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1 Autism-linked CHD gene expression patterns during
2 development predict multi-organ disease phenotypes

3
4 Sahrunizam Kasah*, Christopher Oddy* & M. Albert Basson^

5 Centre for Craniofacial and Regenerative Biology, King's College London, Floor 27,
6 Guy's Hospital Tower Wing, London SE1 9RT, UK and MRC Centre for
7 Neurodevelopmental Disorders, King's College London, London SE1 1UL, UK.

8
9 *Joint first authors

10 ^Correspondence:

11 Dr. M.A. Basson

12 Centre for Craniofacial and Regenerative Biology King's College London

13 Floor 27, Guy's Hospital Tower Wing

14 London SE1 9RT

15 UK

16 Tel: +44-207 188 1804

17 email: albert.basson@kcl.ac.uk

18
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20 organogenesis

21 Short title: CHD gene expression during mouse development

1 **Abstract**

2 Recent large-scale exome sequencing studies have identified mutations in several
3 members of the CHD (Chromodomain Helicase DNA-binding protein) gene family in
4 neurodevelopmental disorders. Mutations in the *CHD2* gene have been linked to
5 developmental delay, intellectual disability, autism and seizures, *CHD8* mutations to
6 autism and intellectual disability, whereas haploinsufficiency of *CHD7* is associated
7 with executive dysfunction and intellectual disability. In addition to these
8 neurodevelopmental features, a wide range of other developmental defects are
9 associated with mutants of these genes, especially with regards to *CHD7*
10 haploinsufficiency, which is the primary cause of CHARGE syndrome. Whilst the
11 developmental expression of *CHD7* has been reported previously, limited information
12 on the expression of *CHD2* and *CHD8* during development is available. Here we compare
13 the expression patterns of all three genes during mouse development directly. We find
14 high, widespread expression of these genes at early stages of development that gradually
15 becomes restricted during later developmental stages. *Chd2* and *Chd8* are widely
16 expressed in the developing central nervous system (CNS) at all stages of development,
17 with moderate expression remaining in the neocortex, hippocampus, olfactory bulb and
18 cerebellum of the postnatal brain. Similarly, *Chd7* expression is seen throughout the
19 CNS during late embryogenesis and early postnatal development, with strong
20 enrichment in the cerebellum, but displays low expression in the cortex and neurogenic
21 niches in early life. In addition to expression in the brain, novel sites of *Chd2* and *Chd8*
22 expression are reported throughout the developing mouse. These findings suggest
23 additional roles for these genes in organogenesis and predict that mutation of these
24 genes may predispose individuals to a range of other, non-neurological developmental
25 defects.

26

1 **Introduction**

2

3 Chromatin remodelling factors have emerged as key regulators of gene expression and
4 are often mutated in human disease (Iwase et al, 2018; Hendrich and Bickmore, 2001;
5 Ronan, Wu & Crabtree et al, 2013). Mammalian chromatin remodelling factors can be
6 subdivided into four families: SWI/SNF (mating type Switching/Sucrose Non-
7 Fermenting), ISWI (Imitation Switch), INO80 (Inositol requiring 80) and CHD
8 (Chromodomain Helicase DNA-binding protein) (Ho and Crabtree, 2010).

9

10 The CHD gene family consists of nine genes (*CHD1-CHD9*). The encoded proteins
11 utilise the energy from ATP hydrolysis to alter nucleosome positioning, thereby causing
12 local changes in the structure of the chromatin (Marfella & Imbalzano, 2007). CHD1
13 and CHD2, which belong to CHD1-2 subfamily, are characterised by the presence of
14 tandem chromodomains and a Snf2 helicase domain – both motifs common to all CHD
15 proteins – in addition to DNA-binding domains at the C-terminus (Marfella &
16 Imbalzano, 2007; Liu, Ferreria & Yusufzai, 2015). CHD3 and CHD4 are structurally
17 similar but each contain a PHD (Plant Homeo Domain) Zn-finger-like domain rather
18 than a DNA binding region, forming the second subfamily (Marfella & Imbalzano,
19 2007). Alongside signature sequence motifs of the CHD family, members of the CHD5-
20 9 subfamily contain a DNA binding region alongside various other C-terminal sequences
21 that alter their function (Marfella & Imbalzano, 2007). The present study focuses on the
22 spatiotemporal pattern of expression of CHD2, CHD7 and CHD8.

23

24 The ATP-dependent activity of CHD2 leads to assembly of chromatin into periodic
25 nucleosome arrays by deposition of various histone proteins, thereby modifying the
26 expression and structure of target sites (Liu, Ferreria & Yusufzai, 2015; Luijsterburg et
27 al., 2016). Functionally, CHD2 has been reported to maintain pluripotency of stem cells,
28 influence cell fate during myogenesis and interneuron development and facilitate DNA
29 repair through interaction with histone variant H3.3 (Harada et al, 2012; Luijsterburg
30 et al., 2016; Meganathan et al, 2017; Rajagopalan, Nepa & Venkatachalam et al., 2012;
31 Semba et al, 2017).

1 De novo loss-of-function mutations in *CHD2* have been reported in Autism Spectrum
2 Disorder (ASD) patients alongside developmental delay, intellectual disability,
3 increased risk of epileptic seizures and additional behavioural problems (Allen et al,
4 2013; Chérnier et al, 2014; Lebrun et al, 2017; O’Roak et al, 2014; Pinto et al, 2016).
5 The association between *CHD2* haploinsufficiency and epileptic encephalopathy, or
6 Lennox-Gastaut or Dravet syndrome, is also well-established and variants of *CHD2* are
7 recognised risk factors for photosensitivity in epilepsy (Carvill et al, 2013; Galizia et
8 al, 2015; Lund et al, 2014; Suls et al, 2013). *CHD2* mutations are commonly identified
9 in patients with chronic lymphocytic leukaemia, frequently in conjuncture with
10 alterations in functional pathways associated with brain development (Rodríguez et al,
11 2015).

12

13 Homozygous *Chd2* mutant mice die around birth due to unknown causes (Marfella et al,
14 2006). Heterozygous mice exhibited reduced growth and viability and range of
15 phenotypic abnormalities which include extramedullary haematopoiesis, susceptibility
16 to lymphomas, cardiomyopathy, liver inflammation, glomerulopathy and various other
17 renal defects (Marfella et al, 2006; Marfella et al, 2008; Nagarajan et al, 2009;
18 Rajagopalan, Nepa & Venkatachalam et al., 2012). More recently *Chd2* knockdown has
19 been demonstrated to decrease Pax6⁺ radial glial cell numbers, a cell type in which it is
20 highly expressed, and to promote neuronal and intermediate progenitor production,
21 implying an important balancing role for CHD2 in progenitor renewal and cortical
22 development (Shen et al, 2015). At present limited expression data for *Chd2* is available.
23 Quantitative analyses of *Chd2* in the adult mouse demonstrate that *Chd2* is widely
24 expressed by a multitude of functional tissue groups including the heart, brain, lungs,
25 thymus, lymphoid tissue and skeletal muscle (Marfella et al, 2006; Nagarajan et al,
26 2009). Macroscopic analysis of whole embryos stained for *Chd2* showed expression in
27 the developing heart, forebrain, eye, dorsal facial region and limbs between E10.5 and
28 E15.5 (Kulkarni et al, 2008). These data show that *Chd2* expression is apparent in many
29 tissues during development and in the adult mouse although a true spatiotemporal
30 pattern of expression is yet to be defined.

31

1 CHD7 is thought to maintain an open chromatin conformation at putative regulatory
2 elements (Feng et al, 2017; Whittaker et al, 2017b). CHD7 facilitates neural stem cell
3 (NSC) multipotency in the developing brain and quiescence in the adult as both
4 differentiation potential and stem cell depletion rates are correlated with the levels of
5 CHD7 (Feng et al, 2015; Fujita, Ogawa & Ito, 2016; Jones et al, 2015; Yamamoto et al,
6 2018). As well as maintaining multipotency, CHD7 has also been shown to directly
7 control lineage identity in NSCs through coordination of transcription factors in the
8 neural crest (Chai et al, 2018). In a similar vein, CHD7 is required for the formation of
9 migratory neural crest cells and, accordingly, induced pluripotent stem cells (iPSCs)
10 derived from patients with CHD7 mutations exhibit defective delamination, migration
11 and motility (Bajpai et al, 2010; Prasad et al, 2012; Okuno et al, 2017). Finally, CHD7
12 has been shown to have multiple roles in cerebellar development; consistent with the
13 observation that individuals harbouring CHD7 mutations may exhibit vermis hypoplasia
14 (Yu et al, 2013; Whittaker et al, 2017a; Whittaker et al, 2017b; Donovan et al, 2017).

15

16 Haploinsufficiency of the *CHD7* gene is the major cause of CHARGE syndrome
17 (Coloboma of the eye, Hear defects, Atresia of the choanae, Retardation of growth
18 and/or development, Genitalia and/or urinary abnormalities and Ear abnormalities and
19 deafness) and mutations have also been reported in patients with Kallmann syndrome
20 (Jongmans et al, 2009; Kim et al, 2008). Some of the *CHD7* mutations in patients with
21 CHARGE syndrome have been shown to result in defective nucleosome remodelling
22 activity in-vitro, directly linking chromatin remodelling defects with disease
23 (Bouazoune and Kingston, 2012). *Chd7*^{-/-} embryos do not survive beyond E11,
24 indicating early requirements for this gene during embryonic development, whereas
25 heterozygotes exhibit features similar to those associated with CHARGE syndrome
26 (Bosman et al, 2005; Hurd et al, 2007). Akin to *Chd2*, *Chd7* expression during
27 development is not limited to one tissue type. *Chd7* has been shown to be expressed in
28 the developing eye, inner ear, olfactory epithelium, dorsal root ganglia, lung, kidneys,
29 gut and throughout the neural ectoderm, including the neural crest (Aramaki et al, 2007;
30 Bosman et al, 2005; Engelen et al, 2011; Fujita et al, 2014; Fujita Ogawa & Ito, 2016;
31 Gage, Hurd & Martin, 2015; Hurd et al, 2007). More recently, preserved expression of
32 *Chd7* has been seen in the adult cerebellum (Whittaker et al, 2017a).

