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***MEF2C* Haploinsufficiency features consistent hyperkinesis, variable epilepsy, and has a role in dorsal and ventral neuronal developmental pathways**

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Abstract

MEF2C haploinsufficiency syndrome is an emerging neurodevelopmental disorder associated with intellectual disability, autistic features, epilepsy, and abnormal movements. We report 16 new patients with MEF2C haploinsufficiency, including the oldest reported patient with MEF2C deletion at 5q14.3. We detail the neurobehavioral phenotype, epilepsy, and abnormal movements, and compare our subjects with those previously reported in the literature. We also investigate Mef2c expression in the developing mouse forebrain. A spectrum of neurofunctional deficits emerges, with hyperkinesia a consistent finding. Epilepsy varied from absent to severe, and included intractable myoclonic seizures and infantile spasms. Subjects with partial *MEF2C* deletion were statistically less likely to have epilepsy. Finally, we confirm that *Mef2c* is present both in dorsal primary neuroblasts and ventral gamma-aminobutyric acid(GABA)ergic interneurons in the forebrain of the developing mouse. Given interactions with several key neurodevelopmental genes such as *ARX*, *FMR1*, *MECP2*, and *TBR1*, it appears that *MEF2C* plays a role in several developmental stages of both dorsal and ventral neuronal cell types.

Keywords

MEF2C haploinsufficiency; Intellectual disability; Autism; Infant-onset myoclonic epilepsy; Infantile spasms; Hyperkinesia; Deletion 5q14.3

Introduction

A number of patient series have described the major symptoms of haploinsufficiency of *MEF2C* at 5q14.3 (OMIM No. 613443) [1–8]. These include epilepsy, intellectual disability (ID), and autistic features such as absent speech, impaired social reciprocity, and stereotypical movements [9]. The details and variability of the major features, particularly the epilepsy, in *MEF2C* haploinsufficiency have not been systematically determined. Children with *MEF2C* mutations also have abnormal movement patterns, but video examples of these movements and their evolution across the lifespan have not been published. Finally, although patients with *MEF2C* haploinsufficiency have neurobehavioral deficits, there has not been a broad survey of neurologic function in a large number of these patients to date. Therefore, key aspects of this disorder remain incompletely characterized.

MEF2C is a transcription factor with changing expression patterns during brain development that correlate with roles in both early neuroprogenitor development [10, 11] and later during neuronal maturation [12–14]. There are three alternative exons capable of generating six *Mef2c* isoforms in the mouse, and the *Mef2c*¹ isoforms are expressed in neurons [15]. Complete loss of *Mef2c* is embryonic lethal, but conditional knockouts have demonstrated a role for *Mef2c* in both gamma-aminobutyric acid (GABA)ergic and glutamatergic pyramidal neuron migration [16], and in maintenance of synapse stability and function [17]. At early embryonic periods, *Mef2c* is a subplate and cortical plate marker [18], and postnatally, it is present throughout the cortical plate.

The expression of *Mef2c* has been monitored in a number of developmental gene knockout models. *Mef2c* expression is reduced in the *Tbr1*-knockout mouse, indicating that *Mef2c* plays a role in *Tbr1* modulation of cortical layering [18]. *Mef2c* expression in the mouse forebrain is also reduced in the absence of *Arx* [19] and *Dlx1/2* [20] suggesting that *ARX* and *MEF2C* are members of a transcriptional regulatory network in GABAergic interneurons. Additionally, the expression of two other important neurodevelopmental genes—*CDKL5* and *MECP2*—is altered in children with mutations in *MEF2C* [7]. *MEF2C* plays a role in forebrain development—both dorsally and ventrally—but knowledge about its specific role in multiple neuronal developmental pathways remains incomplete.

We seek to clarify several of these outstanding issues by reporting 16 new subjects with *MEF2C* haploinsufficiency syndrome and detailing their neurologic phenotype. We report epilepsy subtypes, response to treatment and outcome, illustrate a consistent hyperkinetic movement disorder with video, and detail the extent of neurofunctional deficits in our subjects. We present our data in the context of the 27 *MEF2C* haploinsufficiency subjects in the published literature and where possible identify common and discordant features from this combined expanded cohort. To further clarify the potential mechanisms of the neurodevelopmental disorder in *MEF2C* haploinsufficient individuals, we re-demonstrate that *Mef2c* is present in both neuroblasts and GABAergic inhibitory interneurons in the forebrain of the developing mouse, with significant expression in GABAergic interneurons at embryonic day 14.5. Then, we perform a meta-analysis on publicly available data on the interactions of *Mef2c* with the neurodevelopmental genes *Arx*, *Dlx1/2*, *Mecp2*, and *Tbr1*. From these data, we conclude that *MEF2C* plays a role in multiple neuronal developmental pathways, and that the clinical phenotype likely reflects abnormalities of both dorsal and ventral forebrain development.

