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Severe Mental Retardation, Seizures, and Hypotonia Due to Deletions of *MEF2C*

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We present four patients, in whom we identified overlapping deletions in 5q14.3 involving *MEF2C* using a clinical oligonucleotide array comparative genomic hybridization (CGH) chromosomal microarray analysis (CMA). In case 1, CMA revealed an ~140 kb deletion encompassing the first three exons of *MEF2C* in a 3-year-old patient with severe psychomotor retardation, periodic tremor, and an abnormal motor pattern with mirror movement of the upper limbs observed during infancy, hypotonia, abnormal EEG, epilepsy, absence of speech, autistic behavior, bruxism, and mild dysmorphic features. MRI of the brain showed mild thinning of the corpus callosum and delay of white matter myelination in the occipital lobes. In case 2, an ~1.8 Mb deletion of *TMEM161B* and *MEF2C* was found in a child with severe developmental delay, hypotonia, and seizures. Patient 3 had epilepsy, hypotonia, thinning of the corpus callosum, and developmental delay associated with a de novo ~2.4 Mb deletion in 5q14.3 including *MEF2C* and five other genes. In case 4, a de novo ~5.7 Mb deletion of *MEF2C* and five other genes was found in a child with truncal hypotonia, intractable seizures, profound developmental delay, and shortening of the corpus callosum on brain MRI. These deletions further support that haploinsufficiency of *MEF2C* is responsible for severe mental retardation, seizures, and hypotonia. Our results, in combination with previous reports, imply that exon -targeted oligo array CGH, which is more efficient in identifying exonic copy number variants, should improve the detection of clinically significant deletions and duplications over arrays with probes spaced evenly throughout the genome.

Key words: epilepsy; mental retardation; array CGH; MEF2C; haploinsufficiency

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INTRODUCTION

Several microscopically visible interstitial deletions involving chromosome band 5q14 have been described in patients with developmental delay/mental retardation (DD/MR) and dysmorphic features [Stoll et al., 1980; Silengo et al., 1981; Ohdo et al., 1982; Rodewald et al., 1982; Harprecht-Beato et al., 1983; Krishna et al.,

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1997]. Recently, 11 deletions in 5q14.3, ranging in size from 216 kb to 17 Mb, have been identified using array comparative genomic hybridization (CGH) [Cardoso et al., 2009; Engels et al., 2009; Le Meur et al., 2010]. In five unrelated patients with severe DD/MR, stereotypic movements, hypotonia, cerebral malformations, and dysmorphic features, Le Meur et al. [2010] determined a minimal commonly deleted region encompassing only the *myocyte enhancer-binding factor 2C* (*MEF2C*) gene. They also found a de novo nonsense mutation in a sixth patient with a similar phenotype. Cardoso et al. [2009] described three patients with periventricular heterotopia, mental retardation, epilepsy, and de novo deletions in the 5q14.3q15 region. The deletions ranged in size from 6.3 to 17 Mb and shared a common deleted region spanning 5.8 Mb and 14 genes, among which four candidate genes have been proposed: *GPR98*, *CETN3*, *NR2F1*, and *MCTP1*. One deletion contained *MEF2C* and in the other two the proximal breakpoints of the deletions mapped close but distal to *MEF2C*. Engels et al. [2009] reported three unrelated patients with severe psychomotor retardation, febrile seizures, muscular hypotonia, and variable brain, and other minor anomalies. Array CGH analysis revealed de novo microdeletions involving 5q14.3q15 with the smallest deletion overlap of 1.64 Mb spanning five genes: *CETN3*, *AC093510.2*, *POLR3G*, *LYSMD3*, and *GPR98/MASS1*. *MEF2C* was deleted in two patients and in the third, the proximal breakpoint of the mutation mapped close to *MEF2C*.

We present four patients with severe mental retardation, seizures, and hypotonia, in whom, using a clinical-targeted oligonucleotide array CGH, we identified overlapping deletions in 5q14.3 disrupting *MEF2C*. Our data further support that *MEF2C* is a dosage sensitive gene and its haploinsufficiency is responsible for severe mental retardation, seizures, and hypotonia.

