Down Syndrome Developmental Brain Transcriptome Reveals Defective Oligodendrocyte Differentiation and Myelination

Highlights

- Genome-wide spatiotemporal dysregulation of gene expression is found in DS brains
- Transcriptome changes reflect altered oligodendrocyte development and myelination
- Oligodendrocyte differentiation and myelination is altered in DS model mice (Ts65Dn)
- Speed of action potential propagation is decreased in Ts65Dn neocortical white matter

In Brief

Olmos-Serrano et al. identify defects in oligodendrocyte differentiation and white matter development over the timespan in Down syndrome (DS) brain and in Ts65Dn trisomic mice. Their developmental gene expression study provides a new framework for investigating DS neuropathogenesis.

Accession Numbers

GSE59630
Down Syndrome Developmental Brain Transcriptome Reveals Defective Oligodendrocyte Differentiation and Myelination

Jose Luis Olmos-Serrano,1,9 Hyo-Jung Kang,2,3,9 William A. Tyler,1,9 John C. Silbereis,3,9 Feng Cheng,5,4 Ying Zhu,2 Mihovil Pletikos,2 Lucija Jankovic-Rapan,2 Nathan P. Cramer,5 Zygmunt Galdzicki,5 Joseph Goodliffe,1 Alan Peters,1 Claire Sethares,1 Ivana Delalle,6 Jeffrey A. Golden,7 Tarik F. Haydar,1,* and Nenad Sestan2,8,*

1Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118, USA
2Department of Neuroscience and Kavli Institute for Neuroscience, Yale School of Medicine, New Haven, CT 06510, USA
3Department of Life Science, Chung-Ang University, Seoul 06974, Korea
4Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL 33620, USA
5Department of Anatomy, Physiology, and Genetics, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA
6Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, MA 02118, USA
7Department of Pathology, Brigham and Women’s Hospital, Boston, MA 02115, USA
8Departments of Genetics and Psychiatry, Section of Comparative Medicine, Program in Cellular Neuroscience, Neurodegeneration and Repair, Child Study Center, Yale School of Medicine, New Haven, CT 06510, USA
9Co-first author
*Correspondence: thaydar@bu.edu (T.F.H.), nenad.sestan@yale.edu (N.S.)
http://dx.doi.org/10.1016/j.neuron.2016.01.042

SUMMARY

Trisomy 21, or Down syndrome (DS), is the most common genetic cause of developmental delay and intellectual disability. To gain insight into the underlying molecular and cellular pathogenesis, we conducted a multi-region transcriptome analysis of DS and euploid control brains spanning from mid-fetal development to adulthood. We found genome-wide alterations in the expression of a large number of genes, many of which exhibited temporal and spatial specificity and were associated with distinct biological processes. In particular, we uncovered co-dysregulation of genes associated with oligodendrocyte differentiation and myelination that were validated via cross-species comparison to Ts65Dn trisomy mice. Furthermore, we show that hypomyelination present in Ts65Dn mice is in part due to cell-autonomous effects of trisomy on oligodendrocyte differentiation and results in slower neocortical action potential transmission. Together, these results identify defects in white matter development and function in DS, and they provide a transcriptional framework for further investigating DS neuropathogenesis.

INTRODUCTION

Trisomy for human chromosome 21 (HSA21) causes Down syndrome (DS) in one of every 732 live births (Canfield et al., 2006), making it the most common genetic cause of developmental delay and intellectual disability. DS is characterized by a constellation of phenotypes affecting many organ systems, including CNS abnormalities such as cognitive and motor impairments, microcephaly, and the early appearance of neuropathological characteristics of Alzheimer’s disease (AD; Hartley et al., 2015; Haydar and Reeves, 2012; Letourneau et al., 2014; Lott, 2012). Prior insights into DS neuropathology have come from studies of human individuals and trisomic mouse models. Morphometric and cellular studies on human brain indicate that trisomy 21 causes complex spatiotemporal disturbances in neural development (Golden and Hyman, 1994; Larsen et al., 2008; Haydar and Reeves, 2012). Mouse models have provided additional insights into the developmental progression of DS, elucidating specific defects in neurogenesis, neuronal differentiation, and impaired synaptic plasticity and learning (Chakrabarti et al., 2007, 2010; Cramer et al., 2015; Hyde et al., 2001; Kleschevnikov et al., 2004; Siarey et al., 1997, 1999; Tyler and Haydar, 2013; Wang et al., 2013). While it is clear that trisomy of HSA21 is the root cause of DS, which biological processes are affected and which HSA21 and non-HSA21 genes are associated with those processes are not fully understood. Furthermore, the extent to which human postmortem neuropathology is reflected in the mouse models (and vice versa) remains one of the key problems hampering further understanding of DS brain development and function.

Transcriptome profiling has provided insight into the pathophysiological mechanisms underlying neurodevelopmental brain disorders (Mitchell and Mirnics, 2012; State and Geschwind, 2015; Tebbenkamp et al., 2014). While several reports have investigated gene expression in postmortem human DS brains or derived pluripotent stem cells (Letourneau et al., 2014; Lockstone et al., 2007; Mao et al., 2003, 2005), these studies are limited by sparse genome coverage, numbers of samples or developmental time points, and potential variances between in vitro and in vivo gene regulation. We therefore set out to characterize the spatiotemporal dynamics of gene expression in the...
DS and matched euploid brains and, subsequently, to decipher the biological processes affected over the course of prenatal and postnatal development.

Genome-wide transcriptional profiling was performed in different regions of male and female postmortem brains spanning from mid-fetal development to adulthood. This approach uncovered a highly dynamic disruption of the DS transcriptome across all chromosomes, with alterations in gene expression in different brain regions over time. Bioinformatic analysis revealed genes and gene networks for which expression was affected throughout the DS brain, revealing key molecular and cellular processes previously implicated in neurological dysfunction in DS as well as many that had not been well characterized in DS brains. Notably, we found transcriptome differences indicating that oligodendrocyte differentiation and myelination are altered in DS. We further identified cell-autonomous deficits in oligodendrocyte differentiation and the production of neocortical myelin, which were accompanied by slower action potential transmission between cerebral hemispheres. Altogether, the data illustrate disruption of distinct gene co-expression networks in DS, providing an extensive framework to identify, prioritize, and test gene candidates and biological processes underlying the neuropathophysiology of DS. To facilitate the usefulness and accessibility of the DS transcriptome, we also have generated an internet database (http://medicine.yale.edu/lab/sestan/resources/).

RESULTS

Genome-wide Spatiotemporal Alterations of Gene Expression in DS Brains

We used the Affymetrix exon array platform, which features comprehensive genome-wide coverage of the human transcriptome across entire transcripts and each individual exon, to characterize gene expression in different regions of DS brains over the course of development (see Experimental Procedures). Transcriptome profiling was performed using total RNA extracted from 11 regions, including multiple regions of the cerebral neocortex, the hippocampus (HIP), and the cerebellar cortex (CBC), using a standardized protocol from high-quality postmortem human brains of DS and clinically and anatomically unrestrained (CBC), using a standardized protocol from high-quality postmortem brains spanning from mid-fetal development to adulthood. This approach uncovered a highly dynamic disruption of the DS transcriptome across all chromosomes, with alterations in gene expression in different brain regions over time. Bioinformatic analysis revealed genes and gene networks for which expression was affected throughout the DS brain, revealing key molecular and cellular processes previously implicated in neurological dysfunction in DS as well as many that had not been well characterized in DS brains. Notably, we found transcriptome differences indicating that oligodendrocyte differentiation and myelination are altered in DS. We further identified cell-autonomous deficits in oligodendrocyte differentiation and the production of neocortical myelin, which were accompanied by slower action potential transmission between cerebral hemispheres. Altogether, the data illustrate disruption of distinct gene co-expression networks in DS, providing an extensive framework to identify, prioritize, and test gene candidates and biological processes underlying the neuropathophysiology of DS. To facilitate the usefulness and accessibility of the DS transcriptome, we also have generated an internet database (http://medicine.yale.edu/lab/sestan/resources/).

Principal component analysis showed segregation between DS and control samples from all 58 paired samples and matched control brains (Table S4). However, the majority of DEX genes were found on other chromosomes in DS compared to their matched control. Together, these studies demonstrate that many genes throughout the genome are altered in DS, confirming and extending previous findings that non-HSA21 genes may contribute significantly to trisomy 21 phenotypes (Letourneau et al., 2014; Lockstone et al., 2007). Most importantly, our results clearly show that many of these DEX genes display dynamic temporal and spatial differences in expression, and they highlight specific biological processes potentially disrupted in the developing DS brain.

Co-dysregulation of Genes Associated with Oligodendrocyte Differentiation and Myelination in DS

To integrate the expression differences observed between DS and matched control samples from all 58 paired samples and
each brain region into a systems-level context, we performed weighted-gene co-expression network analysis (WGCNA) (Zhang and Horvath, 2005) and identified modules of co-expressed genes. We defined 60 gene modules exhibiting altered expression during DS brain development in the CBC, HIP, and multiple neocortical regions (Tables S6 and S7). We then carried out gene ontology enrichment analysis of each module to gain insight into the biological significance of these clusters of co-expressed genes (Table S6). This analysis confirmed a number of cellular and molecular processes previously found to be altered in DS or mouse model brains, including cell morphogenesis (Module [M] 24; Figure S5A), RNA processing (M31), gene transcription (M54; Figure S5B), neuronal differentiation (M4 and M45; Figures S6A and S6D), synaptic transmission and regulation (M10 and M34; Figures S6B and S6C), and electron transport (M14) (Table S6; Bahn et al., 2002; Lockstone et al., 2007; Sahuén et al., 2014; Yoshida et al., 2013; Zampieri et al., 2014).

WGCNA also identified co-expression modules enriched for genes associated with biological processes previously not well characterized in DS. Notably, a module of co-expressed genes, number 43 (M43), was enriched for gene ontology categories related to regulation of action potential (BHA p = 1.7 × 10−2) and axon ensheathment (BHA p = 3.7 × 10−2), and it was markedly downregulated throughout the DS neocortex and in the HIP over development (Figures 2A and 2B; Table S6). Differences in this module accelerated over the course of DS neocortical development (Figures 2A and 2B), coinciding with the onset and progression of myelination. Note that the absence of an M43 expression phenotype in the CBC of DS brains (Figures 2A and 2B) is likely because the most numerous cells in CBC, granule cells, have unmyelinated axons.

