KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome

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Contributed by Fred H. Gage, December 15, 2015 (sent for review August 17, 2015; reviewed by Hongjun Song and Anthony N. van den Pol)

Rett syndrome is a severe form of autism spectrum disorder, mainly caused by mutations of a single gene methyl CpG binding protein 2 (MeCP2) on the X chromosome. Patients with Rett syndrome exhibit a period of normal development followed by regression of brain function and the emergence of autistic behaviors. However, the mechanism behind the delayed onset of symptoms is largely unknown. Here we demonstrate that neuron-specific K⁺−Cl⁻ cotransporter2 (KCC2) is a critical downstream gene target of MeCP2. We found that human neurons differentiated from induced pluripotent stem cells from patients with Rett syndrome showed a significant deficit in KCC2 expression and consequently a delayed GABA functional switch from excitation to inhibition. Interestingly, overexpression of KCC2 in MeCP2-deficient neurons rescued GABA functional deficits, suggesting an important role of KCC2 in Rett syndrome. We further identified that RE1-silencing transcriptional factor, REST, a neuronal gene repressor, mediates the MeCP2 regulation of KCC2. Because KCC2 is a slow onset molecule with expression level reaching maximum later in development, the functional deficit of KCC2 may offer an explanation for the delayed onset of Rett symptoms. Our studies suggest that restoring KCC2 function in Rett neurons may lead to a potential treatment for Rett syndrome.

Rett syndrome | MeCP2 | human iPSC | disease modeling | KCC2

Results

We have previously demonstrated that neurons derived from iPSCs from patients with Rett syndrome showed significant glutamatergic deficits (13). Here we investigated GABA function.

Significance

Rett syndrome is a devastating neurodevelopmental disorder that currently has no cure. In this work, we demonstrate that human neurons derived from patients with Rett syndrome show a significant deficit in neuron-specific K⁺−Cl⁻ cotransporter2 (KCC2) expression, resulting in a delayed GABA functional switch. Restoring KCC2 level rescues GABA functional deficits in Rett neurons. We further demonstrate that methyl CpG binding protein 2 regulates KCC2 expression through inhibiting RE1-silencing transcriptional factor. Our data suggest a potential therapeutic approach for the treatment of Rett syndrome through modulation of KCC2.

Author contributions: X.T. and G.C. designed research; X.T., J.K., L. Zhou, E.W., L. Zhang, and Z.W. performed research; C.C., A.R.M., M.C.N.M., and F.H.G. contributed new reagents/analytic tools; X.T., J.K., and G.C. analyzed data; J.K., L. Zhang, and Z.W. helped with cell culture and electrophysiology recording; L. Zhou performed the Western blot analysis; E.W. profiled the developmental timeline of MeCP2 and KCC2 expression; and X.T. and G.C. wrote the paper.

Reviewers: H.S., Johns Hopkins University; and A.N.v.d.P., Yale University School of Medicine. The authors declare no conflict of interest.

1To whom correspondence may be addressed. Email: gongchen@psu.edu or gage@salk.edu. This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1524013113/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1524013113
PNAS | January 19, 2016 | vol. 113 | no. 3 | 751–756
in human iPSC-derived neurons from patients with Rett syndrome. Human neurons were derived from iPSCs obtained from a male patient with Rett syndrome (Q83X, clone 1), which carried a MeCP2 mutation at the amino acid residue 83 from glutamine to a premature stop codon, resulting in truncation and degradation of the MeCP2 protein. Immunostaining for MeCP2 confirmed the absence of MeCP2 signal in neurons derived from Rett patient Q83X, whereas control neurons derived from his father (WT83, clone 7, healthy control) had strong MeCP2 staining in the nuclei (Fig. 1 A and B). When we stained for KCC2 in WT83 neurons, we found a gradual increase in KCC2 expression over 3 mo (Fig. 1 D and E), whereas Q83X neurons showed little KCC2 signal even after 3 mo in culture (Fig. 1 F and G). These results suggest that GABA function may be altered in Rett neurons. We have previously demonstrated that IGF1 can rescue glutamatergic deficits in Rett neurons (13). Therefore, we treated the Q83X Rett neurons with IGF1 and found that, whereas MeCP2 levels were not increased in the nucleus (Fig. 1 C), the KCC2 staining was significantly increased (Fig. 1 H and I), suggesting that IGF1 may up-regulate KCC2 independently of MeCP2. Fig. 1 J shows the developmental change of the KCC2 expression levels in WT83, Q83X, and Q83X + IGF1 groups during 1–3 mo of culture on astrocytes. We also used Western blot to compare KCC2 expression levels among 2-mo-old neurons from different groups (WT83, Q83X, and Q83X + IGF1). Compared with WT83 control, Q83X Rett neurons showed a significant reduction in the expression of KCC2, which was rescued by IGF1 (Fig. 1 K and L).