PATIENTS AND METHODS

Patient 1

A 3-year-old male patient (Fig. 1a), the first child of non-consanguineous parents with no family history of neurodevelopmental disorders, was born at 35 weeks of gestation following premature rupture of amniotic membranes. His birth weight was 2,480 g (50th percentile), length 48 cm (75th percentile), and OFC 33 cm (50th percentile).

Apgar scores were 10 at 1 and 5 min. During the pregnancy, his 33-year-old mother was treated for hyperthyroidism and an active toxoplasmosis infection. Periodic tremor and an abnormal motor pattern with mirror movement of his upper limbs were observed during infancy. By the age of 2 years, he had severe psychomotor delay with absent speech, hypotonia, bruxism, epilepsy, and autistic behavior. He had mild dysmorphic features, including frontal bossing, mild bilateral epicanthus, a broad nose, and full lips with a tendency toward an open mouth. MRI revealed mild thinning of the corpus callosum and delay of white matter myelination in the occipital lobes (Fig. 1b). The EEG revealed abnormal sleep architecture and generalized discharges localized to the posterior regions. Congenital toxoplasmosis was excluded by immunologic investigations performed in the newborn period. Metabolic screening investigations, including gas chromatography–mass spectroscopy, urine oligosaccharides, a lactic acid level, an analysis for congenital disorders of glycosylation, and SAICAR levels in blood were normal.

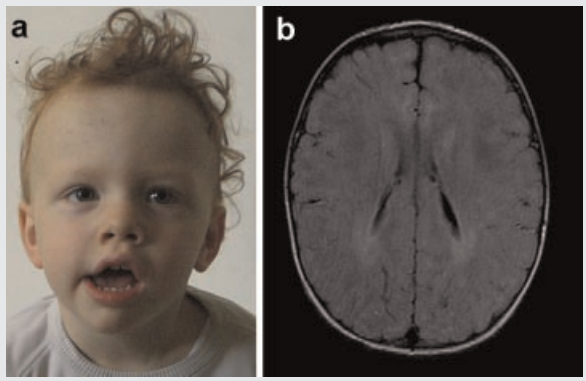

FIG. 1. a: Frontal view of patient 1. b: MRI of the brain in patient 1—FLAIR sequence, axial plane. Note hyperintense areas around the posterior parts of the lateral ventricles, representing delayed myelination of the white matter. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The initial cytogenetic analyses of the patient's G-banded metaphase chromosomes, subtelomeric MLPA, and methylation pattern in the 15q11-q13 region were reportedly normal.

Patient 2

Thirty-month-old girl was referred to genetics clinic due to severe developmental delay. Her length was 85.9 cm (10th percentile), weight 9.1 kg (<1% percentile, 50th percentile for a 10-month old), and OFC 48.2 cm (50th percentile). She rolled over and could replace her pacifier into her mouth but could not sit unsupported. She was extremely hypotonic. Inner canthal and outer canthal distances were 2.3 cm (1st percentile) and 6.5 cm (3.3 standard deviations below the mean), respectively, and she appeared hypoteloric. Visual tracking was poor; she mostly looked up at the ceiling. There was very little interaction although she smiled when tickled or jostled. She had generalized seizures starting at the age of 15 months and now has episodes of startling. She had been on Keppra (levetiracetam) but it was discontinued when it was noted that she slept better and was less fussy when not on medication. She has a tendency to opisthotonic posturing. This girl had marked truncal hypotonia with more increased tone distally with scissoring. She will bear weight minimally when held in a standing position. Reflexes are brisk and toes are downgoing. A period of failure to thrive with no weight gain prompted G-tube placement. Brain MRIs revealed colpocephaly and an incidental pineal cyst. There was neither agenesis of the corpus callosum nor periventircular heterotopias. Ventricles were borderline large. The myelination pattern was normal.

Patient 3

The patient is a 34-month-old female born at 36 weeks gestation to healthy non-consanguineous parents with no family history of epilepsy, birth defects, or mental retardation. An older male sibling, born at 24 weeks, died shortly after birth. Pregnancy was complicated by maternal hypertension and gestational diabetes necessitating insulin treatment. At 14 months she had two bilateral tonic–clonic seizures in association with a temperature of 37.9°C secondary to otitis media. Shortly thereafter, she developed extensor myoclonus on awakening. An EEG at 15 months revealed multiple, generalized spike and poly-spike, and slow wave discharges. Initially she responded to Depakote (divalproex sodium), but the effect waned and Keppra (levetiracetam) was added. An EEG at 19 months revealed generalized semi-rhythmic bursts of polyspike and wave activity in runs of 2–3 sec.