1

2 In vitro evidence has suggested a central role of CHD8 in transcription and
3 transcriptional elongation (Nishiyama et al, 2009; Rodriguez-Paredes et al, 2009; Yates
4 et al, 2010; Yuan et al, 2007). CHD8S, a partial N-terminal fragment of CHD8, also
5 referred to as Duplin, acts as a regulator of β -catenin mediated transcription – largely
6 causing transcriptional repression (Durak et al, 2016; Kobayashi et al, 2002; Nishiyama,
7 2004; Nishiyama et al, 2012; Platt et al, 2017; Sakamoto et al, 2000; Thompson et al,
8 2008).

9

10 Recurrent de novo mutations in *CHD8* have been linked to ASD. A significant body of
11 literature, including case reports and large exome sequencing studies, have identified
12 *CHD8* mutations in individuals with ASD (Bernier et al, 2014; Neale et al, 2012; Merner
13 et al, 2016; O' Roak et al, 2012; Sanders et al, 2012; Stolerman et al, 2016; Talkowski
14 et al, 2012; Wang et al, 2016; Wilkinson et al, 2015; Zahir et al, 2007). It is one of the
15 highest confidence risk genes for autism identified to date. ASD is highly heterogeneous
16 but can be identified by a repertoire of behavioural features in patients: social
17 impairment, communication impairment, repetitive behaviours and sometimes
18 accompanied by an array of other conditions such as epilepsy, dyslexia, dyspraxia and
19 attention deficit hyperactivity disorder (ADHD) (Brieber et al, 2007; Canitano, 2007;
20 Dziuk et al, 2007; Helbig et al, 2009; Leyfer et al, 2006; Taurines et al, 2012). The
21 effects of *CHD8* mutation may also manifest as characteristic physical features
22 including macrocephaly, facial dysmorphism and gastrointestinal disturbance, perhaps
23 defining *CHD8*-related ASD as a distinct subtype (Bernier et al, 2014).

24

25 CHD8 is recruited to promoters of highly expressed genes in NSCs and reduced
26 expression of CHD8 in mouse and human cells has been shown to precipitate
27 dysregulation of ASD related genes and alter cortical neurogenesis (Cotney et al, 2015;
28 Durak et al, 2016; Sugathan et al, 2014; Wang et al, 2015; Wilkinsion et al, 2015). In
29 *Chd8*^{+/-} mice behavioural changes have been documented alongside characteristic
30 neurodevelopmental changes pertaining to altered neurogenesis and long-range
31 connectivity, brain overgrowth and craniofacial anomalies (Gompers et al, 2017;

1 Katayama et al, 2016; Platt et al, 2017; Suetterlin et al, 2018). *Chd8*^{-/-} embryos die by
2 E7.5 of development. The early embryonic lethality associated with CHD8 loss has been
3 proposed to be caused by aberrant p53-mediated apoptosis as a consequence of loss of
4 CHD8-mediated repression of p53 target genes (Nishiyama et al, 2004). As the mutants
5 do not survive, the developmental roles after E7.5 are not known. The expression pattern
6 of *Chd8* has been described between E7.5 and E10.5 in the mouse using a CHD8s/Duplin
7 antisense riboprobe (Nishiyama et al, 2004). Whole embryo analysis showed expression
8 predominantly in the brain, face and limb buds. Since, microarray data has been used to
9 quantify the level of *Chd8* expression in developing mouse, macaque and human brains.
10 A regional expression heatmap showed widespread expression, highest in the early pre-
11 natal period (Bernier et al, 2014). Platt et al (2017) demonstrated a similar temporal
12 pattern of quantitative expression in the mouse brain and further showed that *Chd8* is
13 expressed in almost all neuronal populations. Despite these insights, no study to date
14 has characterised the macroscopic expression pattern of *Chd8* in all tissues of the
15 developing mouse from mid-gestation and through early life. Given the strong
16 association of CHD8 mutations with ASD and other physical abnormalities, determining
17 a comprehensive spatiotemporal expression pattern of CHD8 during development is of
18 great interest.

19

20 In the present study, we investigated the spatiotemporal patterns of three CHD genes
21 with strong evidence for important functions in brain development and
22 neurodevelopmental disorders. The expression pattern of *Chd8* was compared with *Chd7*
23 and *Chd2*. As these genes tend to be widely expressed during early development, we
24 focused on later embryonic stages to identify novel expression sites during
25 organogenesis. We report novel expression sites for all three genes during development,
26 with examples of overlapping, complementary and distinct expression patterns.

27

28

1 **Material and Methods**

2

3 **Animals**

4 Timed-mated CD1 embryos and pups were produced in our in-house facility. Noon on
5 the day a vaginal plug was detected was designated as embryonic day 0.5 (E0.5). The
6 day of birth was designated as postnatal day (P)0. All experimental procedures were
7 approved by the institutional Local Ethical Review Panel and the UK Home Office.

8

9 **Primer design and probe synthesis**

10 Primers were designed to amplify a 455 bp fragment of exon 37 of *Chd8* from mouse
11 genomic DNA: forward 5'-TCTCTGCCTTTTATGCCGTTTG-3'; reverse 5'-
12 CACCTCCTGAAGTCTTGGGTTTC-3' with T7 recognition sequence added to the
13 reverse primers in a PCR reaction. The resulting DNA template was used for the
14 synthesis of digoxigenin (DIG)-labelled antisense or sense mRNA probes. A *Chd7* probe
15 template was made with primer pairs that amplify a 222 bp fragment of *Chd7* exon 3
16 from mouse genomic DNA: forward 5'-TTGGTAAAGATGACTTCCCTGGTG-3';
17 reverse 5'-GTTTTGGCGTGACAGTTTTTGC-3'. A *Chd2* 625bp probe template was
18 amplified from mouse brain cDNA using primer pairs: forward 5'-
19 AGAAGAGCGTCCTCACAAAGACTG-3'; reverse 5'-
20 TTTTTCCTCAGGGTCCACAGG-3'.

21

22 **Sample preparation**

23 Embryos and brains were dissected in ice-cold diethylpyrocarbonate-treated phosphate
24 buffered saline (DEPC PBS) and fixed in 4% paraformaldehyde (PFA) overnight. After
25 several washes in DEPC PBS, embryos or brains were placed in cassettes immersed in
26 70% ethanol. The samples were processed in a Leica ASP300 tissue processor following
27 a standard protocol. The processed samples were embedded in wax, sectioned sagittally
28 at 10 µm using a Leica RM2145 microtome, placed on Superfrost Plus slides and left to
29 dry at 42°C for 48 hours.

1 **In situ hybridisation**

2 E12.5, E14.5, P0, P7 and P20 sagittal sections on slides were deparaffinised in Xylene
3 and rehydrated in decreasing series of ethanol concentrations. This was followed by
4 DEPC PBS washes. Proteinase K (50 µg/ml in DEPC PBS) was added and sections were
5 incubated for 10 minutes at 37 °C.

6

7 The slides were then washed in DEPC PBS, refixed in 4% PFA for 10 minutes and
8 washed again in DEPC PBS. Sections were acetylated (acetic anhydride, 0.1M
9 Triethanolamine, DEPC water at pH 7.5) for 10 minutes after which they were again
10 washed in DEPC PBS thrice. Sections were dehydrated in 70% ethanol (5 minutes) and
11 95% ethanol (a few seconds) and left to air dry for a few minutes. 300 µl probe-
12 hybridisation mix (2 µl of probe per ml hybridisation solution) (50% Dextran Sulfate,
13 50% Formamide, 1% Denhardt's solution, 0.3M NaCl (sodium chloride), 20mM Tris-HCl
14 (pH8), 10mM NaPO₄ (sodium phosphate), 5mM EDTA (Ethylenediaminetetraacetic
15 acid), 250µg/ml Yeast tRNA, 1% sarcosyl, sterile water) pre-heated to 80°C were added
16 to each slide and covered with parafilm. The slides were then arranged in a humid
17 chamber (50% formamide/water) and incubated overnight at 65°C.

18

19 The following day the slides were washed in high stringency (HIS) (formamide, 0.1%
20 SSC (saline-sodium citrate), sterile distilled water) wash for 30 minutes at 65°C
21 followed by RNase buffer (0.5M NaCl, 10mM Tris-HCl pH 7.5, 5mM EDTA, distilled
22 water) at 37 °C for 10 minutes (3x). Slides were treated with RNase buffer with 20
23 µg/ml RNase A at 37°C for 30 minutes followed by a single wash in RNase buffer at
24 37°C for 15 minutes. The slides were again washed twice in HIS at 65°C for 20 minutes
25 each. 2x SSC and 0.1x SSC washes for 15 minutes were performed twice followed by
26 PBT (PBS, 0.1% Tween 20) washes at room temperature. Sections were blocked with
27 10% heat inactivated goat serum in PBT for one hour at room temperature before a 3-
28 hour incubation in alkaline phosphatase coupled with anti-dioxigenin antibody (1:500
29 dilution, Roche) and 1% heat-inactivated goat serum in PBT. At the end of incubation,
30 slides were washed four times with PBT for 15 minutes each at room temperature
31 followed by freshly prepared NTMT buffer (5M NaCl, 1M Tris-HCl at pH 9.5, 1M

1 MgCl₂, 0.1% Tween-20, sterile distilled water and 0.5 mg/ml levamisole) twice at room
2 temperature. Finally, the slides were incubated in darkness in BM purple (Roche) and
3 0.5 mg/ml levamisole at room temperature overnight.