If the lack of KCC2 in Q83X Rett neurons is due to the absence of MeCP2, we reasoned that overexpressing MeCP2 in Q83X Rett neurons would rescue the KCC2 deficit. Indeed, whereas expression of GFP as a control had no effect on KCC2 expression (Fig. 2 A–D), overexpression of MeCP2 in Q83X neurons significantly restored the KCC2 expression level (Fig. 2 E–H). As another control, we overexpressed KCC2 itself in Q83X neurons and verified that the KCC2 level was dramatically increased (Fig. 2 I–L; quantified data in Fig. 2M). Therefore, the absence of MeCP2 in Rett neurons induces a significant deficit of KCC2, which can be rescued by MeCP2 reexpression or IGF1 treatment.

KCC2 has been shown to play an important role during neural development (23, 31). KCC2 functions in transporting Cl− from intracellular to extracellular space to maintain low intracellular Cl− concentration ([Cl−]) in mature neurons (32). Because GABA A−Rs are also Cl− channels, the Cl− reversal potential for GABA A−Rs (E GABA A) is typically governed by KCC2 (23, 33). Because KCC2 expression has a delayed onset during early brain development, immature neurons often have high [Cl−], and then switch to low [Cl−] after KCC2 level increases in mature neurons (33, 34). Such [Cl−] changes lead to a well-studied phenomenon

Fig. 1. Human neurons differentiated from iPSCs from patients with Rett syndrome show deficits in KCC2 expression and GABA functional switch. (A–C) MeCP2 immunostaining in MAP2-positive human neurons differentiated from WT83 (A, clone 7, healthy control, father of Q83X) or Q83X (B, clone 1, patient with Rett) iPSC cell lines. Note the absence of MeCP2 in the nucleus of Q83X neurons, IGF1 treatment of Q83X Rett neurons (C) could not rescue MeCP2 expression. (D–I) Representative micrographs showing KCC2 immunoreactivity (red) in MAP2-positive WT83 neurons (D and E), Q83X neurons (F and G), or Q83X neurons treated with IGF1 (H and I) at different culture stages. Columns of E, G, and I are enlarged views of boxed areas in D, F, and H, respectively. (J) Quantification of the time course of KCC2 expression in WT83, Q83X, or Q83X neurons treated with IGF1 during 3 mo of culture on astrocytes. (K) Representative Western blot probing KCC2 levels among WT83 neurons, Q83X neurons, and Q83X neurons treated with IGF1. (L) Quantified data showing that comparing to WT83 neurons, Q83X Rett neurons had a significant reduction in KCC2 expression level (49 ± 7% of WT83 level, n = 3 independent repeats; P < 0.02, Student’s t test). The KCC2 deficit in Q83X neurons was rescued by IGF1 treatment (P < 0.03 comparing to Q83X neurons; P > 0.2 comparing to WT83 neurons). (Scale bars: A, D, and E, 10 μm.)
of GABA functional switch from excitation to inhibition, which is crucial for normal brain development and function (23).

