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Expression at the Imprinted Dlk1-Gtl2 Locus Is Regulated by Proneural Genes in the Developing Telencephalon

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Abstract

Imprinting is an epigenetic mechanism that restrains the expression of about 100 genes to one allele depending on its parental origin. Several imprinted genes are implicated in neurodevelopmental brain disorders, such as autism, Angelman, and Prader-Willi syndromes. However, how expression of these imprinted genes is regulated during neural development is poorly understood. Here, using single and double KO animals for the transcription factors Neurogenin2 (Ngn2) and Achaete-scute homolog 1 (Ascl1), we found that the expression of a specific subset of imprinted genes is controlled by these proneural genes. Using in situ hybridization and quantitative PCR, we determined that five imprinted transcripts situated at the Dlk1-Gtl2 locus (Dlk1, Gtl2, Mrg, Rian, Rtl1) are upregulated in the dorsal telencephalon of Ngn2 KO mice. This suggests that Ngn2 influences the expression of the entire Dlk1-Gtl2 locus, independently of the parental origin of the transcripts. Interestingly, 14 other imprinted genes situated at other imprinted loci were not affected by the loss of Ngn2. Finally, using Ngn2/Ascl1 double KO mice, we show that the upregulation of genes at the Dlk1-Gtl2 locus in Ngn2 KO animals requires a functional copy of Ascl1. Our data suggest a complex interplay between proneural genes in the developing forebrain that control the level of expression at the imprinted Dlk1-Gtl2 locus (but not of other imprinted genes). This raises the possibility that the transcripts of this selective locus participate in the biological effects of proneural genes in the developing telencephalon.

Introduction

The cerebral cortex is populated by two main neuronal classes, pyramidal neurons and interneurons that represent respectively 85% and 15% of cortical neurons. Two distinct, though tightly linked processes, control the development and placement of these neurons within the cortex. On one hand, ‘spatial patterning’ by which the telencephalon is regionally subdivided into defined morphological and molecular progenitor territories, thereby underlying the generation of future interneurons and pyramidal neurons [1,2]. Hence pyramidal neurons and interneurons originate through two distinct embryonic structures, the dorsal and ventral telencephalon respectively [1,3]. On the other hand, the process of ‘temporal specification’ describes the capacity of cortical progenitors to evolve over time to produce different types of neurons and give rise to the typical laminar organization of the cortex [1,4]. The proneural genes Ngn2 and Ascl1 are respectively expressed in the dorsal and ventral telencephalon, where they determine the regional identity of neural progenitors [3,6]. Interestingly, in Ngn2 KO mice, dorsal progenitors are partially respecified towards a ventral phenotype as a result of Ascl1 ectopic expression in the presumptive cortex [5]. Moreover, Ngn2 and Ascl1 regulate temporal patterning [7,8] and coordinate a wide range of basic cellular processes such as cell cycle exit, migration or neuronal connectivity [9,10].

During recent years a series of transcriptional targets of Ngn2 and Ascl1 that could mediate their functions have been reported [11,12,13,14]. However, the list of these molecular effectors is still far from completion.

Parental genomic imprinting is an epigenetic mechanism that restrains the expression of about 100 mammalian genes to one parental allele [15]. In other words, in contrast to the vast majority of genes that display biallelic expression, imprinted genes (IGs) are differentially transcribed depending on the parental origin of the allele; for example the imprinted H19 gene is transcribed from the paternal allele only (maternally expressed gene; MEG), whereas its imprinted neighbor Igf2 is a paternally expressed gene (PEG).
[15]. The differential transcription depending on the parental origin of the allele is due to methylation marks that are selectively deposited on differentially methylated regions (DMRs) during male and female gametogenesis by specific de novo DNA methyltransferases (Dnmt) [15,16].

Both MEGs and PEGs are required during mouse embryonic development as uniparental embryos (inheriting 2 copies of maternal or paternal genomes) that express only PEGs or only MEGs, die during early development [17,18,19]. Furthermore, mouse mutants for individual IGs display altered phenotypes, including embryonic growth restriction or overgrowth [15]. As IGs are transcribed from a single parental allele, the loss of the transcriptionally active allele results in a complete loss of gene expression while loss of imprinting results in an abnormal biallelic expression [15,20,21,22]. There is growing experimental and clinical evidence that misregulated expression of IGs is involved in human pathologies affecting brain function [15,21,22]. At least one IG (Ube3a) is linked to Angelman disease and several imprinted loci are linked to Autism Spectrum Disorders (ASD) and to Prader-Willi, two syndromes in which cerebral functions are altered [21,23]. Very recently the PEG Dlk1 was shown to be required for neurogenesis in the postnatal brain SVZ [24], a process that depends on Ascl1 [25,26]. However, to date there is no reported link between imprinted genes expression and proroneural genes.