A physical exam at 30 months revealed failure to thrive, with height 83.7 cm (2nd percentile), weight 8.6 kg (<1st percentile, 50th percentile for a 9-month old) and head circumference 45.3 cm (2nd percentile), mild bilateral ptosis, a thin nose, asymmetric ears, a short philtrum, micrognathia, and a pectus excavatum. She had bilateral esotropia but had normal pupillary reflexes and could fixate and follow objects. She had truncal hypotonia but more normal tone distally and 2+ symmetric reflexes. She could roll over, but could not sit unsupported, or crawl. She would not bear weight on her legs and she showed scissoring of the legs when held. She often threw her head back and assumed a rigid posture. Coordination of fine motor movements was limited. Although there were some vocalizations, there was no evidence of meaningful receptive or expressive language despite a normal audiologic evaluation.

Although she can take pureed foods by mouth, she had very poor weight gain and a G-tube was placed at 32 months of age. Her parents reported problems with bruxism and constipation. A heart murmur was heard by several physicians but an echocardiogram was normal.

Brain MRIs at 14 and 19 months revealed thinning of the corpus callosum, most prominent in the splenium, and mild global white matter loss, but no periventircular heterotopias. Although an MRI of the cervical cord suggested atlanto-occipital assimilation, a cervical CT was normal. Both studies revealed possible basilar invagination.

Patient 4

Eighteen-month-old girl was referred to genetics because of abnormal urine organic acid analysis obtained in work-up of developmental delay and seizures. The family history was non-contributory with no consanguinity, no seizures, no recurrent miscarriages, stillbirths, or early or sudden deaths, no learning difficulties or mental retardation, and no birth defects. The pregnancy was complicated only by preterm labor. She was born to a 24-year-old prima gravida mother via vacuum assisted vaginal delivery at 40 weeks. Her birth weight was 3,006 g (20th percentile). At 3–4 months of age she developed infantile spasms. She has been maintained since that time on a ketogenic diet and three antiseizure medications, Vigabatrin, Klonopin, and Keppra. Despite these measures she continues to have generalized tonic–clonic seizures about once a month and an episode of infantile spasms weekly. She also has quick jerking movements up to 20 times a day.

Developmentally she showed no regression but had significant delay in acquiring milestones. At 18 months she babbles but had no words. She maintains her head in midline when sitting supported but, otherwise, has significant head lag. She lifts her head when prone. She observers her hands and grasps objects placed in them but will not reach. She rolls prone to supine but not supine to prone. She sleeps well and has no temperament problems.

She has minor dysmorphic features including brachycephaly, a wide nasal bridge, down-turned corners of her mouth with a cupid-bow upper lip. Her growth parameters were normal showing 10th–25th percentile for weight, 25th–50th percentile for length but severe microcephaly with a head circumference at the 50th percentile for a 9-month-old. She had upper extremity and truncal hypotonia with increased tone in her lower extremities.

Workup included a chromosome analysis which showed a normal 46,XX chromosomal complement and normal plasma amino acids. An MRI of the brain at 17-months of age showed microcephaly, a shorter than expected corpus callosum, prominent lateral, third, and fourth ventricles, slightly wide sylvian fissures, and small frontal lobes with a paucity of the cerebral gyri. Focal increased T2 signal was detected within the globus pallidi. The gray–white matter interface within the temporal lobes appeared ill defined, suggesting either delayed myelination or cortical dysplasia. PET scanning showed hypermetabolism in the right cerebellum with hypometabolism in the left hemisphere and was diffusely suggestive of a cortical dysplasia. EEG showed frequent and spike and wave activity in the left temporal-occipital and left central temporal regions as well as spikes in the right occipital area. Renal ultrasound was normal. Lactic acid was normal at 0.7 mmol/L, ammonia was normal at 36 μmol/L. Urine organic acids showed 3-hydroxybutyric and acetoacetic acids and a pattern of dicarboxylic acids likely secondary to the ketogenic diet.

