Cortical and Commissural Defects Upon HCF-1 Loss in Nkx2.1-Derived Embryonic Neurons and Glia

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ABSTRACT: Formation of the cerebral cortex and commissures involves a complex developmental process defined by multiple molecular mechanisms governing proliferation of neuronal and glial precursors, neuronal and glial migration, and patterning events. Failure in any of these processes can lead to malformations. Here, we study the role of HCF-1 in these processes. HCF-1 is a conserved metazoan transcriptional co-regulator long implicated in cell proliferation and more recently in human metabolic disorders and mental retardation. Loss of HCF-1 in a subset of ventral telencephalic Nkx2.1-positive progenitors leads to reduced numbers of GABAergic interneurons and glia, owing not to decreased proliferation but rather to increased apoptosis before cell migration. The loss of these cells leads to development of severe commissural and cortical defects in early postnatal mouse brains. These defects include mild and severe structural defects of the corpus callosum and anterior commissure, respectively, and increased folding of the cortex resembling polymicrogyria. Hence, in addition to its well-established role in cell proliferation, HCF-1 is important for organ development, here the brain. © 2019 The Authors. Developmental Neurobiology Published by Wiley Periodicals, Inc. Develop Neurobiol 79: 578–595, 2019

Keywords: Nkx2.1; cortex; anterior commissure; corpus callosum; polymicrogyria; glia; GABAergic neurons

INTRODUCTION

Proper development of the cerebral cortex and commissures is achieved by a long and controlled process of proliferation, differentiation, migration, and organization of neuronal and glial cells (Marin and Rubenstein, 2001; Schuurmans and Guillemot, 2002; Mochida and Walsh, 2004; Barkovich et al., 2005; Guillemot et al., 2006; Guerrini and Parrini, 2010). Defects in any of these developmental steps can lead to developmental disorders due to (i) insufficient production of cortical cells or increased cell death, as in microcephaly (Mochida, 2009; Thornton and Woods, 2009; Kaindl et al., 2010), (ii) delayed, excessive, or arrested migration, as in periventricular heterotopia, subcortical band heterotopia, pachgyria, or cobblestone lissencephaly (Francis et al., 2006; Kerjan and Gleeson, 2007; Guerrini and Parrini, 2010), (iii) defective migration of commissural projections owing to improper neuronal/glial positioning and guidance, as in agenesis of corpus callosum, a malformation of the anterior commissure (Silver, 1993; Lindwall et al., 2007; Niquille et al., 2009; 2013; Benadiba et al., 2012; Minocha et al., 2015b), and/or (iv) migration defects combined with defective cortical organization, as in schizencephaly or polymicrogyria (Francis et al., 2006; Barkovich, 2010).

Here, we study the role of the X-linked Hcfcl gene in these processes. Hcfcl encodes HCF-1, a conserved transcriptional co-regulator that binds to the transcriptional start sites of many genes (Dejosez et al., 2010; Michaud et al., 2013) and associates with both
sequence-specific DNA-binding proteins (e.g., Myc, E2F1 and E2F4, Thap11/Ronin, ZNF143) and chromatin-modifying enzymes (e.g., the MLL and Seti histone H3 Lysine 4 methyltransferases and Sin3 histone deacetylase) (reviewed in (Zargar and Tyagi, 2012); see also (Thomas et al., 2016)). Genetic studies in mammalian cell culture, early mouse embryos, and liver have shown that HCF-1 is important for multiple aspects of cell proliferation (Goto et al., 1997; Reilly and Herr, 2002; Julien and Herr, 2003; Minocha et al., 2016b), and very early epiblast-specific embryonic loss of HCF-1 is lethal before gastrulation (Minocha et al., 2016a; 2016b). Nevertheless, in humans, there are mutations in the HCFCL1 gene that are associated with X-linked intellectual disability (ID) and cobalamin metabolism; these disorders point toward an important role of HCF-1 in brain development (Huang et al., 2012; Yu et al., 2013; Gerard et al., 2015; Jolly et al., 2015; Koufaris et al., 2016).

In this study, we investigated the functions of Hcfc1 in the mouse brain. Conditional loss of HCF-1 in ventral telencephalic Nkx2.1+ progenitors did not appear to affect their proliferation, and yet fewer Nkx2.1-derived GABAergic interneurons and glia arose upon loss of HCF-1, owing to increased apoptosis. Reduced migration of GABAergic interneurons and glia was accompanied with corpus callosum defects and abnormal formation of the anterior commissure as well as severe cortical defects that resembled polymicrogyria.

**MATERIALS AND METHODS**

**Mice**

All experimental studies have been performed in compliance with the EU and national legislation rules, as advised by the Lemanic Animal Facility Network (Resal), concerning ethical considerations of transportation, housing, strain maintenance, breeding, and experimental use of animals. Mice were housed four to five per cage at 23°C with ad libitum food and water access. For staging of embryos, midday of the day of vaginal plug formation was considered as embryonic day 0.5 (E0.5). WT mice maintained in a C57BL/6 genetic background were used. We used heterozygous GAD67-GFP knock-in mice, described in this work as GAD67-GFP mice (Tamamaki et al., 2003). GAD67-GFP embryos could be recognized by their GFP fluorescence. PCR genotyping of these lines was performed as described previously (Niquille et al., 2009). We used Hcfc1lox/lox (Minocha et al., 2016b), Nkx2.1-Cre (Xu et al., 2008), and GLAST-Cre:ERT2 (The Jackson Laboratory, Bar Harbor, Maine, USA, Tg(Slc1a3-cre/ERT1Nat/J)) (Minocha et al., 2015b) transgenic mice described previously. The reporter Rosa26R–GFP mouse line was used to reliably express GFP under the control of the Rosa26 promoter upon Cre-mediated recombination. The control Nkx2.1-Cre/Rosa26-GFP+ and Hcfc1lox/lox/Rosa26-GFP+ did not show any GFP labeling. The control GLAST-Cre:ERT2+/Rosa26-GFP+ brains did not show any GFP labeling without tamoxifen treatment. For the induction of CreERT, tamoxifen (20 mg ml−1, Sigma, St Louis, MO) was dissolved at 37°C in 5 ml corn oil (Sigma) pre-heated at 42°C for 30 min. A single dose of 4 mg (250–300 μl) was administrated to pregnant females.