Methods

Patients

Subjects were identified through the Infantile Spasms Registry & Genetic Studies (ARP), search of a database of clinically obtained array CGH (aCGH) results at Signature Genomic Laboratories (LGS), or review of positive aCGH findings at collaborating pediatric neurology, genetics, and neurogenetics clinics. Retrospective records from 16 patients with *MEF2C* haploinsufficiency were reviewed, including EEG data, brain imaging, and physician descriptions of abnormal movements. Care was taken to ensure that non-epileptic movements were appropriately documented as such by the patient's primary neurologist. Video of behavior and movements was reviewed in eight of the subjects. Movements were classified using standardized methods [21, 22]. Neuro-behavioral and neurofunctional deficits were reviewed in 14 of the 16 families by telephone, or by using available records if parents were not available. The study was approved by the institutional review boards of Washington University, University of Rochester Medical Center, Seattle Children's Hospital, and IRB-Spokane, including written consent to publish photographs and video.

Statistical analysis

t test comparing deletion size (partial or complete *MEF2C* deletion) and the presence of epilepsy, abnormal movements, or specific neurofunctional deficits was performed using R version 2.13.1.

Microarray-based comparative genomic hybridization (aCGH)

Microarray analysis was performed on genomic DNA between subjects LR11-305, LR11-306, LR11-307, LR11-308, and LR11-309 on a custom 105K-feature whole genomic microarray (Agilent Technologies, Santa Clara, CA, USA) as previously described [23]. Other subjects were assayed on a variety of clinically available platforms including Illumina BeadChip 6.0 (LR11-312), Illumina HumanQuad610 BeadChip SNP array (LR11-387), Affymetrix Whole-Genome 2.7M SNP array (LR11-325), GeneDx "GenomeDx" v1.0 oligo array (LR11-388), a custom Agilent oligo array with 40K features (LR12-031), 44K oligo array (LR12-013 and LR12-275), 180K oligo array (LR12-022), and 244K oligo array (LR12-021) according to the manufacturer instructions. Chromosome 5q14.3 deletions detected by aCGH were visualized with metaphase fluorescence in situ hybridization (FISH) using one or more BAC clones located within the abnormal regions as determined by aCGH, according to standard clinical protocols. Confirmatory FISH was not performed in subject LR11-325 with a large 6-Mb deletion. The deletion in subject LR11-388 was confirmed by quantitative PCR (qPCR) for exon 45 of *GPR98*. All studies were obtained as part of routine clinical evaluations, except for IS09-024, who was studied on an Affymetrix SNP array as part of a research protocol.

Animal assurance

All experiments were approved by and carried out in accordance with the Animal Care and Use Committee of the Children's Hospital of Philadelphia. All mice were housed in a room with 12-h light–dark cycle and had continuous access to food and water.

Mef2c staining methods

The immunohistochemical experiments were performed on either C57/B16 mice or Dlx5/6 cre-reporter mice (Dlx5/6^{CRE-ires-GFP}) that were on a C57/B16 background. Timed breeding between C57/B16 mice was performed, and embryos and pups were harvested at embryonic day 14.5 (e14.5). The brains were dissected out of the embryos and fixed in 4 % paraformaldehyde for 4–8 h. For older animals of P14 and adult ages (>P45), the animals

were transcardially perfused, and then the brains were post-fixed overnight. The brains at all ages were cut on a cryostat at 14–18 μ . Sections were processed for immunohistochemistry as previously published [24]. For Mef2c staining, goat anti-Mef2c antibody (Santa Cruz Cat No. sc-13268 and Lot No. K2009) was used. The antibody (1:1,000 dilution in 10 % donkey serum) was incubated overnight at 4 °C. After three washes in PBS, a biotinylated (anti-goat) secondary antibody (1:2,000) dilution was applied for 30 min, followed by an avidin-conjugated to Cy3 fluorophore (1:500 dilution), both at room temperature. Tissue was visualized using a Leica DMR microscope (Leica Microsystems, Bannockburn, IL, USA) equipped with epifluorescence and light microscopy. Images were acquired on an Orca digital camera (Hamamatsu, Hamamatsu City, Japan) and processed with Image-Pro software (Media Cybernetics, Bethesda, MD, USA). White balance, auto contrast, and stitching of images were performed with Adobe Photoshop (Adobe Systems).

Gene expression arrays

The methods for the microarray data were published previously and are publicly available via the NIH neuroscience microarray consortium (<http://np2.ctrl.ucla.edu/np2/home.do>). Briefly, a Dlx5/6 reporter mouse line with GFP expression in migrating interneurons was used. Fluorescence-activated cell sorting (FACS) of GFP+ cells from the ganglionic eminence (GE) and cortex was performed. The array data were processed in Partek, and a false discovery rate (FDR) calculation was performed using a standard *p* value of 0.01. For this study, the *Mef2c* probes were queried for presence in migrating interneurons (Cortex+) versus non-interneurons (Cortex-) and both compared to GFP+ cells in the GE (GE+).

Analysis of publicly available gene expression array data

Data from Arx and Dlx1/2 knockout experiments were obtained by reviewing the published papers [19, 25, 26] and querying the relative expression and *p* values for the *Mef2c* probes in the available source arrays (see Online resources). We also reviewed the publicly available data from the *Tbr1* knockout mouse [18] and mouse models of *Mecp2* under- and over-expression [27].

Results

Patients

The key phenotype and genotype characteristics of the 16 subjects are shown in Table 1. They include 15 subjects with deletions of 5q14.3 containing *MEF2C* and one patient with a novel frameshift mutation in *MEF2C*. The genomic breakpoints of the subjects with 5q14.3 deletions are illustrated in Fig. 1.

