies: consideration of layer-specific marker expression. *Cereb Cortex*. 2011; 21:588–596. [PubMed: 20624841]
- Sanders SJ, Ercan-Sencicek AG, Hus V, Luo R, Murtha MT, Moreno-De-Luca D, Chu SH, Moreau MP, Gupta AR, Thomson SA, et al. Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. *Neuron*. 2011; 70:863–885. [PubMed: 21658581]
- Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, et al. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature*. 2012; 485:237–241. [PubMed: 22495306]
- Schaaf CP, Sabo A, Sakai Y, Crosby J, Muzny D, Hawes A, Lewis L, Akbar H, Varghese R, Boerwinkle E, et al. Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorders. *Hum Mol Genet*. 2011; 20:3366–3375. [PubMed: 21624971]
- Schanze I, Schanze D, Bacino CA, Douzgou S, Kerr B, Zenker M. Haploinsufficiency of SOX5, a member of the SOX (SRY-related HMG-box) family of transcription factors is a cause of intellectual disability. *Eur J Med Genet*. 2013; 56:108–113. [PubMed: 23220431]
- Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, et al. Strong association of de novo copy number mutations with autism. *Science*. 2007; 316:445–449. [PubMed: 17363630]
- Serajee FJ, Zhong H, Mahbubul Huq AH. Association of Reelin gene polymorphisms with autism. *Genomics*. 2006; 87:75–83. [PubMed: 16311013]
- Sessa A, Mao CA, Colasante G, Nini A, Klein WH, Broccoli V. Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. *Genes Dev*. 2010; 24:1816–1826. [PubMed: 20713522]
- Shim S, Kwan KY, Li M, Lefebvre V, Sestan N. Cis-regulatory control of corticospinal system development and evolution. *Nature*. 2012; 486:74–79. [PubMed: 22678282]
- Shukla DK, Keehn B, Lincoln AJ, Müller RA. White matter compromise of callosal and subcortical fiber tracts in children with autism spectrum disorder: a diffusion tensor imaging study. *J Am Acad Child Adolesc Psychiatry*. 2010; 49:1269–1278. 1278.e1261–1262. [PubMed: 21093776]
- Skaar DA, Shao Y, Haines JL, Stenger JE, Jaworski J, Martin ER, DeLong GR, Moore JH, McCauley JL, Sutcliffe JS, et al. Analysis of the RELN gene as a genetic risk factor for autism. *Mol Psychiatry*. 2005; 10:563–571. [PubMed: 15558079]
- Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, Nurnberger JI, Ripke S, Santangelo S, Sullivan PF, Consortium C.-D.G.o.t.P.G. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013; 381:1371–1379. [PubMed: 23453885]
- Srinivasan K, Leone DP, Bateson RK, Dobrev G, Kohwi Y, Kohwi-Shigematsu T, Grosschedl R, McConnell SK. A network of genetic repression and derepression specifies projection fates in the developing neocortex. *Proc Natl Acad Sci U S A*. 2012; 109:19071–19078. [PubMed: 23144223]
- State MW, Sestan N. Neuroscience. The emerging biology of autism spectrum disorders. *Science*. 2012; 337:1301–1303. [PubMed: 22984058]

- Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, Stephan DA, Morton DH. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med*. 2006; 354:1370–1377. [PubMed: 16571880]
- Stumm M, Klopocki E, Gasiorek-Wiens A, Knoll U, Wirjadi D, Sarioglu N, Wegner RD, Tönnies H. Molecular cytogenetic characterisation of an interstitial deletion 12p detected by prenatal diagnosis. *Prenat Diagn*. 2007; 27:475–478. [PubMed: 17330228]
- Sultana R, Yu CE, Yu J, Munson J, Chen D, Hua W, Estes A, Cortes F, de la Barra F, Yu D, et al. Identification of a novel gene on chromosome 7q11.2 interrupted by a translocation breakpoint in a pair of autistic twins. *Genomics*. 2002; 80:129–134. [PubMed: 12160723]
- Sundaram SK, Kumar A, Makki MI, Behen ME, Chugani HT, Chugani DC. Diffusion tensor imaging of frontal lobe in autism spectrum disorder. *Cereb Cortex*. 2008; 18:2659–2665. [PubMed: 18359780]
- Tabata H, Kanatani S, Nakajima K. Differences of migratory behavior between direct progeny of apical progenitors and basal progenitors in the developing cerebral cortex. *Cereb Cortex*. 2009; 19:2092–2105. [PubMed: 19150920]
- Takatsuki S, Nakamura R, Haga Y, Mitsui K, Hashimoto T, Shimojima K, Saji T, Yamamoto T. Severe pulmonary emphysema in a girl with interstitial deletion of 2q24.2q24.3 including ITGB6. *Am J Med Genet A*. 2010; 152A:1020–1025. [PubMed: 20358620]
- Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A, Ernst C, Hanscom C, Rossin E, Lindgren AM, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell*. 2012; 149:525–537. [PubMed: 22521361]
- Tarabykin V, Stoykova A, Usman N, Gruss P. Cortical upper layer neurons derive from the subventricular zone as indicated by Svet1 gene expression. *Development*. 2001; 128:1983–1993. [PubMed: 11493521]
- The Boulder Committee. Embryonic vertebrate central nervous system: revised terminology. The Boulder Committee. *Anat Rec*. 1970; 166:257–261. [PubMed: 5414696]
- Thornton GK, Woods CG. Primary microcephaly: do all roads lead to Rome? *Trends Genet*. 2009; 25:501–510. [PubMed: 19850369]
- Tissir F, Goffinet AM. Reelin and brain development. *Nat Rev Neurosci*. 2003; 4:496–505. [PubMed: 12778121]
- Traylor RN, Dobyns WB, Rosenfeld JA, Wheeler P, Spence JE, Bandholz AM, Bawle EV, Carmany EP, Powell CM, Hudson B, et al. Investigation of TBR1 Hemizygosity: Four Individuals with 2q24 Microdeletions. *Mol Syndromol*. 2012; 3:102–112. [PubMed: 23112752]
- Uppal, N.; Hof, PR. Discrete Cortical Neuropathology in Autism Spectrum Disorders. In: Buxbaum, JD., editor. *The Neuroscience of Autism Spectrum Disorders*. San Diego: Academic Press; 2013. p. 313–325.
- Valiente M, Marín O. Neuronal migration mechanisms in development and disease. *Curr Opin Neurobiol*. 2010; 20:68–78. [PubMed: 20053546]
- Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, Salyakina D, Imielinski M, Bradfield JP, Sleiman PM, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. *Nature*. 2009; 459:528–533. [PubMed: 19404256]
- Wegiel J, Kuchna I, Nowicki K, Imaki H, Marchi E, Ma SY, Chauhan A, Chauhan V, Bobrowicz TW, de Leon M, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol*. 2010; 119:755–770. [PubMed: 20198484]
- Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Ben Itzhak E, Artzi M, Tarrasch R, Eksteine PM, Hendler T, Ben Bashat D. Abnormal white matter integrity in young children with autism. *Hum Brain Mapp*. 2011; 32:534–543. [PubMed: 21391246]
- Weiss LA, Escayg A, Kearney JA, Trudeau M, MacDonald BT, Mori M, Reichert J, Buxbaum JD, Meisler MH. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Mol Psychiatry*. 2003; 8:186–194. [PubMed: 12610651]
- Wolff JJ, Gu H, Gerig G, Elison JT, Styner M, Gouttard S, Botteron KN, Dager SR, Dawson G, Estes AM, et al. Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. *Am J Psychiatry*. 2012; 169:589–600. [PubMed: 22362397]

- Wonders CP, Anderson SA. The origin and specification of cortical interneurons. *Nat Rev Neurosci*. 2006; 7:687–696. [PubMed: 16883309]
- Xu Q, Cobos I, De La Cruz E, Rubenstein JL, Anderson SA. Origins of cortical interneuron subtypes. *J Neurosci*. 2004; 24:2612–2622. [PubMed: 15028753]
- Yokota Y, Gashghaei HT, Han C, Watson H, Campbell KJ, Anton ES. Radial glial dependent and independent dynamics of interneuronal migration in the developing cerebral cortex. *PLoS ONE*. 2007; 2:e794. [PubMed: 17726524]
- Zhang L, Song NN, Chen JY, Huang Y, Li H, Ding YQ. Satb2 Is Required for Dendritic Arborization and Soma Spacing in Mouse Cerebral Cortex. *Cereb Cortex*. 2011

Figure 1.

Developmental expression of 15 select ASD risk genes in the human neocortex and other human brain regions. A collective upregulation in ASD risk gene expression (red arrowhead) is present during mid-fetal development in the neocortex but not other brain regions (data from Kang et al., 2011).

Figure 2.

Neocortical development in the mouse. Prior to neurogenesis (<E10), neuroepithelial progenitors (NPs) divide mostly symmetrically in the ventricular zone (VZ). Starting at <E11, NPs assume radial glial morphology to become radial glia cells (RGCs), which divide asymmetrically to generate neurons and guide their migration to the mantle layers. The first projection neurons settle in the preplate (PP) to form the nascent cortical plate (CP), from which L2 to L6 would emerge. Incoming CP neurons segregate the PP into the marginal zone (MZ) and subplate (SP). Projection neurons are then generated sequentially through successive divisions of RGCs in the VZ, as well as neurogenic divisions of intermediate progenitors (IPs) in the SVZ. The generation of projection neurons and their migration into the CP occurs in an inside-first, outside-last manner; early-born neurons form the deep layers (SP, L6, L5), whereas later-born neurons migrate past older neurons to form more superficial layers (L4, L3, L2). At the end of neurogenesis, the radial scaffold is dismantled and gliogenesis occurs (>E17).

Figure 3. Summary of neocortical gene expression and function for *Tbr1*, *Sox5*, *Fezf2*, and *Satb2*.

Figure 4.
Summary of *TBR1* genetic findings.

Figure 5.
Summary of *SOX5* genetic findings.

Figure 6.
Summary of *FEZF2* genetic findings.

Figure 7.
Summary of *SATB2* genetic findings.