**Tissue Immunohistochemistry and Histology**

Embryos were collected after Caesarean section and quickly killed by decapitation. Their brains were dissected out and fixed by immersion overnight in a solution of 4% paraformaldehyde in 0.1 M of phosphate buffer (pH 7.4) at 4°C. Postnatal mice were profoundly anesthetized and perfused with the same fixative and their brains post-fixed for 4 h. Brains were cryoprotected in a solution of 30% sucrose in 0.1 M phosphate buffer (pH 7.4), frozen and cut in 50-μm-thick coronal sections for fluorescence immunostaining. For diamino benzidine (DAB) immunostaining, the brain tissues were paraffin-embedded and sectioned into 8 μm thick sections using a MICROM HM325 microtome. For each immunostaining, we made use of several mice (between three and six) for both control and mutant strains analyzed. The method for paraffin- and cryosections staining was as follows:

1. a DAB and fluorescence immunostaining:
2. The paraffin-embedded sections were first (i) deparaffinized in xylene, (ii) rehydrated through graded alcohol washes, and (iii) rinsed twice with PBS. For DAB immunostaining, endogenous peroxidase activity was quenched at this stage with 6% hydrogen peroxide in methanol for 10 min and rapidly washed once with H2O. Subsequently antigens were revealed by heating in a 750 W microwave oven until boiling for approximately 10 min in citrate buffer (10 mM, pH 6.0), allowed to slowly cool to 4°C, washed twice with PBS, and then blocked for 30 min with 2% normal goat serum (NGS) (Sigma-Aldrich, cat. # G9023) in PBS at room temperature (RT).
3. For fluorescence immunostaining, cryosections were (i) rinsed thrice with PBS, (ii) rinsed thrice with PBS containing 0.3% Triton X-100, and (iii) Developmental Neurobiology
blocked for 1 hr with 2% NGS in PBS solution with 0.3% TritonX-100 at RT.

After blocking, primary immunostaining was performed by the incubation of the slices with specific primary antibody (see below) diluted in 2% NGS overnight at 4°C followed by three washes with PBS.

For DAB immunostaining, the primary antibodies were detected by incubating the sections for 30 min with anti-mouse (Dako cat. # K4000) or anti-rabbit (Dako cat. # K4002) horseradish peroxidase (HRP) secondary antibody. Visualization was performed with DAB substrate (Dako cat. # K3468) before being counterstained with Mayer’s hematoxylin.

For fluorescence immunostaining, incubation with the appropriate secondary antibody (see below) was for 30 min in the dark at RT for paraffin sections and 90 min in the dark at RT for cryo-sections, followed by (i) three PBS washes, (ii) counterstaining with 4’,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, CAS # 28718-90-3), (iii) two PBS washes, and (iv) embedding with Mowiol mounting medium (Sigma-Aldrich, CAS # 9002-89-5).

The DAB-stained sections were subsequently imaged using an Axiolmager M1 microscope with AxioCam MRm monochrome and AxioCam MRc color cameras (Carl Zeiss AG, Oberkochen, Germany), or a Zeiss CLSM 710 spectral confocal laser scanning microscope. Images were processed using AxioVision 4.8.2 (Carl Zeiss AG, Oberkochen, Germany). Fluorescent immunostained sections were imaged using confocal microscopes (Zeiss LSM 510 or Zeiss LSM 710). Z-stacks were acquired for each coronal section in a multitrack mode avoiding crosstalk. All three-dimensional (3D) Z-stack reconstructions and image processing were performed using Imaris 8.2 (Bitplane Inc.) software. To create real 3D data sets, we used the mode “Surpass.” Figures were processed in Adobe Photoshop CS6, and schematic illustrations were produced using Adobe Illustrator CS6.

The primary antibodies used were: rat anti-Ki67 (1:60; eBioscience), anti-Ctip2 (1:500; Abcam), and anti-L1 (1:200; Chemicon) antibodies; rabbit anti-HCF-1 (1:1000, H12; (Wilson et al., 1993)), anti-Cux1 (1:200; Santacruz), anti-Nxx2.1 (1:2,000; Biopat), anti-GFAP (1:500; DAKO), anti-Calbindin (1:2500; Swant), and anti-Calretinin (1:2000; Swant) antibodies; chicken anti-GFP (1:500; Aves) antibody; and mouse anti-SatB2 (1:500; gift from V. Tarabykin) and anti-GFAP (1:500; Chemicon) antibodies.

The secondary antibodies used were: goat anti-rabbit Alexa 488 (1:400; Molecular Probes cat. # A11034), goat anti-mouse Alexa 568 (1:500; Molecular Probes cat. # A11091), goat anti-rabbit Alexa 568 (1:1000; Molecular Probes cat. # A21069), goat anti-mouse Alexa 488 (1:400; Molecular Probes cat. # A11029), donkey anti-mouse Alexa 594 (1:500; Molecular Probes cat. # A11005), and goat anti-mouse Alexa 655 (1:300; Molecular Probes cat. # A31575) antibodies.

b Colorations:

Nissl staining

Standard Nissl staining was performed on both deparaffinized and rehydrated paraffin sections and cryo-sections as described (Paul et al., 2008).

Hematoxylin and eosin staining

Standard hematoxylin and eosin staining were performed on deparaffinized and rehydrated lung sections (Fischer et al., 2008).

TUNEL Assay

Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) was performed on brain sections with the in situ cell death detection kit (Roche Applied Science, cat. # 11684795910), according to the manufacturer’s directions.