Overall, dysmorphic features were not predominant, but some patients exhibited subtle hypertelorism, a “cupid-bow” shape to the upper lip, and hypotonic facies (Fig. 2a–g). The deletions in three subjects included *RASA1*, implicated in the “5q14.3 neurocutaneous syndrome” [2], but only one subject in our cohort (LR12-031) had characteristic capillary malformation of the skin (Fig. 2h). This subject also had atrophic skin adjacent to the suprasternal notch. All subjects had global developmental delay, and none had a phase of regression. All subjects had a normal head circumference.

On brain imaging, there were no pathognomonic features, but several of the subjects had mild asymmetry of the lateral ventricles, increased periventricular white matter signal, dysgenesis of the corpus callosum, and mild cerebellar vermis hypoplasia (Fig. 3). Subject LR11-325, with a 6.0-Mb deletion of 5q14.3 extending into the critical region reported for peri-ventricular nodular heterotopia [28], had a normal brain MRI.

The most consistent neurologic features of *MEF2C* haploinsufficiency reported in the literature are (1) epilepsy, (2) abnormal movement patterns, and (3) a neurobehavioral “autism-plus” phenotype. We therefore focused our attention on these outcomes as the core features of *MEF2C* haploinsufficiency syndrome. Other clinical details of the 16 subjects are found in the Supplementary Clinical Data.

Epilepsy types and ages of onset

Ten of the 16 subjects (63 %) had epilepsy (defined as two or more unprovoked seizures), and six of these had onset of epilepsy in either infancy or soon after the first year of life. Representative EEG abnormalities are shown in Fig. 4. Two subjects had infantile spasms (classic epileptic spasms with hypsarrhythmia) alone, two subjects had myoclonic seizures with onset during infancy (myoclonic seizures, multifocal spikes on EEG, without hypsarrhythmia), and two subjects (LR11-307 and LR11-312) first presented with myoclonic seizures that then evolved into infantile spasms. The other four subjects with epilepsy had onset during childhood (defined as after 18 months of age), and these were intractable in all. Six of the 16 subjects had no diagnosis of epilepsy, including LR11-308 who at the age of 46 years had never had a seizure. Subjects LR11-387 and LR12-013 had multiple febrile seizures, but did not develop epilepsy. The four subjects with partial deletion of *MEF2C* (LR11-387, LR12-013, LR12-021, and LR12-022) did not have epilepsy, and this was statistically significant compared to the prevalence of epilepsy among our subjects with full deletion ($p < 0.0005$).

When combined with the previously reported subjects with *MEF2C* haploinsufficiency, we confirm the frequent presence of infantile spasms and infant-onset myoclonic epilepsy (Table 2). The majority (54 % of all subjects) had an infant-onset epilepsy, either myoclonic seizures (33 %) or infantile spasms (21 %). Other subjects reported here and in the literature had childhood-onset epilepsy (24 %), with 12 % having intractable epilepsy and 12 % controlled by medication. A few patients (12 %) had febrile seizures, and across all patients reported to date, 23 % had no diagnosis of epilepsy.

Abnormal movement patterns

Nearly all (93 %) of the subjects in this report presented with hypotonia as a prominent feature during infancy, and then developed abnormal movements with paroxysms of excess motion best classified as hyperkinesia [22]. During infancy, this pattern may be subtle, but still discernible in some patients (Video 1). By later childhood, the hyperkinesia manifests as a series of rapid stereotypies that are prominent when the child is excited and may interrupt ongoing activity (Video 2). As nearly all of the children in this series had severe ID, it was difficult to assess whether the stereotypies could be voluntarily suppressed. However, the hyperkinetic movements varied in response to external stimuli. At times, the stereotypies were accompanied by dystonia or chorea. Parents and care providers commented on the omnipresence and often constant nature of the stereotypies. The hyperkinesia appears to moderate over time, as several care providers reported they lessened as the children aged. Video from 46-year-old subject LR11-308 was notable for hypokinetic spasticity with intermittent hand-wringing stereotypies (not shown).

Neurobehavioral and neurofunctional outcomes

We obtained detailed data on neurobehavioral and neurofunctional impairments in 14 of the 16 subjects with *MEF2C* haploinsufficiency, and the common features are shown in Table 3. Most subjects had significant abnormalities of gastrointestinal motility, including gastroesophageal reflux disease, dysphagia, and constipation. All subjects were a verbal with normal hearing, indicating severe impairment of language development. Most parents reported their children were prone to inappropriate laughter. The exception was subject

LR11-307 who was easily agitated and engaged in self-mutilating behaviors. Several subjects had a phase of early irritability during infancy, replaced by a generally happy demeanor. Most parents also reported their children had high pain tolerance, manifested as not crying during intramuscular immunizations or after injuries. Poor reciprocal behaviors were common, with lack of or avoidance of eye contact, poor visual tracking, and limited engagement with or recognition of other people. Six subjects had poor sleep initiation and/or maintenance. Breathing rhythm, satiety, and temperature regulation were reported as normal in all subjects, aside from subject LR11-307 who had persistent episodes of hyper/hypoventilation and LR12-013 who had frequent breath-holding behavior.

