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Resource

Cell

A High-Resolution Enhancer Atlas of the Developing Telencephalon

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SUMMARY

The mammalian telencephalon plays critical roles in cognition, motor function, and emotion. Though many of the genes required for its development have been identified, the distant-acting regulatory sequences orchestrating their in vivo expression are mostly unknown. Here, we describe a digital atlas of in vivo enhancers active in subregions of the developing telencephalon. We identified more than 4,600 candidate embryonic forebrain enhancers and studied the in vivo activity of 329 of these sequences in transgenic mouse embryos. We generated serial sets of histological brain sections for 145 reproducible forebrain enhancers, resulting in a publicly accessible web-based data collection comprising more than 32,000 sections. We also used epigenomic analysis of human and mouse cortex tissue to directly compare the genome-wide enhancer architecture in these species. These data provide a primary resource for investigating gene regulatory mechanisms of telencephalon development and enable studies of the role of distant-acting enhancers in neurodevelopmental disorders.

INTRODUCTION

The telencephalon houses the cerebral cortex and basal ganglia, structures that are pivotal for human brain functions (Wilson and Rubenstein, 2000). Impaired telencephalic development and function are associated with major neurological and neuropsychiatric disorders, including mental deficiency, cerebral palsy, epilepsy, schizophrenia, and autism (Lewis and Sweet, 2009; Walsh et al., 2008a). Significant progress has been made toward defining spatially resolved gene expression patterns in the developing and adult brains of mouse and human on a genomic scale (Diez-Roux et al., 2011; Gong et al., 2003; Gray et al., 2004; Lein et al., 2007; Portales-Casamar et al., 2010; Visel et al., 2004; Zeng et al., 2012). In contrast, the distant-acting gene regulatory sequences that are critical for orchestrating the spatial and temporal expression of genes in the developing and adult brain remain poorly defined despite evidence from large-scale human genetic studies demonstrating the contribution of regulatory sequences to a wide spectrum of human traits and disorders (Durbin et al., 2010; Maurano et al., 2012) and anecdotal direct evidence for a critical requirement for enhancers in brain development (Kurokawa et al., 2004; Shim et al., 2012).

Unlike protein-coding genes, enhancers involved in specific biological processes are difficult to identify because they reside in the vast and poorly characterized noncoding portion of the genome and can be located hundreds of thousands of base pairs away from the promoters of the target genes that they regulate (Lettice et al., 2003). The introduction of enhancer prediction methods based on extreme evolutionary conservation (Nobrega et al., 2003; Pennacchio et al., 2006; Visel et al., 2008) and chromatin immunoprecipitation sequencing (ChIP-seq) (Visel et al., 2009a) increased the efficiency of identifying enhancers. Importantly, ChIP-seq experiments that are performed directly on tissues can provide accurate predictions of the broad, general anatomical region in which an enhancer is

active (Visel et al., 2009a). Nevertheless, the spatial resolution of these methods is limited, and detailed in vivo studies are required to precisely define the activity patterns of enhancers at high resolution.

To address the need for an improved understanding of the *cis*-regulatory architecture and gene networks active during telencephalic development, we combined sequence conservation- and ChIP-seq-based enhancer prediction with large-scale histological activity analysis of human telencephalon enhancers in transgenic mice. We demonstrate how the high-resolution neuroanatomical annotation of enhancer activities can be used to develop computational sequence classifiers for enhancers active in different subregions of the telencephalon. We also directly compare the genome-wide enhancer architecture active in the mouse and human cortex using ChIP-seq from these tissues, and we provide examples of downstream applications for enhancers identified through this work.

RESULTS

Genome-wide Identification of Candidate Forebrain Enhancers

To generate a genome-wide set of forebrain enhancer candidate sequences, we collected forebrain tissue from embryonic day 11.5 (e11.5) mouse embryos and performed tissue-ChIP-seq using an antibody for the enhancer-associated protein p300. Results were analyzed alongside previously described data to increase sampling depth (see Extended Experimental Procedures, available online). Genome-wide enrichment analysis led to the identification of 4,425 noncoding regions genome wide that are distal from transcription start sites and significantly enriched in p300 binding in the e11.5 forebrain (Table S1A). Because p300 was previously shown to be associated with active tissue-specific enhancers (Blow et al., 2010; Visel et al., 2009a), these sequences were predicted to be distant-acting forebrain enhancers. As a complementary approach to identifying candidate enhancers, we also used extreme sequence conservation in conjunction with genomic location. Thus, we scrutinized sequences under extreme evolutionary constraint (Siepel et al., 2005; Visel et al., 2008) in the genomic vicinity of 79 genes with a known role in forebrain development or function (Table S1B), and we identified 231 additional candidate forebrain enhancer sequences (Table S1C). Combined, these two data sets comprised a total of 4,656 noncoding sequence elements that we hypothesized to be enriched in forebrain enhancers.

Transgenic Validation and Characterization of Enhancers

To validate candidate telencephalon enhancer sequences and define their in vivo activities in greater detail, we selected 329 elements predicted to be enhancers by conservation and/or ChIP-seq for experimental testing (Table S1D). Nearly all of these selected elements were located near genes with a known function in the forebrain (Table S1B). In order to focus on the most conserved core regulatory architecture of mammalian telencephalon development, only ChIP-seq peaks that were detectably conserved between the human and mouse genome were tested. Regardless of the identification method, all tested sequences showed evidence of significant evolutionary constraint (phastCons scores ranging from 415 to 931, median 798; Table S1D). The selected candidate enhancer sequences were amplified from human genomic DNA, cloned into an enhancer reporter vector (Hsp68-LacZ), and used to generate transgenic mice by pronuclear injection (see Experimental Procedures). Transgenic embryos were stained for reporter gene (LacZ) activity at e11.5, and reporter expression was annotated using established reproducibility criteria (Pennacchio et al., 2006). Only elements that drove expression in the forebrain in at least three embryos, each of them corresponding to an independent transgenic integration event, were considered as reproducible forebrain enhancers. In total, 105 of the 329 (32%) candidate sequences tested were reproducible forebrain enhancers at e11.5, of which 36 showed reproducible expression exclusively in the forebrain (Table S1D). For comparison, in previous transgenic assays of p300-binding sites in two different nonneuronal tissues, limb buds and the heart, only 4 of the 155 (2.6%) tested sequences had reproducible forebrain enhancer activity at e11.5 (Blow et al., 2010; Visel et al., 2009a). Enhancer candidate sequences that overlapped p300 ChIP-seq peaks were more enriched in verifiable in vivo forebrain enhancers than extremely conserved sequences that showed no evidence of p300 binding (58% compared to 23%, Table S1D). Selected examples of reproducible forebrain enhancers whose in vivo activity was confirmed in transgenic mice are shown in Figure 1, and whole-mount images for all validated enhancers are accessible online through the Vista Enhancer Browser (Visel et al., 2007).

High-Resolution Analysis of Telencephalon Enhancer Activity Patterns

To define the precise spatial expression patterns of telencephalic enhancers active at e11.5, we performed high-resolution analysis on a set of 145 enhancers (Table S1E). These sequences were selected from the 105 forebrain enhancers discovered in the present study and from complementary sets of forebrain enhancers identified at whole-mount resolution in previous enhancer screens (Pennacchio et al., 2006; Visel et al., 2008, 2009a). For each enhancer, a full set of contiguous coronal paraffin sections (average: 220 sections) was obtained. Full-resolution digital images of more than 32,000 sections are available through the Vista Enhancer Browser (Visel et al., 2007). Selected sections of patterns driven by different enhancers in subregions of the pallium (cortex), subpallium (basal ganglia), and eminentia thalami (telencephalic-diencephalic connection) are shown in Figures 2 and 3, illustrating the diversity of spatial specificities observed.

In addition to the spatial activity patterns of all 145 enhancers studied at e11.5, we also examined the temporal activities of a subset of these enhancers at later prenatal stages of telencephalon development (Figures 2S, 3F, and 3G). These temporal comparisons showed that the spatial patterns of enhancer activity were largely constant. In two cases, enhancers active in subregions of the subpallium at e11.5 displayed characteristic features of subpallial cell populations (interneurons) that tangentially migrate to the pallium. At e13.5, these cells had just arrived

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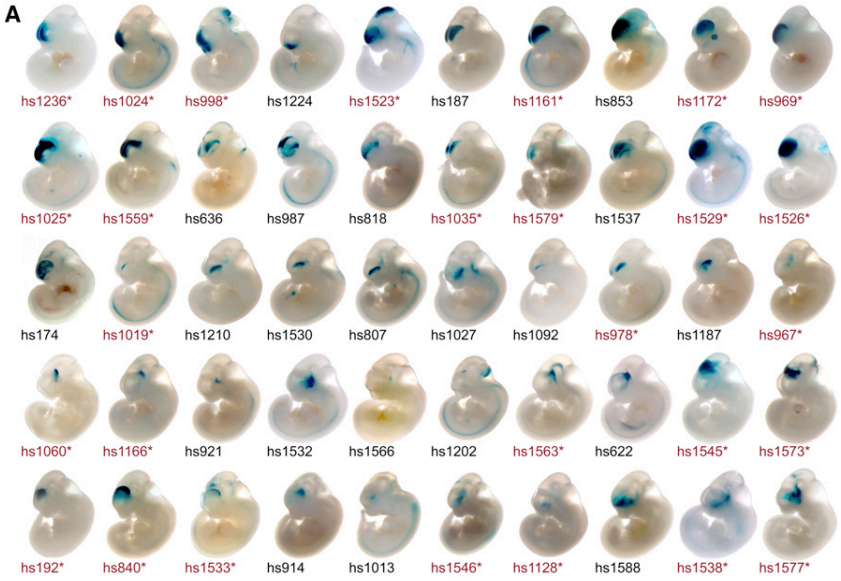

Figure 1. Expression of a Subset of Forebrain Enhancers Identified by Conservation or p300 Binding at Whole-Mount Resolution

(A) A selection of 50 reproducible forebrain enhancers at e11.5 identified in this study. In each case, only one of several (minimum of three) embryos with the same pattern is shown. Additional embryos obtained with each enhancer construct can be viewed at http://enhancer.lbl.gov. Enhancer elements are sorted by broad similarities of patterns as evident at whole-mount resolution.