Molecular Analysis

Microarrays. Genomic DNA was extracted from patients' peripheral blood cells using a Genomic DNA purification kit (Puregene, Gentra Systems, Minneapolis, MN) according to the manufacturer's instructions.

Array CGH was performed using a custom-designed 105 K oligonucleotide V7.4 (in patients 1, 2, and 4) or V7.2 (in patient 3) OLIGO microarray (Agilent Technologies, Santa Clara, CA) as described [El-Hattab et al., 2009]. Labeling and hybridization were performed following the manufacturer's protocols. Briefly, 1 μg of DNA from the patient and a non-commercial control were digested with *Rsa*I and *Alu*I for 2 hr at 37°C. After 20 min at 65°C, DNA was labeled by random primers for 2 hr, using Cy5-dUTP for the patient DNA and Cy3-dUTP for the control DNA. Labeled products were purified on column centrifugal filters (Millipore Corporation, Bedford, MA, USA, Microconr Devices Centrifugal Filter) according to the manufacturer's instruction. After probe denaturation and prehybridization with Cot-1 DNA, hybridization was performed at 65°C with rotation for 72 hr. After washing the array was analyzed with an Agilent scanner and Feature Extraction software (Agilent Technologies) and text file outputs from the quantification analysis

were imported to the Baylor College of Medicine in-house analysis package for copy number analysis, as described [Ou et al., 2008].

In patient 3, the initial analysis was performed using an Affymetrix GeneChip 6.0 SNP array in accordance with the manufacturer's instructions. Fine mapping of the deletion in patient 4 was performed using whole-genome high-resolution oligonucleotide microarray CGH analysis with a 2.1 M NimbleGen oligonucleotide array (NimbleGen Systems, Madison, WI), in accordance with the manufacturer's instructions.

FISH analysis. FISH analyses of the 5q14.3 region were performed according to a standard protocol, using BAC clones RP11-117A24, RP11-44N15, RP11-946O16, and RP11-265O6 in patients 1, 2, 3, and 4, respectively. Additionally, FISH analysis with BAC clone RP11-91D4, specific for chromosome 18p11.23-p11.31, was performed in patient 2. Briefly, a BAC clone was grown in TB media with 20 mg/ml chloramphenicol. DNA was extracted using an Eppendorf plasmid Mini Prep Kit (Eppendorf, Hamburg, Germany) and labeled with SpectrumRed or SpectrumGreen dUTP by nick translation (Vysis, Downer Grove, IL) according to the manufacturers' protocols. Slides were viewed on a Zeiss Axioplan2 fluorescence microscope and images were captured and analyzed using Applied Spectral Imaging Acquisition 5.0 analysis system (Applied Spectral Imaging, Inc. Vista, CA).

Long range PCR and DNA sequencing. Genomic sequences defined on the basis of oligonucleotide coordinates from the array CGH experiments were downloaded from the UCSC genome browser (Build 36, March 2006) and assembled using Sequencher software (Gene Codes Corporation, Ann Arbor, MI). The junction fragments of the deletion were amplified via long-range PCR (Takara Bio, Inc., Shiga, Japan) according to manufacturer's instructions. PCR products were visualized on 1% agarose gel, purified with the PCR Purification Kit (Qiagen, Valencia, CA) and ExoSAP (USB Corp., Cleveland, OH) and sequenced (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw and Lone Star, Houston, TX).

RESULTS

Microarrays

In the first case, clinical-targeted oligonucleotide array (chromosomal microarray analysis, CMA V7.4) analysis revealed an ~140 kb deletion encompassing the first three exons of *MEF2C* (Fig. 2a). The proximal breakpoint was mapped between 88,104,594 and 88,121,748 (build hg18) and the distal breakpoint between 88,232,276 and 88,252,348. In case 2, an ~1,8 Mb deletion was identified (CMA V7.4) which encompassed two genes: *TMEM161B* and *MEF2C* (Fig. 2b). The proximal breakpoint was mapped between 87,086,357 and 87,135,938 and the distal breakpoint between 88,896,344 and 88,912,534. In addition, an ~1.1 Mb duplication of 18p11.23-p11.31 involving the *LAMA1*, *LRRC30*, and *PTPRM* genes was found. The proximal breakpoint was mapped between 6,912,017 and 6,932,050 and the distal breakpoint between 8,064,232 and 8,082,092. In case 3, Affymetrix 1.8 million SNP array (LabCorp, Houston, TX) revealed an ~2.4 Mb deletion (87,807,115–90,158,137). A CMA V7.2 confirmed the deletion with the proximal breakpoint mapping between 87,788,099 and 87,841,596 and the distal breakpoint between 90,142,763 and 90,160,597 (Fig. 2c). No additional CNVs were identified. In case 4, an ~5.7 Mb deletion encompassing six genes: *EDIL3*, *COX7C*, *RASA1*, *CCNH*, *TMEM161B*, and *MEF2C* was identified using CMA V7.4 (Fig. 2d). Using a whole-genome 2.1 M NimbleGen array, the proximal breakpoint was mapped between 83,139,263 and 83,140,336 and the distal breakpoint was mapped between 88,798,204 and 88,799,227.