Immunoblotting

For immunoblotting, approximately 100 mg of brain tissue was homogenized in RIPA buffer (50 mM Tris-HCl ph 7.4, 150 mM NaCl, 1 mM EDTA, 0.2% sodium deoxycholate, 1 mM DTT, 1 mM PMSF, and 1% Triton X) containing protease inhibitors (Roche). Samples (10–20 μg) were boiled for 5 min before PAGE and transfer to nitrocellulose membrane. Membranes were blocked for 60 min with 5 ml of LI-COR blocking buffer, incubated with primary antibody in 50% LI-COR blocking buffer and 50% PBST (PBS containing 0.1% Tween 20) overnight at 4°C, washed three times and incubated with secondary antibody (dilution 1:10,000) for 30 min at RT. The membranes were washed three times and scanned with an Odyssey infrared imager (LI-COR).

The primary antibodies were as follows and used at the following dilutions: rabbit anti-HCF-1 (1:1000,
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H12, (Wilson et al., 1993)) and anti-actin (1:5000, Sigma-Aldrich) antibodies. The secondary antibodies used were: donkey anti-rabbit IRDye 680RD (1:10,000; LI-COR Inc. cat. # 926-68073), donkey anti-mouse IRDye 680RD (1:10,000; LI-COR Inc. cat. # 926-68072), donkey anti-rabbit IRDye 800CW (1:10,000; LI-COR Inc. cat. # 926-32213), and donkey anti-mouse IRDye 800CW (1:10,000; LI-COR Inc. cat. # 926-32212).

Quantitation and Statistical Analyses

For each analysis, all cells in a representative field of either 8 µm-thick paraffin sections or 50 µm cryosections were counted. Mutant embryos/pups were always compared with controls originating from the same litter. In each case, entire fields acquired at same magnification were quantitated. For all analyses, values from at least three independent experiments were first tested for normality and the variance of independent populations were tested for equality. Student’s t-test was performed using the R package (www.r-project.org). To show the degree of significance for quantitation included in the study, we added the number of asterisks based on the following standard P-value criteria: *** P < 0.001; ** P < 0.01; * P ≤ 0.05.

Atlas and Nomenclature

The neuroanatomical nomenclature is based on the “Atlas of the prenatal mouse brain” (Schambra et al., 1991).

RESULTS

Broad Hcfc1 Expression in Mouse Brain

Before investigating the effects of loss of Hcfc1 function in brain cells, we assayed its expression in the developing mouse brain. The human HCF1 and mouse Hcfc1 genes are highly expressed in actively dividing tissue culture cells, and in embryonic and placental tissues and in adult tissues (Wilson et al., 1995; Frattini et al., 1996; Christie, 1997; Huang et al., 2012; Minocha et al., 2016b). Previously, using a well-characterized antibody generating little to no non-specific reactivity (H12), we have shown that HCF-1 is ubiquitous and predominantly nuclear in E6.5-to-E12.5 embryos, postnatal day 0 (P0) brains, and 10-week-old young adult brains (Minocha et al., 2016b). Here, we further investigated in detail the cellular and subcellular localization of HCF-1 in the early mouse brain by immunostaining wild-type C57BL/6 postnatal day 0 (P0) brains. We focused on the cortex (Ctx), corpus callosum (CC), and anterior commissure (AC) — brain regions affected by the Nkx2.1-Cre-engineered...
loss of HCF-1 described here. In these three regions, HCF-1 was found to be ubiquitous (Fig. 1A–C) and predominantly nuclear in the GFAP-positive astroglia (Fig. 1D), NeuN-positive neurons (Fig. 1E), and Olig2-positive oligodendrocytes (Fig. 1F), with astroglial-cell processes showing additionally faint staining (Fig. 1D, see arrow). Immunoblotting analysis of postnatal and adult mouse brains ranging from P1 to 1.2 years old demonstrated continued expression of Hcfc1 into adulthood (Supp. Fig. 1A1) (Minocha et al., 2016b), but with a progressive reduction in relative HCF-1 protein levels with age (Supp. Fig. 1A2). Such a broad and long-term expression profile of Hcfc1 suggests that HCF-1 plays roles in both young and adult mouse brains.

Induced Loss of HCF-1 in Nkx2.1-Positive Cells in the Ventral Telencephalon

We wished to probe such roles for HCF-1 in brain development and decided to use Nkx2.1-Cre mediated Hcfc1-gene inactivation for this purpose. In the embryonic brain, the Nkx2.1 homeobox gene is expressed in a region of the forebrain that develops into the ventral telencephalon which includes the medial ganglionic eminence (MGE), the anterior entopeduncular area (AEP), the anterior preoptic area (POA), the septum (SEP) and parts of the amygdala (Lazzaro et al., 1991; Kimura et al., 1999; Sussel et al., 1999; Puelles et al., 2000; Flames et al., 2007). In the MGE, Nkx2.1 is expressed in the rapidly dividing ventricular (VZ) and sub-ventricular zones (SVZ) as well as the mantle zone comprised of migratory cells (Sussel et al., 1999), and is important for the generation of both neuronal (GABAergic interneurons) and glial (astroglia and oligodendrocytes) cell types (Sussel et al., 1999; Anderson et al., 2001; Corbin et al., 2001; Marin and Rubenstein, 2001; Kessaris et al., 2006; Minocha et al., 2015a; 2015b). These cells ensure proper cortical development and function, and commissure formation (Wonders and Anderson, 2006; Lindwall et al., 2007; Minocha et al., 2015b). Importantly, as shown in Figure 1, Hcfc1 is highly expressed in these cell types.