(B) Examples of genes implicated in forebrain development that were screened for enhancers in the present study and for which enhancers are shown in (A).

A full list of all 329 constructs tested in this study, including annotations of enhancer activity patterns and information about neighboring genes, is provided in Table S1D. See also Tables S1A–S1C and S2.

B

* enhancers near genes implicated in telencephalon development or function:

enhancer element(s)	gene symbol	gene name
hs967, hs998	ASCL1	achaete-scute complex homolog 1
hs1025	EMX1	empty spiracles homeobox 1
hs1236	EMX2	empty spiracles homeobox 2
hs840	EPHA5	Eph receptor A5
hs1523	FOXG1	forkhead box G1
hs1563	HES1	hairy and enhancer of split 1
hs1533	ID4	inhibitor of DNA binding 4
hs1545	LEF1	lymphoid enhancer binding factor 1
hs1161	LMO4	LIM domain only 4
hs1024, hs1060, hs1172, hs1577	NR2F1	nuclear receptor subfamily 2, group F, member 1 (COUP-TFI)
hs1579	OTX2	orthodenticle homeobox 2
hs1546	POU3F1	POU domain, class 3, transcription factor 1 (OCT-6)
hs978, hs1035, hs1128	POU3F2	POU class 3 homeobox 2 (BRN-2)
hs969, hs1526, hs1529	POU3F3	POU class 3 homeobox 3 (BRN-1)
hs192	SOX2	SRY-box containing gene 2
hs1019	SP4	trans-acting transcription factor 4
hs1166, hs1538	NKX2-1	NK2 homeobox 1 (TITF1)
hs1559	WNT7B	wingless-related MMTV integration site 7B
hs1573	ZIC1	zinc finger protein of the cerebellum 1

in the ventrolateral pallium (hs692 and hs799), and by e15.5 they were in the dorsal pallium (hs799, arrowheads in Figures 3F and 3G). These results support the notion that enhancers regulate both spatial and temporal aspects of telencephalic gene expression in patterns consistent with the biology of these regions and cell types.

To facilitate analysis by computational methods, we devised a standardized neuroanatomical annotation scheme for the e11.5 stage of telencephalon development (Figures 2A, 3C, and S1 and Table S1E). All telencephalon enhancer activity patterns examined in this study were annotated using this standardized annotation scheme, in some cases complemented by descriptions that further subdivide the standardized domains or are restricted to subsets of cells (Table S1E). The standardized annotations assigned to enhancers enable computational analysis of their activity patterns as well as a comparison to expression patterns of their presumptive target genes at this stage of development.

Comparison of Enhancer Activities to Gene Expression Patterns

To test whether the telencephalon enhancers examined at high resolution generally recapitulate the spatial expression patterns of their presumptive target genes, we compared their LacZ reporter activities to RNA in situ hybridization data. For example, the *Arx* gene is expressed in both subpallial and pallial regions, with increasing expression in pallial regions from e11.5 to e13.5 (Figure 4A). We found that there are at least four distant-acting telencephalic enhancers in this extended locus, two of which drive subpallial and two of which drive pallial expression, indicating that developmental *Arx* regulation is more complex than initially suggested (Colasante et al., 2008). In addition, comparison of other genes with well-established roles in telencephalon development (*Lef1*, *Wnt8b*, *Gsx2*, *Nr2f1*) to nearby enhancers also revealed examples of spatially concordant enhancer activity and RNA expression (Figures 4B–4E). A recurring feature of these comparisons is the restriction of individual enhancer activities to subregions of the respective gene expression patterns, supporting the modular structure of telencephalic enhancer architecture. For instance, hs687 activity in the lateral ganglionic eminence (LGE) matches *Gsx2* RNA expression, whereas the latter is also expressed in the medial ganglionic eminence (MGE); hs1172 activity in the pallium matches *Nr2f1* RNA expression, whereas the gene is also expressed in the subpallium.

To assess whether these illustrative examples are representative of a general congruence between enhancer activity patterns and the expression of nearby genes, we performed a quantitative correlation analysis across the available data set (see Extended Experimental Procedures for details). Overall, we found a highly

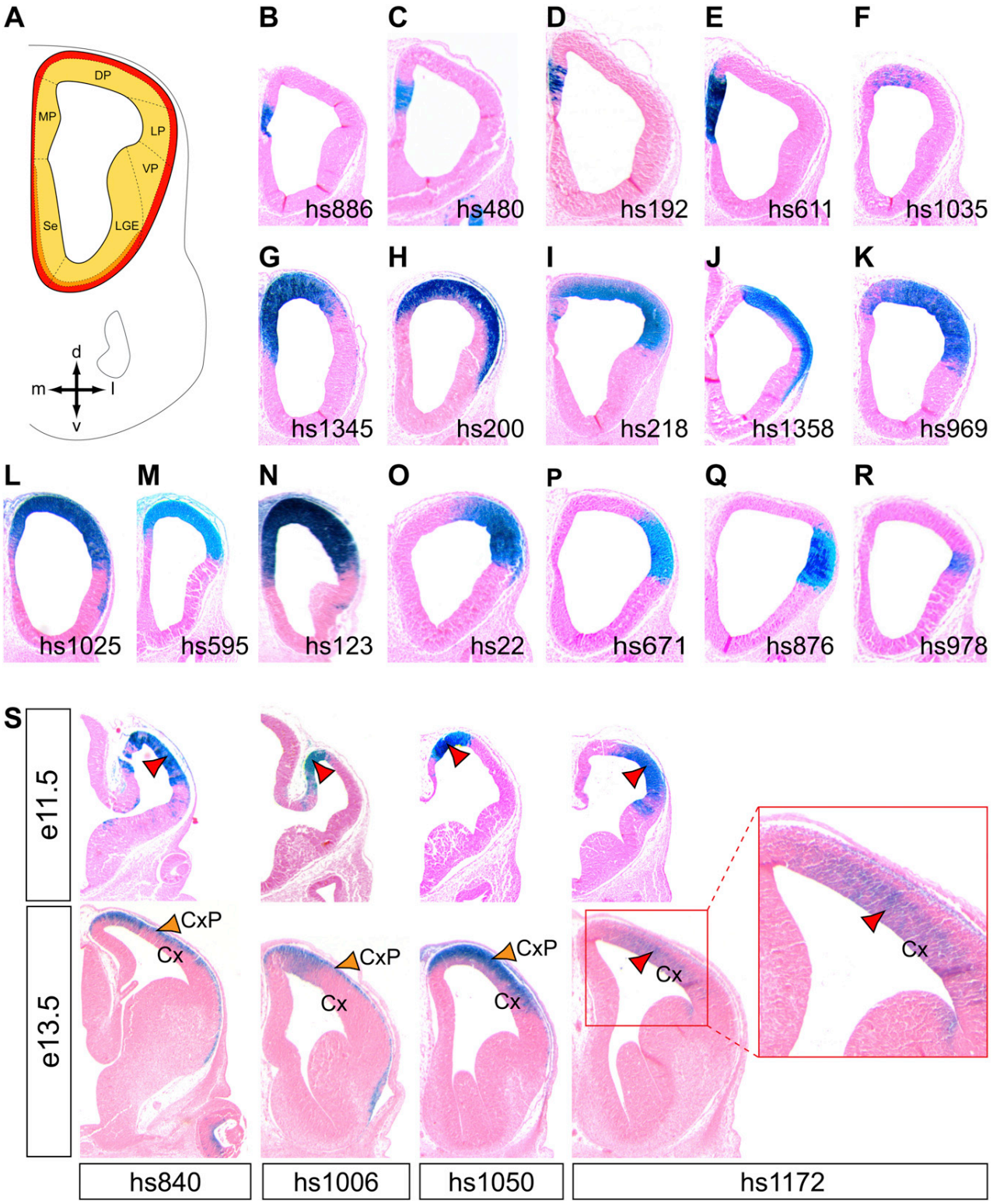

Figure 2. Subset of Forebrain Enhancers with Activity in Different Dorsoventral Subregions of the Developing Mouse Pallium

(A) Overview of annotated structures in the approximate coronal sectioning plane shown in (B–R).