4

5 When signal appeared on sections, the reaction was stopped by washing in PBS at room
6 temperature for 5 minutes. Slides were dehydrated with an increasing series of ethanol
7 washes followed by Xylene before being mounted with Di-N-Butyle Phthalate in Xylene
8 (DPX) and left to air dry.

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1 **Results**

2

3 ***Chd2*, *Chd7* and *Chd8* gene expression in mouse embryos at E12.5 and** 4 **E14.5.**

5 At E12.5 *Chd7* and *Chd8* expression was apparent throughout the neuroepithelium of
6 the developing central nervous system (CNS) (Figure 1A, B). *Chd8* transcript signals
7 were observed throughout the ventricular and subventricular regions of the neocortex
8 and in the hindbrain (Figure 1Aa, Ab), including the cerebellum where expression was
9 evident in the ventricular zone (VZ), rhombic lip (RL) and the isthmus (Figure 1Aa).
10 Both VZ and RL are germinal centres where progenitor cells are born that later migrate
11 and populate the cerebellum (White and Sillitoe, 2012). Notably, *Chd8* expression could
12 also be observed at the lower rhombic lip and floor plate region of the hindbrain,
13 extending to the spinal cord and dorsal root ganglia (Figure 1A, Supplementary Figure
14 1A). *Chd8* expression can be observed throughout the neural tube with no evident
15 mediolateral nor dorsoventral gradient (Supplementary Figure 1A-D). Other regions of
16 interest showing high *Chd8* expression included the diencephalon and areas adjacent to
17 the hypothalamus and pituitary gland (Figure 1A). *Chd8* expression was observed
18 throughout the craniofacial region including the tongue and olfactory epithelium (Figure
19 1A). Elsewhere, other organs of the embryo also showed substantial *Chd8* expression
20 with signals present in the intersomitic regions, lungs, gut, genital tubercle and tail
21 (Figure 1A).

22

23 As with *Chd8*, *Chd7* mRNA transcripts were observed throughout the embryo (Figure
24 1B). Expression was found in the ventricular region of the developing brain and spinal
25 cord. *Chd7* mRNA transcripts were present in both the ventricular and subventricular
26 zones of the neocortex (Figure 1Bb). In the hindbrain, *Chd7* was expressed in all
27 regions including the upper rhombic lip of the cerebellum, the lower rhombic lip and
28 floor plate (Figure 1Ba). *Chd7* expression was also observed in the diencephalon and
29 the pituitary (Figure 1B). Within the neural tube expression was present in both cranial
30 and caudal poles (Supplementary Figure 1E, F). Additionally, in transverse sections
31 *Chd7* at this stage was noted to be enriched in the ventricular zone and displayed a

1 ventral to dorsal gradient within the developing spinal cord (Supplementary Figure 1G,
2 H). Extensive expression was also observed outside the CNS. In the head region, diffuse
3 *Chd7* expression was present in the tongue. Other organs with expression included the
4 dorsal root ganglia, intersomitic regions, gut, lungs, and the tail (Figure 1B).

5
6 At E12.5, *Chd2* mRNA transcript signals could be found in many tissues in the
7 developing mouse (Figure 1C), differentiated from background by use of a sense control
8 (Supplementary Figure 2). Diffuse *Chd2* expression was observed within the brain
9 (Figure 1Ca, Cb), intersomitic regions and the spinal cord. Despite this increased signal
10 density in brain tissue, the level of expression compared to other regions was low,
11 suggesting that, at this stage, *Chd2* is expressed ubiquitously at low levels throughout
12 the embryo.

13
14 As several tissues outside of the CNS expressed both *Chd7* and *Chd8* strongly, these
15 were compared directly at higher power. Sites of expression included the cochlea, lungs,
16 eyes and kidneys (Figure 2A-D, F-I). For both, distinct expression levels were observed
17 at the vestibulocochlear ganglion and cochlear epithelium in the ear (Figure 2A, F). In
18 the kidney, expression levels were high in the mesenchyme and metanephric tubule
19 epithelium (Figure 2B, G) whereas in the lung, expression was observed in the
20 pulmonary epithelium (Figure 2C, H). Both transcripts were also observed in the neural
21 retina/optic cup and retinal pigmented epithelium of the eyes, with *Chd8* transcripts
22 present widely throughout the surrounding mesenchyme and craniofacial tissues (Figure
23 2D, I). Interestingly, *Chd8* also showed high expression in the incisor primordium,
24 where no *Chd7* expression was seen (Figure 2E).

25
26 In E14.5 embryos, several sites of prominent *Chd8* expression could be seen (Figure
27 3A). In the head, abundant *Chd8* transcripts were observed in the forebrain, midbrain,
28 rhombic lip and ventricular zone of the cerebellum (Figure 3Aa). In the neocortex,
29 significant expression was revealed in the ventricular, subventricular and mantle zones
30 (Figure 3Ab). Prominent expression was also seen in the basal forebrain, including the
31 ganglionic eminences, suggesting a role for *Chd8* in the generation of GABA-ergic

1 interneurons (Figure 3A). Diffuse or low *Chd8* expression was observed in the
2 diencephalon and midbrain region. Extending from the hindbrain region, the spinal cord
3 also showed low expression. Elsewhere in the head, expression was seen in the olfactory
4 epithelium, the tongue and the ventral incisor. Other organs continued to show *Chd8*
5 expression as at E12.5, including the lungs, gut and kidneys. In addition, at E14.5, *Chd8*
6 transcripts were detected within the heart, thyroid, thymus, liver, gastric epithelium,
7 trigeminal ganglion and digits of the hind limb (Figure 3A).

8

9 Comparable to its expression at E12.5, high levels of *Chd7* mRNA transcripts were
10 present most prominently in the ventricular region of the neocortex (Figure 3Bb) in
11 accordance with previous expression analyses (Engelen et al, 2011). Signals were also
12 observed in the midbrain region extending to the hindbrain (Figure 3B). Within the
13 cerebellum, significant *Chd7* signals were observed at the rhombic lip and ventricular
14 zone of the fourth ventricle (Figure 3Ba). Widespread *Chd7* expression was also present
15 in the diencephalon (Figure 3B). Extending from the hindbrain, the spinal cord showed
16 widespread *Chd7* signal. In the oral region, the tongue and incisor primordium showed
17 *Chd7* expression. Note that its expression in the tooth appears to occur later in
18 development, at E14.5, than its family member *Chd8* (Figure 2J). Other organs such as
19 the lungs, thymus, heart, kidneys and liver, which showed significant *Chd8* expression,
20 also displayed *Chd7* expression (Figure 3B).

21

22 *Chd2* expression at E14.5 was still low and widespread but was markedly elevated in
23 certain regions compared to E12.5 (Figure 3C). Strong signals were detected in the
24 neocortex (Figure 3Cb) and rhombic lip of the cerebellum (Figure 3Ca), enriched in the
25 ventricular zone of the cerebellum. In the craniofacial region the tongue, incisor
26 primordium and olfactory epithelium all stained for *Chd2*. Specific expression signals
27 outside of the head were noted in the kidney, liver, thymus, lung, thyroid, gut, digits of
28 the hindlimb and myogenic tissue (Figure 3C).

29

30 Much like at E12.5, both *Chd8* and *Chd7* transcripts could be detected in the kidneys,
31 lungs and eyes (Figure 3Ac-Ae, Bc-Be). Additionally, however a strong *Chd2* signal

1 could also be detected in these tissues at this stage (Figure 3Cc-Ce). mRNA transcripts
2 of all three genes were detected at the condensing mesenchyme of the kidney (Figure
3 3Ae, Be, Ce), epithelium of the lung (Figure, 3Ad, Bd, Cd) and neural retina, optic cup
4 and lens of the eyes, with particularly strong expression of *Chd7* seen in the retina
5 (Figure, 3Ac, Bc, Cc). Notably, a *Chd2* signal was also detected in the anatomical space
6 containing the optic nerve and its surrounding structures at this stage (Figure 3Cc).

7

8 **Distinct *Chd2*, *Chd7* and *Chd8* expression patterns in the postnatal** 9 **mouse brain**

10

11 In order to define the domains of *Chd8* expression in the postnatal brain, in situ
12 hybridisation on brain sections at P0 were carried out. At this stage, widespread
13 expression of *Chd8* was observed (Figure 4A, A'), in agreement with previous studies
14 suggesting that *Chd8* expression peaks during mid-gestation in the embryo (Bernier et
15 al, 2014; Platt et al, 2017). Closer examination revealed expression throughout the
16 cerebellum (Figure 4Aa) and a slight enrichment of *Chd8* expression towards the outer
17 neocortex (Figure 4Ab). Other *Chd8*-expressing regions of interest include the
18 hippocampus, hypothalamus and olfactory bulb (Figure 4A, A').