To further characterize M43 genes, we investigated whether they were associated with the developmental program of specific neural cell types (i.e., astrocytes, endothelial cells, microglia, neurons, oligodendrocyte progenitor cells, newly formed oligodendrocytes, or myelinating oligodendrocytes). We queried whether M43 genes that were highly expressed (fragments per kilobase per million mapped reads [FPKM] > 20) in different populations of neural cell types purified from mouse cerebral cortex

Figure 1. Genes Dysregulated in DS Brains Are Globally Distributed and Developmentally Dynamic
(A) The percentages of differentially expressed (DEX) genes on each chromosome are shown, indicating global disruptions in gene expression.
(B) The numbers of genes on each chromosome that are DEX in Down syndrome (DS). Note that the majority of DEX genes are not located on HSA21.
(C) The numbers of upregulated (white shading) and downregulated (gray shading) DEX genes per chromosome are shown.
(D and E) A permutation analysis of DEX genes in the dorsal frontal cortex (DFC) (D) and cerebellar cortex (CBC) (E) across four sliding windows corresponding to periods from mid-fetal development to adulthood. Periods of human brain development and adulthood are defined as previously described (Kang et al., 2011). Note that the number of DEX genes rises over development for DFC, but not CBC. No CBC samples were available for periods 5–7.
We found that, of the 121 mouse homologs of genes in M43, 67 met this cutoff for high expression (FPKM > 20) and 82.1% (55/67) of this subset were found to be highly expressed by oligodendrocyte lineage cells (gene names labeled in red in Figures 2B–2D). Importantly, eight of the top ten genes with high expression in M43 (TMEM63A, MYRF, PLD1, RTKN, ASPA, OPALIN, ERBB3, and EVI2A) are primarily expressed by mature oligodendrocytes and tightly correlated with other oligodendrocyte-associated M43 genes. Oligodendrocyte-enriched genes are shown in red. Note their central position in the network, suggesting high intramodular connectivity.

Figure 2. A Co-expression Module Enriched in Genes Associated with Oligodendrocyte Development and Myelination Is Decreased over Development in DS

(A) Gene network analysis identifies distinct modules of co-expressed genes dysregulated in DS, including module (M)43 that is significantly enriched in genes associated with oligodendrocyte development and myelination. Plots show relative expression of the PC1 of M43 over development in the DFC, primary visual cortex (V1C), hippocampus (HIP), and CBC, indicating higher expression in euploid control versus DS brain that increases in degree over development.

(B) A heatmap showing the expression of M43 genes in DFC (left) and CBC (right), which confirms and details the gene expression trajectories shown in (A). Note that a high proportion of mouse homologs of the M43 genes are expressed in mouse oligodendrocyte lineage cells (gene symbols are highlighted in red).

(C) A network plot of M43 genes and their intramodular connections (cutoff, Pearson correlation > 0.7). Eight of the ten hub genes (the top ten genes with highest intramodular connectivity; TMEM63A, MYRF, PLD1, RTKN, ASPA, OPALIN, ERBB3, and EVI2A) are primarily expressed by mature oligodendrocytes and tightly correlated with other oligodendrocyte-associated M43 genes. Oligodendrocyte-enriched genes are shown in red. Note their central position in the network, suggesting high intramodular connectivity.

(D) The number of mouse homologs of M43 genes that are highly expressed in major cell types from mouse cerebral cortex. The majority of the M43 genes are highly expressed specifically in oligodendrocytes. Red bars denote the oligodendrocyte lineage; gray bars denote other cell lineages.

(See the Supplemental Experimental Procedures.) We found that, of the 121 mouse homologs of genes in M43, 67 met this cutoff for high expression (FPKM > 20) and 82.1% (55/67) of this subset were found to be highly expressed by oligodendrocyte lineage cells (gene names labeled in red in Figures 2B–2D). Importantly, eight of the top ten genes with
highest intramodular connectivity (hub genes) in M43 were primarily expressed by mature oligodendrocytes and tightly correlated with other oligodendrocyte-associated M43 genes (Figure 2C). To ensure that DEX genes were not detected due to variability in tissue dissections, we analyzed genes highly enriched in astrocytes, neurons, and oligodendrocytes (Zhang et al., 2014; see the Supplemental Experimental Procedures). We found that intra-individual gene expression for these three cell types was highly correlated in all samples (see Table S8). We further found by pairwise analysis that gene expression levels for markers of all three cell types were highly correlated for the same brain regions in all control (r > 0.94) or DS individuals (r > 0.95), indicating uniform tissue collection procedures. However, in agreement with our DEX analysis, expression of oligodendrocyte-specific genes was less correlated and significantly different between DS and control DFC (r = 0.89, p = 0.018 Wilcoxon signed-rank test; Table S8).

We analyzed individual expression trajectories of several genes within the M43 module known to be expressed in oligodendrocytes and involved in myelination. Lower levels of gene expression spanning from birth to adulthood for myelin-associated glycoprotein (MAG; Figure 3A) and from mid-fetal development to early childhood for myelin basic protein (MBP; Figure 3B) were observed. To validate these, we used digital droplet PCR (ddPCR) (Figure 3C) and immunoblotting (Figures 3D–3F). Together, both DEX and WGCNA analyses provide evidence for alterations in neocortical oligodendrocyte differentiation and myelination in developing DS brains at transcript and protein levels.

Given these substantial changes in oligodendrocyte lineage genes and proteins, we sought to confirm previous reports of reduced myelination in the DS forebrain. We imaged tissue sections of the DFC from five pairs of human DS and matched control specimens, ranging in age from 1 to 70 years old, using the spectral confocal reflectance microscopy (SCoRe) technique (Schain et al., 2014). Consistent with previous reports, we found that the overall density of myelinated fibers is reduced in DS brain across all ages (Figures S6A and S6B). In addition, we found that myelinated axons in control brains form a grid-like lattice that resembles a rectilinear grid, as described previously (Ang et al., 2003; Wedeen et al., 2012; Figure S6C). However, DS brains did not exhibit evidence of grid-like myelinated fiber orientations, suggesting reduced complexity of fiber pathways in the trisomic human brain.

White Matter Abnormalities in a Trisomic Mouse Model of DS

To determine whether the protein- and cellular-level consequences of altered oligodendrocyte/myelination-associated gene expression in DS are also reflected in mouse models, we examined oligodendrocyte lineage progression, myelination, and neuronal conductivity over development in the Ts65Dn mouse brain. This commonly used DS model is trisomic for ~100 orthologs of the 364 genes on HSA21 and displays many DS-specific phenotypes, including delays in brain development and cognitive defects (Rueda et al., 2012). However, changes in white matter have not been previously described in the Ts65Dn mouse brain. By immunoblotting of neocortex tissue samples (n = 10) (Figure 3G) and immunostaining of the anterior cingulate cortex and underlying corpus callosum (n = 9) (Figure 3H), we found a reduction of MAG (Figures 3G and 3H) and MBP (Figures 3I and 3J) protein levels and immunostaining intensity in postnatal day (P)30 Ts65Dn mice. Reduced immunostaining intensity for 2’,3’-cyclic nucleotide 3’ phosphodiesterase (CNP), an oligodendrocyte- and myelin-associated protein, was also observed in the P60 neocortex in Ts65Dn brains (Figure S7). Importantly, these cellular and biochemical changes are first detected during the period of myelin development and refinement and are maintained at later ages (e.g., P60).

Myelin, Axon, and Action Potential Conduction Deficits in Trisomic White Matter

Alterations in white matter-associated proteins measured within the trisomic human and mouse brains indicate possible changes in the amount or allocation of myelin. We tested this hypothesis with ultrastructural and immunofluorescent studies in Ts65Dn and euploid mice at P60. Electron microscopy revealed that axon profiles in the Ts65Dn corpus callosum are on average larger than in controls (Figures 4A and 4B), and there was a trend for fewer myelinated axons in the body of the Ts65Dn corpus callosum (p = 0.1, n = 6; Figure 4C). Furthermore, when myelinated axons were binned by axon area, we found a significant decrease in the number of small-bore myelinated axons (0–0.5 µm², p = 0.046; Figure 4D). We also found that myelinated axons in Ts65Dn brains have significantly thinner myelin sheaths (g ratio, p < 0.011; Figures 4E and 4F). Axons were then binned by size to determine whether a correlation exists between axon diameter and myelin sheath thickness. Specifically, small-bore myelinated Ts65Dn axons within 0–0.5 and 0.5–1 µm² have thinner myelin sheaths than corresponding controls (p = 0.084 and p = 0.076, respectively; Figure 4G). Altogether, these results indicate a decrease in the percentage of small-bore myelinated axons and myelin sheath thickness in Ts65Dn white matter.

Deficits in myelination and oligodendrocyte maturation also can lead to impaired formation of the nodes of Ranvier (Kaplan et al., 2001; Rasband et al., 1999; Tanaka et al., 2009). Therefore, we immunostained coronal brain sections from Ts65Dn and controls for NF186 (an NFASC isoform) and CNTNAP1 (Thaxton et al., 2010), which mark nodal regions. Quantification of nodal protein profiles in the Ts65Dn brain at P60 revealed a striking decrease in the number of nodes of Ranvier in the corpus callosum, and we found similar decreases in the external capsule (Figures 5A–5C; p < 0.005). Ultrastructural analysis confirmed this result, revealing a trend for fewer paranode cross-sections in Ts65Dn (Figures 5D and 5E; p = 0.059). To determine if these findings implicate changes in node formation as a novel human DS brain phenotype, we evaluated expression levels of both NFASC and CNTNAP1 in the exon array dataset. These genes were significantly downregulated from birth to adulthood (Figures 5F and 5G), and subsequent ddPCR analysis confirmed these differences in the DS brain samples (Figure 5H).
To determine the functional consequences of these white matter defects, we measured compound action potentials in the corpus callosum of P30–P55 Ts65Dn forebrain slices (Figure 6A). Our results revealed that action potential transmission is significantly slower in myelinated Ts65Dn axons (N1) compared to euploid controls (Figure 6B; p = 0.04, n = 10). In contrast, the conduction velocity in unmyelinated axons (N2) is similar between groups (Figure 6B; p = 0.4, n = 11). Examining the relationship between stimulus intensity and response magnitude (input-output curve) revealed that both types of axons in Ts65Dn are less excitable than those in euploid mice (Figures 6C and 6D; p < 0.001 for N1 and p < 0.007 for N2). However, the action potential refractory periods were not different between groups, indicating that sodium channel kinetics are less likely to be responsible for the shift in the input-output relationship (data not shown). These data, combined with our cellular and electron microscopy results, indicate that white matter abnormalities found in the trisomic human and Ts65Dn mouse brains contribute to structural and functional defects in neurotransmission.