Here we identify 5 imprinted genes, 2 PEGs (Dlk1, Rtl1) and 3 MEGs (Gtl2, Rian, Mirg) situated at an imprinted locus at 12qF1 [15] as potential targets of Ngn2 and Ascl1, raising the possibility that they participate in brain development.

Results

In a microarray screen to identify Ngn2 targets [JS, TB and PV, unpublished data], we observed that the non-coding RNA encoded by the imprinted gene Gtl2 (Gene trap locus 2, also known as Maternally expressed gene 3) was highly upregulated in the dorsal telencephalon of Ngn2 KO mice. First, to shed light on Gtl2 localization and infer about its function in the normal developing telencephalon, we performed in situ hybridizations (ISH) on WT animals. At E12.5, Gtl2 is mostly expressed in postmitotic neurons of the ventral telencephalon where it remains strongly expressed up to E17.5 (Fig.1A and Fig.S1). From E13.5 onward, Gtl2 starts to be expressed in the nascent cortical plate in the dorsal telencephalon (Fig.1A–C), where it is expressed mostly in early generated neurons forming the marginal zone (MZ) and subplate (SP) at E14.5 (Fig.1B). Gtl2 RNA is also abundant in the neighboring thalamus (Fig.1A). As previously reported for the muscle [27], Gtl2 RNA is localized mostly in the nucleus in the developing forebrain (Fig.1B).

In addition, by using reciprocal hybrid cortex issued from crosses between M. m. domestus and M. m. molossinus, we were able to distinguish between parental alleles and to determine that Gtl2 is maternally expressed in the developing dorsal telencephalon (T.B. and L.J., unpublished observation), confirming that Gtl2 is a MEG at this developmental stage in this structure. We then confirmed by ISH (Fig.1C) and quantitative PCR (qPCR) (Fig.1D) the upregulation of Gtl2 in Ngn2 KO cortex. In Ngn2 KO mice, we did not observe co-localization of Gtl2 RNA with the postmitotic neuronal markers TuJ1 and Thr1, indicating that the cells expressing ectopically Gtl2 located in the ventricular and intermediate zones are likely progenitor cells (Fig.1E and Fig.S1B,D). We didn’t detect any changes in Gtl2 expression in the ventral telencephalon at any developmental stages in Ngn2 KO mice (data not shown). This data thus shows that in the developing telencephalon, the imprinted gene Gtl2 encodes for a nuclear RNA expressed mostly in post-mitotic neurons of the ventral and dorsal telencephalon and thalamus (Fig.1A). Upon loss of Ngn2, Gtl2 becomes ectopically and precociously expressed in the dorsal telencephalon. We also found that the cells overexpressing Gtl2 in Ngn2 KO are not yet differentiated (TuJ1 negative) at early embryonic stages (E12.5, Fig.1E and located predominantly in the intermediate zone (Fig. S1B, D).

Gtl2 is part of an imprinted locus at chromosomal position 12qF1 that contains several additional MEGs and PEGs including Dlk1, Rian (also known as Meg9), Mirg and Rtl1 (also known as Peg11) (Fig.2A) [15]. To determine whether the level of expression of these IGs is also affected in the cortex of Ngn2 KO mice, we performed qPCR on cDNA samples from Ngn2 WT and Ngn2 KO cortex at E13.5. As shown in Figure 2B, Dlk1, Gtl2, Rtl1, Rian, and Mirg were all significantly upregulated upon Ngn2 loss. By contrast Dio3, which is situated at the extremity of the locus (Fig.2A), was unaffected. We confirmed by ISH that expression of Dlk1, Rian and Rtl1 are upregulated and that they are ectopically expressed in Ngn2 KO cortex (Fig.2C,D). We also observed by immunohistofluorescence an increase in the protein product of Dlk1 gene in Ngn2 KO cortex (Fig.2C), an increase that parallels those of RNA. These data thus show that 5 IGs of the Dlk1-Gtl2 locus are upregulated in the cortex of Ngn2 KO mice. This upregulation seems to be independent of the parental origin of the transcripts as on these 5 IGs, 3 are established MEGs (Gtl2, Mirg, Rian) while 2 are PEGs (Dlk1, Rtl1) (Fig.2A).