19

20 At this stage, a comparable widespread pattern of expression was seen for *Chd7* (Figure
21 4B, B', C, C') with *Chd7* exhibiting particularly strong expression in the cerebellum
22 and pons (Figure 4Ba, Ca). *Chd7* was highly expressed within the cerebellum in contrast
23 to *Chd2* and *Chd8* for which moderately strong and more diffuse expression was seen
24 (Figure 4Aa-Ca). *Chd2* transcripts were enriched in the outer neocortex, hypothalamic
25 area, superior olivary complex and basal pons (Figure 4C).

26

27 The expression patterns of these genes in the P7 brain were similarly widespread with
28 continued expression in the cerebellum, neocortex and hippocampus (Figure 5A-C, A'-
29 C'). Interestingly, all three genes appear to be expressed within the rostral migratory

1 stream (RMS) suggesting a role for the CHD family in coordinating the formation of
2 the infant olfactory system. High power images demonstrated prominent expression of
3 all three genes in the cerebellum (Figure 5Aa-Ca), the dentate gyrus (DG) and cornu
4 ammonis 1-3 (CA1-3) of the hippocampus (Figure 5Ab- Cb) and neocortex, enriched in
5 layers II-III of the neocortex (Figure 5Ac- Cc). This cortical distribution is particularly
6 marked for both *Chd7* and *Chd8* where a distinct band of high signal density can be
7 appreciated. Much like in the P0 brain, *Chd7* was most strongly expressed in the
8 cerebellum.

9

10 At P20, *Chd8* and *Chd2* expression was prominent in the cerebellum, neocortex,
11 hippocampus, RMS and olfactory bulb (Figure 6A, A', C, C'). *Chd7* was most prominent
12 in the cerebellum, with low expression in the hippocampus, RMS and olfactory bulb
13 (Figure 6B, B', Bc). All three genes were expressed in the maturing granule cell layer
14 (GCL) of the cerebellum (Figure 6Aa-Ca) and the DG and CA1-3 of the hippocampus
15 (Figure 6Ab-Cb). *Chd2* and *Chd7* expression in the hippocampus was much lower and
16 more diffuse compared to the prominent expression of *Chd8* (Figure 6Ab-Cb). Clear
17 expression of *Chd2* and *Chd8* was noted in the neocortex, whilst *Chd7* expression was
18 very low in comparison (Figure 6Ac-Cc).

19

20 **Discussion**

21

22 The results of the current study demonstrate that all three genes are widely expressed
23 and show little evidence of restricted temporal and spatial expression patterns during
24 embryonic development. Although expression seemingly occurs in many different
25 tissues in-utero it can be noted that neurological tissue in particular expresses these
26 members of the CHD family at a high level; an observation that is not wholly
27 unsurprising considering the phenotypic manifestations of mutations of these genes.

28

29 **CHD gene expression in the embryo**

1 *Chd8* is widely expressed in embryonic stages E12.5 and E14.5, consistent with a
2 continued role for CHD8 during early stages of development, after E7.5 when *Chd8*^{-/-}
3 mouse embryos were demonstrated to die due to apoptosis (Nishiyama et al, 2004).
4 Recent work also implicated a role for CHD8 in suppressing p53 and the transactivation
5 of genes under p53 control by preventing the process of apoptosis (Nishiyama et al,
6 2009). This could explain the early embryonic lethality observed. Moreover, the
7 suggested role of CHD8 in transcription and elongation together with its role in
8 controlling the expression of CCNE2 and TYMS which are involved in the G1/S phase
9 of cell cycle reinforce its possible role in normal gene regulation and cell proliferation
10 respectively (Rodriguez-Paredes et al, 2009), hence normal development.

11

12 Similar to *Chd8*, the widespread *Chd2* and *Chd7* expression suggests they also have
13 important roles in early developmental processes and organogenesis. These data are
14 consistent with the evidence that neither *Chd2* nor *Chd7* homozygotes thrive past early
15 development (Bosman et al, 2005; Hurd et al, 2007; Marfella et al, 2006). The *CHD7*
16 gene is the dominant cause of CHARGE syndrome which is characterised by defects in
17 the eye, brain, ear, heart and genitalia; areas in which we observed high levels of *Chd7*
18 expression (Janssen et al, 2012; Vissers et al, 2004). There are also reports of scoliosis
19 caused by *CHD7* mutations (Gao et al, 2007) which might relate to the expression we
20 observed in the inter-somitic mesoderm. FAM124B was reported to be a component of
21 a CHD7 and CHD8-containing complex (Batsukh et al, 2012) suggesting that this multi-
22 protein complex could be functional in cells where *Chd7* and *Chd8* are co-expressed.
23 Whereas *CHD7* mutations are clearly linked to multi-organ defects in the context of
24 CHARGE syndrome (Gao et al, 2007; Janssen et al, 2012; Patten et al, 2012; Van de
25 Laar et al, 2007; Vissers et al, 2004), a clear role for CHD8 in organogenesis has not
26 been reported. Here, however, we show that *Chd8* is expressed in many developing
27 organs including the lumen of stomach and midgut; an observation which may explicate
28 the gastrointestinal complications associated with *CHD8* mutations in ASD patients
29 (Bernier et al, 2014).

30

31 In the case of *Chd2*, heterozygous mice most notably display an array of gross kidney
32 abnormalities, which might pertain to the high levels of expression of this gene we

1 observed in the developing kidney (Marfella et al, 2008). Despite this association, the
2 absence of reported renal dysgenesis in humans harbouring *CHD2* mutations might
3 indicate divergent functions for this gene in the human kidney, or a degree of functional
4 redundancy with other CHD genes. Notably, at E14.5 the *Chd2* expression pattern was
5 markedly similar to *Chd8* and indeed, several regions of the embryo at this stage
6 expressed these two genes in exclusion of *Chd7*, suggesting the possibility that they
7 may serve similar functions. Some such regions include the dorsal hindlimb, thyroid,
8 gut and olfactory epithelium.

9

10 **CHD genes in brain development**

11 *CHD2* and *CHD8* mutations share a well-established link with ASD, a disorder that is
12 widely regarded to be caused by aberrant neurodevelopment (Lebrun et al, 2017; O'
13 Roak et al, 2012; Neale et al, 2012; Sanders et al, 2012). Our study demonstrates high
14 levels of both *Chd2* and *Chd8* expression in the developing brain, especially during
15 embryonic development. Additionally, preserved expression of both was revealed in key
16 areas of the perinatal (P0) and postnatal brain (P7 and P20) including the cerebellum,
17 hippocampus and neocortex – regions of the brain that are implicated in ASD (Allen,
18 2005; de Anda et al, 2012; Donovan & Basson, 2017; Riedel and Micheau, 2001). Within
19 the neocortex expression of *Chd2* and *Chd8* appears to be particularly prominent within
20 the outer layers, distinctly layers II-III of the postnatal brain, areas in which high
21 numbers of other ASD risk genes are also enriched (Parikshak et al, 2013).

22

23 Taken together, and bolstered by evidence of aberrant neurodevelopmental phenotypes
24 associated with mutants of these genes (Allen et al, 2013; Chérnier et al, 2014; Gompers
25 et al, 2017; Katayama et al, 2016; Lebrun et al, 2017; O'Roak et al, 2014; Pinto et al,
26 2016; Platt et al, 2017; Suetterlin et al, 2018), our data suggest that *Chd2* and *Chd8* are
27 expressed in a spatiotemporally appropriate way such that impairment in their
28 expression might precipitate some of the neurological changes seen in patients with
29 ASD. With both genes expressed in the SGZ of the hippocampus our data further support
30 the notion that CHD2 and CHD8 might regulate neurogenesis (Shen et al, 2015; Durak

1 et al, 2016), akin to the reported role of their counterpart CHD7 (Feng et al, 2013; Jones
2 et al, 2015).

3

4 In addition to its role in adult neurogenesis, the diverse, temporally distinct functions
5 of CHD7 during cerebellar development (Yu et al, 2013; Whittaker et al, 2017a;
6 Whittaker et al, 2017b; Donovan et al, 2017), are consistent with its pronounced
7 expression in the postnatal cerebellum. In view of the role of CHD7 in
8 neurodevelopment our study supports the notion that its mutation might account for the
9 cerebellar hypoplasia associated with CHARGE syndrome. Furthermore, its expression
10 in the olfactory bulb and RMS throughout postnatal development further bolsters the
11 link between Kallmann syndrome, characterised by anosmia and hypogonadism, and
12 CHD7 mutation (Jongmans et al, 2009).

13

14 Finally, the reported expression of *Chd2* in the postnatal hippocampus invites a potential
15 link between the dysfunction and deficiency of hippocampal interneurons documented
16 in epileptic encephalopathies, temporal lobe epilepsy and seizures associated with ASD
17 and its proposed role in interneuron development (Fyre et al, 2016; Lado et al, 2013;
18 Liu et al, 2014; Meganathan et al, 2017). Furthermore, *Chd2* expression in the eye and
19 related structures during early development might also pertain to the association of
20 *CHD2* mutation with photosensitivity in epilepsy (Carvill et al, 2013; Galizia et al,
21 2015; Lund et al, 2014; Suls et al, 2013).

22

23 In conclusion, in addition to their established roles in early brain development, our
24 expression analyses also implicate *Chd2* and *Chd8*, alongside *Chd7*, in organogenesis.
25 Our data also implicate all three genes in the process of postnatal neurogenesis due to
26 their expression in the neurogenic niches of the adult brain. Additional studies will be
27 necessary to further define the function of these genes in these developmental processes.
28 The gene expression data reported here will provide invaluable information and
29 reference points to guide these future studies.

30

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4

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Figure legends

Figure 1. Distinct *Chd8*, *Chd7* and *Chd2* expression patterns at E12.5.