Figure 3. Expression of the Essential Myelin Components MAG and MBP Is Diminished in Developing DS and the Ts65Dn Mouse Brains
(A) Log2 values of the array signal intensity in human euploid control (Ctrl) and DS DFC show that MAG expression is decreased from birth onward (periods 8–14) in DS. Periods 5–9, p = 0.28; periods 8–12, p = 0.023; periods 9–13, p = 0.025; periods 10–14, p = 0.0024; all periods, p = 0.014 (paired t test).
(B) Log2 values of the array signal intensity in human Ctrl and DS DFC show that MBP expression is decreased from mid-fetal development to early childhood (periods 5–9) in DS. Periods 5–9, p = 0.078 (one-tailed test, p = 0.039); periods 8–12, p = 0.192; periods 9–13, p = 0.52; periods 10–14, p = 0.72; all periods, p = 0.10 (paired t test).
(C) The ddPCR analysis of MBP and MAG expression in human Ctrl and DS DFC samples confirms decreased expression in developing DS brains.
(D) Representative western blots for MAG and MBP in human Ctrl and DS DFC over development are shown (DS samples in red type).
(E and F) Sliding-window analysis of MAG (E) and MBP (F) protein levels identify significant reductions in developing and adult DS brains. *p < 0.03 (paired t test).
(G and H) Western blotting of neocortex tissue samples (n = 10) (G) and immunostaining of the anterior cingulate cortex (n = 9) (H) in postnatal day (P)30 Ctrl and trisomic Ts65Dn mice identify a reduction of MAG protein expression in Ts65Dn brains. *p ≤ 0.05 and **p ≤ 0.001 (paired t test).
(I and J) Western blotting for MBP in the neocortex (n = 3 pairs) (I) and immunostaining for MBP in P30 cingulate cortex (J) of Ctrl and Ts65Dn mice show a trend toward reduced expression of MBP in the white matter of Ts65Dn mice (n = 3 pairs). *p = 0.009 (paired t test).
Alterations in Oligodendrocyte Lineage Progression in DS and Trisomic Mouse

To gain further insight into possible underlying mechanisms for myelin and white matter defects in DS, we examined the expression of genes associated with the development of human oligodendrocyte precursor cells and mature oligodendrocytes by generating sets of genes predicted to be selectively enriched in these cell types during human development. These gene sets were generated by overlapping lists of oligodendrocyte lineage candidate genes from previous mouse (Zhang et al., 2014) and human (Kang et al., 2011) studies (for details on the gene selection and analysis, see the Supplemental Experimental Procedures and Table S9). We then assessed if there was differential expression of the oligodendrocyte precursor and myelinating oligodendrocyte gene sets in DS versus control DFC. We observed that expression of putative human oligodendrocyte precursor cell-related genes gradually increases in DS compared to controls, persisting until periods 12–14 (Figure 7A). In contrast, putative human myelinating oligodendrocyte genes in the DFC are expressed at lower levels from birth into adulthood (Figure 7C). These data support and extend our DEX and WGCNA analyses (Figure 2; Tables S5 and S6), suggesting an overall and long-lasting impairment of oligodendrocyte maturation in DS, which is detectable after birth in the neocortex.

To characterize these changes at the gene network level, we determined the overlap between WGCNA modules and sets of genes that we predicted to be selectively enriched in oligodendrocyte precursor cells or myelinating oligodendrocytes (Table S9). We found that human oligodendrocyte precursor cell genes were significantly enriched in modules M9, M36, M43, and M47 (Figure 7B); exon array trajectories for the two most significantly enriched modules (i.e., M9 and M47) indicated elevated expression in DS brain samples over the course of development (Figure 7E). Conversely, human mature oligodendrocyte genes were found to be significantly enriched primarily in two modules, M8 and M43, and their trajectories indicated decreased expression in DS brains during development (Figures 7D and 7F). Thus, the relevant WGCNA modules exhibit similar trajectories when compared with the expression changes of individual oligodendrocyte lineage genes, demonstrating that these cell-type-specific defects in oligodendroglial development are associated with complex gene network disturbances in the DS brain. Altogether, these data indicate that aberrations in the process of oligodendrocyte maturation to the myelinating stage may underlie the white matter abnormalities in DS.

To test whether differences observed through our transcriptomic analyses identify changes in the oligodendrocyte lineage at the cellular level, we performed a developmental study of...
the Ts65Dn corpus callosum from P7 to P60 (Figures 7G–7I). We first immunostained for OLIG2 to mark the oligodendrocyte lineage and CC1 or NG2 to identify myelinating oligodendrocytes or oligodendrocyte precursor cells, respectively. At P7, around the onset of myelination, we found equivalent numbers of OLIG2+, CC1+, and NG2+ cells in Ts65Dn and control brain white matter. However, during the ensuing periods of active myelination and thereafter (after P7 and up to P60), there were fewer OLIG2+ cells in the Ts65Dn white matter compared to controls, reaching significance at P60; this decline in numbers was not due to higher than normal apoptotic rates (data not shown). More importantly, compared to controls and as predicted by the human transcriptome study, there was a higher percentage of OLIG2+/NG2+ oligodendrocyte precursor cells and a decreased percentage of OLIG2+/CC1+ oligodendrocytes in Ts65Dn white matter from P15 on. These results demonstrate that changes in the ratio of oligodendrocyte precursor cells to mature oligodendrocytes develop during the period of active myelination.

Finally, we sought to determine if there were cell-autonomous effects of Ts65Dn chromosomal triplication on oligodendrocyte development by carrying out proliferation and maturation assays of purified oligodendrocyte progenitor cells isolated by immunopanning with an antibody against alpha-type platelet-derived growth factor receptor (PDGFRα) (Figure 8). Effects on oligodendrocyte progenitor cell proliferation were assayed by plating euploid control and Ts65Dn oligodendrocyte progenitor cell proliferation and differentiation.
compared to euploid cells (Figure 8H). Taken together, these findings suggest that Ts65Dn-derived MBP+ cells displayed a simple morphology for 48 hr. We found no difference in the number of OLIG2+/PDGFRA oligodendrocyte progenitor cells in cultures derived from euploid and Ts65Dn mice (Figures 8A, 8B, and 8E).

To test for effects of trisomy on oligodendrocyte maturation, oligodendrocyte progenitor cells were differentiated for 72 hr. The cells were stained for MBP and OLIG2 (Figures 8C and 8D). MBP+/OLIG2+ cells were considered mature, whereas MBP- cells were considered immature. We found that the total number of OLIG2 cells was diminished in Ts65Dn cultures after 72 hr, suggesting that Ts65Dn cell viability is reduced in these conditions (Figures 8C, 8D, 8F, and 8G). In addition, the percentage of OLIG2+/MBP+ cells was significantly diminished. To further assess this possibility, we characterized the morphology of MBP+ cells in each culture. We found that a greater proportion of Ts65Dn-derived MBP+ cells displayed a simple morphology compared to euploid cells (Figure 8H). Taken together, these cell culture experiments indicate that Ts65Dn oligodendrocytes exhibit cell-autonomous impairments in oligodendrocyte maturation and viability, but not proliferation.

**DISCUSSION**

In this study, we provide the most comprehensive developmental analysis of gene expression in postmortem human DS brains and their respective age-matched controls to date, and, thereby, we establish a novel framework for the study of neural development in DS. This effort has elucidated three key aspects of gene expression differences between DS and euploid control brains. First, dysregulated genes are found throughout the genome and are not present solely on HSA21. Second, many of the dysregulated genes exhibit highly specific temporal and regional expression profiles. Third, these dysregulated genes form distinct co-expression networks associated with distinct biological categories, providing novel and unbiased insight into the multiple biological processes affected in developing and adult DS brains. Viewing DS brain development through this new lens, we have uncovered novel and robust abnormalities in the expression of genes associated with oligodendrocyte development and myelination.

Our assessment of Ts65Dn mice provided strong confirmation of the human oligodendrocyte and white matter defects, and it identified cell-autonomous impairments in oligodendrocyte maturation and viability, but not proliferation, as the underlying mechanism. These close similarities allowed us to further assess the cellular and functional consequences of these abnormalities, identifying changes in action potential conductance velocity in the forebrain white matter. These results elucidate a continuum of myelination-associated defects at the molecular, structural, and functional levels that likely contribute to developmental delays and life-long intellectual disability in DS.

The chromosomal location of the dysregulated genes identified in our study indicates a scattered, widespread distribution throughout the genome. It has been shown recently by Letourneau et al. (2014), in fibroblasts from monozygotic twins discordant for trisomy 21, that the DS-altered gene expression follows a consistent pattern with increased and decreased gene expression levels alternating across large chromosomal segments, which they named gene expression dysregulation domains (GEDDs). These GEDDs were not observable when comparing grouped samples due to gene expression differences between individuals, and this is consistent with our results (Figure S4). It therefore remains unclear whether and how these individual-specific GEDDs underlie cardinal features of DS brain development and function. Nevertheless, in the present study, we show that dysregulated genes, despite being present throughout the genome, are organized into multiple gene networks that are robust enough to be measured across multiple samples of unrelated individuals. Most importantly, by elucidating the in vivo impact of the white matter-associated gene modules as an example, we show that these systems-level changes impact cellular development and play a functional role in brain maturation.