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significant correlation between the activity patterns of enhancers and telencephalic expression patterns of nearby annotated genes ($p = 0.0003$, Mann-Whitney test, Figure 4F). In addition to the high-resolution comparisons of enhancer and gene activity patterns, we also examined whether the genome-wide set of 4,425 forebrain enhancer candidate sequences identified by ChIP-seq from embryonic mouse forebrain tissue is associated with genes with known functions in the telencephalon. Unbiased genome-wide assessment (McLean et al., 2010) showed highly significant enrichment in genes that cause forebrain-related phenotypes when deleted in mouse models (Table S2). These observations support on a genomic scale that the large set of forebrain candidate enhancers predicted by ChIP-seq in this study is enriched near genes that are involved in telencephalon development.

Sequence Analysis of Subregion-Specific Enhancers

A large set of telencephalon enhancers, analyzed at high spatial resolution and annotated to a standardized scheme, offers the possibility to examine sequence features that are associated with in vivo activity in different telencephalic subregions. To explore this regulatory code, we trained a random forest (RF) classifier (Breiman, 2001; Bureau et al., 2005; Cummings and Segal, 2004; Lunetta et al., 2004) to discriminate between random genomic sequences and enhancers active in (1) pallium only, (2) pallium and subpallium (compound pattern), or (3) subpallium only (see Figures 5, Figures S2–S5, and Extended Experimental Procedures). Classification is based on the presence or absence of combinations of sequence motifs matching known transcription factor binding sites (Bryne et al., 2008; Matys et al., 2006). The five most relevant motifs distinguishing the three classes of enhancers and their respective importance are shown in Figure 5B (for additional motifs, see Figure S2 and Table S1G). We did not observe any single motif that was sufficient to accurately discriminate between the different classes of enhancers, suggesting that only the combinatorial binding of multiple transcription factors determines the observed spatial regulatory activity. The majority of the most discriminatory motifs (at least 60% of the top 15 motifs characterizing enhancers active in each of the telencephalic subregions considered) correspond to predicted binding sites for homeodomain-containing transcription factors, consistent with the known critical role of these proteins in telencephalon development (Hébert and Fishell, 2008). Figure S3 summarizes the enrichment of the 15 most relevant motifs for enhancer activity in the three different telencephalic subregions considered. Despite possible ambiguities associated with computational transcription factor binding site predictions, the RF classifier accurately predicts ~80% of the sequences (see Extended Experimental Procedures and Table S3). Sequence motifs with high quantitative importance for discriminating between different classes of telencephalon enhancers are overall more conserved in evolution compared to nonimportant motifs, supporting their functional relevance (Figure S4).

These computational predictions of relevant sequence motifs provide a starting point for experimental studies aimed at understanding the transcription factor binding site content of telencephalon enhancers in greater detail. To illustrate the value of a large set of enhancers with known sequences and activity patterns for studying genetic dependencies in telencephalon development, we tested a subset of subpallial enhancers for their direct regulation by two major subpallial transcription factors, Dlx2 and Ascl1 (see Extended Experimental Procedures). In a cell-based luciferase assay, we observed that Dlx2 and/or Ascl1 significantly increased reporter expression when cotransfected with 13 of 20 tested enhancers (Figure 5C). Of note, these enhancers are located near several genes with known roles in subpallium development, and the results are consistent with previous studies demonstrating that Dlx2 regulates the expression of *Arx*, *Meis2*, and *Sp8* and that Ascl1 regulates the expression of *Sox4* (Castro et al., 2011; Colasante et al., 2008; Long et al., 2009). Considering the expected complexity of the spectrum of transcription factors binding to different subsets of telencephalon enhancers (Figure 5B and Table S1G), complementary scalable methods will be required to experimentally validate all binding sites within each of the enhancers identified. Our cell-based studies of a small subset of these sequences highlight, however, that the combined knowledge of the genomic location, the spatial activity, and the upstream transcription factors of discrete, distant-acting regulatory sequences generates hypotheses that are directly testable in genetic in vivo systems.

Human Brain ChIP-Seq

Our large-scale transgenic testing and high-resolution analysis of telencephalon enhancers focused on sequences that are highly conserved in evolution, with the goal being to characterize the most conserved core regulatory architecture of mammalian telencephalon development. However, epigenomic methods also enable the systematic discovery of poorly conserved and lineage-specific enhancers (Schmidt et al., 2010). To explore possible differences between human and mouse telencephalon enhancers in greater detail, we determined the genome-wide occupancy of the enhancer-associated proteins p300/CBP in human fetal (gestational week 20) cortex (Figures 6A and 6B). ChIP-seq analysis identified 2,275 peaks (candidate enhancers) genome wide that were located at least 2.5 kb from the nearest transcript start site. Comparison with transcriptome data from human fetal cortex tissue revealed a 2.7-fold enrichment in candidate enhancers within 2.5–20 kb of the transcript start sites

(B–R) Selected enhancers that reproducibly label subregions of the developing pallium. Enhancers are arranged by their spatial specificities in the medial, dorsal, lateral, and ventral pallium. Detailed annotations of all patterns, as well as additional enhancers that drive expression in these subregions, are provided in Table S1E. Full serial sets of sections for each enhancer can be viewed at http://enhancer.lbl.gov, using the enhancer IDs indicated in the figure panels.

(S) Comparison of enhancer activities between e11.5 and e13.5. Red arrowheads indicate activity in neuronal precursor/differentiation zones, and orange arrowheads indicate immature neurons in the cortical plate.

Cx, cortex; CxP, cortical plate; DP, dorsal pallium; LGE, lateral ganglionic eminence; LP, lateral pallium; MP, medial pallium; VP, ventral pallium; Se, septum. See also Figure S1 and Tables S1A–S1D.

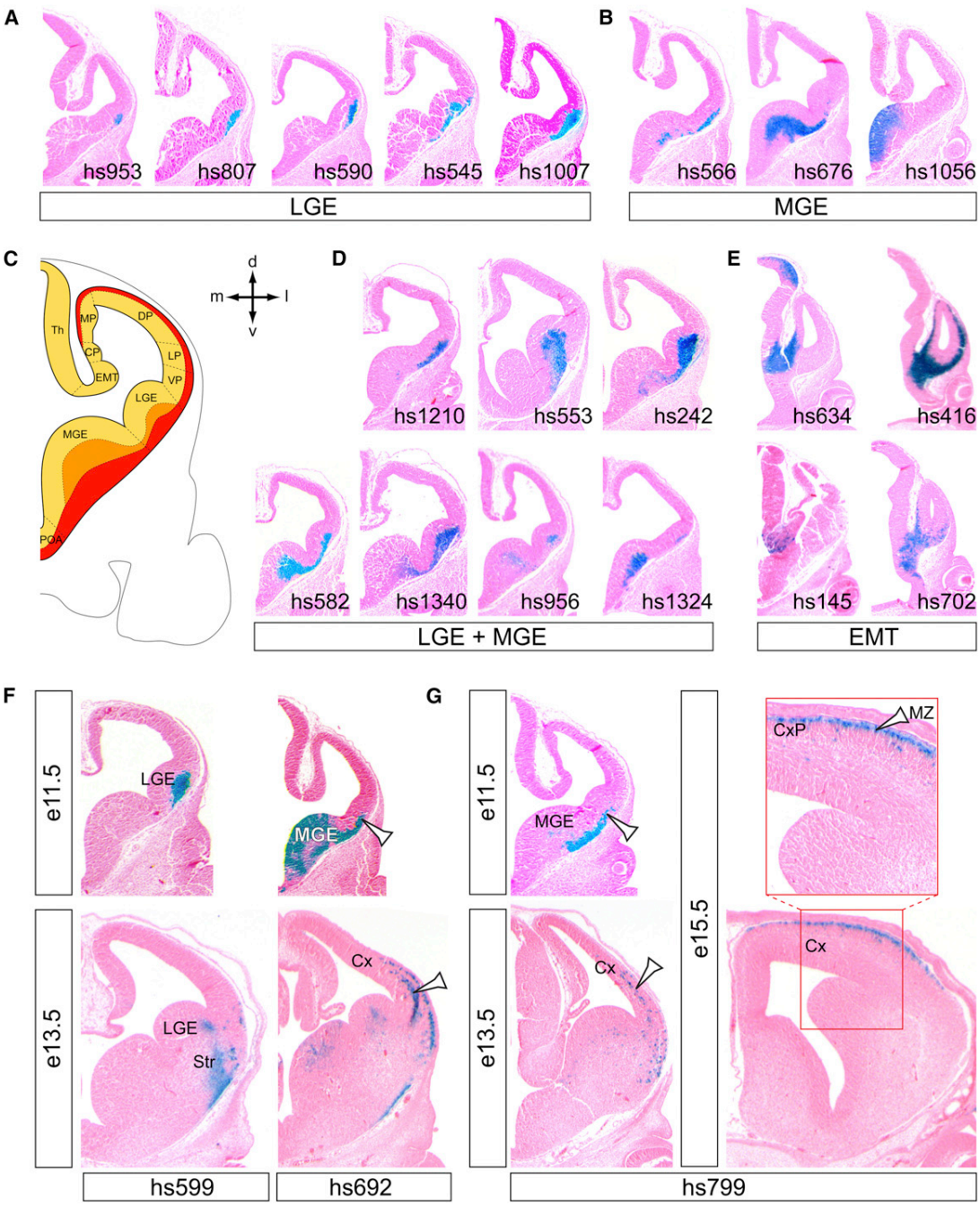

Figure 3. Subset of Forebrain Enhancers with Activity in Different Subregions of the Mouse Subpallium and Eminentia Thalami

(A–E) Selected enhancers that target LacZ expression (A) predominantly or exclusively to subregions of the LGE, (B) predominantly to the MGE, (D) both to the LGE and to MGE, and (E) to the EMT. (C) Schematic overview of structures in the approximate sectioning plane shown in (A), (B), (D), and (E). Depending on the rostrocaudal extent of staining, for some enhancers, more rostral or caudal planes were chosen to illustrate salient features of the respective patterns.