The lack of KCC2 expression in Q83X Rett neurons led us to examine whether GABA function was altered by measuring $E_{\text{GABA}}$, an index for $[\text{Cl}^-]$, that is controlled by KCC2. For control WT83 neurons, $E_{\text{GABA}}$ showed a clear developmental shift from $-50$ mV to $-70$ mV when neurons gradually matured during 3-mo of culture on astrocytes (Fig. 3 A and D), indicating a normal GABA functional switch from excitation to inhibition. In contrast, Q83X Rett neurons did not show a significant change in $E_{\text{GABA}}$ even after 3-mo of culturing on astrocytes (Fig. 3 B and D). Interestingly, IGF1 treatment significantly rescued the alterations of $E_{\text{GABA}}$ in Q83X Rett neurons (Fig. 3 C and D), consistent with its rescue of KCC2 expression level (Fig. 1 H–J). Thus, the lack of KCC2 expression in Rett neurons significantly altered GABA function during early neuronal development. To ensure that the KCC2 deficit was not specific to the Q83X clone used, we further investigated KCC2 levels in different iPSC clones derived from the same patient (Q83X clone 6) and his father (WT83 clone 6). We found that similar to Q83X clone 1 (Fig. 1), KCC2 level was also significantly reduced in the Q83X Rett neurons derived from clone 6, and rescued by IGF1 treatment (Fig. 3 E–H). Accordingly, the $E_{\text{GABA}}$ in Rett neurons derived from the new clone 6 of Q83X did not shift toward hyperpolarization like that in WT83 clone 6 after 2-mo of culture, but was rescued by IGF1 treatment (Fig. 3 I–L).

To further test whether our finding is consistent across different patients with Rett syndrome, we derived neurons from a different patient with Rett syndrome (a female carrying a different MeCP2 mutation N126I). Compared with control neurons derived from a different human iPS cell line WT126, we found that N126I Rett neurons had no MeCP2 signal in the nucleus and a significant reduction of KCC2 expression level in the soma and dendrites (Fig. 4 A and B). The IGF1 treatment rescued the KCC2 deficit but not MeCP2 signal in the N126I Rett neurons (Fig. 4 C and D). Furthermore, gramicidin-perforated patch-clamp recordings revealed that, whereas $E_{\text{GABA}}$ showed a normal shift from $-48$ mV to $-68$ mV in WT126 control neurons, there was no developmental shift of $E_{\text{GABA}}$ in the N126I Rett neurons (Fig. 4 E, F, and H). IGF1 treatment also rescued the $E_{\text{GABA}}$ deficit in N126I Rett neurons (Fig. 4 G and H). Therefore, KCC2 deficit and the consequent GABA functional alteration is a general feature associated with Rett neurons and can be rescued by IGF1 treatment.

To further investigate the mechanisms underlying MeCP2 regulation of KCC2, we used cultured mouse cortical neurons to

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**Figure 2.** Rescue of KCC2 deficit in Q83X Rett neurons. (A–D) Control experiment showing that transfection of GFP into Q83X neurons had no effect on the KCC2 expression level. (Scale bar, 10 μm.) (E–H) Transfection of MeCP2 in Q83X neurons significantly increased the KCC2 expression level. Arrowhead indicates transfected neuron; * indicates nontransfected neuron. (I–L) Transfected KCC2 in Q83X neurons also increased the KCC2 expression level. (M) Quantified data showing a significant increase in KCC2 immunoreactivity in Q83X neurons after overexpressing MeCP2 or KCC2 (GFP, 49 ± 4 a.u., n = 16; MeCP2, 98 ± 6 a.u., n = 31; KCC2, 146 ± 9 a.u., n = 36; ***P < 0.001, one-way ANOVA with Bonferroni correction).

**Figure 3.** Q83X Rett neurons from different clones show deficits in GABA functional switch. (A–C) Representative traces showing GABA-evoked currents under various holding potentials in 3-mo-old WT83 (A), Q83X (B), or Q83X neurons treated with IGF1 (C). Dashed lines indicate GABA reversal potential ($E_{\text{GABA}}$). (D) Quantified data illustrating the time courses of $E_{\text{GABA}}$ changes during neuronal maturation. Note that Q83X neurons did not show typical $E_{\text{GABA}}$ shift as that of WT83 neurons. (E–G) Representative micrographs showing KCC2 immunostaining in 2-mo-old WT83 CL6 (E), Q83X CL6 (F), or Q83X neurons treated with IGF1 (G). Enlarged views of neurons are presented below each micrograph. (Scale bars, 10 μm.) (H) Quantified results showing that KCC2 expression level is reduced in clone 6 Q83X Rett human neurons. (I–L) Representative GABA-evoked responses recorded from 2-mo-old neurons derived from a different pair of Q83X and WT83 clones. Dashed lines indicate GABA reversal potential ($E_{\text{GABA}}$). (I) Quantified data illustrating the $E_{\text{GABA}}$ levels recorded at 2-mo time point. Note that Q83X clone 6 neurons did not show typical $E_{\text{GABA}}$ hyperpolarizing shift as that of WT83 clone 6 neurons. Data are presented as mean ± SEM.
molecularly manipulate the MeCP2 level and monitor consequent KCC2 changes. We first compared both MeCP2 and KCC2 expression levels between mouse and human neurons during development. Interestingly, in both human and mouse neurons, MeCP2 and KCC2 showed highly correlative increase during neuronal maturation, although human neurons developed much slower than mouse neurons (Figs. S1 and S2). We then knocked down MeCP2 in mouse neurons and confirmed that KCC2 was consequently reduced and $E_{\text{GABA}}$ shifted from $-70$ mV toward $-50$ mV (Fig. S3), consistent with our findings in human Rett neurons. Therefore, our experiments in mouse neurons essentially recapitulate the results in human Rett neurons that the absence of MeCP2 leads to a decrease of KCC2, which in turn causes alteration of GABA signaling.