We uncovered substantial differences between brain regions in the number and identities of altered genes, and we demonstrated
Figure 7. Oligodendrocyte Maturation Is Impaired in DS
(A) Developmental changes in genes associated with oligodendrocyte progenitor cells, expressed as a ratio of DS versus Ctrl, are shown. *p ≤ 0.05 (paired t test).
(B) The −log10 p values of expression of oligodendrocyte progenitor cell-enriched genes in weighted-gene co-expression network modules reveal significant enrichment in the M9, M36, M43, and M47 modules (y axis, −log10 [p value]). Lower dashed line corresponds to p = 0.05; upper dashed line corresponds to p = 0.01.
(C) Developmental changes in genes associated with myelinating oligodendrocytes, expressed as a ratio of DS versus Ctrl, are shown. *p ≤ 0.05 (paired t test).
(D) The −log10 p values of enrichment analysis for expression of mature oligodendrocyte-enriched genes in gene network co-expression modules demonstrate significant enrichment in modules M8 and M43 (y axis, −log10 [p value]). Lower dashed line corresponds to p = 0.05; upper dashed line corresponds to p = 0.01.
(E) PC1 plots of the co-expression modules enriched in oligodendrocyte progenitor cell-specific genes (M9 and M47) demonstrate that they are increased in DS and that the differences between Ctrl and DS samples increase over postnatal development.
(F) PC1 plots of the co-expression modules enriched in specific myelinating oligodendrocyte-specific genes (M8 and M43) demonstrate that they are decreased in DS and that the differences between Ctrl and DS samples increase over postnatal development.
(G) Representative immunofluorescent stains of P60 Ctrl corpus callosum for OLIG2 (purple), CC1 (green), NG2 (red), and nuclei (blue). Yellow arrows point to NG2-labeled oligodendrocyte progenitor cells and white arrows point to CC1-labeled mature oligodendrocytes.
(H) Representative immunofluorescent stains of P60 Ts65Dn corpus callosum for OLIG2 (purple), CC1 (green), NG2 (red), and nuclei (blue). Fewer CC1-labeled mature oligodendrocytes are apparent.
(I) The numbers of OLIG2 immuno-positive cells in the corpus callosum were counted in image volumes from P7 to P60 (n = 4 pairs at each age). There was a general trend of fewer OLIG2+ cells in the Ts65Dn white matter, which becomes significant at P60. In addition, as a proportion of the total OLIG2+ population, the percentage of NG2+ oligodendrocyte progenitor cells is higher in Ts65Dn corpus callosum from P15 to P60. In contrast, the percentage of mature CC1+ oligodendrocytes is reduced in Ts65Dn from P15 to P60. *p ≤ 0.05 and **p ≤ 0.005.

Please cite this article in press as: Olmos-Serrano et al., Down Syndrome Developmental Brain Transcriptome Reveals Defective Oligodendrocyte Differentiation and Myelination, Neuron (2016), http://dx.doi.org/10.1016/j.neuron.2016.01.042
Figure 8. Impaired Maturation and Viability of Immunopurified Oligodendrocyte Progenitor Cells In Vitro

(A) Representative micrographs show immunostaining for oligodendrocyte progenitor cells isolated from P7 euploid cortex and proliferated for 48 hr. PDGFRA, green; OLIG2, red; nuclei, blue.

(B) Representative micrographs show immunostaining for oligodendrocyte progenitor cells isolated from P7 Ts65Dn and proliferated for 48 hr. PDGFRA, green; OLIG2, red; nuclei, blue.

(C) Representative micrographs show immunostaining for oligodendrocyte progenitor cells isolated from P7 euploid cortex and cultured in pro-maturation conditions for 72 hr. MBP, green; OLIG2, red; nuclei, blue.

(D) Representative micrographs show immunostaining for oligodendrocyte progenitor cells isolated from P7 Ts65Dn cortex and cultured in pro-maturation conditions for 72 hr. MBP, green; OLIG2, red; nuclei, blue.

(E) Graph indicates there were no differences between euploid control and Ts65Dn in the number of OLIG2+ cells observed after 48 hr in proliferative conditions.

(F) Graph indicates the number of OLIG2+ cells was reduced by ~30% after 72 hr in pro-maturation conditions.

(G) Graph indicates the percentage of OLIG2+ cells co-expressing MBP was reduced by ~40% after 72 hr in pro-maturation conditions.

(H) Graph indicating a greater percentage of MBP+ cells from Ts65Dn mice cultured in pro-maturation conditions exhibited a simple morphology (less than six processes) than MBP+ euploid cells, which tended to have a more complex (six or more processes) or membranous morphology. *p ≤ 0.05 and **p ≤ 0.01 (unpaired Student’s t test).
temporal changes in these region-specific expression profiles. When considered together, these data indicate that the neurobiology of DS cannot be characterized by static lists of dysregulated genes or gene networks. Instead, the spatiotemporal nature of the disturbance plays a large role over the course of development and adulthood. Our transcriptome analyses support a cascade hypothesis in which dysregulation of genes on HSA21 is likely the first genome-level disturbance and gene expression changes on other chromosomes follow quickly thereafter. Indeed, HSA21 genes were already robustly upregulated compared to other chromosomes in the youngest fetal DS samples analyzed in our study, while changes in other regions of the genome increase with age (Figure S3). Moreover, we found that the temporal dynamics of gene module disruptions track the time periods relevant to specific developmental processes. For example, changes in modules 8, 9, 43, and 47 appear during late fetal development and the first several years of postnatal development (Figures 2 and 7), a time in development that coincides with dramatic upregulation of oligodendrocyte/myelination genes (Kang et al., 2011) and rapid expansion of oligodendrocytes in the human brain (Yeung et al., 2014). The record of these expression changes can now be used for the design and application of therapeutics tailored to particular biological process and brain regions at relevant developmental periods.

Myelination is one of the most prolonged neurodevelopmental processes, continuing until the third decade of life (Benes et al., 1994; Flichsig, 1901; Miller et al., 2012; Yakovlev and Lecours, 1967). Notably, human studies have shown that development and maturation of the white matter correlates with increased motor skills and cognitive functions (Casey et al., 2000; Gibson, 1991; Nagy et al., 2004; Paus et al., 1999; Schmithorst et al., 2000). The present results clearly show a profound deficit in white matter maturation in individuals with DS during infancy and adolescence in DFC, a brain region of late myelination that performs a critical role in the organization of behavioral, linguistic, and cognitive functions (Fuster, 2002; Makinodan et al., 2012). Recent evidence in animal models indicates that ongoing myelin remodeling may be important for learning, behavior, and cognition throughout adulthood (Liu et al., 2012; McKenzie et al., 2014). This is in agreement both with previous studies showing that DS children manifest learning and memory problems in late infancy, which often worsen in adolescence (Koo et al., 1992; Lanfranchi et al., 2010), and with postmortem studies that show reduced myelin content (Abraham et al., 2012; Wisniewski, 1990; Wisniewski and Schmidt-Sidor, 1989) and fewer oligodendrocytes in DS striatum (Karlsen and Pakkenberg, 2011).

Alterations in white matter or myelin components also may play a role in neuropathology in the aging DS brain with early-onset Alzheimer’s-like pathology (Powell et al., 2014). In addition, adults with DS have higher incidence of dementia (Sheehan et al., 2014). While triplication of APP is likely at the root of AD neuropathology in DS, our results raise the intriguing possibility that abnormalities in oligodendrocytes and myelin might contribute to early onset of Alzheimer’s-like pathology in DS. Consistent with this possibility, disturbances in myelin have been associated with an increased rate of AD progression, and by-products of homeostatic myelin maintenance can promote the formation of amyloid plaques (Bartozkis, 2007, 2011). Characterizing the axon-oligodendrocyte interactions in DS throughout development and aging will thus be an important topic of future work.

The concordance between the human DS and Ts65Dn mouse samples at the gene, protein, and cellular levels clearly demonstrates the value of the Ts65Dn mouse in reflecting the cellular and functional consequences of trisomy 21. Our finding that the Ts65Dn white matter phenotype mirrors that occurring in the human DS brain so robustly suggests that it will be a good model to identify neuronal cues that influence the onset and completion of myelination. Both intrinsic and extrinsic factors have been shown to influence myelination. For example, recent studies have shown that electrical activity drives myelination (Gibson et al., 2014; Ishibashi et al., 2006; Wake et al., 2011) and that synaptogenesis and myelination are co-regulated. However, we also show that myelin abnormalities in DS are at least in part due to a cell-autonomous phenomenon in oligodendrocyte development. Teasing out the relative contributions of cell-autonomous effects and neuron-glial signaling, or other cell-extrinsic mechanisms, will be an important goal of future work. These and other queries can now be approached using the developmental expression profiling and multi-species analysis presented here.

In summary, our findings indicate that strategies to enhance myelination may serve as therapeutic targets to attenuate the cognitive and neurological symptoms of DS. Our results also implicate spatiotemporal disturbances in other molecular pathways in DS brain, and they provide a powerful dataset for further computational and functional analyses. We anticipate that the resources provided by this study will inspire and facilitate future studies of the mechanistic basis for impaired neural development in DS and other cognitive disorders, while also lending insight into the genetic and transcriptional underpinnings of previously described DS phenotypes and the relevance of mouse model studies to the human disease.

**EXPERIMENTAL PROCEDURES**

**Human Tissue**

This study was conducted using de-identified postmortem human brain specimens from tissue collections at the Department of Neuroscience, Yale School of Medicine; the University of Maryland Brain and Tissue Bank; Brigham and Women’s Hospital Pathology Department; and Boston University Pathology Department. Appropriate informed consent was obtained and all available non-identifying information was recorded for each specimen. The ages of specimens, sex, ethnicity, postmortem interval (PMI), RNA integrity number (RIN), and dissection protocol are detailed Tables S1 and S2 and the Supplemental Experimental Procedures. Details of the brain regions analyzed and the dissection technique are detailed in Tables S1 and S2 and the Supplemental Experimental Procedures.