We induced conditional deletion of the Hcfc1 gene in these cell types by crossing males heterozygous for an Nkx2.1-Cre transgene (referred to as Nkx2.1-Cre[+]) (Xu et al., 2008) with Hcfc1lox/+ females (Minocha et al., 2016b). The X-linked Hcfc1lox allele contains two loxP sites, one in intron 1 and another in intron 3 that undergo recombination in the presence of Cre recombinase, deleting exons 2 and 3 to generate the conditional knockout (cKO) allele encoding a highly truncated 66 amino acid long N-terminal HCF-1 protein (Minocha et al., 2016b). Thus, hemizygous Hcfc1lox/+ males carrying the Nkx2.1-Cre[+] allele were expected to generate a complete embryonic Nkx2.1-specific knock out. The resulting male progeny of the aforementioned cross generated the control strain Nkx2.1-Cre[+]; Hcfc1lox/lox and the Nkx2.1-Cre-induced knockout strain Nkx2.1-Cre[+]; Hcfc1lox/+. The Nkx2.1-Cre[+]; Hcfc1lox/+ postnatal mice appeared to be ill, probably owing to improper lung formation (compare Supp. Fig. 2A to B and C; see [Lazzaro et al., 1991]), and often suffered from maternal cannibalism or died during early postnatal ages around P5.

To follow the generation of Nkx2.1-Cre-induced conditional knockout allele, we used a Cre-recombination inducible Rosa-GFP reporter, which can mark Nkx2.1-Cre-expressing cells and hence identify likely conditional knockout allele-containing cells. As expected, the control Rosa-GFP[+]; Hcfc1lox/+ embryos lacking the Nkx2.1-Cre allele did not show specific GFP labeling (Supp. Fig. 3A). As Nkx2.1 expression can be seen as early as embryonic (E) day 10.5 (Sussel et al., 1999), we began by investigating the generation of conditional knockout allele-containing cells in embryonic Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1lox/+ brains at E12.5 and E14.5. For clarity, here, the control strain is referred to as Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1+/Y and the Nkx2.1-Cre-induced knockout strain is referred to as Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1lox/+. Similar to the control Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1+/Y embryos, the Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1lox/+ embryos displayed temporally and spatially specific Nkx2.1-Cre-mediated recombination activity, demonstrated by the presence of GFP only in the Nkx2.1-derived zones such as the MGE and POA (Fig. 2A, B, I and J; Supp. Fig. 3B–C). Coherently, the GFP-positive cells were found to be HCF-1-positive in control Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1+/Y embryos and HCF-1-negative in Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1lox/+ embryos at E12.5 (compare Fig. 2C and E) and at E14.5 (compare Fig. 2K and M); compare also the single confocal layers shown in Figure 2O and P. The GFP-positive cells continued to be HCF-1-positive in control Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1+/Y embryos (Fig. 3A) and HCF-1-negative in Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1lox/+ embryos (Fig. 3B) after exiting the MGE region and migrating to the cortex. We note, however, that GFP-negative cells remained and that in Nkx2.1-Cre[+]; Rosa-GFP[+]; Hcfc1lox/+ embryos these were HCF-1 positive, indicating that the Nkx2.1-Cre transgene was not active in all cells (Supp. Fig. 3D). Nevertheless, the very high correspondence between GFP-positive and HCF-1-negative cells (Supp. Fig. 3E) — owing to the cell autonomous function of HCF-1 — allowed us to follow GFP fluorescence as a marker for HCF-1-negative cells.

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Figure 2 Nkx2.1-Cre mediated loss of HCF-1 leads to decreased generation of ventral telencephalic cells. Immunofluorescence analysis of cryo-sections from control Nkx2.1-Cre; Rosa-GFP; Hcfc1lox/lox (A, C, D, I, K, and O) and knockout Nkx2.1-Cre; Rosa-GFP; Hcfc1lox/lox (B, E, F, J, M, and P) embryonic brains at E12.5 (A-to-F) and E14.5 (I-to-P) stained with antibodies against HCF-1 (red) and GFP (green). The boxed region of medial ganglionic eminence (MGE) shown in A, B, I, and J is shown as higher magnification in C, E, K, and M, respectively. The HCF-1 (red) and GFP (green) staining in boxed region in A3, B3, I3, and J3 is shown at higher magnification in D, F, L, and N, respectively. A single layer from the confocal stack shown in K and M is shown in O and P, respectively. (G-H) Immunofluorescence analysis of cryo-sections from control Nkx2.1-Cre; Rosa-GFP; Hcfc1lox/lox (G) and knockout Nkx2.1-Cre; Rosa-GFP; Hcfc1lox/lox (H) embryonic brains at E12.5 stained with antibodies against HCF-1 (red) and proliferation marker, Ki67 (green). CP, cortical plate; IZ, intermediate zone; MZ, marginal zone; SVZ, sub-ventricular zone; VZ, ventricular zone. Scale bars are indicated in the figure.
Paucity of HCF-1 Negative Cells in the Developing Cortical Region

With the induction of loss of HCF-1 in the MGE, we noted that the number of GFP-positive (i.e., HCF-1-negative) cells in the developing cortical region appeared to be reduced in Nkx2.1-Cre<sup>+</sup>; Rosa26-GFP<sup>+</sup>; Hcfc1<sup>lox/+</sup> embryos compared to control Nkx2.1-Cre<sup>+</sup>; Rosa26-GFP<sup>+</sup>; Hcfc1<sup>+/+</sup> embryos, both at E12.5 (compare Fig. 2A to B; also see higher magnification in Fig. 2D and F) and E14.5 (compare Fig. 2I to J; also see higher magnification in Fig. 2L and N). This reduction in GFP-positive cells probably reflects a paucity of HCF-1-negative migratory cells in the developing cortex. Quantification of GFP-positive cells showed a 70% and 55% decrease at E12.5 and E14.5, respectively, in Nkx2.1-Cre<sup>+</sup>; Rosa26-GFP<sup>+</sup>; Hcfc1<sup>lox/+</sup> vs. control Nkx2.1-Cre<sup>+</sup>; Rosa26-GFP<sup>+</sup>; Hcfc1<sup>+/+</sup> embryos (Fig. 4A).