Analysis of published array data

In order to better understand the role of MEF2C in the developing forebrain, we examined the microarray data files from several publications that used expression arrays in mice designed to study genes putatively upstream of *Mef2c* expression [19, 20, 25]. We confirmed that *Mef2c* expression was diminished in the *Dlx1/2* knockout mouse. Consistent with the report that *MECP2* expression is diminished in *MEF2C* haploinsufficient patients [7], we found that *Mecp2* expression is similarly reduced in the *Dlx1/2* knockout mouse forebrain, suggesting a network relationship between *Dlx1/2*, *Mef2c*, and *Mecp2* (Supplementary Tables 1 and 2). Unfortunately, no probes for *Cdk15* were present on the Affymetrix arrays used, so we could not evaluate *Cdk15* expression in the *Dlx1/2* knockout mouse.

Mef2c expression and localization in mouse forebrain neuronal populations

Due to evidence that *Mef2c* is involved in the development of both dorsal glutamatergic neurons and ventral GABAergic interneurons, we assayed the neuronal cell-type specific *Mef2c* gene expression and staining pattern in the mouse forebrain. *Mef2c* expression was significant in migrating interneurons versus non-interneurons in the cortex at e14.5, when compared to GFP-positive control cells in the ganglionic eminence (GE), as summarized in Table 4. However, *Mef2c* staining was seen in both *Dlx5/6*(+) interneurons in the GE, as well as in neurons elsewhere in the cortex at e14.5 (Fig. 5). Staining for *Mef2c* at P14 revealed scattered neuronal labeling, but more prominent in the upper layers of the cortex. The immunohistochemical staining at both ages was in agreement with in situ data from published sources (<http://developingmouse.brain-map.org/data/search/gene/index.html?term=Mef2c>).

Discussion

MEF2C haploinsufficiency syndrome is an emerging neuro-developmental disorder with only 27 previously published cases. One patient with deletion of a putative regulatory region upstream of *MEF2C* [29] and another patient with an unbalanced chromosomal translocation [30] have also been reported. We describe an additional 16 subjects with the goal of furthering the characterization of the neurologic phenotype seen in this disorder. The core phenotype includes consistent hyperkinesia, hypotonia, intellectual disability with deficits in verbal language, and alterations in GI motility, mood, pain tolerance, and social reciprocity. Surprisingly, though epilepsy is a major feature, it is not uniform but variable in both its severity and age of onset and not present in one-fifth of the patients.

From the combined phenotype data that includes our new patient series and those already published, a spectrum can be described ranging from those with severe epilepsy/consistent hyperkinesia to those with no epilepsy or milder epilepsy who nevertheless have consistent hyperkinesia. Only six out of 16 subjects in this report had infant-onset myoclonic seizures and/or electroclinically defined infantile spasms. An important observation from our data is

that, despite the frequency of early-onset epilepsies, those children with later onset, milder seizures, or no epilepsy at all also had severe neurobehavioral and neurofunctional outcomes. While there was no obvious correlation between severity of the neurologic phenotype and deletion size, children in our cohort with partial deletion of *MEF2C* were significantly more likely not to have epilepsy. It is possible that effects on alternative splicing of the neuronal *MEF2C* isoform may affect the severity of epilepsy in subjects with *MEF2C* haploinsufficiency.

Hyperkinesia was seen consistently among the patients in this report, regardless of deletion size and whether epilepsy was present or not. The hyperkinesia observed in *MEF2C* haploinsufficiency patients is similar to the movement disorders described in children with a number of other developmental disorders due to a variety of genes. For example, children with *ARX* mutations often have a notable movement disorder [31, 32], and hyperkinesia and stereotypic movements have been documented in patients with both duplications and deletions of *FOXG1* [33, 34]. Additionally, although patients with Rett syndrome have characteristic stereotyped movements [35, 36], the quality of these movements is distinct from those seen in individuals with *MEF2C*-related disorder. Together, we hypothesize that certain types of abnormal movements are a common feature among children with abnormalities of forebrain-expressed transcription factors, and the movement disorders seen in these patients may arise from deficits in a shared gene regulatory network.

MEF2C appears to be a marker neither for the development of dorsal-restricted nor ventral-restricted neuronal populations. Our meta-analysis of the gene expression data, as well as our immunostaining data across multiple stages of mouse forebrain development, suggest a more complex picture. While *Mef2c* expression is diminished in the context of *Arx* and *Dlx1/2* deficiency, *Mef2c* staining is not restricted to ventral GABAergic interneuron populations. *Mef2c* expression during forebrain development therefore appears to begin in both dorsal primary neuroblasts and ventral GABAergic interneurons, but then during development is more prevalent among mature glutamatergic neurons. This is consistent with its known role as a marker of *Tbr1*-mediated cortical lamination [18]. This also confirms other forebrain gene expression studies where, although *Mef2c* was associated with two modules of interneuron development, the strength of these associations was insufficient to formally assign *Mef2c* to either of those modules [26]. The extent of interaction of *Mef2c* in the *Dlx1/2–Arx* pathway in GABAergic interneurons, the *Tbr1* cortical neuron developmental pathway, *Mecp2*-associated pathways, and reported *MEF2C* interactions with *CDKL5* and *MECP2* are summarized in Fig. 6. Additionally, *MEF2C* interactions with the causative gene for Fragile X syndrome *FMR1* appear to be important for post-developmental synaptic maturation [37]. The array and immunohistochemical data presented confirm the involvement of *MEF2C* in both dorsal and ventral neuronal development, and suggest a compound role for *MEF2C* in the generation of a complex neurodevelopmental disorder.