FISH Analysis

FISH analyses confirmed all four deletions (Fig. 2h,i) and parental studies showed that they occurred de novo. The duplication 18p11.23-p11.31 in patient 2 was found to be inherited from the phenotypically normal father.

To determine whether the identified 5q14.3 deletions and 18p11.23-p11.31 duplications were benign CNVs, we have screened our database of oligonucleotide array CGH studies performed in ~10,000 patients (Versions 6.2-8.0) and found no similar changes in 5q14.3. In addition, no CNVs in the 5q14.3 genomic region were found in the Database of Genomic Variants (http://projects.tcag.ca/variation/). These data indicate that this genomic region is not polymorphic and further support its potential causative role in the phenotype in the described patients.

We also found that the 18p11.23-p11.31 duplication was inherited from the phenotypically normal mothers in two unrelated families. Therefore, we consider this CNV as likely non-pathogenic. All other changes identified in these patients were known copy number polymorphisms.

PCR and Sequence Analysis

In patients 1, 2, and 3 the junction fragments of the deletions were amplified using LR-PCR, and the amplicons were sequenced (Fig. 2f). In patient 1, the proximal breakpoint maps at 88,107,302 and the distal breakpoint at 88,249,531. Thus, the deletion is 142,229 bp in size. The DNA sequence analysis of a PCR product that spans the junction revealed a 2 bp TG microhomology (Fig. 2e). In patient 2, the proximal breakpoint maps at 87,133,893 and the distal breakpoint at 88,897,214. Thus, the deletion is 1,763,321 bp in size. Additionally, between the breakpoints, a 15 bp insertion AATTAACACAGTGTT was identified (Fig. 2f). No microhomology was found between the breakpoint regions. The deletion in patient 3 is 2,356,271 bp in size with proximal breakpoint mapping at 87,803,790 and the distal breakpoint at 90,160,061. No microhomology was present at the junction. Nine base pairs of DNA ATAAAAGTT, was inserted at the junction (Fig. 2g).

DISCUSSION

We present four patients with severe psychomotor retardation, truncal hypotonia, and seizures, in whom we identified overlapping deletions in 5q14.3 that deleted or disrupted the *MEF2C* gene. Recently, *MEF2C* was reported to be deleted in eight out of 11 patients with similar phenotypic features and deletions of 5q14.3 (Fig. 3) [Cardoso et al., 2009—patient 2; Engels et al., 2009—patients 1 and 2; Le Meur et al., 2010—patients 1–5] (Table I).