(F and G) Comparison of enhancer activities between e11.5, e13.5, and e15.5. White arrowheads indicate cell populations whose location is consistent with migration from the MGE, through the LGE, and to the cortex.

CP, choroid plexus; Cx, cortex; CxP, cortical plate; EMT, eminentia thalami; DP, dorsal pallium; LGE, lateral ganglionic eminence; LP, lateral pallium; MGE, medial ganglionic eminence; MP, medial pallium; MZ, marginal zone; POA, preoptic area; Str, striatum; VP, ventral pallium; Th, thalamus. See also Figure S1 and Tables S1A–S1D.

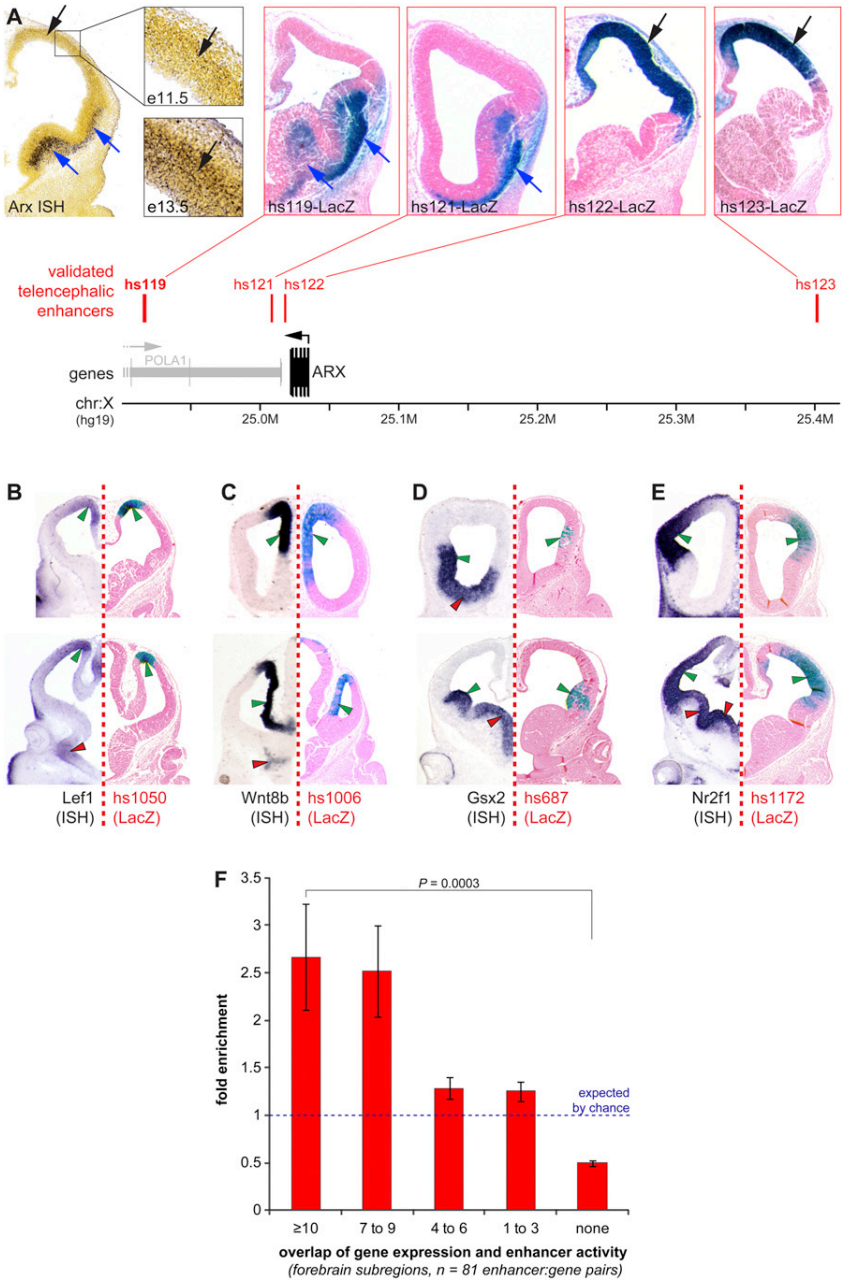

Figure 4. Correlation of Spatial Enhancer Activity Patterns with RNA Expression Patterns of Nearby Genes

(A–E) Examples of individual enhancers recapitulating aspects of the gene expression patterns. (A) The *Arx* gene is expressed in subpallial (blue arrows) and pallial (black arrows) regions. Pallial expression increases from e11.5 to e13.5 (insets). At least four enhancers in the extended locus drive subpallial (hs119, hs121) or pallial expression (hs122, hs123) at e11.5. (B–E) Additional examples of overlap in enhancer activity with expression of nearby genes in rostral (top) and more caudal (bottom) areas of the telencephalon at e11.5. In all four cases, there was spatial overlap in activity (green arrowheads), as well as gene expression in additional regions that did not show enhancer activity (red arrowheads). (F) To assess overall correlations, the annotated activity patterns of telencephalic enhancers were compared to RNA expression patterns of nearby genes. Compared to randomly assigned pairs of genes and enhancers, there is a highly significant enrichment of cases in which concordant enhancer activity and gene expression are observed in one or multiple telencephalic subregions ($p = 0.0003$, Mann-Whitney test; error bars represent SEM). *Arx* RNA in situ hybridization images in (A): Allen Developing Mouse Brain Atlas (http://developingmouse.brain-map.org), reproduced with permission from Allen Institute for Brain Science. See also Tables S1A–S1D.

of genes highly expressed in fetal human cortex ($p < 1 \times 10^{-14}$, binomial distribution), with significant enrichment up to 220 kb away from promoters ($p < 0.001$, binomial distribution, Figure 6C). In contrast, no enrichment of p300/CBP binding sites was observed near genes highly expressed in other tissues. Similar to candidate enhancers predicted from mouse e11.5 forebrain, unsupervised statistical enrichment analysis of functional gene annotations (McLean et al., 2010) showed significant association with genes implicated in nervous-system-related phenotypes (Table S2). Although many extremely conserved noncoding sequences in the human genome are enhancers active in the developing nervous system (Pennacchio et al., 2006), we observed that one-third (36.5%) of ChIP-seq-predicted human brain candidate enhancers are under weak (phastCons < 350) or no detectable evolutionary constraint, suggesting that subsets of human brain enhancers may not be functionally conserved in mice.

At gestational week 20, the human cortex is considerably further developed than the mouse pallium at e11.5 and instead corresponds broadly to early postnatal stages in mouse (Clancy et al., 2007). To enable a direct experimental comparison between the two species, we performed p300/CBP ChIP-seq on mouse postnatal (P0) cortex tissue. Using identical methods to those used for human tissue, we identified 1,132 candidate enhancers (distal ChIP-seq peaks). The majority (58%) of human-derived peaks showed significant or suggestive (subsignificant) enrichment in ChIP-seq reads at the orthologous site in the mouse genome (Figure 6D). The remaining 42% either showed no enrichment in the orthologous mouse region or were not alignable to the mouse genome. Though the lower sequencing coverage in the mouse data set may lead to an underestimation of mouse- compared to human-specific peaks (compare Figures 6D