MeCP2 is a global transcription regulator and binds with DNA in the nucleus, whereas KCC2 is a membrane transporter and also found in the cytoplasm of soma and dendrites. How does MeCP2 regulate KCC2? Previous studies have reported that MeCP2 can regulate the transcriptional repressor REST, a master regulator of neuronal gene expression (35, 36). Interestingly, KCC2 has been reported to be regulated by REST (37). We therefore hypothesized that MeCP2 might regulate KCC2 through REST. To test this hypothesis, we overexpressed REST in mouse neurons and found that KCC2 expression level was significantly reduced (Fig. 5A and B). Interestingly, coexpression of MeCP2 with REST rescued the KCC2 deficit induced by REST alone (Fig. 5C), suggesting that MeCP2 suppressed the inhibitory effect of REST on KCC2 expression. In contrast, the IGF1 treatment failed to rescue the KCC2 deficit induced by REST overexpression (Fig. 5D), suggesting that transcriptional repression of KCC2 by REST is independent of IGF1 signaling. To further test the interactions among MeCP2, REST, and KCC2, we expressed a dominant negative mutant of REST (REST DN) in mouse neurons and found that the KCC2 expression level was not altered (Fig. 5E). Knockdown of MeCP2 induced a significant decrease of KCC2 expression in mouse neurons, as shown above (Fig. 5F). Interestingly, coexpressing REST DN with MeCP2 shRNA significantly rescued the KCC2 deficit induced by MeCP2 shRNA alone (Fig. 5G, quantified data shown in Fig. 5H). Consistent with the KCC2 changes, we found that overexpression of REST shifted $E_{\text{GABA}}$ from $-70$ mV toward $-50$ mV, which was reversed by coexpression with MeCP2 (Fig. 5I–L). These results demonstrate that MeCP2 regulates KCC2 through modulating REST activity.

### Discussion

In this study, we demonstrate that MeCP2 regulates KCC2 expression through REST and ultimately controls GABA functions in neurons. Using human iPSC-derived neurons from different patients with Rett syndrome, we have discovered significant KCC2 deficit in Rett neurons, which hinders the normal GABA functional switch from excitation to inhibition. Because KCC2 is a late onset molecule during early brain development, our discovery may explain why Rett syndrome shows a delayed onset in developing infants.

Deficits in KCC2 expression have been linked to a number of human neuropsychiatric disorders. A disruption in KCC2 mRNA level has been reported in patients with schizophrenia (27, 28). Difference in the expression levels of specific KCC2 transcripts has been linked to schizophrenia and affective disorders (38). We have previously discovered a significant decrease of KCC2 expression induced by a neuregulin 2 mutation found in patients with schizophrenia (39, 40), suggesting a potential role of KCC2 in the pathogenesis of schizophrenia. Altered KCC2 expression has also been implied in stress (41). Recent studies found that inhibiting NKCC1, a chloride transporter with opposite function to KCC2, can be used to treat autism and fragile X syndrome.