**Transcriptome Analyses**

Full detailed procedures for RNA isolation and cDNA synthesis can be found in the Supplemental Experimental Procedures and were as previously described (Kang et al., 2011). Affymetrix Human 1.0 ST arrays and the Affymetrix GeneChip platform were used to obtain transcriptome data, as described in the Supplemental Experimental Procedures. Data normalization, quality control, and analysis were performed as detailed in the Supplemental Experimental Procedures. Briefly, the Partek Genomics Suite was used for data normalization and to determine gene level intensities. The expression level...
of a gene (transcript cluster) was estimated using the median of all exons within the gene.

Principal component analysis was applied to visualize the relatedness of DS and their matched control samples. A paired t test was used to identify DEX genes between paired DS and matched control samples during all development periods. FDR-adjusted p value < 0.1 was used as a cutoff. Signed co-expression networks were built using the WGCNA package in R (Zhang and Horvath, 2005). Modules were generated by hybrid dynamic tree-cutting. For DEX genes and co-expression modules, functional enrichment was assessed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resource 6.7 (https://david.ncifcrf.gov/).

For ddPCR, the same human tissue RNA samples used for exon arrays were used for CDNA synthesis.

**Mice, Tissue Culture, and Relevant Procedures**

Ts65Dn (RRID: MGI_2178111) and euploid control B6EiC3Sn mice were generated by backcrossing Ts65Dn females to B6EiC3SnBl6AF1/J F1 hybrid (B6EiC3) males. The qPCR genotyping was performed on genomic DNA extracted from tail tips (Chakrabarti et al., 2007). All procedures regarding the care and death of these animals were approved by the Institutional Animal Care and Use Committee of Boston University School of Medicine, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. For procedures relevant to all animal studies, including histology, electron microscopy, image analysis, electrophysiology, immunopanning, oligodendrocyte cultures, and immunoblotting, refer to the Supplemental Experimental Procedures.

**ACCESSION NUMBERS**

The accession number for all transcriptome data reported in this paper is GEO: GSE59630.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and nine tables and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2016.01.042.

**AUTHOR CONTRIBUTIONS**


**ACKNOWLEDGMENTS**

We thank Robert Johnson, Andre Sousa, and Ronald Zelke for assistance with tissue acquisition and processing. We also thank Manzoor Bhat and William Stallcup for providing antibodies. We thank members of the T.F.H. and N.S. laboratories for their comments. This work was supported by grants NS076503, MH103339, MH105972, and MH106934 from the NIH, intramural USUHS grants, and by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI, H1142C461). Additional support was provided by the Kavli Foundation, the James S. McDonnell Foundation scholar award, the Ruth Kirschstein National Research Service Award (NRSA) fellowship (NIH), and the China Scholarship Council fellowship. The views expressed in this scientific presentation are those of the author(s) and do not reflect the official policy or position of the U.S. government or the Department of Defense.

**References**


Received: January 28, 2015

Revised: November 24, 2015

Accepted: January 21, 2016

Published: February 25, 2016


Supplemental Information

Down Syndrome Developmental Brain Transcriptome Reveals Defective Oligodendrocyte Differentiation and Myelination

Supplemental Information

INVENTORY OF SUPPLEMENTAL INFORMATION

Supplemental Figures
Figure S1. Related to Figure 1
Figure S2. Related to Figure 1
Figure S3. Related to Figure 1
Figure S4. Related to Figure 1
Figure S5. Related to Figure 2
Figure S6. Related to Figures 3 and 7
Figure S7. Related to Figures 3 and 7

Supplemental Tables
Table S1. Descriptions of anatomical regions analyzed. Related to Figure 1
Table S2. Demographic, clinical, and tissue quality details for all samples. Related to Figures 1, 3, and S6
Table S3. DEX genes on each chromosome. Related to Figure to Figures 1
Table S4. DEX genes across the sliding window periods. Related to Figures 1, 3, and 5
Table S5. GO analysis of DEX genes. Related to Figures 1 and 2
Table S6. List of WGCNA modules and GO categories. Related to Figures 2, 7, and S5
Table S7. List of genes within each WGCNA module. Related to Figures 2, 7, and S5
Table S8. Expression analysis of cell type-specific marker genes across tissues. Related to Figures 2 and 7
Table S9. Lists of OPC and OL enriched genes highly co-expressed in the developing human brain. Related to Figure 7

Extended Experimental Procedures

Supplemental References
Figure S1. Robustness and Reproducibility of the Exon Microarray Protocol. Related to Figure 1

(A) Box plots showing the log2 intensity of 100 segments divided along the longest transcript of each gene from the 5’-end to 3’-end compared to the expression of the whole gene indicating array hybridization uniformity.

(B) Spearman correlation analysis of eight samples, which were re-tested to confirm technical reproducibility.

(C-D) Hierarchical clustering of all genes (C) or chromosome 21 (HSA21) genes (D) indicating genes cluster most strongly according to region and developmental period rather than disease status or other factors. Disease status (light blue, euploid control; red, Down syndrome), period (blue to red representing younger to old), region (blue, neocortex [NCX; all neocortical areas/regions combined]; white, hippocampus [HIP]; red, cerebellar cortex [CBC]), postmortem interval (PMI; low to high representing blue to red), RNA integrity number (RIN; low to high representing blue to red), ethnicity (African-American, light blue; Caucasian, brown; Hispanic, yellow), and sex (blue, male; pink, female).
Figure S2. Principle Component Analysis Reveals that Brain Region and Age Contribute More to Transcriptional Differences than Disease Status. Related to Figure 1

(A) Three-dimensional plots of the principle component analysis (PCA) of Down syndrome (DS) samples and their matched euploid controls (Ctrl) rotated in 3 different views for better visualization and colored according to disease status. Note fairly minimal differences between DS versus Ctrl clusters.

(B) Three-dimensional plots of the PCA of DS samples and their matched controls rotated in 3 different views for better visualization and colored according to brain region (NCX [pooled neocortical regions/areas], HIP, and CBC). Note the large separation of CBC clusters from NCX and HIP.

(C) Three-dimensional plots of the PCA of DS and Ctrl samples rotated in 3 different views for better visualization and colored according to developmental period. Note that there are clear patterns defining distinct developmental periods, that display larger separation than disease state, but more moderate separation than CBC versus NCX/HIP clusters.
Figure S3. Developmental Dynamics in the Proportion of Dysregulated Genes Across Chromosomes. Related to Figure 1

(A and B) The distribution of differentially expressed (DEX) genes percentages in each chromosome across four sliding window periods calculated from 20 cycle permutations for the dorsolateral prefrontal cortex (DFC) (A) and CBC (B). The percentage of DFC expression rose across the sliding window periods in nearly all chromosomes, while the developmental dynamics in the proportion of DEX genes was variable between chromosomes in the CBC, with most chromosomes exhibiting no change in the percentage of DEX genes across development. For the permutation test, DS samples were randomly matched to control samples within the same sliding window and with the same sex to identify DEX genes.
Figure S4. Gene Expression Fold Change is not Organized in Chromosomal Domains in the Down Syndrome Brain Transcriptome. Related to Figure 1
Representative plots of a period-specific genome-wide expression variations (log2 fold change [log2FC]) for the DFC indicate a lack of widespread and contiguous genomic regions of up-regulated or down-regulated gene expression in DS brain. A LOWESS smoothing function (red trace) demonstrates minimal deviation from zero.
Figure S5. Representative Co-Expression Modules with Differential Expression in Down Syndrome Brains. Related to Figure 2
(A) Plots of relative expression of the principal component 1 (PC1) of gene co-expression module (M) 24 over development in the DFC, primary visual cortex (V1C), HIP, and CBC, indicating higher expression in DS versus Ctrl forebrain that increases over development. Subsequent gene ontology
analysis revealed that this module was enriched in genes associated with cell morphogenesis/adhesion.

(B) Plots of relative expression of the PC1 of M54 over development in the DFC, V1C, HIP, and CBC, indicating higher expression in DS versus Ctrl forebrain that increases over development. Subsequent gene ontology analysis revealed that this module was enriched in genes associated with immune responses.

(C) Plots of relative expression of the PC1 of M4 over development in the DFC, V1C, HIP, and CBC, indicating decreased expression in DS versus Ctrl brain over development. Subsequent gene ontology analysis revealed that this module was enriched in genes associated with neuron differentiation.

(D) Plots of relative expression of the PC1 of M10 over development in the DFC, V1C, HIP, and CBC, indicating decreased expression in DS versus Ctrl brain over development. Subsequent gene ontology analysis revealed that this module was enriched in genes associated with synaptic transmission.

(E) Plots of relative expression of the PC1 of M34 over development in the DFC, V1C, HIP, and CBC, indicating decreased expression in DS versus Ctrl brain over development. Subsequent gene ontology analysis revealed that this module was enriched in genes associated with synaptic transmission/regulation.

(F) Plots of relative expression of the PC1 of M45 over development in the DFC, V1C, HIP, and CBC, indicating decreased expression in DS versus Ctrl brain over development. Subsequent gene ontology analysis revealed that this module was enriched in genes associated with neuron differentiation.
Figure S6. Myelin Laser Reflectance Signatures are Reduced in Down Syndrome Neocortex. Related to Figure 3 and Figure 7

(A) Spectral confocal reflectance microscopy of 70 year old Ctrl and DS tissue sections of the DFC.

(B) Quantification of myelinated fiber density neocortical images from five DS cases captured from the pial surface down to the compacted white matter. Each brain is compared to its respective control brain (black line).

(C) Representative examples of myelinated fiber orientations at two different depths within 70 year old Ctrl and DS brains. Polar plots depict the fiber orientations.
Figure S7. Oligodendrocyte-Related Genes and Proteins are Reduced in Down Syndrome and Ts65Dn Mouse Neocortex. Related to Figures 3 and 7

(A) ddPCR analysis of human euploid Ctrl and DS DFC shows down-regulation of SOX10 expression in developing and adult DS brain.

(B) Western blot measurements of SOX10 protein levels in the Ctrl and Ts65Dn mouse corpus callosum (CC) at P30. **, p<0.03 (paired t-test).

(C) Representative immunofluorescence labeling of the corpus callosum in P60 euploid Ctrl and Ts65Dn mice using the RIP antibody raised against CNP.