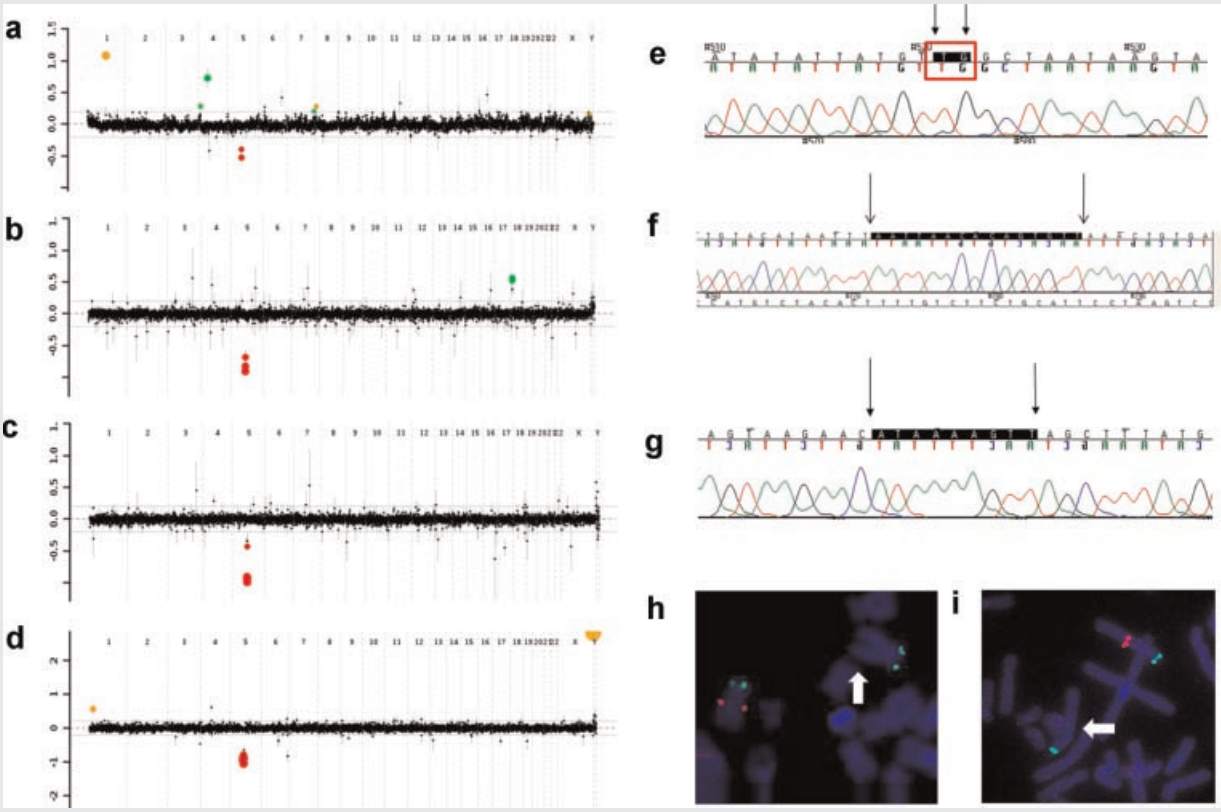

FIG. 2. a: Oligonucleotide array CGH profile in patient 1 showing an ~140 kb deletion in the 5q14.3 region. b: Array CGH analysis in patient 2 showing deletion of the 5q14.3 region. c: Array CGH data in patient 3 showing our patient's de novo ~2.4 Mb deletion on chromosome 5q14.3. d: CMA revealed an ~5.7 Mb deletion on 5q14.3 in patient 4. Red dots denote the deleted region. e: Chromatogram of the junction fragment of patient 1. The red frame shows the microhomology, the vertical black arrows indicate the breakpoints. f: Chromatogram showing patient 2's junction fragment sequence. A 15 base pair insertion is indicated in black. g: Chromatogram of the junction fragment in patient 3. The vertical black arrows indicate the breakpoints. A 9 bp insertion is indicated in black. h: Results of patient's 1 FISH analysis with clones RP11-117A24 (red) and RP11-94J21 (green) used as a control. White arrow indicates the deleted chromosome. i: FISH of patient 2 with the BAC clone RP11-44N15 (red) and Vysis 5p telomere (green) verified the 5q14 deletion. White arrow indicates the deleted chromosome. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MEF2C plays an important role in the development and maintenance of multiple organ systems in humans [Janson et al., 2001]. MEF2C acts in brain as an effector of neurogenesis, which regulates excitatory synapse number [Flavell et al., 2006] and differentiation of postsynaptic structure [Shalizi et al., 2006]. MEF2C has a crucial function in the homeostatic control of activity-dependent synaptogenesis that plays a role in the establishment of functional neuronal circuits during development and memory storage [Barbosa et al., 2008] and in programming early neuronal differentiation and proper distribution within layers of the neocortex [Flavell et al., 2006]. The causal role of *MEF2C* haploinsufficiency in the reported patients is in agreement with the biological function of the Mef2c and the murine models of the gene inactivation. Li et al. [2008] reported that conditional knockout of murine *Mef2c* in the neural progenitors causes abnormal aggregation and compaction of neuronal migration into the lower layers of the neocortex during development. Knockout mice that survived to adulthood manifested smaller, apparently less mature neurons, smaller whole brain size with immature electrophysiological network properties and severe behavioral deficits reminiscent of an autism-related disorder or Rett syndrome like phenotype (e.g., paw-clasping behavior mimicking stereotypic hand movements in girls with Rett syndrome due to mutation in *MECP2*) [Li et al., 2008; Morrow et al., 2008; Lipton et al., 2009]. Of note, Chahrour et al. [2008] showed that MeCP2 directly represses *Mef2c*. Interestingly, Rett syndrome was initially suspected in two of five patients described by Le Meur et al. [2010] (case 4 with partial deletion of *MEF2C* and case 5 with entirely removed *MEF2C*), and the point mutation inactivating *MEF2C* was identified in a patient with a Rett-like phenotype. These data indicate that isolated disruptions of *MEF2C* can be responsible for a Rett-like phenotype.