![Fig. 4.](image)

**Fig. 4.** Rett neurons derived from a different patient N126I also show deficits in KCC2 and GABA functional switch. (A–C) Representative micrographs showing KCC2 immunoreactivity (red) in WT126 neurons (clone 6, A), human neurons carrying a MeCP2 missense mutation N126I (clone 6, B), or N126I neurons treated with IGF1 (C) at 2-mo culture on astrocytes. Enlarged views of neurons are presented below each micrograph. (D) Quantification of the KCC2 expression level in WT126 CL6, N126I CL6, or N126I CL6 neurons treated with IGF1. (E–G) Representative traces showing GABA-evoked currents under various holding potentials in 3-mo-old WT126 (F), N126I (F), and N126I neurons treated with IGF1 (G). Dashed lines indicate $E_{\text{GABA}}$. (H) Quantified data illustrating the developmental changes of GABA reversal potential during neuronal maturation. Note that N126I neurons did not show typical $E_{\text{GABA}}$ shift as that of WT126 neurons. Data are presented as mean ± SEM **$P < 0.01$, one-way ANOVA with Bonferroni correction.

![Fig. 5.](image)

**Fig. 5.** MeCP2 regulates KCC2 through the transcriptional repressor REST. (A–G) Representative micrographs showing KCC2 immunoreactivity (red) in mouse cortical neurons after different manipulations of REST and MeCP2. Scale bar, 10 μm.) (H) Quantified KCC2 staining intensity in various groups. Note that overexpression of REST significantly decreased the KCC2 expression level, which was rescued by coexpression of MeCP2. (I–K) Representative recording traces illustrating GABA-evoked currents under various holding potentials recorded in neurons transfected with GFP (I), REST (J), or REST + MeCP2. (L) Quantified $E_{\text{GABA}}$ changes after different manipulations of REST and MeCP2 expression levels in mouse neurons. Note that MeCP2 shRNA–induced $E_{\text{GABA}}$ shift was rescued by coexpression with dominant negative mutant of REST (REST DN). Data are presented as mean ± SEM ***$P < 0.001$, ****$P < 0.0001$, determined by one-way ANOVA with Bonferroni correction.
MeCP2 leads to a deficit of KCC2 to inhibition. However, in patients with Rett syndrome, the lack of KCC2 expression takes place in the first postnatal year in humans (32). During human brain development, the KCC2 protein level at birth is very low in the cortical and hippocampal regions and order (20, 32). In the neonatal mouse brain, the KCC2 expression level is very low in the cortical and hippocampal regions and gradually increases to adult level around 2 wk after birth (32). During human brain development, the KCC2 protein level at birth is only about 20% of the adult level. A significant increase in KCC2 expression takes place in the first postnatal year in humans (22, 23). In this study, we discovered that human Rett neurons derived from different patients with Rett syndrome showed a consistent KCC2 deficit and altered GABA functions. Therefore, KCC2 may be an important developmental marker for neuronal maturation. The late onset of KCC2 expression during human brain development coincides with the delayed onset of Rett syndrome in human patients. In fact, this may not be a simple coincidence, given the critical role of KCC2 in neuronal maturation and brain development (29). The developmental time course of KCC2 follows a general rule: KCC2 is late onset, gradually expressed in a caudal-to-rostral order (20, 32). In the neonatal mouse brain, the KCC2 expression level is very low in the cortical and hippocampal regions and gradually increases to adult level around 2 wk after birth (32).

In Rett neurons, MeCP2 deficiency may be a potential drug target for developing a therapy to treat Rett syndrome. Rett syndrome is a neuropsychiatric disorder caused by loss-of-function mutations in the MECP2 gene on the X chromosome. Affected females have severe, progressive cognitive and motor deficits, as well as failure to express normal language and fine motor skills (21, 25). Rett syndrome affects 1 in 15,000 females, and this genetic change is not present in males because they have a single X chromosome. MeCP2 is a protein that binds to DNA sequences called methyl-CpG-binding regions (26). It is involved in the regulation of gene expression and is essential for brain development (27).

In Rett neurons, MeCP2 deficiency leads to a deficit of KCC2, which causes an anomalous depolarizing GABA response (8, 21). GABA is a neurotransmitter that inhibits neuronal activity, and its activity is modulated by KCC2, a chloride/acid exchanger that regulates chloride concentration in neurons (28). The loss of KCC2 expression in Rett neurons causes brain development to stall. Consistent with our study in human Rett neurons, a delayed GABA functional switch has also been reported in mouse models of autism and fragile X syndrome (47, 48). Interestingly, we demonstrate that elevating KCC2 level can reverse the functional deficits caused by MeCP2 deficiency. Therefore, Rett syndrome is potentially treatable with appropriate drugs that can boost the function of KCC2.