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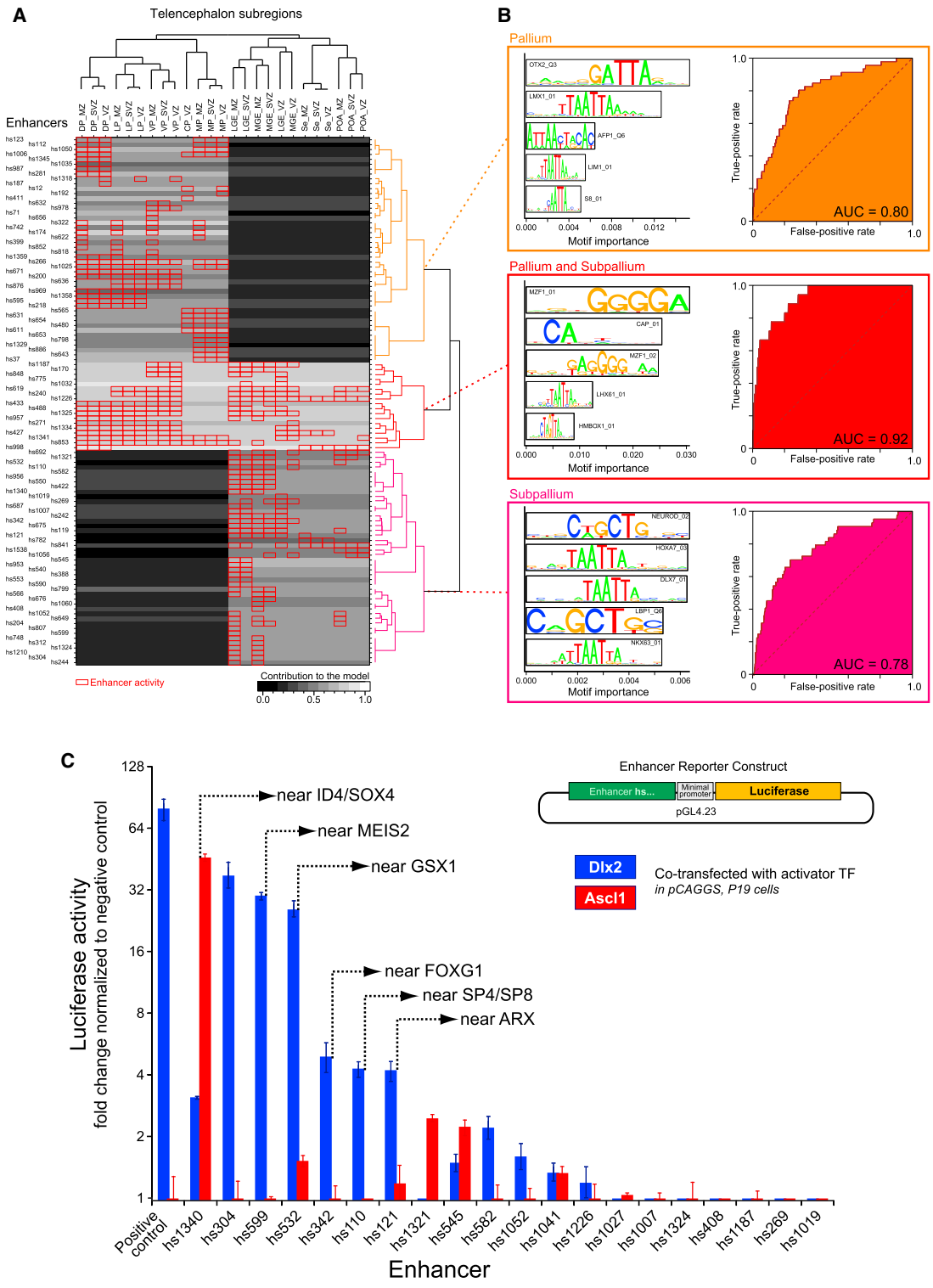

Figure 5. Relating Sequence Motif Content to High-Resolution Activity Annotations

(A) Red squares indicate enhancers (rows) active in different telencephalic subregions (columns). Unsupervised clustering (Jaccard's coefficient, average linkage) of telencephalic subregions by similarity of enhancer activity profiles (top dendrogram) largely follows known developmental, functional, and topological relations of telencephalic subregions. Clustering (Euclidean distance, Ward's method) of enhancers by similarity of observed activity in telencephalic subregions suggests

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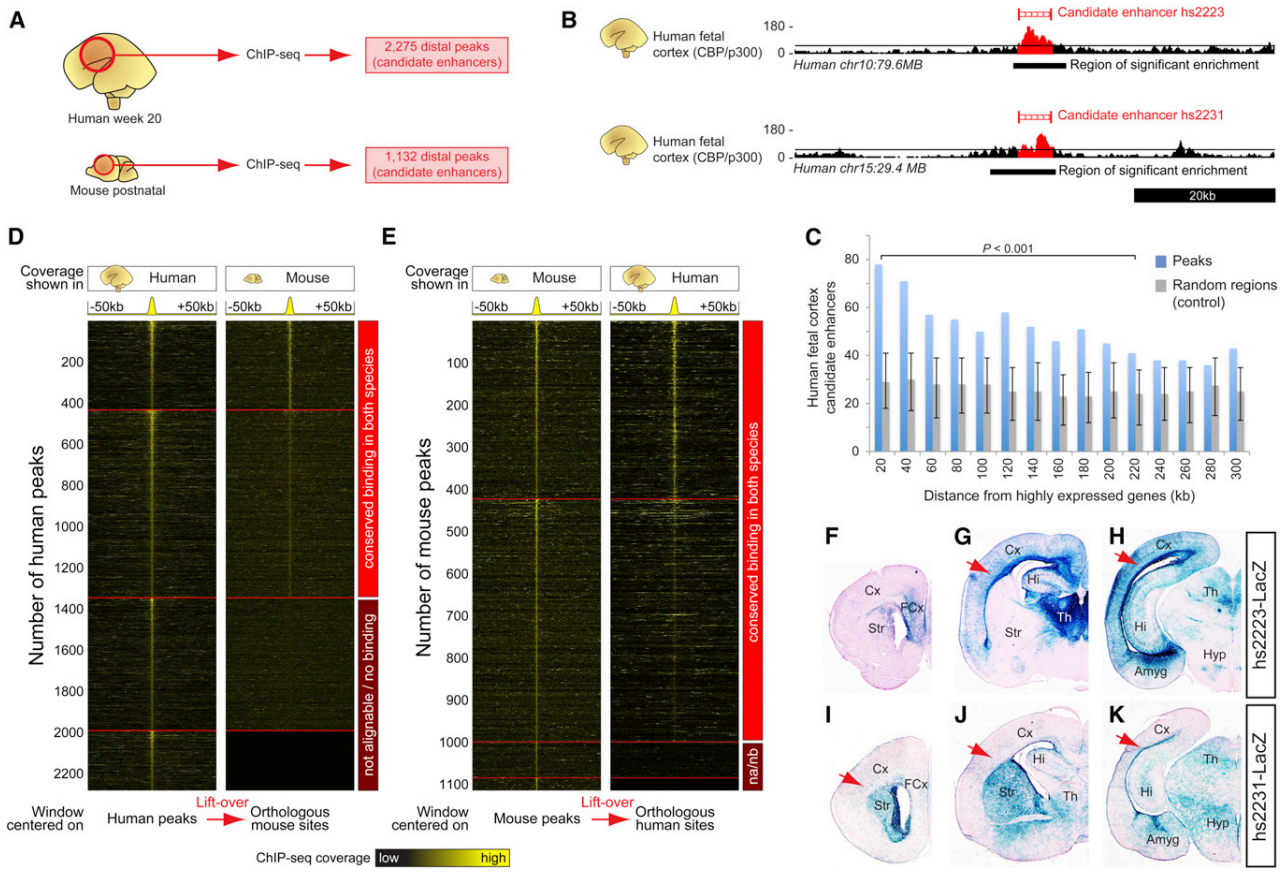

Figure 6. Genome-wide Experimental Comparison of Enhancers Active during Human and Mouse Cortex Development

(A) ChIP-seq analysis was performed on human gestational week 20 and mouse postnatal day 0 cortex tissue using an antibody directed against the enhancer-associated p300/CBP proteins.

(B) Two representative peaks (candidate enhancers) identified from the human fetal data set.

(C) Predicted human fetal cortex enhancers are significantly enriched in the larger vicinity (up to 220 kb away) of genes highly expressed in the human fetal cortex. Error bars represent the 90% confidence interval on the basis of 1,000 iterations of randomized distribution (see Extended Experimental Procedures).

(D) The majority of candidate enhancers identified from human fetal cortex show evidence of p300/CBP binding at orthologous sites in the mouse genome (top two sectors of heat map). However, a substantial proportion of human peaks either shows no evidence of p300/CBP binding at orthologous sites in the mouse genome (third sector) or falls into regions of the human genome that have no known orthologous sequence in the mouse (fourth sector).

(E) A substantially larger proportion of mouse P0 cortex candidate enhancers was found to be bound by p300/CBP at orthologous sites in the human genome.

(F–K) Transgenic activity analysis of two candidate enhancers (B) in transgenic mice at postnatal day 1. Each pattern was reproducible in a minimum of three F_0 animals; three sectioning planes from one representative brain per enhancer are shown. Red arrows indicate expression in the cortex.

and 6E), the presence of 307 peaks in nonalignable regions of the human genome (Figure 6D) supports that a nonnegligible proportion of human brain enhancers emerged in evolution after the divergence of primates and rodents from their last common ancestor.

Similar to the large collection of telencephalon enhancers identified and characterized at e11.5, ChIP-seq peaks derived from human fetal cortex are expected to include enhancers with a variety of in vivo activity patterns. To illustrate this, we examined the in vivo activities of candidate enhancers from

functional subgroups (right dendrogram). Shades of gray indicate the proportion of decision trees assigning each enhancer to the pallium or subpallium class (for pallium and subpallium enhancers) or to the compound pallium/subpallium class (for compound enhancers).