Increased Apoptosis in the Ventral Telencephalon after Induction of HCF-1 Loss

The decrease in proportion of GFP-positive cells in the developing cortex could be attributed to either a reduction in HCF-1 negative cells, either owing to defective proliferation or increased cell death, or a defect in migration. We addressed the former possibility here. To investigate a defect in cell proliferation, we performed immunostaining for the cell proliferation marker Ki67. At E12.5, no statistically significant difference in the percentage of Ki67-positive cells was found between the VZ of control Nkx2.1-Cre<sup>+</sup>; Rosa26-GFP<sup>+</sup>; Hcfc1<sup>lox/+</sup> and Nkx2.1-Cre<sup>+</sup>; Rosa26-GFP<sup>+</sup>; Hcfc1<sup>lox/+</sup> embryos (compare Fig. 2G to H; see quantitation Fig. 4B). Indeed, most HCF-1-negative cells were Ki67-positive consistent
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Figure 4 Nkx2.1-Cre mediated loss of HCF-1 causes decreased migration and increased cell death of ventral telencephalic cells. (A) Graph showing the relative number of GFP+ migratory cells outside MGE region in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> (n = 2 at E12.5; n = 2 at E14.5; shown as Hcfc1<sup>+/Y</sup>) and mutant Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> (n = 2 at E12.5; n = 3 at E14.5; shown as Hcfc1<sup>lox/Y</sup>) brains at E12.5 and E14.5. The number of GFP+ migratory cells outside MGE region in Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> control brains was calculated, and the mean value was set as 100%. The percentage of GFP+ migratory cells outside MGE region in Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> mutant brains was then calculated relative to the value in control sections. The difference between relative percentage of GFP+ migratory cells outside MGE region in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> brains at E12.5 was significant (P-value 0.05). The difference between relative percentage of GFP+ migratory cells outside MGE region in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> brains at E14.5 was significant (P-value 0.003). (B) Graph showing the number of Ki67+ cells in ventricular zone (VZ) of control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> (n = 3; shown as Hcfc1<sup>+/Y</sup>) and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> (n = 2; shown as Hcfc1<sup>lox/Y</sup>) brains at E12.5. The difference between number of Ki67+ cells in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> brains was not significant. (C) Graph showing the number of TUNEL+ cells in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> (n = 3 at E12.5; n = 2 at E14.5; shown as Hcfc1<sup>+/Y</sup>) and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> (n = 5 at E12.5; n = 3 at E14.5; shown as Hcfc1<sup>lox/Y</sup>) brains at E12.5 and E14.5. The difference between number of TUNEL+ cells in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> brains at E12.5 was significant (P-value 0.008). The difference between number of TUNEL+ cells in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> brains at E14.5 was significant (P-value 0.002). (D) Graph showing the relative number of cortical GFP+ cells in cortices of control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> (n = 3; shown as Hcfc1<sup>+/Y</sup>) and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> (n = 3; shown as Hcfc1<sup>lox/Y</sup>) brains at P0. The number of cortical GFP+ cells in Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> control brains was calculated, and the mean value was set as 100%. The percentage of cortical GFP+ cells in Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> mutant brains was then calculated relative to the value in control sections. The difference between relative percentage of cortical GFP+ cells in control Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>+/Y</sup></i></sup> and knockout Nkx2.1-Cre<sup>+</sup>; Rosa-GFP<sup><i>Hcfc1<sup>lox/Y</sup></i></sup> brains was significant (P-value 0.001).
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with active or recent proliferation. Furthermore, Ki67-positive cells appeared to be evenly distributed in the MGE of Nkx2.1-Cre+; Rosa26-GFP+; Hcfc1lox/Y embryos (Fig. 2H). Similar results were also observed at E14.5 (data not shown). Thus, although HCF-1 has been implicated in cell proliferation in vitro (Goto et al., 1997) and in liver regeneration (Minocha et al., 2016b), these results indicate that HCF-1 is not required for proliferation of MGE neuronal and glial progenitor cells in the developing embryonic mouse brain.

Next, we assayed for the possibility of increased cell death in the ventral telencephalon of Nkx2.1-Cre+; Rosa26-GFP+; Hcfc1lox/Y vs. control embryos. Indeed, TUNEL assays revealed a significantly increased number of apoptotic cells, specifically in the SVZ and the mantle zone, both at E12.5 (A-to-D) and E14.5 (E-to-H) co-stained with DAPI (blue) and antibody against Nestin (red). TUNEL-positive apoptotic cells are shown in green. The medial ganglionic eminence (MGE) region shown in A, B, E, and F is shown as higher magnification in C, D, G, and H, respectively. SVZ, sub-ventricular zone; VZ, ventricular zone. Scale bars are indicated in the figure.

Postnatal Brains Display a Severe Reduction of Interneurons and Glia Upon Induced HCF-1 Loss

To determine the effects of the aforementioned loss of Nkx2.1-lineage cells owing to the loss of HCF-1 on brain development, we turned to postnatal mice. Because, however, postnatal Nkx2.1-Cre+; Hcfc1lox/Y mice are ill, (Supp. Fig. 2), and because they suffered from maternal cannibalism, we studied brains from early postnatal (i.e., neonatal) P0 and P1 pups. Consistent with the loss of HCF-1-negative cells during embryonic development, as shown in Figure 6, throughout the brain there were many fewer (approximately 75% less) GFP-positive cells in knockout Nkx2.1-Cre+; Rosa26-GFP+; Hcfc1lox/Y vs. control Nkx2.1-Cre+; Rosa26-GFP+; Hcfc1+/Y brains. Regions affected included the entire cortical region (compare Fig. 6A to B; see quantitation Fig. 4D), the corpus

Figure 5 Hcfc1-conditional knockout embryonic brains display increased incidence of cell death. TUNEL assay was performed on cryo-sections from control Nkx2.1-Cre+; Rosa-GFP+; Hcfc1+/Y (A, C, E, and G) and knockout Nkx2.1-Cre+; Rosa-GFP+; Hcfc1lox/Y (B, D, F, and H) embryonic brains at E12.5 (A-to-D) and E14.5 (E-to-H) co-stained with DAPI (blue) and antibody against Nestin (red). TUNEL-positive apoptotic cells are shown in green. The medial ganglionic eminence (MGE) region shown in A, B, E, and F is shown as higher magnification in C, D, G, and H, respectively. SVZ, sub-ventricular zone; VZ, ventricular zone. Scale bars are indicated in the figure.
callosum (CC), striatum (Str), indusium griseum (IG), and septum (SEP) (compare Fig. 6C to D), and anterior commissure (AC) and surrounding areas including the anterior entopeduncular area (AEP) and anterior preoptic area (POA) (compare Fig. 6E to F). The panels show confocal images obtained by tiling and stitching nine adjacent regions, acquired with 10x objective, together to display larger areas. AEP, anterior endopeduncular area; IG, indusium griseum; LV, lateral ventricles, SEP, septum; Str, striatum; POA, anterior preoptic area. Scale bars are indicated in the figure.