In situ hybridisation on sagittal sections of mouse embryos at developmental stage E12.5 using antisense riboprobes to detect *Chd8*, *Chd7* and *Chd2* mRNA, anterior to the right (A-C). Gene expression is indicated by purple/blue staining. Note the widespread *Chd8* expression in most embryonic tissues (A), the high, localized expression of *Chd7*, specifically in the developing nervous system (B), and very low, widespread expression of *Chd2* (C). High magnification images of the developing cerebellum (Aa-Ca) demonstrate the presence of *Chd8* (Aa) and *Chd7* (Ba) transcripts throughout the neuroepithelium, with little *Chd2* expression evident (Ca). High magnification images through the neocortex show widespread *Chd8* expression (Ab), note that *Chd7* expression tends to be higher on the ventricular side (Bb) and that there is little discernible *Chd2* expression (Cb). Other regions with relatively strong signals were the nasal epithelium, tail, genital tubercle, intersomitic mesoderm, spinal cord, mid brain, diencephalon, tongue and pituitary. Scale bars represent 100 µm.

Cb = cerebellum; Di = diencephalon; Drg = dorsal root ganglia; FP = floor plate; Gt = genital tubercle; Gu = gut; H = heart; Is = isthmus; Iso = intersomitic mesoderm; Lu = lungs; LRL = lower rhombic lip; MB = midbrain; MZ = molecular zone; NC = neocortex; Pi = pituitary; Sc = spinal cord; SVZ = subventricular zone; Ta = tail; To = tongue; URL = upper rhombic lip; VZ = ventricular zone.

Figure 2. *Chd7* and *Chd8* are expressed in multiple organs at E12.5.

1 *In situ* hybridisation images of *Chd8* (A-E) and *Chd7* (F-I) transcripts around the
2 cochlea of the inner ear (A, F), kidney (B, G), lung (C, H) eye (D, I) and tooth (E, J).
3 Scale bars represent 100 μ m.

4 C = cornea; CD = cochlear duct; CVG = cochlea-vestibular ganglia; CM = condensing
5 mesenchyme; LE = lung epithelium; LN = lens; MT = metanephric tubule; NR =
6 neuroretina; PI = primordium of incisor; PO = pre-optic cup; RPE = retinal pigmented
7 epithelium; SB = segmental bronchus.

8

9 **Figure 3. Distinct nervous system and organ-specific expression patterns of *Chd8*,**
10 ***Chd7* and *Chd2* in E14.5 mouse embryos.**

11 *In situ* hybridisation on sagittal sections of E14.5 mouse embryos (A-C), anterior to
12 the right. Note distinct *Chd8*, *Chd7* and *Chd2* expression patterns throughout the
13 embryos with notably higher level in the developing nervous system. Beyond the
14 nervous system, other notable regions of expression included various organs and
15 glands, for example the thymus and thyroid, heart and kidneys. High magnification
16 images (Aa-Ce) revealed specific expression patterns in the cerebellum (Aa-Ca),
17 Neocortex (Ab-Cb), Eye (Ac-Cc), Lung (Ad-Cd) and kidney (Ae-Ce). Scale bars
18 represent 100 μ m.

19

20 B = bronchus; C = cornea; Cb = cerebellum; CD = collecting duct; CM = condensing
21 mesenchyme; CP = choroid plexus; C-PL = cortical plate; Di = diencephalon; Dh =
22 digit of hindlimb; GE = gastric epithelium; GEm = ganglionic eminence; Gu = gut; H
23 = heart; K = kidney; LC = lens capsule; LE = lung epithelium; Li = liver; LN = lens;
24 Lu = lung; Mb = mid brain roof plate; MZ = marginal zone; NC = neocortex; NR =
25 neural retina; OE = olfactory epithelium; ON = optic nerve and surrounding
26 structures; PO = pre-optic cup; RL = rhombic lip; RPE = retinal pigmented
27 epithelium; Sc = spinal cord; T = thyroid ; To = tongue; Ta = tail; Th = thymus; TG =
28 trigeminal ganglion; vI = ventral incisor; VZ = ventricular zone.

29

1 **Figure 4. Comparative *Chd8*, *Chd7* and *Chd2* expression patterns in the newborn**
2 **mouse brain.**

3 Sagittal sections through newborn mouse brain (anterior to the right), hybridised with
4 antisense RNA probes to detect *Chd8* (A, A'), *Chd7* (B, B') and *Chd2* (C, C') transcripts
5 in blue. Note wide-spread expression of *Chd8*, highly localised *Chd7* expression in the
6 cerebellum and pons, and *Chd2* in the neocortex, midbrain and cerebellum. High
7 magnification views of the cerebellum (Aa-Ca) and neocortex (Ab-Cb) are shown. Scale
8 bars represent 100 µm.

9 Cb = cerebellum; EGL = external granule cell layer; HC = hippocampus; Hy =
10 hypothalamus; I = cortical layer I; II-III = cortical layers II-III; IC = inferior colliculus;
11 IGL, = internal granule cell layer; Me = medulla; NC = neocortex; OB = olfactory bulb;
12 Pn = pons; SC = superior colliculus; SOC = superior olivary complex.

13

14 **Figure 5. *Chd8*, *Chd7* and *Chd2* are expressed in the early postnatal cerebellum,**
15 **hippocampus, neocortex and rostral migratory stream.**

16 Sagittal sections through postnatal day 7 (P7) mouse brain (anterior to the right),
17 hybridised with antisense RNA probes to detect *Chd8* (A, A'), *Chd7* (B, B') and *Chd2*
18 (C, C') transcripts, visualised in blue. Higher magnification images to visualise specific
19 expression domains in the cerebellum (Aa-Ac), hippocampus (Ba-Bc), and neocortex
20 (Ca-Cc) are shown. Scale bars represent 100 µm.

21 CA1-3 = cornu ammonis 1-3; Cb = cerebellum; DG = dentate gyrus; EGL = external
22 granule cell layer; GL = glomerular layer; HC = hippocampus; I = cortical layer I; II-
23 III = cortical layers II-III; IGL = internal granule cell layer; IPL = internal plexiform
24 layer; ML = molecular layer; NC = neocortex; OB = olfactory bulb; RMS = rostral
25 migratory stream; SGZ = subgranular zone; WM = white matter.

26

27

28 **Figure 6. Comparison of *Chd8*, *Chd7* and *Chd2* expression patterns in the P20 mouse**
29 **brain.**

1 Sagittal sections through P20 mouse brain (anterior to the right), hybridised with
2 antisense RNA probes to detect *Chd8* (A, A'), *Chd7* (B, B') and *Chd2* (C, C') transcripts,
3 visualised in blue. Note high expression of all three genes in the cerebellum, with
4 widespread *Chd8* and *Chd2* expression remaining in the neocortex. High magnification
5 images of the cerebellum (Aa-Ca); hippocampus (Ab-Cb) and neocortex (Ac-Cc) are
6 shown. Scale bars represent 100 µm.

7 CA1-3 = cornu ammonis 1-3; Cb = cerebellum; DG = dentate gyrus; GCL = granule cell
8 layer; HC = hippocampus; I = cortical layer I; II-III = cortical layers II-III; ML =
9 molecular layer; NC = neocortex; OB = olfactory bulb; RMS = rostral migratory stream;
10 SGZ = subgranular zone; WM, = white matter.

11

12 **Supplementary Figure 1. Distinct *Chd8* and *Chd7* expression patterns within the**
13 **neural tube at E12.5.**

14 *In situ* hybridisation on sagittal (anterior to the right) and transverse sections of mouse
15 embryos at developmental stage E12.5 using antisense riboprobes to detect *Chd8* and
16 *Chd7* mRNA (A-H). Gene expression is indicated by purple/blue staining. Note that both
17 *Chd8* and *Chd7* are expressed throughout the length of the neural tube (A, B, E, F).
18 Whilst *Chd8* displays no mediolateral or dorsoventral gradient in transverse sections
19 *Chd7* shows distinct enrichment in the ventricular zone of the developing CNS and a
20 ventral to dorsal gradient within the spinal cord.

21 Drg = dorsal root ganglia; Iso = intersomitic region; LGE = lateral ganglionic eminence;
22 MGE = medial ganglionic eminence; No = notochord; NT = neural tube; SE = surface
23 ectoderm.

24

25 **Supplementary Figure 2. Sense control sections at E12.5.**

26 *In situ* hybridisation on sagittal (anterior to the right) sections of mouse embryos at
27 developmental stage E12.5 using sense riboprobes to *Chd8*, *Chd7* and *Chd2* mRNA. Note
28 that for all three genes there is little to no hybridisation or staining using the sense
29 riboprobe in contrast to what is seen when using the anti-sense probe.