To determine whether the absence of Ngn2 selectively affects the Dlk1-Gtl2 locus or whether it also impacts on IGs located at other genomic regions, we measured RNA levels of 14 additional IGs present on 4 imprinted loci. These loci are situated at chromosomes 6 (Fig.3A) and 7 (Fig.3B). They were selected because they either contain IG involved in brain development (Necdin [28], Mest [29], Igf2 [30]) or because they contain IGs implicated in neurodevelopmental diseases (Angelman: Ube3a, Prader-Willi: Necdin, IPW). These 14 genes therefore represent potential targets for factors involved in brain development such as Ngn2 and Ascl1. Strikingly, as shown in Figure 3, none of these 14 IGs was significantly affected in the presumptive cortex of Ngn2 KO mice. This indicates that loss of Ngn2 in the cortex likely selectively impacts expression on IGs situated at the Dlk1-Gtl2 locus.

We next thought to identify the molecular mechanism responsible for the overexpression of Dlk1, Gtl2, Rian, Mirg and Rtl1 in the Ngn2 KO dorsal telencephalon. One obvious candidate to test is Ascl1 that is known to be highly upregulated in the cortex of Ngn2 KO animals (Fig.4A and [5]). Furthermore Ascl1 ectopic expression in the Ngn2 positive territory is sufficient to respecify the dorsal telencephalon towards a ventral phenotype [5]. To determine whether Ascl1 is required for upregulation of transcripts at the Dlk1-Gtl2 locus, we compared the expression of Dlk1, Gtl2, Rian, Mirg and Rtl1 in the dorsal telencephalon of WT, Ngn2 KO, Ascl1 KO and Ngn2/Ascl1 double KO mice. The observed upregulation of IGs in the dorsal telencephalon of Ngn2 KO mice was counteracted by the absence of a functional copy of Ascl1 in Ngn2/Ascl1 double KO mice as illustrated by an average expression of RNAs for Dlk1, Gtl2, Mirg, Rtl1 and Rian that was lower in double KO mice compared to single Ngn2 KO mice (Fig.4B). The values for Dlk1, Rian and Rtl1 were not significantly different between Ngn2 KO and Ngn2/Ascl1 double KO mice (Mann-Whitney test). We attribute this to variations within the simple and double KO categories and to the small proportion of animals in each of these categories, which is principally due to the fact that double KO animals are difficult to generate.
expression of Ascl1 in the respecified dorsal telencephalon. In Ngn2 KO animals would be a consequence of the ectopic expression in the normal developing ventral telencephalon. Role in regulating the expression of IGs of the Dlk1-Gtl2 locus in the telencephalon is responsible for ectopic expression of those genes during embryogenesis [27], notably during corticogenesis [1]. By scrutinizing the list of genes differentially expressed between WT and Pax6 KO mice [36], we noticed that Pax6 deletion leads to an overexpression of Gtl2, Dlk1, Rian and Mirg. Therefore, Gtl2 is crucial for mouse development as its inactivation causes perinatal death and skeletal muscle defects [31,32]. The mechanisms of action of this non-coding RNA are largely unknown but it was recently shown that there is an increased expression of angiogenic genes in the brain of Gtl2 KO mice [33].

Gtl2 is part of the Dlk1-Gtl2 imprinted locus at chromosomal position 12qF1 that contains several additional MEGs and PEGs [15]. The expression of these IGs is regulated by an imprinted center, the IG-DMR, which is differentially methylated during male and female gametogenesis [15]. Gtl2 deletion affects the methylation of the IG-DMR, yielding to a transcriptional silencing of MEGs while an enhancement of neighboring PEGs situated at 12qF1 [32]. Therefore, Gtl2 itself participates in controlling the expression at its own locus.