Figure 6 Nkx2.1-Cre mediated loss of HCF-1 leads to decreased presence of Nkx2.1-derived cells in the postnatal brains. Immunofluorescence analysis of cryo-sections from control Nkx2.1-Cre\(^+\); Rosa-GFP\(^+\); Hcfc1\(^{+/+}\) (A, C, and E) and knockout Nkx2.1-Cre\(^+\); Rosa-GFP\(^+\); Hcfc1\(^{lox/lox}\) (B, D, and F) brains at P0 stained with DAPI (blue) and antibody against GFP (green). Both anti-GFP and DAPI staining (A2, B2, and C2) and only anti-GFP staining (A1, B1, and C1) is shown in cortex (Ctx; A and B), corpus callosum (CC) and surrounding region (C and D), and anterior commissure (AC) and surrounding region (E and F). The panels show confocal images obtained by tiling and stitching nine adjacent regions, acquired with 10x objective, together to display larger areas. AEP, anterior endopeduncular area; IG, indusium griseum; LV, lateral ventricles, SEP, septum; Str, striatum; POA, anterior preoptic area. Scale bars are indicated in the figure.
telencephalic region apparently fails to reach their target regions.

Secondly, to identify Nkx2.1-derived glia, which populate the corpus callosum and its surrounding regions (Minocha et al., 2017), we probed the astroglia and oligodendrocytes. We assayed the effect of loss of HCF-1 on Nkx2.1-derived astroglia by immunostaining against GFAP, a glial marker, in the Nkx2.1-Cre+; Hcfc1lox/lox male brains at P0 stained with DAPI (blue) and antibodies against GFAP (green) and HCF-1 (red). The boxed region in A1 and B1 is shown at higher magnification in A2 and B2, respectively. The two-sided arrow line in A2 and B2 depicts the thickness of the corpus callosum (CC). IG, indusium griseum; GW, glial wedge; MZG, midline zipper glia. Scale bars are indicated in the figure.

Figure 7 Nkx2.1-Cre mediated loss of HCF-1 leads to decreased presence of glial cells and reduced corpus callosum width in the postnatal brains. (A-B) Immunofluorescence analysis of cryo-sections from Nkx2.1-Cre+; Hcfc1lox/lox (A) and Nkx2.1-Cre+; Hcfc1lox/lox (B) male brains at P1 stained with DAPI (blue) and antibodies against GFAP (green) and HCF-1 (red). The boxed region in A1 and B1 is shown at higher magnification in A2 and B2, respectively. The two-sided arrow line in A2 and B2 depicts the thickness of the corpus callosum (CC). IG, indusium griseum; GW, glial wedge; MZG, midline zipper glia. Scale bars are indicated in the figure. (C) Graph showing the relative percentages of GFAP+ glia within the CC in control Nkx2.1-Cre+; Hcfc1lox/lox (n = 2) and knockout Nkx2.1-Cre+; Hcfc1lox/lox (n = 3) male brains at P1. The number of GFAP+ glia within the CC of Nkx2.1-Cre+; Hcfc1lox/lox control brains was calculated, and the mean value was set as 100%. The percentage of GFAP+ glia within the CC of Nkx2.1-Cre+; Hcfc1lox/lox mutant brains was then calculated relative to the value in control sections. The difference between relative percentages of GFAP+ glia within the CC in control Nkx2.1-Cre+; Hcfc1lox/lox and knockout Nkx2.1-Cre+; Hcfc1lox/lox male brains was significant (P-value 0.005). (D) Graph showing the relative percentages of Olig2+ cells within the CC in control Nkx2.1-Cre+; Hcfc1lox/lox (n = 3) and knockout Nkx2.1-Cre+; Hcfc1lox/lox (n = 3) male brains at P1. The number of Olig2+ cells within the CC of Nkx2.1-Cre+; Hcfc1lox/lox control brains was calculated, and the mean value was set as 100%. The percentage of Olig2+ cells within the CC of Nkx2.1-Cre+; Hcfc1lox/lox mutant brains was then calculated relative to the value in control sections. The difference between relative percentages of Olig2+ cells within the CC in control Nkx2.1-Cre+; Hcfc1lox/lox and knockout Nkx2.1-Cre+; Hcfc1lox/lox male brains was significant (P-value 0.047). (E) Graph showing the width of corpus callosum (CC) of control Nkx2.1-Cre+; Hcfc1lox/lox (n = 3) and knockout Nkx2.1-Cre+; Hcfc1lox/lox (n = 3) brains at P0. The difference between the width of corpus callosum (CC) of control Nkx2.1-Cre+; Hcfc1lox/lox and knockout Nkx2.1-Cre+; Hcfc1lox/lox brains was significant (P-value 0.007).
Hcfc1lox/Y brains. Indeed, as shown in Figure 7, a reduction in the number of GFAP-positive astroglia was seen in the corpus callosum (CC), indusium griseum (IG), midline zipper glia (MZG), and glial sling (GS) of the Nkx2.1-Cre\(^+\); Hcfc1lox/Y knockout brains when compared to control Nkx2.1-Cre\(^+\); Hcfc1lox/Y brains (compare Fig. 7A to B). Quantification revealed a loss of approximately 50% GFAP-positive glia within the anterior commissure region (Supp. Fig. 5E). Thus, these results also suggest a role of HCF-1 in brain astroglia development.