MeCP2 regulates the expression of many neuronal genes (5, 6, 16). In this study, we discovered that KCC2 is a key downstream signaling molecule that determines the functional output of MeCP2. Interestingly, MeCP2 regulation of KCC2 is mediated by suppressing REST, a transcriptional repressor that inhibits neuronal genes (see Fig. 6 for our working model). In normal neurons, MeCP2 can bind to the RE-1 site within the KCC2 promoter and prevents REST binding to KCC2 promoter (37). In Rett neurons, where MeCP2 is deficient, REST can bind with an RE-1 site in the KCC2 promoter region as well as an additional RE-1 site in the intronic region of the KCC2 gene to suppress KCC2 expression (37). On the other hand, MeCP2 is known to regulate BDNF (16, 17), which in turn can regulate KCC2 (49). In this study, our data suggest that IGF1 may rescue KCC2 expression in Rett neurons, providing a potential mechanistic explanation for IGF1 treatment of Rett syndrome (13, 19, 50). Therefore, MeCP2 may regulate many downstream signaling molecules, with different signaling pathways that converge onto KCC2, a master regulator of GABA functions during brain development. In fact, restoring KCC2 is emerging as a valuable therapeutic approach (21, 51). Our study suggests that KCC2 may be a potential drug target for developing a therapy to treat Rett syndrome.
and the Department of Defense Foundation; NIH Grant MH092758; and the Department of Defense (WH13140414) (to F.H.G.).