Consistent with the notion of coregulation, we show here that the level of transcripts for Gtl2 but also those of Dlk1, Mrg, Rian, Rtl1, are all increased in Ngn2 KO mice (Fig.2). To our knowledge, this is the first case report of a situation where all IGs of the Dlk1-Gtl2 locus, independently of their parental origin, are upregulated. Interestingly, a mirror situation, but showing a common downregulation, was recently reported. Fibroblasts that are poorly prone to be reprogrammed during the process of generating induced pluripotent stem cells have an aberrant low level of genes situated at this locus [34]. As other IGs are not markedly affected by Ngn2 deletion in the forebrain (Fig.3), this suggests that proneural genes may specifically target this imprinted locus. This raises the question of what would be the advantage for a proneural gene to target an entire imprinted locus. A possibility would be that, these IGs are not capable to exert a function during development as individual genes but rather need to work in synergy. Few data are available to evaluate this hypothesis. One supporting set of data concerns the Dlk1 gene. Indeed, Dlk1 KO animals show normal embryonic neurogenesis but impaired postnatal SVZ neurogenesis [24], indicating that the loss of this single gene has no impact during prenatal development. A synergy between IGs could take place as meta-analysis of microarray data support the existence of a network of IGs [35]. Supporting this synergistic view, Gtl2 and Dlk1 KO mice have similar growth retardation phenotype and Gtl2 and Dlk1 are frequently co-expressed. Here we show that both transcripts are expressed in the ventral forebrain in WT mice and ectopically expressed in the dorsal telencephalon as a result of Ngn2 deletion. These normal and aberrant expressions likely rely on Ascl1 expression. Indeed, on one hand IGs are principally expressed in the Ascl1 positive territory, the ventral telencephalon (Figs. 1, 4 and S1); on the other hand their upregulation in Ngn2 KO animals is reduced in Ngn2/Ascl1 double KO animals (Fig.4).

Each structure in the developing brain results from the coordinated action of specific combinations of transcriptions factors. In the dorsal telencephalon, Ngn2 and the master control gene Pax6 are co-expressed and are both necessary to complete corticogenesis [1]. By scrutinizing the list of genes differentially expressed between WT and Pax6 KO mice [36], we noticed that Pax6 deletion leads to an overexpression of Gtl2, Dlk1, Rian and of Ascl1. This set of genes is very similar to the one described here with Ngn2 KO mice. The fact that Ngn2 and Pax6 act on the same targets could therefore be a way to reinforce their combined action on patterning and specification. One possible explanation is that Pax6 and Ngn2 control IGs expression indirectly through the joint repression of Ascl1. Finally, it would be exciting in the future to determine the identity of cells ectopically expressing IGs in Ngn2 KO mice and to perform gain and loss of function experiments where the levels of multiple genes from the Dlk1-Gtl2 locus are manipulated in order to investigate their impact on brain development.

Materials and Methods

Maintenance and Genotyping of Ngn2<sup>MGFP</sup> and Ascl1 Mutant Mice

The Ngn2<sup>MGFP</sup> transgenic mice where eGFP has been inserted into the endogenous Ngn2 locus by homologous recombination in ES cells have been described in [10]. The Ascl1 transgenic mice have been described in [37]. To generate WT, heterozygous and...
Figure 2. Upregulation and ectopic expression of imprinted genes at the Dlk1-Gtl2 locus in Ngn2 KO mice. A. Schematic representation of the Dlk1-Gtl2 locus. The active transcribed alleles are colored in blue for PEGs and red for MEGs. The silenced alleles are colored in grey. The control region Ig-DMR is represented by a triangle. Arrows indicate the sense of transcription. Adapted from [15]. B. qPCR analysis of RNAs expression for Dlk1, Gtl2, Rtl1, Rian, Mirg, and Dio3 in Ngn2 WT and Ngn2 KO at E13.5. Data are presented as percentage of change normalized to the mean of WT (taken as 100%) + s.e.m. *P<0.01. Transcription of Dio3 did not change significantly (NS, Mann-Whitney’s test). C. Dlk1 mRNA and D. Rian protein expression.
homozygous (KO) Ngn2 animals, heterozygous Ngn2\textsuperscript{KIGFP}\textsuperscript{+/−} mice were intercrossed. To generate WT, Ascl1 KO, Ngn2 KO and Ascl1/Ngn2 double KO mice, Ngn2\textsuperscript{KIGFP}\textsuperscript{+/−}; Ascl1\textsuperscript{+/−} mice were intercrossed. The morning of vaginal plug detection is considered as the morning of the first day of gestation (E0.5). For analysis, WT and heterozygous animal were pooled as loss on one allele does not markedly alter phenotype [5,6,11]. Animal care and procedures were in compliance with local Ethics Committees (Université Libre de Bruxelles and Belgian National Fund for Scientific Research FRS/FRS) and institutional guidelines. The ‘commission du bien être et de l’éthique animale’ (Welfare and