We present a large series of *MEF2C* haploinsufficiency patients and provide detailed description of the neurologic phenotype. These data illustrate a recurring clinical profile of *MEF2C* haploinsufficiency distinct from other severe neurodevelopmental disorders such as Rett syndrome and *FOXG1*-related disorders. This profile specifically includes a consistent hyperkinetic movement disorder, intellectual disability with impairment of spoken language, reciprocal behavior, GI motility, mood, and pain tolerance. Breathing rhythm abnormalities were uncommon, and there were no obvious impairments of the autonomic nervous system. The types and severity of epilepsy are variable, and the absence of epilepsy is significantly correlated with partial deletion of *MEF2C*. Overall, these data suggest the phenotypic features of *MEF2C* haploinsufficiency may be a reflection of dysregulation of development of multiple neuronal cell populations at the transcriptional level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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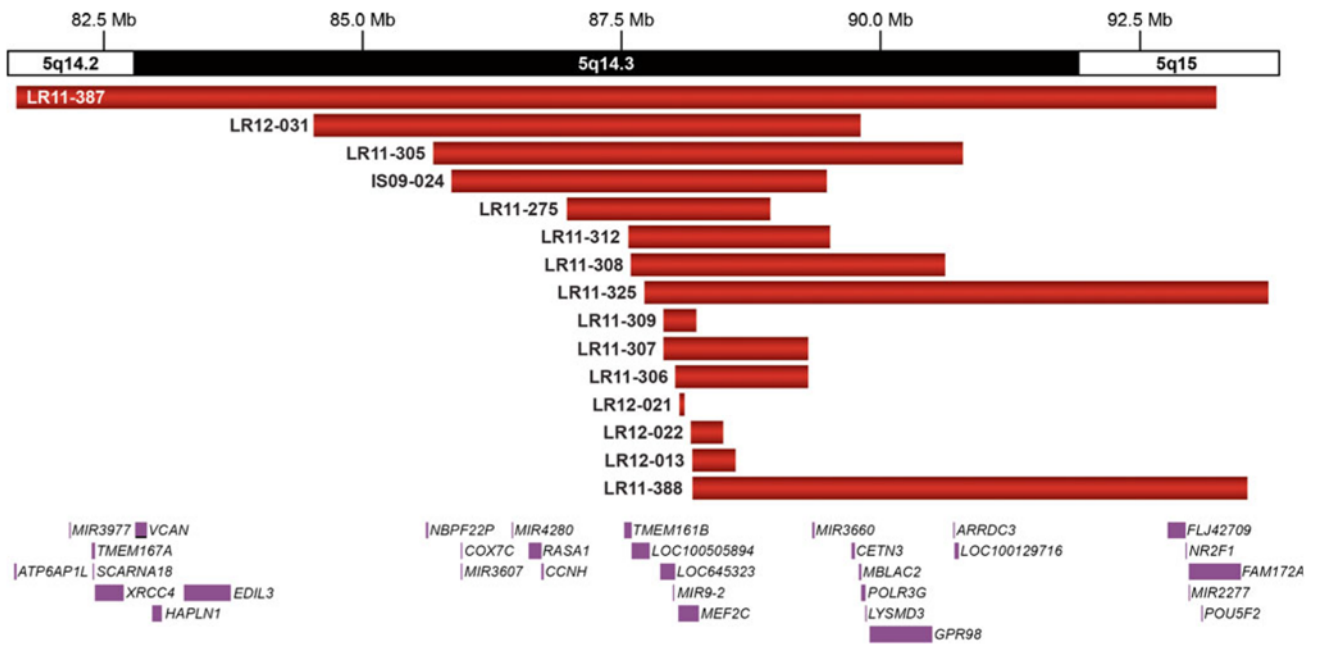


Fig. 1. Genomic data showing deletions (*red*) of 5q14.3 in 15 subjects reported in this paper



Fig. 2. Photographs of subjects. **a** IS09-024 at age 11 years. **b** LR11-305 at 12 months. **c** LR11-306 at 5 years, 3 months. **d** LR11-308 at 46 years. **e** LR11-309 at 17 months. **f** LR11-312 at 5 months. **g** LR11-325 at 6 months. Note characteristics of tenting of upper border of the mouth and hypotonic facies in several subjects. **h** Characteristic capillary malformation on the lower extremity of LR12-031