1. Levy SE, Mandell DS, Schultz RT (2009) Autism. Lancet 374(9701):1627–1638.
2. Cohen S, Greenberg ME (2008) Communication between the synapse and the nucleus in neuron development, plasticity, and disease. Annu Rev Rev 34(1):248–289.
3. Chahrour M, Zoghbi HY (2007) The story of Rett Syndrome: From clinic to neurobiology. Neuron 56(3):422–437.
4. Weaving LS, Ellaway CJ, Gecz J, Christodoulou J (2005) Rett syndrome: Clinical review and update. J Med Genet 42(1):1–7.
5. Skene PJ, et al. (2010) Neuronal MeCP2 is expressed at near histone-ocumer levels and globally alters the chromatin state. Mol Cell 37(4):457–468.
6. Cohen S, et al. (2011) Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. Neuron 72(2):172–85.
7. Tudor M, Akbarian S, Chen RZ, Jaenisch R (2002) Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. Proc Natl Acad Sci USA 99(24):15536–15541.
8. Chahrour M, et al. (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320(5888):1224–1229.
9. Dani VS, Nelson SB (2009) Intact long-term potentiation but reduced connectivity between neocortical layer 5 pyramidal neurons in a mouse model of Rett syndrome. J Neurosci 29(36):11263–11270.
10. Hao HT, Zhang YH, Rosenmund C (2007) MeCP2 controls excitatory synaptic strength by regulating glutamatergic synaptic number. Neuron 56(1):58–65.
11. Krishnan K, et al. (2015) MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. J Neurosci 35(12):4782–4791.
12. Hao HT, et al. (2010) Dysfunction in GABA signaling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 468(7321):263–269.
13. Manchette MC, et al. (2010) A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell 143(6):527–539.
14. Ricciardi S, et al. (2012) CDKL5 ensures excitatory synapse stability by reinforcing NGL-1-PSD95 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. Nat Cell Biol 14(9):911–923.
15. Li Y, et al. (2010) Global transcriptional and translational repression in human embryonic-stem-cell-derived Rett syndrome neurons. Cell Stem Cell 10(3):446–458.
16. Zhou Z, et al. (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bid/g transcription, dendritic growth, and spine maturation. Neuron 52(2):255–269.
17. Chang Q, Khare G, Dani V, Nelson S, Jaenisch R (2006) The disease progression of MeCP2 mutant mice is affected by the level of BDNF expression. Proc Natl Acad Sci USA 103(8):2547–2552.
18. Bleges P, Airaksinen MS, Rivera C, Kaila K (2009) Cation-chloride cotransporters and synaptic inhibition in the brain decreases BDNF levels by REST/CoREST-mediated repression and increases TRKA production. Epigenetics 4(2):214–222.
19. Yeo M, Berglund K, Augustine G, Liedke W (2009) Novel repression of Kcc2 transcription by REST-RE-1 controls developmental switch in neuronal chloride. J Neurosci 29(46):14652–14662.
20. Tao R, et al. (2012) Transcript-specific associations of SLC1A2AS (KCC5) in human prefrontal cortex with development, schizophrenia, and affective disorders. J Neurosci 32(15):5216–5222.
21. Sun C, et al. (2011) Identification and functional characterization of rare mutations of the neurtlin-2 gene (NLGN2) associated with schizophrenia. Hum Mol Genet 20(5):1054–1064.
22. Sun C, Zhang L, Chen G (2013) An unexpected role of neuropilin-2 in regulating KCC2 and GABA functional switch. Mol Brain 6:23.
23. Hewitt SA, Wamsteker Jr, Kurt EU, Bains JS (2009) Altered chloride homeostasis and synaptic inhibition is a constraint of the stress axis. Nat Neurosci 12(4):438–443.
24. Lemmonier E, et al. (2012) A randomised controlled trial of bumetanide in the treatment of autism in children. Transl Psychiatry 2:e202.
25. Lemmonier E, et al. (2013) Treating Fragile X syndrome with the diuretic bumetanide: A case report. Acta Paediatr 102(9):e288–e290.
26. Kim JY, et al. (2012) Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. Cell 148(5):1051–1064.
27. Cancedda L, Fiuzelli H, Chen K, Poo MM (2007) Exocytotic GABA action is essential for morphological maturation of cortical neurons in vivo. J Neurosci 27(19):5224–5235.
28. Horn Z, Ringstedt T, Blesse P, Kaila K, Herenstein E (2010) Premature expression of KCC2 in embryonic mouse perturbs neuronal development by an ion transport-independent mechanism. Eur J Neurosci 31(12):2142–2155.
29. He G, Nomura T, Xu J, Contractor A (2014) The developmental switch in GABA uptake is delayed in fragile X mice. J Neurosci 34(2):446–450.
30. Tyszio R, et al. (2014) Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. Science 343(6171):675–679.
31. Rivera C, et al. (2002) BDNF-induced TrkB activation down-regulates the K+Cl cotransporter KCC2 and impairs neuronal Cl- extrusion. J Cell Biol 159(5):747–757.
32. Castro J, et al. (2014) Functional recovery with recombinant human g1F1 treatment in a mouse model of Rett syndrome. Proc Natl Acad Sci USA 111(27):9941–9946.
33. De Koninck Y (2007) Altered chloride homeostasis in neurological disorders: A new target. Curr Opin Pharmacol 7(1):93–99.
34. Tang X, et al. (2013) Astroglial cells regulate the developmental timeline of human neurons differentiated from induced pluripotent stem cells. Stem Cell Res (Amst) 11(2):743–753.
35. Jiang M, Chen G (2009) Ca2+ regulation of dynamin-independent endocytosis in cortical astrocytes. J Neurosci 29(25):8063–8074.
36. Deng L, et al. (2007) Sequential post synaptic maturation governs the temporal order of GABAergic and glutamatergic synaptogenesis in rat embryonic cultures. J Neurosci 27(40):10860–10869.
37. Cichon J, et al. (2012) Gclfin aggregation blocks intracellular trafficking and induces synaptic loss in hippocampal neurons. J Biol Chem 287(6):3919–3929.
38. Yao L, Qi J, Chen G (2006) Actin-dependent activation of presynaptic silent synapses contributes to long-term synaptic plasticity in developing hippocampal neurons. J Neurosci 26(31):8137–8147.
39. Jiang M, Chen G (2006) High Ca2+-phosphate transfection efficiency in low-density neuronal cultures. Nat Protoc 1(2):595–700.
40. Chen ZF, Paquette AJ, Anderson DJ (1998) NRSF/REST is required in vivo for repression of multiple neuronal target genes during embryogenesis. Nat Genet 20(2):136–142.