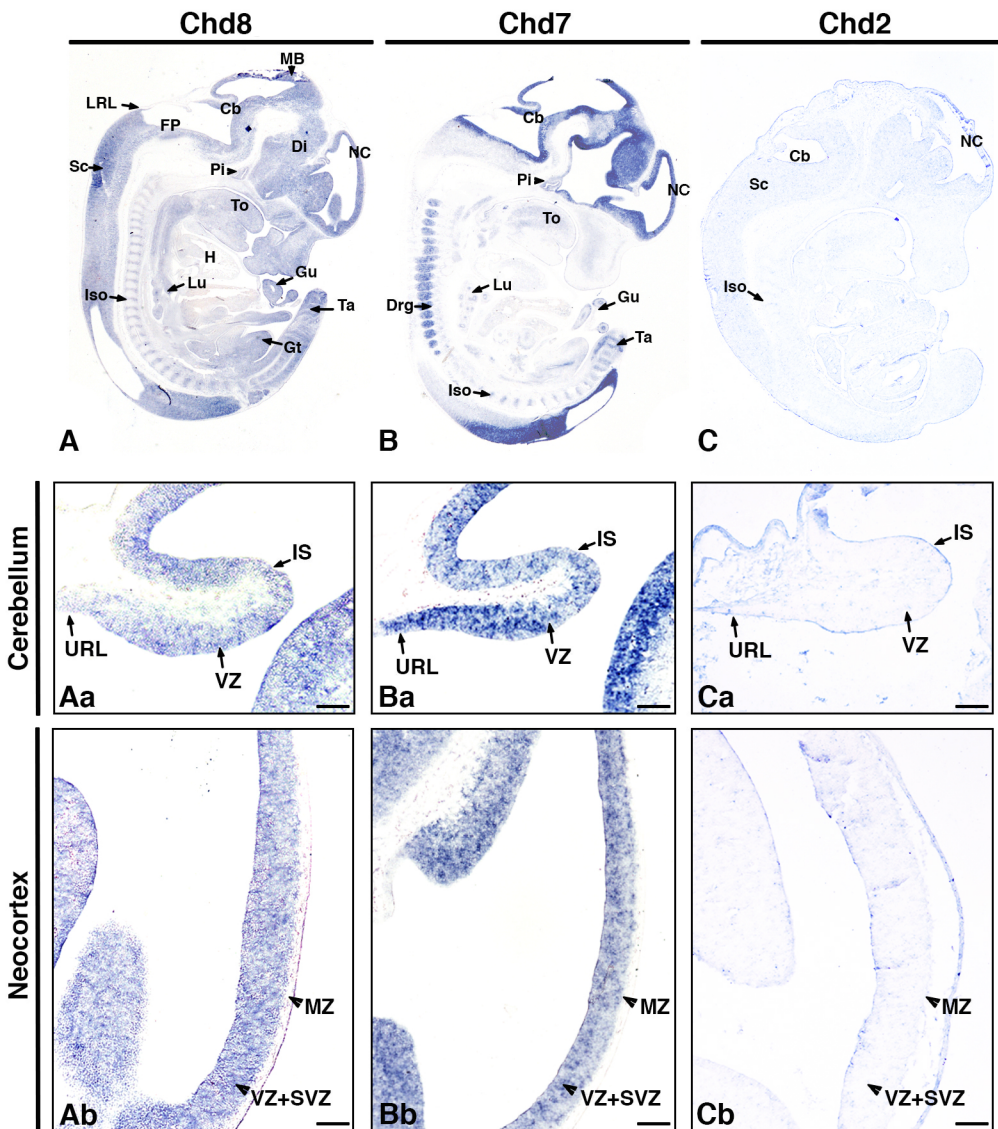


Figure 1

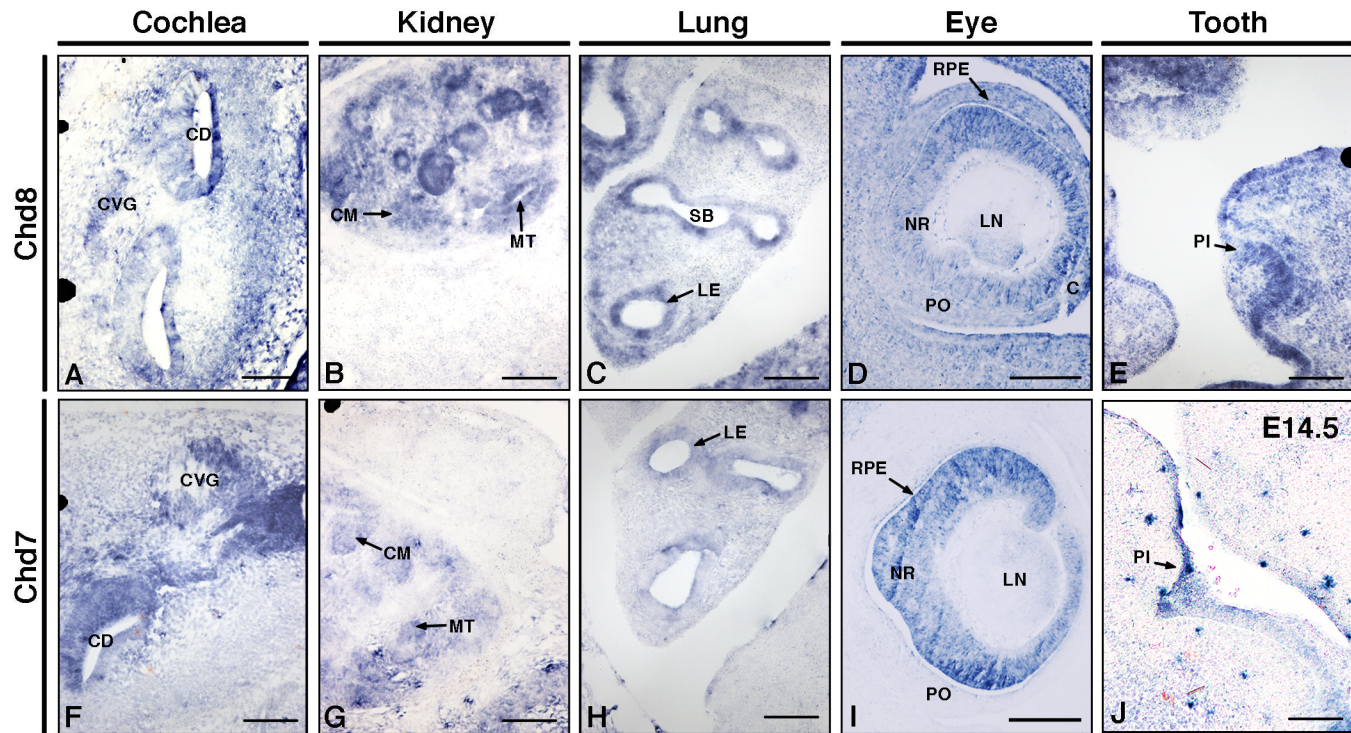


Figure 2

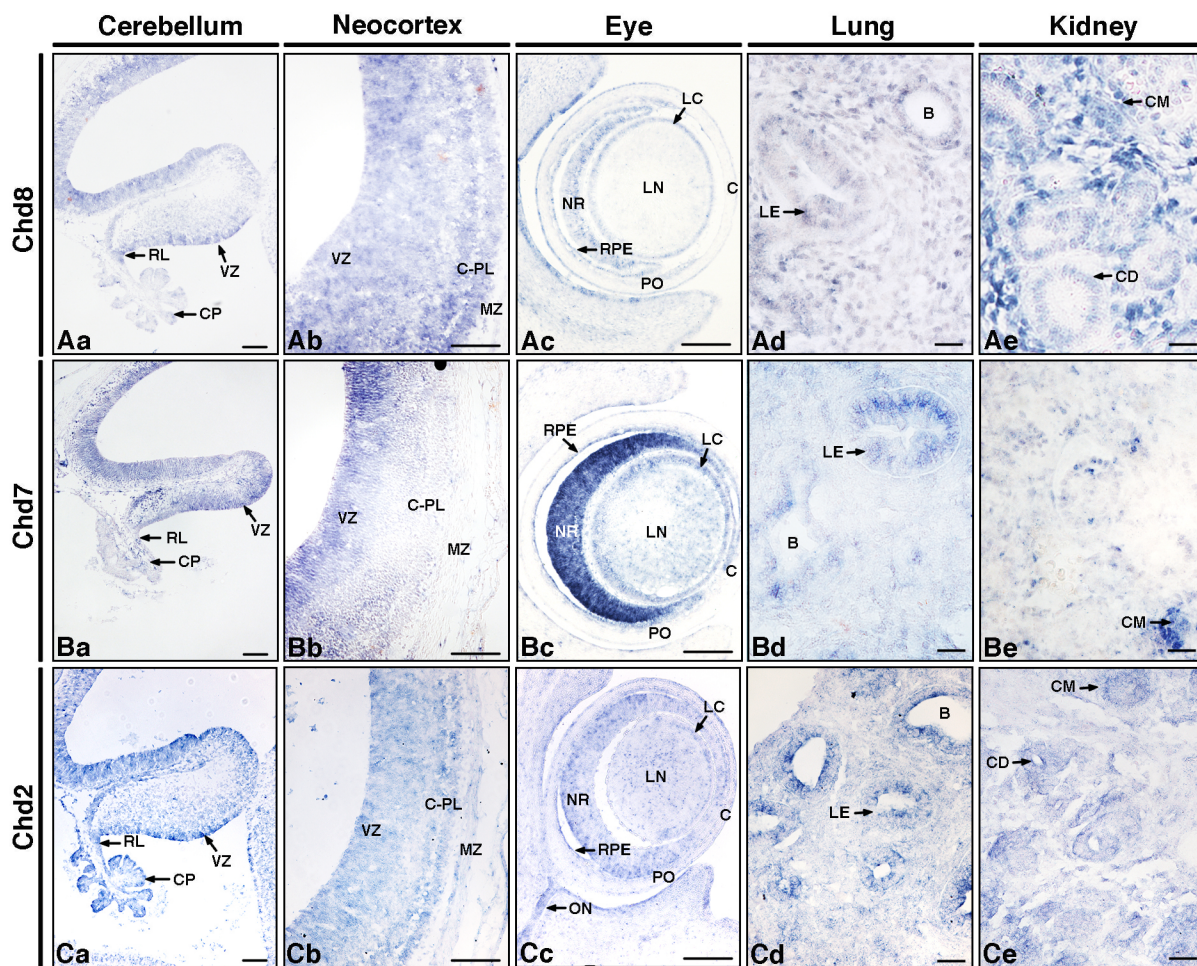
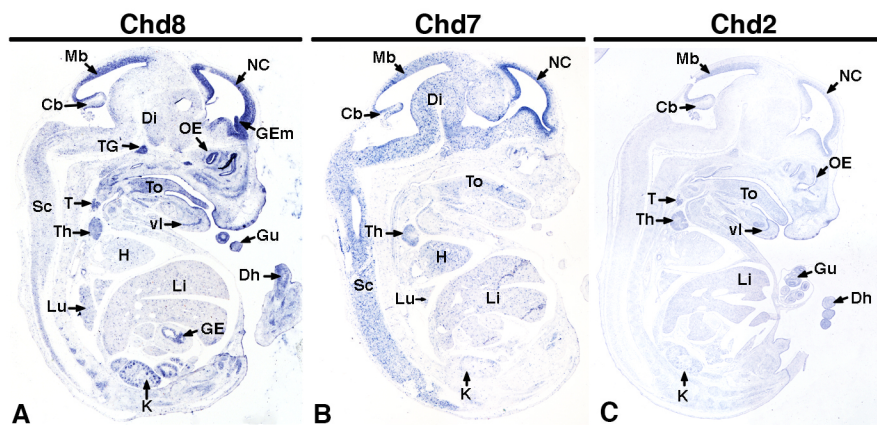
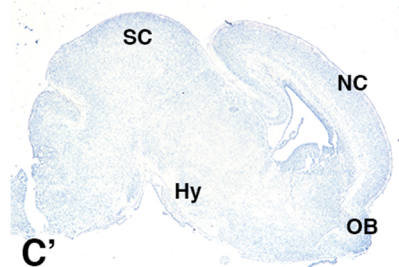
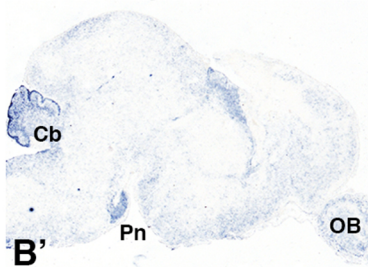
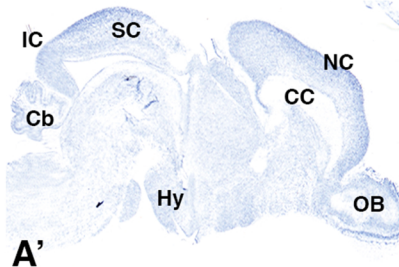
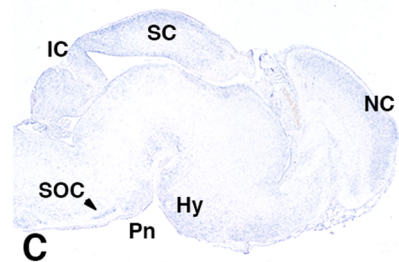