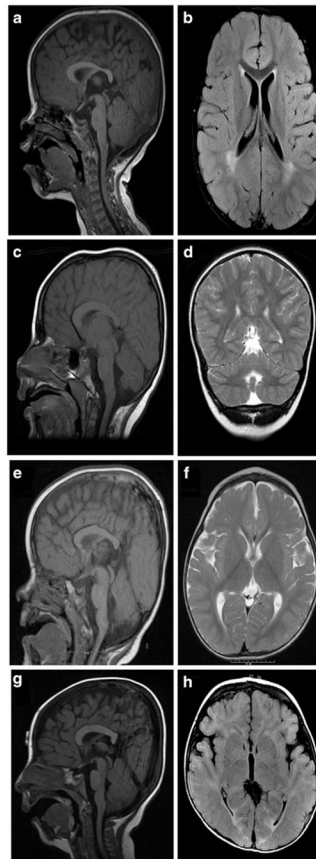


Fig. 3. Representative brain imaging findings. Subject LR11-047 has normal midline sagittal structures (**a**), and posterior periventricular white matter hyperintensities on axial T2 FLAIR (**b**). Subject LR11-307 has a dysmorphic corpus callosum and mild cerebellar vermis hypoplasia (**c**), with the cerebellar vermis disproportionately small compared to the cerebellar hemispheres on T2 coronal (**d**). Subject LR11-310 has normal midline sagittal structures (**e**) and mild thinning of the cortical white matter on T2 axial (**f**). Subject LR11-312 has frontal bossing and brachycephaly (**g**), as well as mild cortical atrophy and thinning of the white matter visible on T2 axial (**h**)

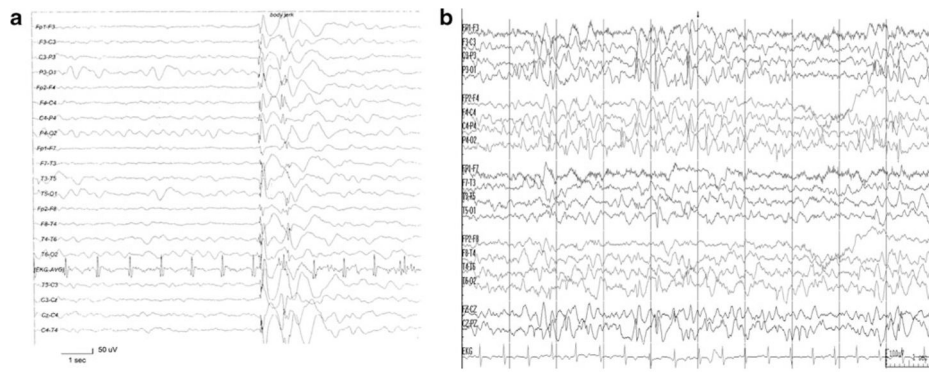


Fig. 4. Representative EEG abnormalities in subjects with *MEF2C* haploinsufficiency showing spike-wave associated with epileptic spasm in subject IS09-024 (a) and multifocal epileptiform activity and poorly developed anterior-posterior gradient in subject LR11-310 (b)

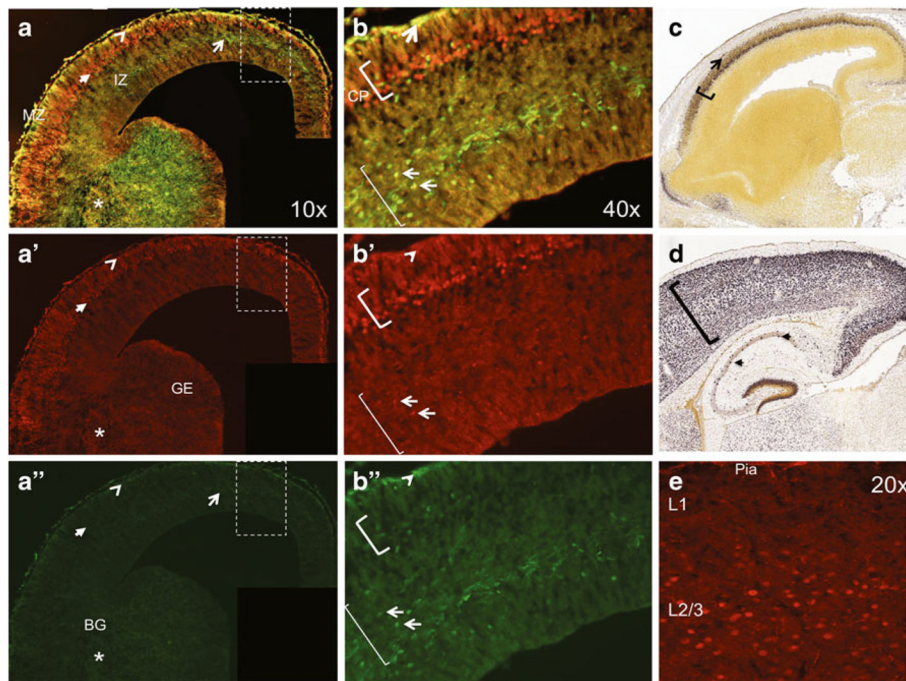


Fig. 5. Mef2c is present in both developing excitatory and inhibitory neurons throughout development. At embryonic day14 (e14.5), Mef2c-positive cells (*red* in *A*, *B*) are found in the developing cortical plate and basal ganglia. Dlx5/6-positive cells (developing interneurons; *green* in *A*, *B*) are present in the SVZ of the ganglionic eminence (GE), migrating into the cortex in the intermediate zone (IZ, *thin bracket* in *B*) and the marginal zone (MZ, labeled in *A*), as well as having entered the cortical plate (CP, *thick bracket* in *B*). There is co-localization between Mef2c(+) cells and Dlx5/6 cells, primarily in the MZ, but also in the CP and SVZ. Expansion of the *boxed area* in *A* (*A* and *A'*) is shown in *B*. There is more substantial colocalization in the forming basal ganglion (*asterisk*). This staining pattern for Mef2c is consistent with the Allen Brain Atlas at e14.5 (*C*) as is mostly found in the CP but also in the MZ (*bracket* and *arrows* in *C*). In the mature brain, Mef2c is located throughout the cortex both by in situ hybridization (*brackets* in *D*) and immunohistochemistry (*E*) and in inter-neurons in the hippocampus (*arrows* in *D*)