(B) RF classifier distinguishes enhancers that are active in pallium only (top), in both pallium and subpallium (center), and in subpallium only (bottom). (Left) Top five sequence motifs characterizing each class of enhancers and their relative contribution to the classification. Additional motifs are shown in Figure S2. (Right) Receiver-operating characteristic (ROC) curves of predictive performances. The area under the curve (AUC) measures the ability of the classifier to limit incorrect predictions while maintaining sensitivity in true predictions. For example, the "pallium and subpallium" classifier correctly identifies ~70% of enhancers in this cluster at a false positive rate of 10%.

(C) Luciferase cotransfection assays of 20 subpallial enhancers with either the transcription factors Dlx2 or Ascl1 in P19 cells. Error bars represent SD.

See also Figures S2–S5 and Tables S1E–G, S2, and S3.

human fetal cortex in postnatal transgenic mice. Two examples of such enhancers driving reproducible expression in a minimum of three independent transgenic animals are shown in Figures 6F–6K. Consistent with the ChIP-seq prediction, both enhancers were active in the cortex (red arrows) as well as in additional but distinct and reproducible regions of the telencephalon.

To illustrate the value of the genome-wide sets of human and mouse candidate enhancers for the interpretation of human genetic data sets, we compared the genomic position of these sequences with different catalogs of regions in the human genome implicated in neurodevelopmental, neurological, or neuropsychiatric diseases. We intersected the genome-wide sets of candidate enhancers identified in the three different ChIP-seq experiments with (1) lead single-nucleotide polymorphisms (SNPs) from genome-wide association studies of relevant traits (Hindorff et al., 2009), (2) catalogs of syndromic microdeletions and microduplications (Firth et al., 2009), and (3) a set of autism-associated rare copy-number variants (Marshall et al., 2008; Szatmari et al., 2007). Fourteen lead SNPs from genome-wide association studies, including SNPs associated with attention deficit hyperactivity disorder, bipolar disease, and schizophrenia, were found to be located within predicted forebrain enhancers. Moreover, 381 enhancers mapped within recurrent microdeletions or microduplications associated with neurological phenotypes, and 421 enhancers overlapped copy-number variants present in autism cases, but not healthy controls. Though further experimental studies will be required to examine possible causal roles of variants affecting enhancer sequences, the genome-wide sets of candidate enhancers identified from human and mouse brain tissue through this study provide a starting point to explore the role of telencephalon enhancers in human diseases.

Telencephalon Enhancers as Molecular Reagents

The enhancers described in our high-resolution atlas can be used as molecular reagents to drive in vivo expression of reporter or effector genes to specific telencephalic subregions of interest, owing to the reproducibility of their activity patterns (Figure 7A). To illustrate some of the resulting applications, we coupled enhancer hs1006, associated with the *WNT8B* gene, to a minimal Hsp68 promoter, followed by a tamoxifen-inducible Cre recombinase ($CreER^{T2}$), an internal ribosomal entry site, and a green fluorescent protein (GFP) reporter (Figure 7B). In stable transgenic mouse lines generated with this construct, termed CT2IG-hs1006, GFP expression at e11.5 was indistinguishable from LacZ reporter expression (Figures 7A and 7B). GFP expression in these stable lines facilitates a temporally resolved mapping of enhancer activity. A comparison of GFP activity at e12.5, e15.5, and e17.5 with *Wnt8b* RNA expression reveals that enhancer activity spatially coincides with *Wnt8b* gene expression, indicating that this enhancer controls region-specific expression of the gene over an extended period of prenatal telencephalon development.

Because expression of the compound effector/reporter transcript in CT2IG-hs1006 mice faithfully resembled *Wnt8b* expression across multiple stages of development, the chemically inducible $CreER^{T2}$ recombinase can be used for spatially and temporally highly restricted genomic recombineering applications such as neuronal fate mapping studies. To demonstrate this, we crossed CT2IG-hs1006 mice with Rosa26-LacZ mice (Figure 7B) (Indra et al., 1999). Tamoxifen induction of $CreER^{T2}$ in pregnant compound CT2IG-hs1006:Rosa26-LacZ mice at e10.5 leads to recombination only in the small proportion of pallial cells in which the enhancer is active at this time point. LacZ staining at later stages revealed the spatial fate of cells in which the enhancer was active at e10.5. For example, hs1006-driven e10.5→ e12.5 fate mapping marked pallial cell populations with a distribution that is clearly distinct from hs1006 activity at this time point (compare e12.5 patterns in Figures 7C and 7D). These data highlight the utility of these enhancers to precisely drive gene expression in the developing brain and their value as a rich resource for a diversity of uses.

DISCUSSION

This work provides a comprehensive resource for basic studies of telencephalon enhancers. Our targeted screen identified the genomic location of thousands of candidate enhancers putatively active in the embryonic forebrain. The mapping and annotation of the activity patterns of nearly 150 human telencephalon enhancers at histological resolution in transgenic mice provide insight into the regulatory architecture of individual genes that are required for forebrain development and will facilitate studies of molecular genetic pathways by identifying the genomic regions to which upstream transcription factors bind.

Our analysis revealed several cases of enhancers that drive similar patterns and are associated with the same gene (e.g., Figure 4A) in a manner reminiscent of the "shadow enhancers" observed in invertebrate models (Frankel et al., 2010; Hong et al., 2008). The data provided through this work will support the identification of minor spatial activity differences between such enhancers, as well as the functional exploration of their apparent redundancies. It is also remarkable that a large proportion of enhancers examined in this study drove patterns that were at least partially different from all other enhancers examined, highlighting the complexity of the developing forebrain, as well as the regulatory sequence code orchestrating its development.

The motif-based classifiers derived from enhancers active in different subregions of the telencephalon demonstrate the value of systematically annotated enhancer activity data sets for computational studies aimed at deciphering the correlation between the transcription factor binding sites present in an enhancer and its precise spatial activity pattern. Beyond such functional genomic studies, the enhancers identified and characterized in this work provide a comprehensive set of molecular reagents that can be used to target gene expression to defined subregions of the developing brain or to defined cell states when differentiating stem cells in vitro. This will enable tissue-specific homologous recombination and deletion strategies or expression of reporter and selectable genes, as illustrated in Figure 7.

Finally, results from this study are expected to enable and facilitate the functional genomic exploration of the role of enhancers in human brain disorders. There is accumulating

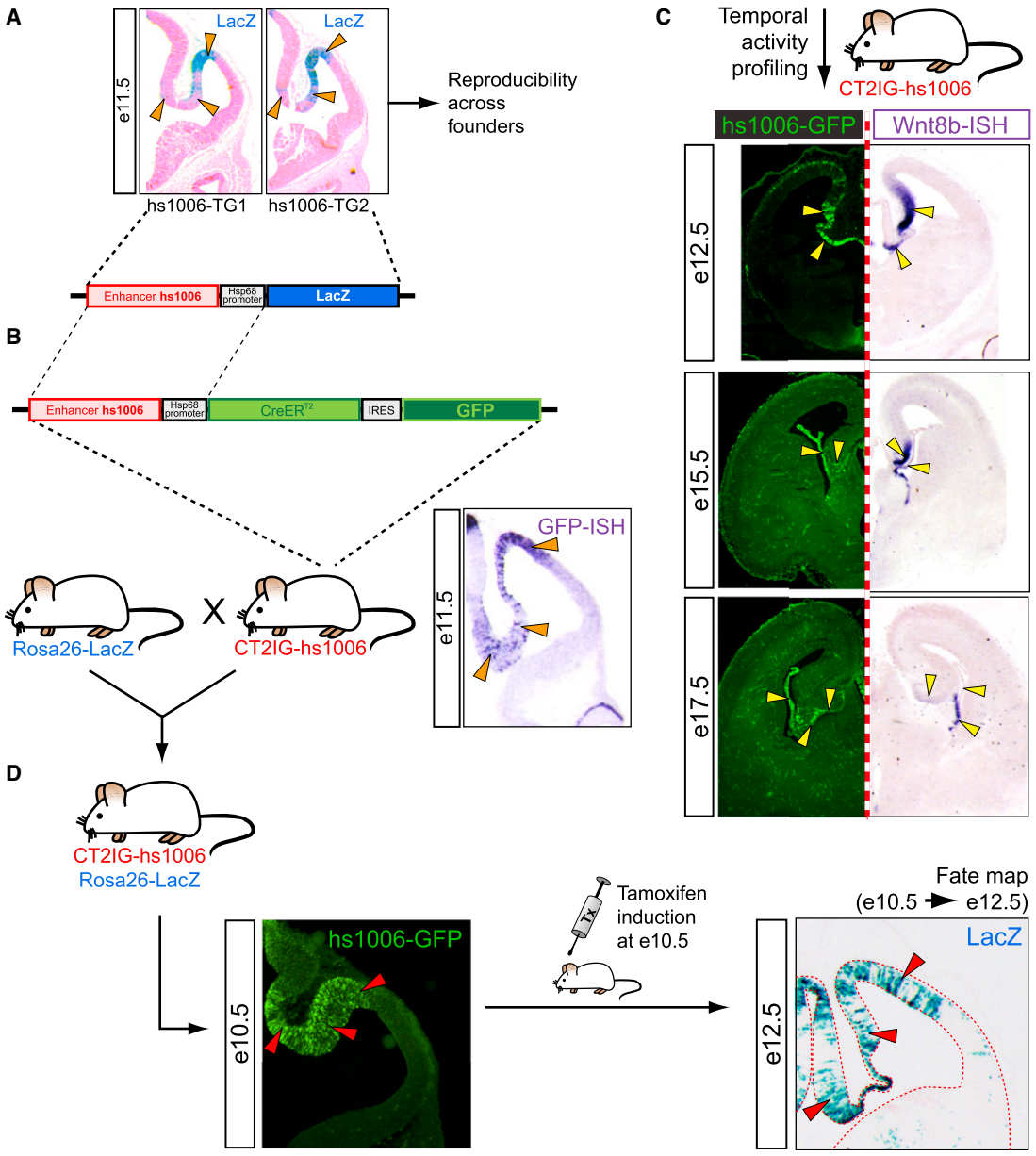

Figure 7. Using Telencephalon Enhancers as Tissue-Specific Reagents

(A) Approach used for the generation of the large-scale high-resolution atlas at e11.5.