Epilepsy is one of the most common neurological disorders and affects ~1% of the general population [Andrade, 2009]. It has been estimated that up to 40% of epilepsies are genetically determined and inherited in a Mendelian, non-Mendelian, or complex trait fashion [Gardiner, 2000]. The clinical symptoms of the disease

TABLE I. Clinical Features of Patients With Deletions or Mutation [#7, Le Meur et al., 2010] Involving *MEF2C* in 5p14.3

Patient	1	2	3	4	Le Meur 1	Le Meur 2	Le Meur 3	Le Meur 4	Le Meur 5	Le Meur 7	Engels 1	Engels 2	Engels 3	Cardoso 1	Cardoso 2	Cardoso 3
Gender	M	F	F	F	F	F	M	M	F	M	F	F	F	M	F	M
Age at evaluation	3 yo	30 mo	34 mo	18 mo	4 yo 9 mo	9 mo	18 mo	3 yo	8 yo	7 yo	6 yo	8 yo	6 yo	7 yo	5 yo	5 yo
Birth/current weight	50%/N/A	N/A/1%	N/A/<1%	20%/10–25%	0 SD/−0.5 SD	0 SD/+2 SD	+3 SD/−2 SD	−1 SD/−1 SD	−3 SD/−1 SD	0 SD/0 SD	−0.4 SD/N/A	−0.41 SD/2–9%	+0.1 SD/25%	N/A	N/A	N/A
Birth/current length	75%/N/A	N/A/10%	N/A/2%	N/A/25–50%	0 SD/−0.5 SD	0 SD/−0.5 SD	+3 SD/N/A	+1.5 SD/+1 SD	−3 SD/−1 SD	0 SD/0 SD	+0.4 SD/−1.1 SD	N/A/−1.6 SD	−0.3 SD/−1.5 SD	N/A	N/A	N/A
Birth/current OFC	50%/N/A	N/A/50%	N/A/2%	N/A/≪5%	0 SD/0 SD	−3 SD/−2 SD	−2.5 SD/+1 SD	N/A/−0.5 SD	−3 SD/−1.5 SD	+1 SD/+2 SD	+2.2 SD/+1.1 SD	N/A/−0.38 SD	+0.1 SD/−1.6 SD	N/A	N/A	N/A
Hypotonia	+	Severe	+	+	+	Severe	Severe	Severe	+	−	+	+	+	+	N/A	+
DD/MR	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe	+	Severe	Severe
Speech	−	N/A	−	−	−	−	−	−	−	−	N/A	N/A	Delayed	−	−	−
Seizures	+	Generalized	Tonic–clonic, myoclonus	Infantile spasms, generalized tonic–clonic seizures	Myoclonic, refractory	Tonic–clonic, refractory	−	−	Tonic–clonic febrile	Tonic–clonic	+	Febrile	+	Tonic–clonic febrile	+	+
EEG	Abn	N/A	Abn	Abn	Abn	Abn	Abn	NI	N/A	N/A	Abn	Abn	Abn	NI	Abn	Abn
Stereotypic movements	Bruxism	N/A	Bruxism	Jerking movements	Head rocking, hand chin rubbing	−	−	Hand clapping	Hand washing, hand-to-mouth movements	Hand and hand–mouth movements	N/A	Myoclonic jerking of arms	Bruxism, dyskonetic hand movements	N/A	N/A	N/A
Brain	Delayed myelination in the occipital lobes	Colpocephaly and an incidental pineal cyst	Mild global white matter loss, but no periventricular heterotopias, possible basilar invagination	Delayed myelination or cortical dysplasia, prominent lateral third and fourth ventricles, slightly wide sylvian fissures, smaller frontal lobes, paucity of cerebral gyri	Fronto parietal atrophy, enlarged pericerebral spaces	Enlarged lateral ventricles, reduced cortical gyration	Verticalization of the cerebellum tent, enlarged 4th ventricle	Enlarged 4th ventricle	N/A	Enlarged lateral ventricles, periventricular white-matter hyperintensities	Aplasia of cerebral vermis, multiple plexus cysts, enlarged occipital horns of lateral ventricles	Prominence of the arachnoid spaces in the perivascular areas	Atrophy of the supra- and infratentorial region, mildly enlarged ventricular region, unspecific leucoencephalopathy	Bilateral PH, mild dilation of frontal horns	Bilateral PH	Bilateral PH, under-rotated hippocampi, irregular thickening, polymicrogyria
Corpus callosum	Thinning	NI	Thinning	Short	NI	Abn	Abn	NI	Abn	N/A	Aplasia of posterior	N/A	N/A	N/A	N/A	N/A
Head	Frontal bossing	N/A	Micrognathia	Microcephaly, brachycephaly	High and broad forehead, small chin	High and broad forehead, small chin	High and broad forehead, small chin	High and broad forehead, small chin	High and broad forehead, small chin	Small chin	N/A	Brachycephaly, low anterior hairline, mild facial dysmorphism	N/A	High forehead	High forehead, fontal bossing, micrognathia	Macrocephaly, triangular shaped
Eyes	Bilateral epicanthus	Hypoteloric, poor contact	Bilatera ptosis, esotropia	N/A	Up-slanted palpebral fissures, poor contact	Up-slanted palpebral fissures, poor contact	Up-slanted palpebral fissures, poor contact, corlical blindness	Poor contact	Deep set, poor contact	Deep set, decreased contact	Bilateral optic atrophy, broad eyebrows	Visual preoccupation with stripes, eyebrows broad, down-slanted palpebral fissures	Hyperopia, strabismus convergens, hyperopia, up-slanted palpebral fissures	High arched eyebrows, down-slanted palpebral fissures, coloboma of left iris, left eye exotropia hypertelorism	High arched eyebrows, hypertelorism	N/A
Nose	Broad	N/A	Thin	Wide bridge	N/A	Short	Short	Short	N/A	N/A	N/A	N/A	N/A	Depressed nasal bridge, thick columella	Anteverted nostrils, depressed nasal bridge, thick columella	N/A