(D) Quantitative analysis of the intensity of RIP immunostaining reveals a significant decrease in Ts65Dn compared to euploid Ctrl mice. *, p<0.02 (paired t-test).
SUPPLEMENTAL TABLES

Table S1. Descriptions of anatomical regions analyzed. Related to Figure 1

Table S2. Demographic, clinical, and tissue quality details for all samples. Related to Figure 1, 3, and S6

Table S3. DEX genes on each chromosome. Related to Figure to Figure 1

Table S4. DEX genes across the sliding window periods. Related to Figure 1, 3, and 5

Table S5. GO analysis of DEX genes. Related to Figures 1 and 2

Table S6. List of WGCNA modules and GO categories. Related to Figures 2, 7, and S5

Table S7. List of genes within each WGCNA module. Related to Figures 2, 7, and S5

Table S8. Expression analysis of cell type-specific marker genes across tissues. Related to Figures 2 and 7

Table S9. Lists of OPC and OL enriched genes highly co-expressed in the developing human brain. Related to Figure 7
EXTENDED EXPERIMENTAL PROCEDURES

Human Tissue
This study was conducted using postmortem human brain specimens from tissue collections at the Department of Neuroscience, Yale School of Medicine, the University of Maryland Brain and Tissue Bank (Baltimore, MD), Brigham and Women’s Hospital Pathology Department, and Boston University Pathology Department. Tissue was collected after obtaining parental or next of kin consent and with approval by the institutional review boards. Tissue was handled in accordance with ethical guidelines and regulations for the research use of human brain tissue set forth by the NIH (http://bioethics.od.nih.gov/humantissue.html) and the WMA Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/index.html).

Appropriate informed consent was obtained and all available non-identifying information was recorded for each specimen. Specimens used for transcriptome analysis ranged in age from 14 post-conception week (pcw) to 40 years. The postmortem interval (PMI) was defined as hours between time of death and time when tissue samples were frozen (Table S2).

Human Tissue Dissection and Neuropathological Evaluation
All clinical histories, tissue specimens, and histological sections were evaluated to assess for hypoxia, cerebrovascular incidents, tumors, microbial infections, neurodegeneration, demyelination, and metabolic disease.

Trisomy 21 was confirmed by karyotyping and/or Illumina Omni-2.5 million SNP arrays. The localization of dissected samples was verified by histology in the postnatal brains and across fetal periods using the same anatomical landmarks. The complete list of periods and samples, including corresponding putative functional brain regions and neocortical areas, analyzed in this study can be found in Tables S1 and S2.

Fresh frozen postmortem brain specimens from donors affected with DS and from neurotypical euploid controls matched on age and sex (see Tables S1 and S2 for the list of brain specimens, tissue samples and brain regions analyzed) were dissected as follows. For fetal brains, the entire neocortical plate and adjacent superficial part of the subplate zone was sampled; for postnatal brains the entire thickness of the cortex, containing all six cortical layers, and the underlying gyral white matter corresponding approximately to white matter segment 4 as defined by von Monakow were sampled. Hippocampal samples were dissected from the middle third of the hippocampus proper. Samples of the cerebellar cortex were dissected from lateral part of posterior lobe of cerebellum and they contained all three layers of cerebellar cortex and underlying white matter, but did not contain cerebellar nuclei. Tissue dissection was done using a dental drill (AnyXing, 300D) with a Lindemann Bone Cutter H162A.11.016 and diamond disk saw (Dental Burs USA; r=11 mm) on an aluminum plate over dry ice.

To prepare tissue sections for microscopic histological and neuropathological examination, small samples (usually the dorsal parietal cortex, striatum with ependymal layer, hippocampus, and the cerebellum) were dissected and fixed in 4% paraformaldehyde and processed for histology and immunohistochemistry as described below. Neocortical cytoarchitecture of each euploid control sample was compared to areal cytoarchitectonic maps to distinguish Brodmann areas (BA). Euploid control specimens with incorrect cytoarchitecture or abnormal microscopical appearance were excluded from the study. Neocortical areas (see below) were grouped according to the lobes from which they were sampled. The same anatomical landmarks were used to dissect DS brain specimens.

Frontal lobe
Dorsolateral prefrontal cortex (DFC) was sampled from approximate border of the anterior and middle third of the medial frontal gyrus. Cytoarchitectonically, DFC corresponds approximately to Brodmann area (BA) 9 and BA46. Orbital prefrontal cortex (OFC) was sampled from the anterolateral two thirds of the orbital gyri. OFC corresponds approximately to BA11. Ventrolateral prefrontal cortex (VFC) was sampled from the posterior third of the inferior frontal gyrus, corresponding to the opercular and triangular part of the inferior frontal gyrus. VFC corresponds approximately to BA44 and BA45. Medial prefrontal cortex (MFC) was sampled from perigenual and subgenual parts of the anterior cingulate gyrus and the anteromedial part of the superior frontal gyrus. MFC corresponds approximately to BA24, BA32, and BA33.

Parietal lobe
Primary somatosensory cortex (S1C) was sampled from the ventrolateral part of the postcentral gyrus adjacent to the M1C area. S1C corresponds to BA1, BA2, and BA3. Posterior inferior parietal cortex (IPC) was sampled from the posterior half of the supramarginal gyrus. IPC corresponds approximately to BA40.

Temporal lobe
Posterior superior temporal cortex (STC) was sampled from the posterior third of the superior temporal gyrus. STC corresponds approximately to BA22. Inferior temporal cortex (ITC) was sampled from the anterior third of the inferior temporal gyrus. ITC corresponds approximately to BA20.

Occipital lobe
Primary visual cortex (V1C) was sampled from the area surrounding the calcarine fissure. Only samples in which the stria of Gennari could be recognized were included. V1C corresponds to BA17. Small pieces of the neighboring BA18 could have been occasionally present in the sample, but the majority of the sample corresponded to BA17.

Hippocampus (HIP) was sampled from the middle third of the retrocommissural hippocampal formation, located on the medial side of the temporal lobe. Sampled areas always contained dentate gyrus and the cornu ammonis. Samples dissected from frozen tissue may contain small quantities of the neighboring choroid plexus.

Cerebellar cortex (CBC) was sampled from the lateral part of the posterior lobe. The sampled area contained all three layers of cerebellar cortex and underlying white matter but not the deep cerebellar nuclei. CBC approximately corresponds to the lateral pontocerebellum.

Spectral Confocal Reflectance Microscopy
Spectral confocal reflectance microscopy (SCoRe) (Schain et al., 2014) was used to quantify myelinated axon segments in tissue sections of the DFC dissected from 4% paraformaldehyde fixed brains. We analyzed tissue from five pairs of matched brains obtained from the University of Maryland Brain and Tissue Bank, Brigham and Women’s Hospital Pathology Department and Boston University Pathology Department: one pair from 1 year old brains, one pair of 46 year old brains, one pair of 49-50 year old brains and two pairs of 70 year old brains. Tissue sections for the 46-50 year old cases were 4µm paraffin sections from Pathology collections at Boston University and Brigham and Women’s Hospital whereas all other cases were 100µm vibratome-cut formalin-fixed sections from the Maryland Brain Bank. We found that SCoRe microscopy parameters did not vary between sections based on how thick they were or how they were processed prior to microscopy. Each pair constituted an experiment and the laser power and detector sensitivity settings were calibrated first to
the euploid control brain section to yield a thresholded image highlighting the myelinated axon segments within the image. The same imaging settings were then used for the Ts21 brain of that pair. Images (1024 x 1024; 425 mm x 42.5 mm) were collected using reflectance from 488 nm, 561 nm and 633 nm laser lines collected with 486-501 nm, 554-563 nm and 630-634 nm filters respectively using a 20X, 0.8 NA objective lens. For each brain, we collected overlapping SCoRe images from the DFC in a radial stripe from the pial surface down to the compacted white matter underneath the cortical grey matter. We calculated the myelinated fiber density within each image as the percent of the image pixels containing a myelin reflectance signal.

RNA Isolation and Exon Array Hybridization
Total RNA was isolated from 30 mg of pulverized frozen brain tissues using a non-phenolic procedure (RNeasy Plus Mini Kit, Qiagen), followed by DNase treatment (TURBO DNase, Ambion). Optical density values at 260/280 were consistently above 1.9 (NanoDrop, Thermo Scientific), and samples with RNA integrity values of at least 5 were used for microarray (RIN>5, Agilent Bioanalyzer). Synthesized cDNA (5.5 µg) was labeled using a WT Expression kit (Ambion) and loaded onto individual Affymetrix Human Exon 1.0 ST arrays. Microarrays were hybridized at 45 °C for 16–24 hours, washed and stained using an Affymetrix FS450 fluidics station, according to manufacturer recommendations. Microarrays were scanned on a GeneChip Scanner 3000 and visually inspected for hybridization artifacts. Exon chip analysis was performed using Affymetrix Power Tools 1.12.0. Probe level data was summarized into probe set level data using the Robust Multichip Average (RMA) background correction algorithm in combination with an R-script. The raw image files (.DAT files) were analyzed using Affymetrix GeneChip Operating Software to generate .CEL files.

Quality Control Measures
Three QC measures were performed to test the quality of exon array data. First, ratio intensity plots were plotted for all exon arrays to detect spatial artifacts that are defined as severe non-random spatial patterns of exon arrays. The construction of these ratio intensity plots was previously described (Kang et al., 2011). Second, exon array hybridization uniformity was estimated by gene expression uniformity from 5'-end to 3'-end (Figure S1A). Microarrays displaying spatial artifacts and altered hybridization uniformity were excluded for further data analysis. Third, 8 samples were re-tested to evaluate technical reproducibility (Figure S1B). The correlations were high for these technical replicates (Spearman correlation, r² = 0.977; N=16, Figure S1B).

Data Normalization
Affymetrix exon array raw data (.CEL files) were normalized using the Partek Genomics Suite version 6.6 to generate probeset-level (exon-level) and transcript cluster (gene-level) intensities. The expression level of a probe set was estimated by averaging the intensities of all core probe sets within the exon. We applied the following default Partek settings: RMA background correction, exclusion of probes containing SNPs, quantile normalization, mean probe set summarization, and log2-transformation. Only core probe sets defined by Affymetrix were included for the calculation. These core probe sets have reliable sequence annotations. The expression level of a gene (transcript cluster) was estimated using the median of all exons within the gene.