(B) Enhancers can be used as drivers of other reporter and effector genes, such as GFP or tamoxifen-inducible Cre recombinase. GFP reporter expression at e11.5 recapitulates the annotated LacZ expression pattern (orange arrowheads). Schematic components of constructs in (A) and (B) are not shown to scale.

(C) Stable transgenic lines facilitate temporal profiling of enhancer activity and comparisons with corresponding gene expression patterns.

(D) Tamoxifen induction at e10.5, followed by LacZ staining at a later time point (shown: e12.5) can be used for developmental fate mapping of neuronal cell populations.

evidence that noncoding sequence variants, as well as copy-number variation in coding and noncoding portions of the genome, have important impacts on a wide spectrum of disorders, including bipolar disorder, schizophrenia, autism, intellectual disability, and epilepsy (Cooper et al., 2011; Durbin et al., 2010; International Schizophrenia Consortium, 2008; Malhotra et al., 2011; Sebat et al., 2007; Vacic et al., 2011; Visel et al., 2009b; Walsh et al., 2008b). However, the functional interpretation of noncoding sequence or copy-number variants remains a major challenge, and few potentially causative connections linking neurological traits to molecular variation in enhancers have been identified (e.g. Poitras et al., 2010). Thus, the

systematic mapping and high-resolution analysis of telencephalon enhancers through this work are expected to provide functional genomic insights to guide studies that will mechanistically relate individual noncoding sequence and copy-number variants to brain disorders.

EXPERIMENTAL PROCEDURES

Chromatin Immunoprecipitation Sequencing

ChIP-seq on forebrain tissue isolated from e11.5 CD-1 strain mouse embryos, using an antibody directed against p300, was performed according to previously described procedures (Visel et al., 2009a). For human tissue ChIP-seq and the matched mouse postnatal cortex data set, an anti-acCBP/p300 pan-specific antibody was used (May et al., 2011).

All procedures of this study involving human tissue samples or animals were reviewed and approved by the respective institutional Human and Animal Regulatory Committees at Lawrence Berkeley National Laboratory and the University of California at San Francisco.

Transgenic Mouse Assays

Enhancer candidate regions were analyzed in transgenic mouse embryos as previously described (Kothary et al., 1988; Pennacchio et al., 2006). Paraffin sections were prepared according to standard protocols. Serial sets of sections were digitally photographed and uploaded to the Vista Enhancer Browser (http://enhancer.lbl.gov).

GFP Reporter Assays and Cell Fate Mapping

A previously described Cre-ERT^2 construct (Feil et al., 1997) was modified to allow Cre recombinase expression to be driven by the hs1006 enhancer (Figure 7B). For fate mapping, CT2IG-hs1006 mice were crossed with Rosa26-LacZ reporter mice (Soriano, 1999).

Luciferase Assays

Dlx2 and Ascl1 were selected for luciferase reporter assays due to their well-established roles in subpallial development and because they are representatives of two major groups of transcription factors found among the top motifs of the subpallium classifier (see Extended Experimental Procedures). P19 cells were grown by previously described methods (Farah et al., 2000).

Data and Reagent Availability

Images of whole-mount-stained embryos and full sets of e11.5 coronal brain sections are available through the Vista Enhancer Browser (http://enhancer.lbl.gov). All enhancer reporter vectors described in this study are freely available from the authors. In addition, archived surplus transgenic embryos for many constructs can be made available upon request for complementary studies. The genome-wide set of ChIP-seq peaks derived from mouse e11.5 forebrain is provided in Table S1A. Raw data and additional ChIP-seq data sets from postnatal mouse and fetal human cortex are available from GEO under accession number GSE42881.

ACCESSION NUMBERS

The GEO accession number for the ChIP-seq data reported in this paper is GSE42881.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, five figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2012.12.041.

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REFERENCES

Blow, M.J., McCulley, D.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., et al. (2010). ChIP-Seq identification of weakly conserved heart enhancers. Nat. Genet. *42*, 806–810.

Breiman, L. (2001). Random Forests. Mach. Learn. *45*, 5–32.

Bryne, J.C., Valen, E., Tang, M.H., Marstrand, T., Winther, O., da Piedade, I., Krogh, A., Lenhard, B., and Sandelin, A. (2008). JASPAR, the open access database of transcription factor-binding profiles: new content and tools in the 2008 update. Nucleic Acids Res. *36*(Database issue), D102–D106.

Bureau, A., Dupuis, J., Falls, K., Lunetta, K.L., Hayward, B., Keith, T.P., and Van Eerdewegh, P. (2005). Identifying SNPs predictive of phenotype using random forests. Genet. Epidemiol. *28*, 171–182.

Castro, D.S., Martynoga, B., Parras, C., Ramesh, V., Pacary, E., Johnston, C., Drechsel, D., Lebel-Potter, M., Garcia, L.G., Hunt, C., et al. (2011). A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets. Genes Dev. *25*, 930–945.

Clancy, B., Finlay, B.L., Darlington, R.B., and Anand, K.J. (2007). Extrapolating brain development from experimental species to humans. Neurotoxicology *28*, 931–937.

Colasante, G., Collombat, P., Raimondi, V., Bonanomi, D., Ferrai, C., Maira, M., Yoshikawa, K., Mansouri, A., Valtorta, F., Rubenstein, J.L., and Broccoli, V. (2008). Arx is a direct target of Dlx2 and thereby contributes to the tangential migration of GABAergic interneurons. J. Neurosci. *28*, 10674–10686.

Cooper, G.M., Coe, B.P., Girirajan, S., Rosenfeld, J.A., Vu, T.H., Baker, C., Williams, C., Stalker, H., Hamid, R., Hannig, V., et al. (2011). A copy number variation morbidity map of developmental delay. Nat. Genet. *43*, 838–846.

Cummings, M.P., and Segal, M.R. (2004). Few amino acid positions in rpoB are associated with most of the rifampin resistance in Mycobacterium tuberculosis. BMC Bioinformatics *5*, 137.

Diez-Roux, G., Banfi, S., Sultan, M., Geffers, L., Anand, S., Rozado, D., Magen, A., Canidio, E., Pagani, M., Peluso, I., et al. (2011). A high-resolution anatomical atlas of the transcriptome in the mouse embryo. PLoS Biol. *9*, e1000582.

Durbin, R.M., Abecasis, G.R., Altshuler, D.L., Auton, A., Brooks, L.D., Gibbs, R.A., Hurles, M.E., and McVean, G.A.; 1000 Genomes Project Consortium. (2010). A map of human genome variation from population-scale sequencing. Nature *467*, 1061–1073.

Farah, M.H., Olson, J.M., Sucic, H.B., Hume, R.I., Tapscott, S.J., and Turner, D.L. (2000). Generation of neurons by transient expression of neural bHLH proteins in mammalian cells. Development *127*, 693–702.

Feil, R., Wagner, J., Metzger, D., and Chambon, P. (1997). Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. Biochem. Biophys. Res. Commun. *237*, 752–757.

Firth, H.V., Richards, S.M., Bevan, A.P., Clayton, S., Corpas, M., Rajan, D., Van Vooren, S., Moreau, Y., Pettett, R.M., and Carter, N.P. (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am. J. Hum. Genet. *84*, 524–533.

Frankel, N., Davis, G.K., Vargas, D., Wang, S., Payre, F., and Stern, D.L. (2010). Phenotypic robustness conferred by apparently redundant transcriptional enhancers. Nature *466*, 490–493.

Gong, S., Zheng, C., Doughty, M.L., Losos, K., Didkovsky, N., Schambra, U.B., Nowak, N.J., Joyner, A., Leblanc, G., Hatten, M.E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature *425*, 917–925.

Gray, P.A., Fu, H., Luo, P., Zhao, Q., Yu, J., Ferrari, A., Tenzen, T., Yuk, D.I., Tsung, E.F., Cai, Z., et al. (2004). Mouse brain organization revealed through direct genome-scale TF expression analysis. Science *306*, 2255–2257.