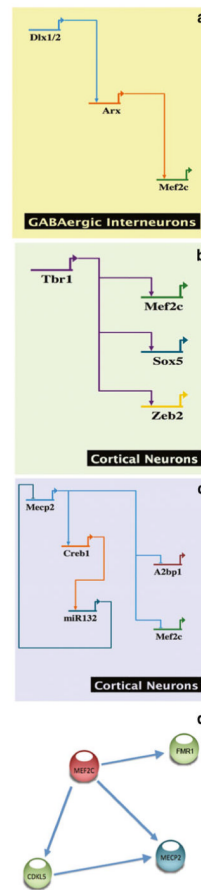


Fig. 6. Summary of the published data showing gene regulatory relationships for *Mef2c* in the *Dlx1/2-Arx* pathway in GABAergic inter-neurons [19, 20] (a), the *Tbr1* cortical neuron developmental pathway [18] (b), *Mecp2*-associated pathways [27] (c), and reported *MEF2C* interactions with *CDKL5* and *MECP2* [7], as well as interactions with *FMR1* during synaptic development [37] (d)

Table 1

Genomic data and key features of movement disorder and epilepsy for 15 subjects with *MEF2C* deletion and one subject (LR11-310) with frameshift mutation in *MEF2C*

Subject	Age/Sex	Genomic event/size (Mb)	Genomic coordinates chr5 (Hg18)	Abnormal movements	Epilepsy type/age onset
IS09-024	13 years/M	Del/3.6	85855118-89474751	Dystonia, stereotypies (hand flapping), nonambulatory	ISS/6 months
LR11-305	11 months/F	Del/5.11	85684257-90798560	Dystonia	Myoclonic/11 months
LR11-306	5 years, 3 months/M	Del/1.0	88018766-89063989	Stereotypies (hand flapping, chin rubbing), nonambulatory	Type unknown/onset after 1 year
LR11-307	13 years/F	Del/1.38	87905325-89289023	Stereotypies (hand-flapping, rocking) abnormal gait with pes planus and valgus deformity	Myoclonic at 4 months/ISS by 9 months
LR11-308	46 years/M	Del/3.02	87591751-90619421	Spasticity, nonambulatory	No epilepsy
LR11-309	17 months/F	Del/0.32	87905325-88220403	Hyperkinesia, bruxism, does not roll or lift head	Myoclonic and generalized/13 months
LR11-310	22 months/F	Frameshift mutation	c833delT	Stereotypies (hand-wringing), began walking at 22 months	Myoclonic and atonic/18 months
LR11-312	5 years, 5 months/F	Del/1.95	87566009-89505509	Hyperkinesia, stereotypies (rocking, side-to-side head movements)	Myoclonic and ISS/3 months
LR11-325	6 months/M	Del/6.0	87719139-93736389	Hyperkinesia	ISS/4 months
LR11-387	6 years/M	Del/11.6	81657245-93240731	Hyperkinesia, dystonia, can take steps with gait trainer	No epilepsy, febrile seizures
LR11-388	5 years, 6 months/F	Del/5.4	88185348-93546896	Hyperkinesia, stereotypies (hand flapping, head shaking)	Myoclonic/2 years
LR12-013	7 years/M	Del/0.41	88177038-88592311	Hyperkinesia, stereotypies (arm flapping, bruxism)	No epilepsy, febrile seizures
LR12-021	30 months/M	Del/0.05	88051970-88104535	Repetitive back arching, stereotypies (hand batting, head shaking, bruxism)	No epilepsy
LR12-022	7 years/F	Del/0.30	88167504-88472051	Hand tremor, stereotypies (hand flapping, waving hands in front of face, bruxism)	Single generalized seizure, no epilepsy
LR12-031	6 years/M	Del/5.2	84520000-89800000	Hyperkinesia, back arching, stereotypies (waving hands in front of eyes)	Myoclonic/6 months
LR12-275	21 months/M	Del/2.0	86972414-88928741	Hyperkinesia, stereotypies (head shaking, leg kicking)	No epilepsy

M male, F female, Del/deletion, ISS infantile spasms

Table 2

Reported epilepsy and seizure phenotypes in 27 published patients with *MEF2C* haploinsufficiency and 16 subjects from this series