Defects Upon HCF-1 Loss in Nkx2.1-Lineage Cells

Loss of HCF-1 Causes Corpus Callosum and Anterior Commissure Defects

Here, we investigated the effects of the loss of Nkx2.1-lineage cells upon the absence of HCF-1 on brain development. In the analysis of the disappearance of Nkx2.1-lineage cells in Nkx2.1-Cre\(^+\); Rosa-GFP\(^+\); Hcfc1lox/Y brains shown in Figure 6, we noted a number of morphological defects, particularly commissural and cortical. We first investigated the commissural and then the cortical defects, and used in each case glial- and neuronal-specific immunofluorescence markers to follow the contribution of glia and neurons to the defective structures observed. To avoid a conflicting GFP fluorescence signal from the Rosa-GFP transgene, it was excluded from the mouse strains used in the experiments described below.

Relative to the commissures, we observed mild to severe midline defects. We noted a tendency toward the occurrence of similar trajectories in control Nkx2.1-Cre\(^+\); Hcfc1lox/Y knockout brains compared to Nkx2.1-Cre\(^+\); Hcfc1lox/Y knockout brains (Fig. 6A–D). Though anterior commissure axons were found near the midline in Nkx2.1-Cre\(^+\); Hcfc1lox/Y knockout brains, only some axons successfully crossed the midline while most seemed to be stalled (compare Fig. 8A and C to B and D). Furthermore, the anterior commissure axons were loosely bundled and adopted abnormal trajectories in Nkx2.1-Cre\(^+\); Hcfc1lox/Y knockout brains compared to the tightly bundled axons of the anterior commissure in control Nkx2.1-Cre\(^+\); Hcfc1lox/Y brains (compare Fig. 8C to D). These defects may be explained by the greatly reduced and disorganized status of Nkx2.1-lineage cells in the anterior commissure and its surrounding regions seen in the Nkx2.1-Cre\(^+\); Rosa-GFP\(^+\); Hcfc1lox/Y knockout versus normal Nkx2.1-Cre\(^+\); Rosa-GFP\(^+\); Hcfc1\(^+/Y\) brains (compare Fig. 6E to F).

As the Nkx2.1-lineage population comprises both neurons and glia, we assayed the organization of these two populations in the anterior commissure region of Nkx2.1-Cre\(^+\); Hcfc1lox/Y knockout brains. The GABAergic interneurons (compare Supp. Fig. 4E to F) and the GFAP-positive glia (compare Fig. 8E to F) were both disorganized in the anterior commissure of Nkx2.1-Cre\(^+\); Gad1-GFP\(^+\); Hcfc1lox/Y knockout brains and its surrounding regions compared to the control Nkx2.1-Cre\(^+\); Gad1-GFP\(^+\); Hcfc1\(^+/Y\) brains.

Hence, loss of HCF-1 in Nkx2.1-lineage cells affects their survival and as a result midline commissures, particularly the anterior commissure, are malformed in mutant brains.

Early Loss of HCF-1 Causes Cortical Defects

Nkx2.1 plays a key role in the specification and production of GABAergic interneurons and glia from the ventral telencephalon that migrate into the striatum and cerebral cortex (Sussel et al., 1999; Anderson et al., 2001; Corbin et al., 2001; Marin and Rubenstein, 2001;
To elucidate the effect on cortical development of loss of migratory neurons and glia upon Hcfc1-gene disruption in Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) knockou brains, we analyzed P0-to-P1 mice. Although less frequent than the commissure defects, cortical defects were still common. Such defects are clearly visible in both hemispheres in the Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) knockout brains, we analyzed P0-to-P1 mice. Although less frequent than the commissure defects, cortical defects were still common. Such defects are clearly visible in both hemispheres in the Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) knockout brains, we analyzed P0-to-P1 mice.

Although less frequent than the commissure defects, cortical defects were still common. Such defects are clearly visible in both hemispheres in the Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) knockout brain serial sectioning shown in Supplemental Figure 6 (black arrows). Indeed, we did not observe a preference for defects in one or the other hemisphere. Eleven of 19 analyzed Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) knockout brains exhibited severe cortical defects in at least one hemisphere (see Fig. 9B and D compared to A and C) whereas the rest exhibited milder cortical defects (see Fig. 9F compared to E) and yet the cortices were thinner when compared to control embryos (Fig. 9K). In comparison to the unaltered six-layered cortex of control Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) brains (Fig. 9G and I), the laminar organization of the Nkx2.1-Cre\(^+\); Hcfc1\(^{lox/}\) knockout brain cortices (Fig. 9H and J) was disturbed, perhaps missing layers.

To probe for layering defects, we performed immunostaining for the cortical markers listed in Figure 10. We used markers Reelin (Fig. 10A,B) and Calretinin (Fig. 10C,D) for layer I; Calretinin (Fig. 10C,D); Parvalbumin (Fig. 10E,F); Cux1 (Fig. 10G,H); SatB2 (Fig. 10I,J); and Calbindin (Fig. 10K,L) for layers II-III; Parvalbumin (Fig. 10E,F) and Cux1 (Fig. 10G,H) for layer IV; Parvalbumin (Fig. 10E,F),

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Calbindin (Fig. 10K,L) and Ctip2 (Fig. 10M,N) for layer V; and Parvalbumin (Fig. 10E,F) and Tbr1 (Fig. 10O,P) for layer VI. From the cortical layer analysis and NISSL staining (Fig. 9), it appeared that layer I though deformed is present, whereas layers II, III, and V though present are relatively thinner in Nkx2.1-Cre+; Hcfc1lox/Y knockout brains when compared to control brains. Interestingly, it appeared that the layer IV and layer VI are either much thinner or absent.