Figure 3. Expression of imprinted genes outside the Dlk1-Gtl2 locus is not affected in Ngn2 KO mice. A. Changes in RNA expression assessed by qPCR in Ngn2 WT and KO mice of 7 IGs on chromosome (Chr.) 6. A schematic representation of the locus is shown above the graphs. B. Changes in RNA expression assessed by qPCR in Ngn2 WT and KO mice of 7 IGs on Chr.7. A schematic representation of the locus is shown above the graphs. For both loci, the active transcribed alleles are colored in blue for PEGs and red for MEGs. The silenced alleles are colored in grey. Arrows indicate the sense of transcription. Expression of none of the 14 IGs was significantly affected in Ngn2 KO mice (Mann-Whitney’s test).

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Figure 4. Role of Ascl1 in the upregulation of genes of the DLK1-Gtl2 locus in Ngn2 KO mice. A. Upregulation of Ascl1 mRNA in the dorsal telencephalon from WT or Ngn2 KO was determined by ISH (E12.5, left panel) and qPCR experiments (E13.5, right panel). B. Expression of imprinted RNAs from the Gtl2-Dlk1 locus (Dlk1, Gtl2, Rtl1, Mirg, Rian) in WT, Ngn2 KO, Ascl1 KO and Ngn2/Ascl1 double KO in E13.5 dorsal telencephalons. Data are presented as percentage of change normalized to the mean of WT (taken as 100% ± s.e.m; *P < 0.01 in Mann-Whitney’s test. C. RNA expression (ISH) of Gtl2 in WT and Ascl1 KO mice at E12.5 and E14.5. D. RNA expression (ISH) of DLK1 in WT and Ascl1 KO mice at E13.5. Red arrows in C and D indicate regions in the ventral telencephalon where expression of Gtl2 and DLK1 are reduced in Ascl1 KO mice. Scale bars: A,B,D = 200 μm, C = 400 μm.

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animal ethics) of Faculty of Medicine, University of Brussels specifically approved this study (protocol number 259N).

RNA Isolation and qRT-PCR

E13.5 dorsal telencephalons were dissected out from embryos obtained from Ngn2GFP+/− or Ngn2GFP−/− Aesc1+/− heterozygous intercrosses. RNA was isolated using RNaseqy mixed according to the manufacturer’s instructions (Qiagen). Alternatively, RNA was extracted using RNA now (Ozyme). All RNA samples were treated with DNase (Qiagen or Ambion for RNaseqy and RNA now methods respectively). Reverse transcription was performed using N6 primers and MMLV-RT (Promega). Quantitative PCR (qPCR) was performed in duplicate in 384 well plates using 2× SybrGreen Mix and a LC480 Real-Time PCR System (Roche). Results are presented as linearized Cp-values normalized to housekeeping genes TBP, Gus2 and Gapdh and the indicated reference value (2−ΔΔCt). The sequence of primers is available upon request.

RNA In Situ Hybridization

All steps and solutions for RNA in situ hybridization (ISH) were achieved in RNase free environment. Embryos were perfused intracardially with a solution of 4% paraformaldehyde (in PBS) and the brains were post-fixed in the same fixative over night. Brains were then cryoprotected in 30% sucrose (in PBS), ISH was carried out on 10 μm cryostat sections (coronal cut) using digoxigenin-labeled (Roche) RNA probes as previously described [38]. We have generated probes antisense to RNAs for Gli2, Rian, Dlk1, Mrg and Rlh1 using embryonic brain cDNA as templates. Sense probes were used as controls and generated no staining (not shown). The sequence of primers are available upon request. For co-labeling of ISH with TuJ1 (1/500, Covance) and Thb1 (1/1000, a gift from Robert Hevner), revelation of the anti-digoxigenin antibody with NBT/BCIP was directly followed by several PBS washes and 10 min post-fixation in 4% paraformaldehyde. To unmask epitopes, slides were treated with 100% EtOH/0.3% hydrogen peroxide for 30 min at RT. This procedure was followed by standard protocol for DAB immunohistochemistry protocol. Counterstaining in 0.5% green methyl (in distilled water) was performed after NBT/BCIP revelation by application of the nuclear staining solution for 5 min at RT followed by immersion in distilled water to stop the reaction.