Reference	Infantile spasms	Infant-onset myoclonic epilepsy	Childhood-onset epilepsy, intractable	Childhood-onset epilepsy, controlled	Febrile seizures	No epilepsy ^b
[28]	1	0	0	0	0	0
[4]	1	2	0	1	0	3
[3]	0	1	0	0	1	0
[7]	1	4	0	0	1	0
[8]	0	0	0	0	0	1
[6]	1	1	1	1	0	0
[5]	1	1	0	0	0	0
[1]	0	0	0	1	0	0
[2]	0	1	0	0	0	0
[38]	0	0	0	1	0	0
[39]	0	0	0	1	1	0
Current series	4 ^a	4 ^a	4	0	2	6
Total (%)	9 (21)	14 (33)	5 (12)	5 (12)	5 (12)	10 (23)

^aSubjects LR11-307 and LR11-312 had both infantile spasms and infant-onset myoclonic epilepsy

^bEpilepsy is defined as two or greater unprovoked (i.e., afebrile) seizures

Table 3
Key neurobehavioral and neurofunctional features for 14 subjects for whom detailed data could be obtained

Subject	Breathing	GI	Language	Mood	Pain response	Reciprocity	Sleep
IS09-024	Normal	GERD	Averbal, no meaningful communication	Generally happy, with inappropriate laughter	High pain tolerance	Poor eye contact	Normal
LR11-305	Normal	Feeding difficulties in infancy	Babbles	ND	ND	No visual fixation	ND
LR11-306	ND	Normal	Averbal, no meaningful communication	ND	Normal	Diminished responses	Normal
LR11-307	Hyperventilation/hypoventilation	Constipation	Averbal, no meaningful communication	Easily agitated, with self-mutilating behaviors	High pain tolerance	Poor attention, inconsistent eye contact, no pointing	Disrupted
LR11-308	ND	ND	Averbal, no meaningful communication	Generally happy	ND	Poor eye contact	ND
LR11-309	ND	ND	Averbal vocalizations only, no meaningful communication	ND	ND	Poor visual tracking	ND
LR11-312	Normal	Slow gastric emptying, GERD, constipation	Averbal, no meaningful communication	Generally happy	ND	Poor visual awareness, limited engagement	Irregular sleep initiation and maintenance
LR11-387	Normal	Severe GERD and dysphagia, constipation	Averbal, no meaningful communication	Generally happy, occasional inappropriate laughter	High pain tolerance	Poor visual tracking	Occasionally irregular sleep maintenance
LR11-388	Normal	Mild GERD and dysphagia, constipation	Averbal, no meaningful communication	Generally happy, occasional inappropriate laughter	High pain tolerance	Poor visual tracking, does not appear to distinguish individuals	Normal
LR12-013	Breath-holding behavior	Severe GERD in infancy	Averbal, no meaningful communication	Generally happy, with inappropriate laughter	High pain tolerance	Poor eye contact in early childhood, but improving, no reciprocal play	Normal
LR12-021	Normal	Normal	Averbal, some babbling	Generally happy, with inappropriate laughter, easily excitable	High pain tolerance	Inconsistent eye contact, no reciprocal play	Very disrupted in infancy, now improving
LR12-022	Normal	Severe GERD in infancy	Has 10 consistent words, some jargon	Generally happy, with some inappropriate laughter	High pain tolerance	Eye contact emerged at 3 years, some reciprocal interactions	Normal
LR12-031	Normal	GERD in infancy, treated with	Averbal, some vocalizations	Irritable until 2.5 years; now generally happy with	Inappropriate pain response, laughs with vaccinations	Poor visual fixation and attention, avoided eye contact until age 3 years	Difficult sleep onset and maintenance

Subject	Breathing	GI	Language	Mood	Pain response	Reciprocity	Sleep
LR12-275	Normal	medication, now resolved Normal	Some babbling	inappropriate nocturnal laughter Generally happy	High pain tolerance	Poor eye contact, poor visual tracking	Difficulty with sleep onset

GERD gastroesophageal reflux disease, *ND* no data

Table 4Results of *Mef2c* gene expression microarray studies in the mouse forebrain at e14.5

Affymetrix ID	Gene symbol	Fold change (cortex + vs. cortex-)	p value (cortex+ vs. cortex-)	Fold change (cortex+ vs. GE+)	p value (cortex + vs. GE+)	Fold change (cortex- vs. GE+)	p value (cortex - vs. GE+)
1424852_at	Mef2c	4.35475	3.87E-07	3.78075	1.05E-06	-1.15182	0.275226
1446484_at	Mef2c	4.39426	2.11E-06	2.90246	3.51E-05	-1.51398	0.0215743
1451506_at	Mef2c	4.30536	2.38E-05	5.02955	1.19E-05	1.16821	0.452434
1451507_at	Mef2c	4.95591	2.16E-05	4.7312	3.08E-05	-1.0475	0.833532
1439946_at	Mef2c	5.62548	9.05E-05	3.5717	9.09E-04	-1.57502	0.136682
1445420_at	Mef2c	3.17248	3.92E-04	3.14179	4.64E-04	-1.00977	0.96674
1421028_a_at	Mef2c	4.79451	3.86E-04	4.02632	9.71E-04	-1.19079	0.583585
1421027_a_at	Mef2c	5.34583	1.78E-03	4.34332	4.38E-03	-1.23082	0.62629

Cortex+ indicates migrating cortical interneurons; Cortex- indicates non-interneurons; GE+ indicates GFP-positive cells in the ganglionic eminence