Although Nkx2.1-Cre+; Hcfc1lox/Y knockout brains exhibit these malformations, the cells within the cortex are essentially all HCF-1 positive and are proliferating as found in normal brains (Supp. Fig. 7). Thus, it is likely that it is the absent Nkx2.1-lineage cells that are causing the cortical aberrations. These aberrations resemble those that occur in asymmetric polymicrogyria, which are typically characterized by the presence of irregular cortical folds and a reduced number of cortical layers (Barkovich, 2010; Guerrini and Parrini, 2010). Such malformations could be one way by which human patients carrying HCFC1 mutations display intellectual disability.

DISCUSSION

We have shown that conditional loss of HCF-1 in Nkx2.1-derived neurons and glia leads to commissural defects affecting primarily the AC as well as asymmetric polymicrogyria-like cortical defects. These defects appear to arise because of a reduced number of embryonic Nkx2.1-derived neurons and glia owing to their increased cell death. This reduced presence of Nkx2.1-derived cells leads to formation of severely malformed AC whose axons deviate from the normal path, being deflected both dorsally and ventrally. Also, the laminar organization of the cortex is locally disturbed generating irregular folds where cortical layers are reduced in number and thickness.
The Activity of HCF-1 Differs Depending on Cell Context

HCF-1 is a transcriptional co-regulator and is important for several aspects of the cell cycle in tissue culture cells and during liver regeneration (Goto et al., 1997; Reilly and Herr, 2002; Julien and Herr, 2003; Minocha et al., 2016a; 2016b). Interestingly, loss of HCF-1 does not seem to affect the proliferation capacity of Nkx2.1-positive precursor cells in the ventral telencephalic region. These results are consistent with a previous report where knockdown of HCF-1 was shown to display increased proliferation of neural precursor cells in an in vitro neurosphere assay (Jolly et al., 2015). Our results show that, instead of inhibiting cell proliferation, loss of HCF-1 leads to increased cell death of Nkx2.1-derived post-mitotic cells (GABAergic interneurons and glia) in the ventral telencephalic region. These results demonstrate that the role of HCF-1, likely as a transcriptional regulator of many genes, can differ depending on the cell context. This finding of differing roles of HCF-1 in different cell contexts complements that in the context of resting adult mouse hepatocytes where loss of HCF-1 leads to hepatocyte malfunction (Minocha et al., 2018). HCF-1 appears to have evolved to play a multitude of cell-specific roles in the regulation of gene expression, probably principally gene transcription but also through protein stabilization as in the case of PGC1α in hepatocytes (Ruan et al., 2012; Minocha et al., 2018). In this manner, it serves as a broad and versatile potentiator of cell function.

Effects of HCF-1 Loss in Nkx2.1-lineage Cells on Embryonic Brain Development

Nkx2.1-derived cells (GABAergic interneurons and glia) have the striking capacity to migrate to numerous areas of the brain including the commissures and cortex after their initial formation primarily in the MGE and POA. Here, owing to their death, Nkx2.1-derived cells cannot migrate apparently causing Nkx2.1-Cre+; Hcfc1lox/Y knockout mice to display the numerous diverse brain defects observed.

These results are consistent with previous reports where it has been shown that polymicrogyria primarily develops due to either reduced proliferation of neural precursors, disturbed neuronal migration or aberrant...
cortical organization (Guerini and Filippi, 2005; Guerini and Parrini, 2010). A role of GABAergic interneurons in cortical development has also been shown in Arx-deficient mice that recapitulate features of cortical malformation called as X-linked lissencephaly with absent corpus callosum and Ambiguous Genitalia (XLAG) in humans (Bonneau et al., 2002; Kitamura et al., 2002). XLAG is characterized by agenesis of CC, poorly laminated cortex, microcephaly, and epilepsy (Bonneau et al., 2002). Together, these results all point to the importance of GABAergic interneurons in cortical development.

Cases of polymicrogyria are known to occur sporadically, though several families have also been observed with loci mapping to the X chromosome, including Xq28 (Geerdink et al., 2002; Villard et al., 2002; Barkovich, 2010). In humans, HCFC1 resides on Xq28 (Frattini et al., 1994; Wilson et al., 1995) and has been strongly implicated in development of intellectual disability (Huang et al., 2012; Yu et al., 2013; Jolly et al., 2015). Intellectual disability is often a clinical manifestation of cortical malformations such as polymicrogyria. HCF-1 is essential for survival of Nkx2.1-lineage cells, GABAergic interneurons and glia, whose absence in turn causes cortical defects strongly resembling polymicrogyria. We suggest that these cortical malformations observed upon loss of HCF-1 in subpopulations of GABAergic interneurons and glia may well also be present in human intellectual disability patients carrying HCFC1 mutations.

Uncovering a New Role of Nkx2.1-Lineage Cells in Polymicrogyria

The effect of Nkx2.1-Cre-induced loss of HCF-1 on anterior commissure formation closely mimics those observed upon ablation of Nkx2.1-derived cells, both neurons and glia, with the help of diphtheria toxin (Minocha et al., 2015b). But, interestingly, ablation of Nkx2.1-derived cells did not show any cortical aberrations (Minocha et al., 2015b), such as those visible in brains lacking HCF-1 in Nkx2.1-derived cells. These previous studies involving ablation of Nkx2.1-derived cells failed to observe an effect on cortical development apparently due to the delayed accumulation of diphtheria toxin where precursors are not affected and also because embryos died before birth probably owing to an effect on lung development. In our study, as we were able to analyze the brains of neonatal pups lacking HCF-1 in Nkx2.1-derived cells, we could observe the cortical aberrations. Hence, our study uncovers a new role of Nkx2.1-derived cells during cortical development.

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AUTHOR CONTRIBUTIONS

The experiments were conceived and designed by S.M. and W.H. The experiments were performed by S.M. S.M. and W.H. analyzed the data and prepared the manuscript. Both authors participated in the discussion of the data and in production of the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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