Hébert, J.M., and Fishell, G. (2008). The genetics of early telencephalon patterning: some assembly required. Nat. Rev. Neurosci. *9*, 678–685.

Hindorff, L.A., Sethupathy, P., Junkins, H.A., Ramos, E.M., Mehta, J.P., Collins, F.S., and Manolio, T.A. (2009). Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc. Natl. Acad. Sci. USA *106*, 9362–9367.

Hong, J.W., Hendrix, D.A., and Levine, M.S. (2008). Shadow enhancers as a source of evolutionary novelty. Science *321*, 1314.

Indra, A.K., Warot, X., Brocard, J., Bornert, J.M., Xiao, J.H., Chambon, P., and Metzger, D. (1999). Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. Nucleic Acids Res. *27*, 4324–4327.

International Schizophrenia Consortium. (2008). Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature *455*, 237–241.

Kothary, R., Clapoff, S., Brown, A., Campbell, R., Peterson, A., and Rossant, J. (1988). A transgene containing lacZ inserted into the dystonia locus is expressed in neural tube. Nature *335*, 435–437.

Kurokawa, D., Takasaki, N., Kiyonari, H., Nakayama, R., Kimura-Yoshida, C., Matsuo, I., and Aizawa, S. (2004). Regulation of Otx2 expression and its functions in mouse epiblast and anterior neuroectoderm. Development *131*, 3307–3317.

Lein, E.S., Hawrylycz, M.J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A.F., Boguski, M.S., Brockway, K.S., Byrnes, E.J., et al. (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature *445*, 168–176.

Lettice, L.A., Heaney, S.J., Purdie, L.A., Li, L., de Beer, P., Oostra, B.A., Goode, D., Elgar, G., Hill, R.E., and de Graaff, E. (2003). A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. Hum. Mol. Genet. *12*, 1725–1735.

Lewis, D.A., and Sweet, R.A. (2009). Schizophrenia from a neural circuitry perspective: advancing toward rational pharmacological therapies. J. Clin. Invest. *119*, 706–716.

Long, J.E., Swan, C., Liang, W.S., Cobos, I., Potter, G.B., and Rubenstein, J.L. (2009). Dlx1&2 and Mash1 transcription factors control striatal patterning and differentiation through parallel and overlapping pathways. J. Comp. Neurol. *512*, 556–572.

Lunetta, K.L., Hayward, L.B., Segal, J., and Van Eerdewegh, P. (2004). Screening large-scale association study data: exploiting interactions using random forests. BMC Genet. *5*, 32.

Malhotra, D., McCarthy, S., Michaelson, J.J., Vacic, V., Burdick, K.E., Yoon, S., Cichon, S., Corvin, A., Gary, S., Gershon, E.S., et al. (2011). High frequencies of de novo CNVs in bipolar disorder and schizophrenia. Neuron *72*, 951–963.

Marshall, C.R., Noor, A., Vincent, J.B., Lionel, A.C., Feuk, L., Skaug, J., Shago, M., Moessner, R., Pinto, D., Ren, Y., et al. (2008). Structural variation of chromosomes in autism spectrum disorder. Am. J. Hum. Genet. *82*, 477–488.

Matys, V., Kel-Margoulis, O.V., Fricke, E., Liebich, I., Land, S., Barre-Dirrie, A., Reuter, I., Chekmenev, D., Krull, M., Hornischer, K., et al. (2006). TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. Nucleic Acids Res. *34*(Database issue), D108–D110.

Maurano, M.T., Humbert, R., Rynes, E., Thurman, R.E., Haugen, E., Wang, H., Reynolds, A.P., Sandstrom, R., Qu, H., Brody, J., et al. (2012). Systematic localization of common disease-associated variation in regulatory DNA. Science *337*, 1190–1195.

May, D., Blow, M.J., Kaplan, T., McCulley, D.J., Jensen, B.C., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., et al. (2011). Large-scale discovery of enhancers from human heart tissue. Nat. Genet. *44*, 89–93.

McLean, C.Y., Bristor, D., Hiller, M., Clarke, S.L., Schaar, B.T., Lowe, C.B., Wenger, A.M., and Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. Nat. Biotechnol. *28*, 495–501.

Nobrega, M.A., Ovcharenko, I., Afzal, V., and Rubin, E.M. (2003). Scanning human gene deserts for long-range enhancers. Science *302*, 413.

Pennacchio, L.A., Ahituv, N., Moses, A.M., Prabhakar, S., Nobrega, M.A., Shoukry, M., Minovitsky, S., Dubchak, I., Holt, A., Lewis, K.D., et al. (2006). In vivo enhancer analysis of human conserved non-coding sequences. Nature *444*, 499–502.

Poitras, L., Yu, M., Lesage-Pelletier, C., Macdonald, R.B., Gagné, J.P., Hatch, G., Kelly, I., Hamilton, S.P., Rubenstein, J.L., Poirier, G.G., and Ekker, M. (2010). An SNP in an ultraconserved regulatory element affects Dlx5/Dlx6 regulation in the forebrain. Development *137*, 3089–3097.

Portales-Casamar, E., Swanson, D.J., Liu, L., de Leeuw, C.N., Banks, K.G., Ho Sui, S.J., Fulton, D.L., Ali, J., Amirabbasi, M., Arenillas, D.J., et al. (2010). A regulatory toolbox of MiniPromoters to drive selective expression in the brain. Proc. Natl. Acad. Sci. USA *107*, 16589–16594.

Schmidt, D., Wilson, M.D., Ballester, B., Schwalie, P.C., Brown, G.D., Marshall, A., Kutter, C., Watt, S., Martinez-Jimenez, C.P., Mackay, S., et al. (2010). Five-vertebrate ChIP-seq reveals the evolutionary dynamics of transcription factor binding. Science *328*, 1036–1040.

Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., Yamrom, B., Yoon, S., Krasnitz, A., Kendall, J., et al. (2007). Strong association of de novo copy number mutations with autism. Science *316*, 445–449.

Shim, S., Kwan, K.Y., Li, M., Lefebvre, V., and Sestan, N. (2012). Cis-regulatory control of corticospinal system development and evolution. Nature *486*, 74–79.

Siepel, A., Bejerano, G., Pedersen, J.S., Hinrichs, A.S., Hou, M., Rosenbloom, K., Clawson, H., Spieth, J., Hillier, L.W., Richards, S., et al. (2005). Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res. *15*, 1034–1050.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet. *21*, 70–71.

Szatmari, P., Paterson, A.D., Zwaigenbaum, L., Roberts, W., Brian, J., Liu, X.Q., Vincent, J.B., Skaug, J.L., Thompson, A.P., Senman, L., et al.; Autism Genome Project Consortium. (2007). Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat. Genet. *39*, 319–328.

Vacic, V., McCarthy, S., Malhotra, D., Murray, F., Chou, H.H., Peoples, A., Makarov, V., Yoon, S., Bhandari, A., Corominas, R., et al. (2011). Duplications of the neuropeptide receptor gene VIPR2 confer significant risk for schizophrenia. Nature *471*, 499–503.

Visel, A., Thaller, C., and Eichele, G. (2004). GenePaint.org: an atlas of gene expression patterns in the mouse embryo. Nucleic Acids Res. *32*(Database issue), D552–D556.

Visel, A., Minovitsky, S., Dubchak, I., and Pennacchio, L.A. (2007). VISTA Enhancer Browser—a database of tissue-specific human enhancers. Nucleic Acids Res. *35*(Database issue), D88–D92.

Visel, A., Prabhakar, S., Akiyama, J.A., Shoukry, M., Lewis, K.D., Holt, A., Plajzer-Frick, I., Afzal, V., Rubin, E.M., and Pennacchio, L.A. (2008). Ultraconservation identifies a small subset of extremely constrained developmental enhancers. Nat. Genet. *40*, 158–160.

Visel, A., Blow, M.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., et al. (2009a). ChIP-seq accurately predicts tissue-specific activity of enhancers. Nature *457*, 854–858.

Visel, A., Rubin, E.M., and Pennacchio, L.A. (2009b). Genomic views of distant-acting enhancers. Nature *461*, 199–205.

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Walsh, C.A., Morrow, E.M., and Rubenstein, J.L. (2008a). Autism and brain development. Cell *135*, 396–400.

Walsh, T., McClellan, J.M., McCarthy, S.E., Addington, A.M., Pierce, S.B., Cooper, G.M., Nord, A.S., Kusenda, M., Malhotra, D., Bhandari, A., et al. (2008b). Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science *320*, 539–543.

Wilson, S.W., and Rubenstein, J.L. (2000). Induction and dorsoventral patterning of the telencephalon. Neuron *28*, 641–651.

Zeng, H., Shen, E.H., Hohmann, J.G., Oh, S.W., Bernard, A., Royall, J.J., Glattfelder, K.J., Sunkin, S.M., Morris, J.A., Guillozet-Bongaarts, A.L., et al. (2012). Large-scale cellular-resolution gene profiling in human neocortex reveals species-specific molecular signatures. Cell *149*, 483–496.