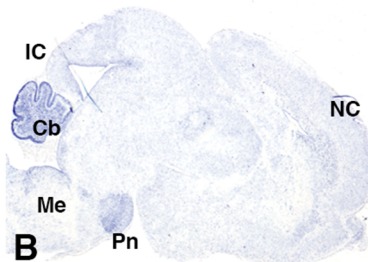
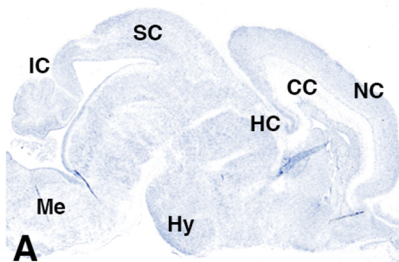
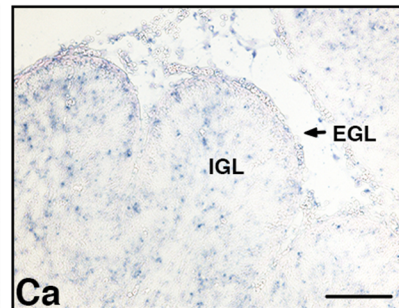
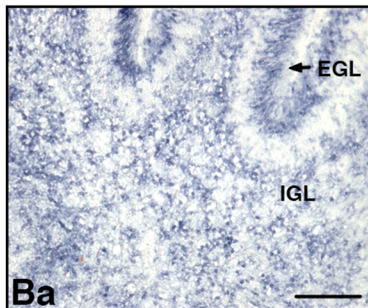
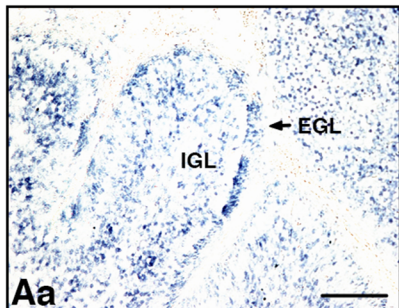
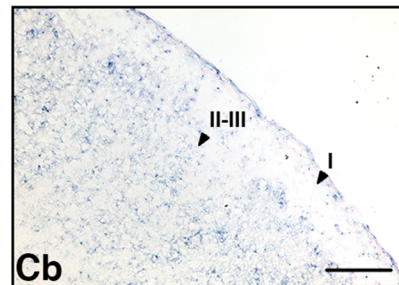
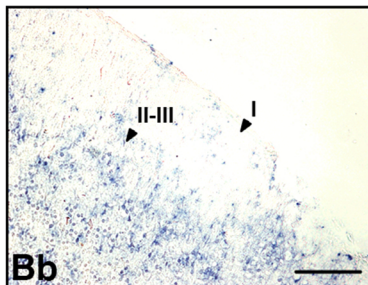
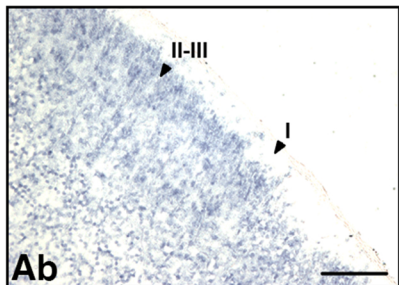