Immunofluorescence Staining

Immunostaining was performed on 20 μm thick cryosections. Blocking solution consisted of PBS supplemented with 5% horse serum (Invitrogen), 0.3% Triton X-100 (Sigma) and 3% Bovine Serum Albumin (BSA; Sigma). Antibody solution consisted of PBS supplemented with 1% horse serum, 0.1% Triton X-100 and 3% BSA. Dlk1 or Thb1 antibodies (a kind gift of Charlotte Harken Jensen and Robert Hevner respectively) were incubated overnight at 4°C and secondary during 2 hours at RT. Nuclei were stained with bisbenzimide (Hoechst#33258; Sigma). Sections were mounted with glycerigel (DAKO).

Imaging

Pictures of the in-situ RNA hybridization and immunofluorescence staining were acquired with an Axioplan2 Zeiss microscope and a Spot RT camera, converted in false colors and overlayered using Adobe Photoshop software.

Statistical Analysis

Data are presented as mean ± standard error of the mean of at least three biologically independent experiments or of WT and KO embryos. A Mann-Whitney’s test was used for comparing the distribution of data of measurements of RT-qPCR experiments.

Supporting Information

Figure S1 Expression of Gtl2 RNA (ISH) at E17.5 in Ngn2 WT and Ngn2 KO mice. (A-E) Co-labeling with Thb1 protein (immunohistochemistry) and a Spot RT camera, in false colors and overlayed using Adobe Photoshop software.

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Author Contributions

Conceived and designed the experiments: JS PV TB. Performed the experiments: JS OA ALD DC VR TB. Analyzed the data: JS TB. Contributed reagents/materials/analysis tools: FG LJ. Wrote the paper: JS PV TB.