Transcriptome Data Analyses
Principal component analysis was applied to visualize the relatedness of DS and their-matched control samples. The first three principal components were calculated using the function “prcomp” in R. All these principal components were plotted using the function “plot3d” in R. Each data point in the picture represents one sample. All samples were colored according to the phenotype of the samples, such as brain region, period, and disease status (Figure S2). To make these pictures clearer, three different directional views are displayed.
A paired t-test was used to identify differentially expressed (DEX) genes between paired DS and matched control samples across all development periods. FDR-adjusted p-value < 0.1 was used as a cutoff. To see how the DEX genes distribute along the human genome, chromosome location of these DEX genes were determined based on the gene annotations provided by Affymetrix. Chromosomes 1 to 22, X and Y were investigated. The percentage of DEX genes and ratio of up-regulated/down-regulated genes in each chromosome were calculated.

To identify differentially expressed (DEX) genes between paired DS and matched control tissue samples at specific developmental periods, a sliding-window approach and paired t-test were used. The window size was set to 3 periods. For each window, a paired t-test was applied to determine if the expression level of a gene in DS brain samples was significantly different from the expression level in the samples from the matched control. Statistical threshold was set at a p-value < 0.05 and minimum fold difference > 2 between DS and control brains. We performed a 20-cycle permutation test to ensure that our results are robust and are not unduly influenced by any particular sample. For the permutation test, DS samples were randomly matched to control samples within the same sliding window and with the same sex to identify DEX genes using the same approach and cutoff. The distributions of the number of DEX genes from different match sets were plotted in a boxplot.

Unsupervised signed co-expression networks were constructed using the weighted gene co-expression network analysis (WGCNA) package in R (Zhang and Horvath, 2005). All genes with core probe sets were included in the analysis. A pair-wise correlation matrix was computed, and an adjacency matrix was calculated by raising the correlation matrix to a power. The power was set to 21 according to a scale-free topology criterion (Zhang and Horvath, 2005). For each pair of genes, a robust measure of network interconnectedness (topological overlap measure) was calculated based on the adjacency matrix. The topological overlap based dissimilarity was then used as input for average linkage hierarchical clustering. Modules were generated by hybrid dynamic tree-cutting. To obtain co-expression patterns, we set the minimum module size to 20 genes, deepSplit to 2, and the minimum height for merging modules to 0.15. Each module was summarized by an eigengene, which is the first principal component of the scaled module expression. To obtain cleaner modules, we defined the module membership measure (also known as module eigengene based connectivity kME) as the correlation between gene expression values and the module eigengene. Genes were iteratively assigned to the module with highest kME as long as they had maximum kME > 0.7. The module membership is also used to rank genes in the module. Top ten genes in the rank were considered as hub genes of module.

For differential expressed genes and co-expression modules, functional enrichment was assessed using the DAVID Bioinformatics Resource 6.7 (http://david.abcc.ncifcrf.gov/).

Analysis of Oligodendrocyte Precursor Cells and Oligodendrocyte Associated Gene Expression

To identify the cell type expression profile of all 121 genes expressed in module (M) 43 (the myelination associated module in Figure 2), we determined if they were highly expressed (FPKM > 20) in acutely purified representative populations of neurons, astrocytes, oligodendrocyte precursor cells (OPCs), newly formed oligodendrocytes, myelinating oligodendrocytes (mOLs), microglia, endothelial cells, and pericytes from mouse cerebral cortex at postnatal day 7 (see Zhang et al. 2014; http://web.stanford.edu/group/barres_lab/brain_rnaseq.html).

Furthermore, to identify genes that are specifically expressed in OPCs and mOLs (Figure 7), but not other cell types, during fetal and early postnatal human brain development we generated a set of genes that were found to both be highly enriched in OPCs and mOLs using the mouse dataset (Zhang et al. 2014) and in a list of OL and OPC related human genes generated in our previous study that categorizes genes according to their correlations with major cell types and neurodevelopmental processes across human development (Kang et al., 2011). Overlapping these two lists allowed us to create a set of human homologs that are most likely to be selectively enriched in OPCs and mOLs.
during human fetal and postnatal development. Note that the list in Zhang et al. (2014) specifically tested purified OPCs and mOLs in mouse at one postnatal stage of development. Because OPCs are highly proliferative at this age, the OPC list may contain genes broadly associated with cell proliferation and progenitor states. Therefore, genes that overlap between the datasets in Zhang et al. and Kang et al. provide a highly stringent set of OPC and mOL specific genes that are dynamically expressed over human development (Table S9).

The following methodology was used to derive the lists of genes highly enriched in OPCs and mOLs. The raw RPKM values from Zhang et al. (2014) were first normalized using quantile normalization. Genes enriched in mouse OPCs or mOLs were identified by the criterion that the fold change of each gene in these respective cell types must be 2 fold greater than in any other cell type (cell types other than OPC, newly formed oligodendrocytes, and mOLs). These lists were then intersected with the lists of genes highly co-expressed with OPC or mOL markers during human brain development (Kang et al., 2011) to form lists of OPC and mOL genes (Table S9). To test changes in OPC and mOL genes, paired t-test were performed between DS and control for each human developmental period. Enrichment of OPC and mOL genes in each module was calculated using Fisher’s exact test (Figures 7B and 7D).

Expression Analysis of Cell Type-Specific Marker Genes Across Tissues
To confirm the accuracy and reproducibility of our dissection technique within each brain and across individuals, we determined if there was high correlation of the expression of genes enriched in neural cell types between each neocortical region across individual brains, and between samples of DFC and CBC (the brain regions for which the most tissue samples were available) from both control and DS individuals. All analyses were done using adult samples, to avoid discrepancies in developmental age that may confound pair-wise analysis. The lists of cell-type enriched genes were obtained from publically available RNA-seq data from purified astrocytes, neurons, and myelinating oligodendrocytes of the mouse cerebral cortex generated by Zhang et al. (2014). For each cell type, genes were ranked by their fold of enrichment (the fold change between this cell type and the maximum expression in the rest of cell types). Human homologs of the top 100 most enriched genes were then defined as cell-type enriched genes. Using the resulting lists of cell-type enriched genes, we calculated the Pearson correlation coefficient of oligodendrocyte, astrocyte, and neuronal human genes between samples of each neocortical area dissected from the same individual brain. If the dissection technique was consistent, high correlation should be observed as the glia/neuron ratio is more consistent throughout the neocortex than between different brain regions (Herculano-Houzel, 2014). In addition, we calculated the Pearson correlation through pairwise comparisons between samples from the CBC and DFC both within and between experimental groups (i.e., control vs control, DS versus DS, and control versus DS) for astrocytes, neurons, and oligodendrocytes. Note the limited number of samples from other brain regions precluded reliable pairwise analysis. To determine statistically significant differences in the pairwise correlation between each cell type across the experimental groups (e.g. oligodendrocyte versus astrocyte genes in DFC of paired control versus a matched control sample, matched DS versus control, and so on), we used the Wilcoxon signed-rank test (Table S8).

Droplet Digital PCR
An aliquot of the total RNA that was previously extracted from each brain region was used for secondary validation by droplet digital PCR analysis. One µg of total RNA was used for cDNA synthesis using oligo dT primers and SuperScript III First-strand synthesis Supermix (Invitrogen), and subsequently diluted with nuclease-free water to 1 ng/µl cDNA. FAM- or VIC-labeled TaqMan® probes were used for detecting copy number of target genes (Applied Biosystems). PCR reactions were conducted on the QX100 Droplet Digital™ PCR system (Bio-Rad) according to manufacturer recommendations. The reaction mixture containing sample cDNA, primers and probe was partitioned into about 20,000 droplets in oil through the QX100 Droplet Generator. After PCR amplification (95°C
10 min; 40 cycles of (94°C 30 sec, 57°C 60 sec); 98°C 2 min), each droplet provided a positive or negative fluorescent signal indicating the target gene was present or not present after partitioning. Positive and negative droplets were counted in the QX100 Droplet Reader and the software calculated the concentration of target gene as copies per microliter. The copy number of each gene was normalized to the housekeeping gene GAPDH, which was counted in the same sample.

List of PCR Primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>ID</th>
<th>RefSeq</th>
<th>Exon boundary</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNTNAP1</td>
<td>Hs00182533_m1</td>
<td>NM_003632.2</td>
<td>18-19</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Hs02758991_g1</td>
<td>NM_001256799.1</td>
<td>6..7</td>
</tr>
<tr>
<td>MAG</td>
<td>Hs01114387_m1</td>
<td>NM_001199216.1</td>
<td>9..10</td>
</tr>
<tr>
<td>MBP</td>
<td>Hs00921945_m1</td>
<td>NM_001025081.1</td>
<td>6..7</td>
</tr>
<tr>
<td>NFASC</td>
<td>Hs00391791_m1</td>
<td>NM_001005388.2</td>
<td>11..12</td>
</tr>
<tr>
<td>SOX10</td>
<td>Hs00366918_m1</td>
<td>NM_006941.3</td>
<td>3..4</td>
</tr>
<tr>
<td>Cntnap1</td>
<td>Mm00489702_m1</td>
<td>NM_016782.2</td>
<td>20..21</td>
</tr>
<tr>
<td>Gapdh</td>
<td>Mm99999915_g1</td>
<td>NM_008084.2</td>
<td>2..3</td>
</tr>
<tr>
<td>Mag</td>
<td>Mm00487538_m1</td>
<td>NM_010758.2</td>
<td>4..5</td>
</tr>
<tr>
<td>Mbp</td>
<td>Mm01266402_m1</td>
<td>nm_001025251.2</td>
<td>3..4</td>
</tr>
<tr>
<td>Nfasc</td>
<td>Mm00813922_m1</td>
<td>NM_001160316.1</td>
<td>10..11</td>
</tr>
<tr>
<td>Sox10</td>
<td>Mm01300162_m1</td>
<td>NM_011437.1</td>
<td>2..3</td>
</tr>
</tbody>
</table>

Mice
Ts65Dn (RRID: MGI_2178111) and euploid B6EiC3 mice were generated by backcrossing Ts65Dn females to B6EiC3Sn.BI1AF1/J F1 hybrid (B6EiC3) males. The parental generation was obtained from Jackson Laboratory. Quantitative PCR genotyping was performed on genomic DNA extracted from tail tips (Chakrabarti et al., 2007). All procedures regarding the care and death of these animals was approved by the Institutional Animal Care and Use Committee of Boston University School of Medicine, in accordance with the NIH guide for the care and use of laboratory animal. Ts65Dn and euploid littermates at P7, P15, P30 and P60 were anesthetized by ketamine/xylacine cocktail and intracardially perfused with 4% paraformaldehyde (PFA) in 0.1M phosphate buffer saline (PBS, pH 7.4). Perfused brains were removed and fixed in 4% PFA overnight at 4°C followed by 30% sucrose. All samples were embedded in Tissue-Tek OCT compound (Sakura Finetek), frozen on dry ice and sectioned (16µm) with a Microm HM 560 cryostat (MO BIO Laboratories, Inc).