Figure 3

Chd8**Chd7****Chd2****Cerebellum****Neocortex****Figure 4**

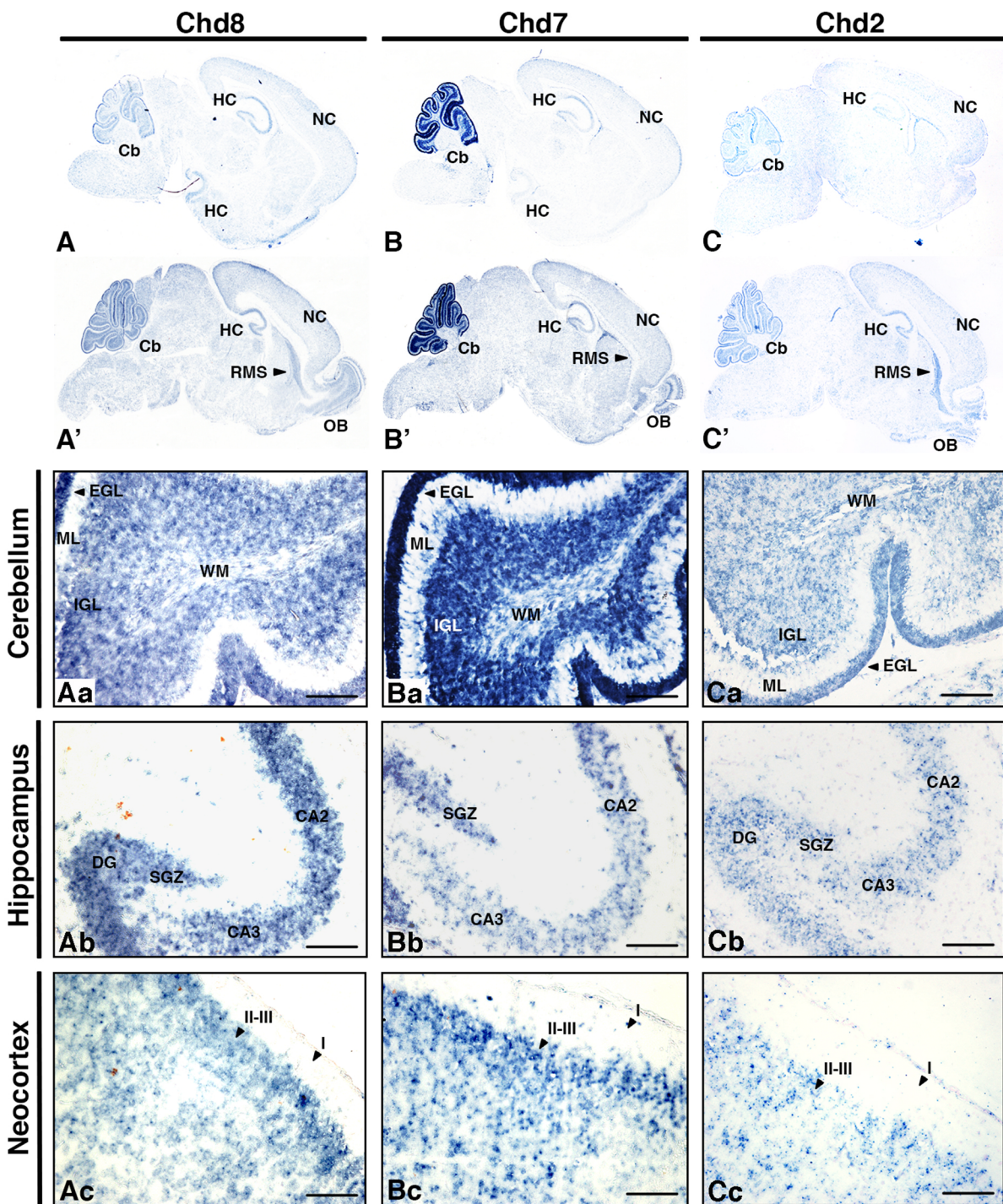
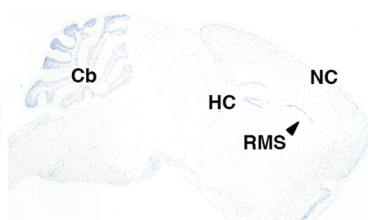
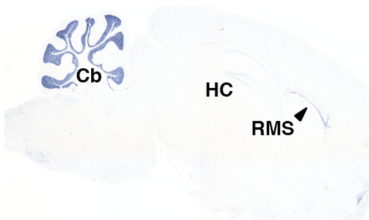
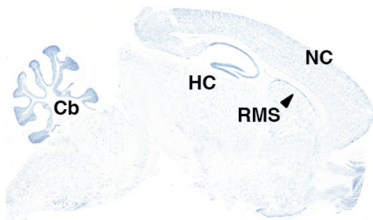
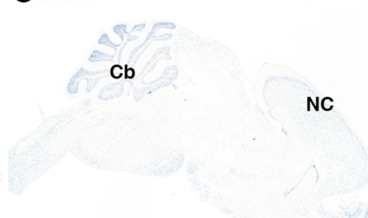
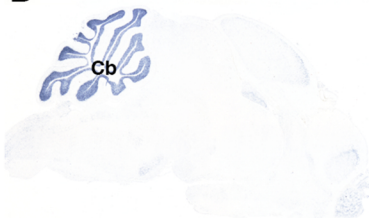
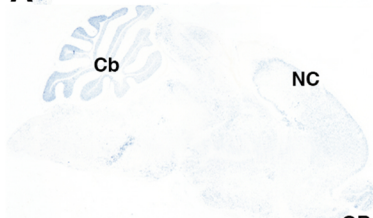
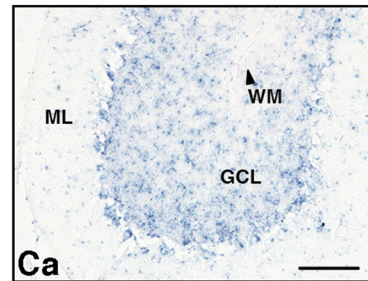
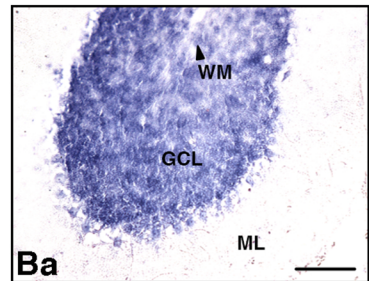
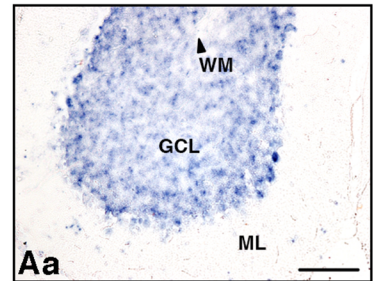
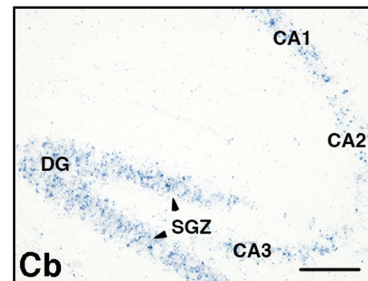
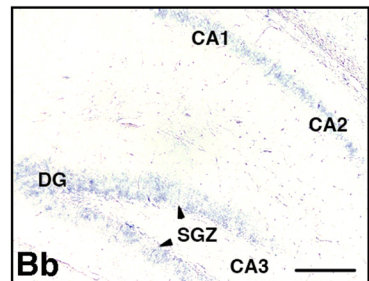
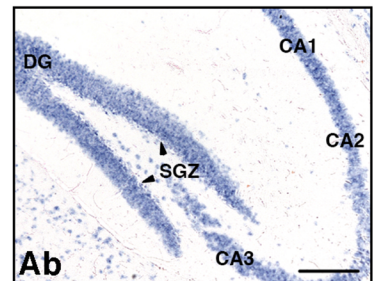
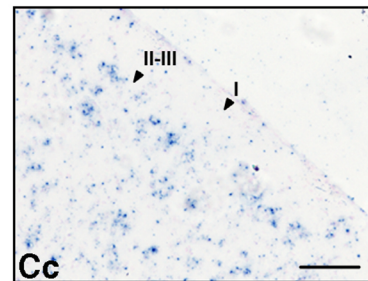
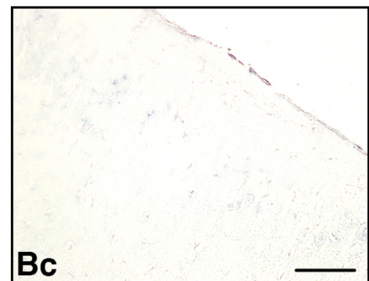
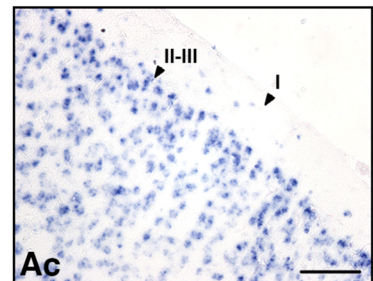
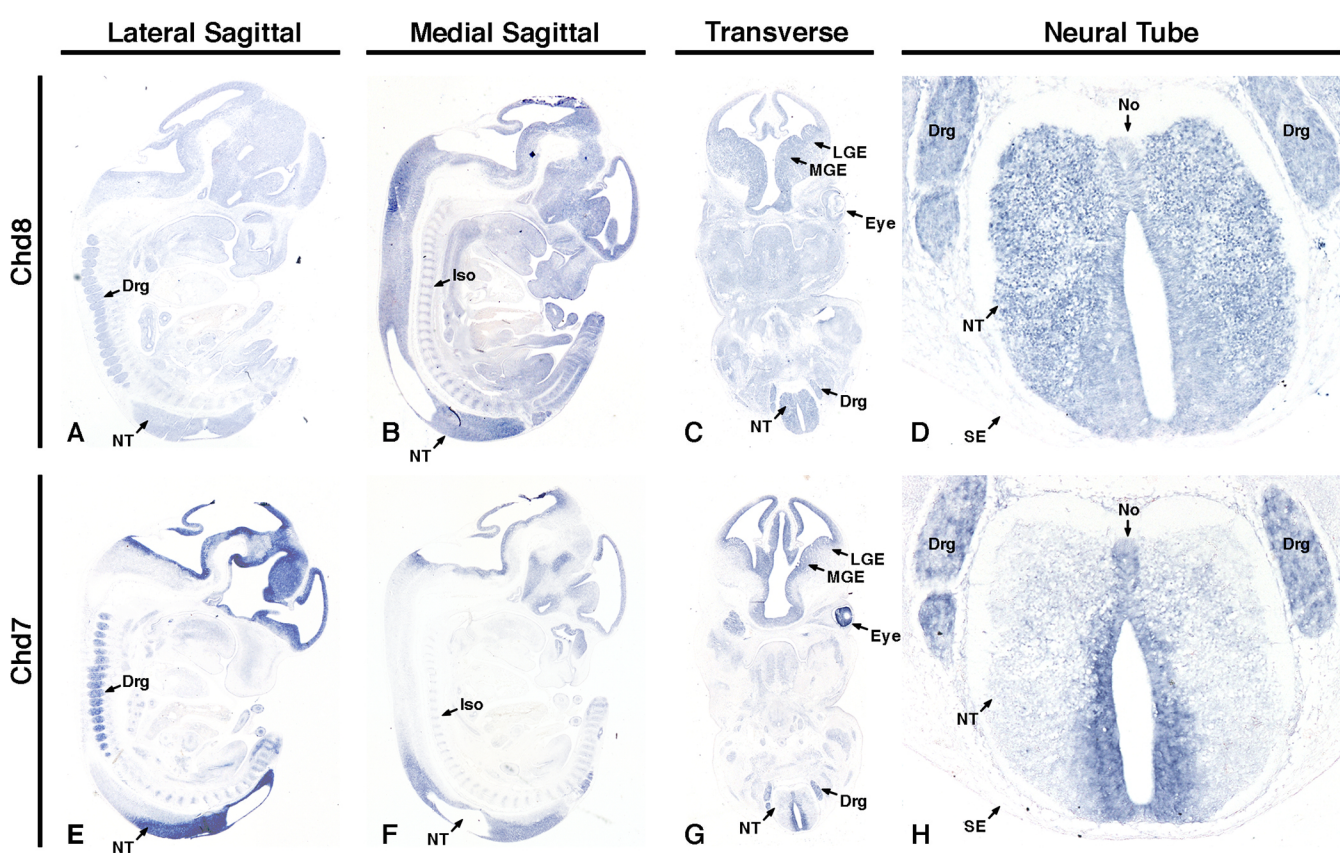


Figure 5

Chd8**Chd7****Chd2****A****B****C****A'****B'****C'****Cerebellum****Aa****Ba****Ca****Hippocampus****Ab****Bb****Cb****Neocortex****Ac****Bc****Cc****Figure 6**



Supplementary Figure 1

Chd8



Chd7



Chd2



Supplementary Figure 2