References

1. Guillemot F, Mohar Z, Tarabykin V, Steylova A (2006) Molecular mechanisms of cortical differentiation. Eur J Neurosci 23: 837–868.
2. Wilson SW, Rubenstein JL (2000) Induction and dorsoverentral patterning of the telencephalon. Neuron 28: 641–651.
3. Wonders CP, Anderson SA (2006) The origin and specification of cortical interneurons. Nat Rev Neurosci 7: 687–696.
4. Molyneaux BJ, Arlotta P, Menezes JR, Macklis JD (2007) Neuronal subtype specification in the cerebral cortex. Nat Rev Neurosci 8: 427–437.
5. Fode C, Ma Q, Casarosa S, Aug SL, Anderson DJ, et al. (2008) A role for neuronal determination genes in specifying the dorsoverentral identity of telencephalic neurons. Genes Dev 14: 67–80.
6. Casarosa S, Fode C, Guillemot F (1999) Mash1 regulates neurogenesis in the ventral telencephalon. Development 126: 525–534.
7. Dixit R, Zimmer C, Waclaw RR, Mattar P, Shaker T, et al. (2011) Axiol Participates in Cajal-Retzius Cell Development in the Neocortex. Cereb Cortex.
8. Britz O, Mattar P, Nguyen L, Langevin LM, Zimmer C, et al. (2006) A role for proneural genes in the maturation of cortical progenitor cells. Cereb Cortex 16 Suppl: 1138–1151.
9. Bertrand N, Castro DS, Guillemot F (2002) Proneural genes and the specification of neural cell types. Nat Rev Neurosci 3: 517–530.
10. Suij H, Schaursman C, Gradwohl G, Dehay C, Vanderhaeghen P, et al. (2003) Neurogenin2 specifies the connectivity of thalamic neurons by controlling axon responsiveness to intermediate target cues. Neuron 39: 439–452.
11. Mattar P, Britz O, Johannes C, Nieto M, Ma L, et al. (2004) A screen for downstream effectors of Neurogenin2 in the embryonic neocortex. Dev Biol 273: 373–389.
12. Heng J, Nguyen L, Castro DS, Zimmer C, Wildner H, et al. (2008) Neurogenin 2 controls cortical neuron migration through regulation of Rnd2. Nature 455: 114–118.
13. Castro DS, Martinez-Palma B, Parras C, Ramesh V, Pacary E, 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.
14. Godlike JM, Armaut O, Parham FM, Smith MV, Zimmer C, et al. (2008) Characterization of the proneural gene regulatory network during mouse telencephalon development. BMC Biol 6: 15.
15. Ferguson-Smith AC (2011) Genomic imprinting: the emergence of an epigenetic paradigm. Nat Rev Genet 12: 565–575.
16. Shai K (2008) A mechanistic view of genomic imprinting. Annu Rev Genomics Hum Genet 9: 197–216.
17. Surani MA, Barton SC, Norris ML (1984) Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 308: 548–550.
18. Barton SC, Surani MA, Norris ML (1984) Role of paternal and maternal genomes in mouse development. Nature 313: 374–376.
19. McGrath J, Solted D (1984) Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 37: 179–183.
20. Lalande M (1996) Parental imprinting and human disease. Annu Rev Genet 30: 175–195.
21. Wilkinson LS, Davies W, Idles AR (2007) Genomic imprinting effects on brain development and function. Nat Rev Neurosci 8: 832–843.
22. Das R, Hampton DD, Jirtle RL (2009) Imprinting evolution and human health. Mamm Genome.
23. Chamberlain SJ, Lalande M (2010) Angelman syndrome, a genomic imprinting disorder of the brain. J Neurosci 30: 9930–9963.
24. Ferron SR, Charalambous M, Radford E, McEwen K, Wildner H, et al. (2011) Postnatal loss of Dlk1 imprinting in stem cells and niche astrocytes regulates neurogenesis. Nature 475: 381–385.
25. Kim Ej, Ables JL, Dickel LK, Eshch AJ, Johnson JE (2011) Ascl1 (Mash1) defines cells with long-term neurogenic potential in subgranular and subventricular zones in adult mouse brain. PLoS One 6: e19472.
26. Parras CM, Galli R, Britz O, Soares S, Galicch C, et al. (2004) Mash1 specifies neurons and oligodendrocytes in the postnatal brain. EMBO J 23: 4495–4505.
27. Schuster-Gossler K, Bilinski P, Sudo T, Ferguson-Smith A, Gossler A (1998) The mouse Gtl2 gene is differentially expressed during embryonic development, encodes multiple alternatively spliced transcripts, and may act as an RNA. Dev Dyn 212: 214–226.
28. Kowajima T, Nishimura I, Yoshikawa K (2006) Necdin promotes GAARergic neuron differentiation in cooperation with Dlx homeodomain proteins. J Neurosci 26: 5383–5392.
29. Sansom SN, Hebert JM, Thammomongkol U, Smith J, Nibet G, et al. (2005) Genomic characterisation of a Fgf-regulated gradient-based neocortical protomap. Development 132: 3947–3961.
30. Lehtinen MK, Zappaterra MW, Chen X, Yang YJ, Hill AD, et al. (2011) The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. Neuron 69: 893–905.
31. Schuster-Gossler K, Simon-Chazottes D, Guernet JL, Zachgo J, Gossler A (1996) GtllacZ, an insertional mutation on mouse chromosome 12 with paternal origin-dependent phenotype. Mamm Genome 7: 20–24.
32. Zhou Y, Chenusschon P, Nakayama Y, Lawler MW, Zhong Y, et al. (2010) Activation of paternally expressed genes and perinatal death caused by deletion of the Gtll gene. Development 137: 2643–2652.
33. Gordon FC, Nutt CL, Chenusschon P, Nakayama Y, Provencher KA, et al. (2010) Increased expression of angiogenic genes in the brains of mouse meg3-null embryos. Endocrinology 151: 2443–2452.
34. Stadtfeld M, Apostolou E, Akotu H, Fukuda A, Follett P, et al. (2010) Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent cells. Nature 465: 175–181.
35. Varrault A, Guerydan C, Delahbre A, Bellmann A, Houssami S, et al. (2006) Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell 11: 711–722.
36. Holm PC, Mader MT, Haubs N, Wizennman A, Sigvardsson M, et al. (2007) Loss- and gain-of-function analyses reveal targets of Pax6 in the developing mouse telencephalon. Mol Cell Neurosci 34: 99–119.
37. Guillemot F, Lo LC, Johnson JE, Auerbach A, Anderson DJ, et al. (1993) Mammalian achaete-scute homolog 1 is required for the early development of olfactory and autonomic neurons. Cell 75: 463–476.
38. Deparpe V, Suarez-Gonzaldez N, Dufour A, Pasante L, Gorski JA, et al. (2005) Ephrin signalling controls brain size by regulating apoptosis of neural progenitors. Nature 435: 1244–1250.