Immunohistochemistry
All immunohistochemical reactions were performed on 16-µm frozen brain sections. Primary antibodies used were: rabbit anti-Olig2 (1:500, Millipore, RRID: AB_2299035), guinea-pig anti-NG2 (gift from William B. Stallcup, Sanford-Burnham Medical Research Institute, La Jolla, RRID: AB_2314937), mouse anti-RIP (1:50, DSHB), mouse anti-CC1 (CellbioChem), rabbit anti-Caspase3 (1:500, Cell Signaling, RRID: AB_2070042), guinea-pig anti-NF186, rabbit anti-CaspR (1:200, gift from Manzoor Bhat, University of North Carolina at Chapel Hill), mouse anti-MBP (1:1000, Covance, RRID:
AB_2314771) and mouse anti-MAG (1:1000, Millipore, RRID: AB_2137847). We used AlexaFluor 488-, AlexaFluor 546- and AlexaFluor 636-conjugated (1:200, Invitrogen) as secondary antibodies. All frozen sections were mounted with Vectashield (Vector Laboratories).

**Immunoblotting**
Dissected cerebral hemispheres were homogenized in RIPA lysis buffer (Santa Cruz Biotechnology). Proteins were loaded into 4-20% gradient gels. Gels were electrotransferred to a 0.2µm nitrocellulose membrane (Millipore Bioscience Research Reagents). Blots were blocked in 5% milk in TBST and incubated in primary antibodies. Bands were detected with appropriate HRP-conjugated secondary antibodies, reacted with chemiluminescent ECL substrate (Pierce) and imaged. Band intensity was measured using Image J program (National Institute of Health).

**Electron Microscopy**
8-9 week-old mice were perfused intra-aortically with a warm solution of 2% of paraformaldehyde and 2.5% of glutaraldehyde in phosphate buffer 0.1M at pH 7.2-7.4. Brains were removed and postfixed in the same fixative. To expose the corpus callosum, cerebral hemispheres were separated in the mid-sagittal plane using a razor blade. This was followed by one longitudinal 3 mm cut from the midline to each of the cerebral hemispheres. Each thick section was further trimmed into two blocks with which corpus callosum was clearly seen. Sections were rinsed in 0.1M Phosphate buffer for 30 minutes and then incubated in 1% osmium tetroxide during 2-3 hours. Once blocks were osmicated, blocks were dehydrated in an ascending series of alcohols for 2 hours and finally embedded in Araldite. Embedded corpus callosum was sectioned in the transverse plane, such that the nerve fibers were cross-sectioned. Semi-thick (1 µm) sections of the entire corpus callosum were first taken and stained with toluidine blue. For electron microscopy, the block was trimmed so thin sections contained only the corpus callosum. All thin sections were stained with uranyl acetate and lead citrate. All thin sections were mounted on 200 mesh grids, and, after they had been examined to ascertain that the quality of preservation was acceptable, electron micrographs were taken using a JEOL 100S electron microscope. All images were taken in a systematic manner, so electron micrographs were taken approximately in the center of the thin section, and thus focusing on the body of the corpus callosum. 6 images per brain were taken at magnification of 3000x and 8000x. 3000x magnification images were used for counting the myelinated axons, while 8000x magnification images were used to assess g-ratios. To calculate g-ratios, the area of axons and axons plus myelin was measured in electron micrographs by first measuring the circumference of each by hand-tracing tool using ImageJ, then by calculating areas. A minimum of 3500 axons was counted per animal for assessing numbers of myelinated axons while a minimum of 600 axons was analyzed per animal for assessing g-ratios. Statistical analyses were performed by two-tailed Student’s t test analysis. Euploid control, n=3; Ts65Dn, n=3.

**Electrophysiology**
Mice 2-4 weeks old, were anesthetized and decapitated and the brain was rapidly removed and placed in ice-cold (~ 4°C) cutting artificial cerebrospinal fluid (ACSF) containing (in mM) sucrose 206, KCl 2, CaCl2 1, NaH2PO4 1.25, MgSO4 2, MgCl-6H2O 2, NaHCO3 26, d-glucose 10, bubbled with a mixture of 95% O2/5% CO2. The mice were then decapitated and the brains placed in a dish of sACSF on ice for blocking. Coronal slices, 400-µm thick, were cut on a Leica VT1200S and transferred to a warmed (~36°C) solution of normal ACSF (nACSF) NaCl 126, KCl 3, CaCl2 2, NaH2PO4 1.25, MgSO4 2, NaHCO3 26, d-glucose 10, bubbled with a mixture of 95% O2/5% CO2 for 45 minutes. After this recovery period the slices were maintained in the same solution at room temperature for at least 1 hour before recording. All recordings were performed at room temperature (~25 °C). Compound action potentials (CAPs) were evoked by electrical stimulation of the corpus callosum with a bipolar tungsten wire electrode and were recorded with a pulled borosilicate glass pipette (~1 MΩ resistance) within the contralateral corpus callosum. Stimulation intensities ranged
from 30 to 3000 µA. Input-output curves were generated by recording the amplitudes of N1 and N2 (see inset Figure 1) as a function of stimulation intensity. The amplitude of each response was taken to be the difference between the corresponding trough and a straight line drawn between the adjacent peaks. Three to five responses were averaged for each measurement. The conduction velocities for myelinated and unmyelinated fibers were calculated as the slope of a straight line fitted through a plot of the distance between the recording and stimulating electrodes versus the response latency (time to N1 or N2 respectively). Refractory periods were measured using a paired pulse protocol where two stimuli were applied with a decreasing time interval (10 to 2 ms) between each pulse. Peak amplitudes and onset latencies were calculated using custom written routines in Igor (WaveMetrics, Lake Oswego, OR). Statistical analysis was performed using SigmaPlot (Systat Software Inc. San Jose, CA) and consisted of repeated measure 2-factor ANOVAs for input-output and refractory period results or a two-tailed Wilcoxon test for conduction velocity data and p < 0.05 was assigned for significance.

**Image Analysis**

All fluorescent images were taken on a LSM710 confocal microscope (Carl Zeiss). Three to four brains per genotype were analyzed at each age studied. A minimum of 4 images and up to 6 images per brain were taken for quantitative analysis. 10-µm confocal z-stacks at 40x magnification were analyzed using LSM software to quantify the number of OLIG2+, CC1+, NG2+ and Caspase 3+ cells. 6-µm confocal z-stacks at 63x magnification with zoomX2 were analyzed to quantify the number of nodes using Volocity software (Improvision). For comparative analysis of RIP staining intensity, single plane images were converted to gray-scale. The intensity as the mean gray value obtained from all pixels within a region of interest was quantified using Image J software (National Institutes of Health). All analyses were done blindly to genotype.

**Proliferation and Maturation Assays in Oligodendrocyte Progenitor Cultures**

Mouse oligodendrocyte precursor cells were isolated by immunopanning through positive selection of cells from postnatal day (P) 7 mouse cerebral cortices that bound to PDGFRA (PDGFRα), as previously described with the exception that Papain treatment was carried out in a 37°C, 5% CO₂ incubator (Emery and Dugas, 2013; Fancy et al., 2011). Cells were plated on poly-D-lysine coated slide wells and allowed to overnight in proliferation conditions with the addition of CNTF, PDGF, and NTF3 (NT-3) (Peprotech) to base medium. For proliferation analysis, cells were allowed to proliferate for an additional 48 hours. For maturation analysis, the media was switched the next morning to contain triiodothyronine (T3; Sigma), but not PDGF or CTNF, and cells were maintained in these conditions for 72 hours in a 5% CO₂ 37°C incubator. Cells were fixed for 15 m in 4% paraformaldehyde at room temperature.

Cells left in proliferative conditions were then immunostained for PDGFRA (PDGFRα) and OLIG2. To count the total number of OPCs an image was taken at 10x of the center of 3 slides wells for each sample and OLIG2+ cells were counted using the Cell counter plug in ImageJ (NIH, Bethesda, MD). Cell counts were additionally assessed for contamination form other cells types. No OLIG2/PDGFRα–negative cells were observed. Images of MPB/OLIG2 immunostaining were obtained by the same method and the total number OLIG2 cells, in addition to the number of MBP/OLIG2 double positive cells was quantified. To further assess maturation, the complexity of the morphology of MBP cells was categorized into three groups and quantified: simple (i.e. less mature) in which there were fewer than 6 MBP+ processes, complex in which there were more than 6 MBP+ processes, and membranous in which a membranous MBP+ lamella without discernible processes extended from the cell body. Student’s t-tests were conducted to assess statistical significance (defined as p < 0.05) between three experimental replicates.

**Statistical Analysis**
All data are presented as mean ± SEM, unless otherwise noted. Comparisons of mean differences between groups were made by unpaired two-tailed Student’s t-test, except as otherwise noted above in the extended experimental procedures. A probability level of p < 0.05 was considered to be statistically significant.

SUPPLEMENTAL REFERENCES