TABLE I. (*Continued*)

Patient	1	2	3	4	Le Meur 1	Le Meur 2	Le Meur 3	Le Meur 4	Le Meur 5	Le Meur 7	Engels 1	Engels 2	Engels 3	Cardoso 1	Cardoso 2	Cardoso 3
Ears	N/A	N/A	Asymmetric	N/A	Dysplastic	N/A	N/A	N/A	N/A	N/A	Large	N/A	Large, simple, sensitivity to noise	N/A	N/A	N/A
Mouth	Full lips, open mouth	N/A	Short philtrum	Down-turned corners, cupid-bow upper lip	Lobulation of the tongue, short prominent philtrum	Short prominent philtrum	Thin upper lip	Large open	Large open, short prominent philtrum	N/A	N/A	N/A	Frequently open	Flat long philtrum	Thin lips, long philtrum, thin lips	N/A
Other features	N/A	Opisthotonic posturing	Pectus excavatum, constipation	N/A	Sus-sternal fistula	Edema of hands and feet	Sleep disturbance, clinodactyly of both 5th fingers, bilateral 2–3 toe syndactyly, ectopic testis, Simian creases	Left 2-3 toe syndactyly, right distal phalanx Agenesis of toes 2–5	Episodic hyper-ventilation and apnea	Feeding difficulties, wide-based gait	Transverse palmar creases, long fingers, mygocardial hypertrophy, café-au-lait spots, frequent infections, chronic constipation, bilateral pes equines	Fetal increased nuchal translucency	Long fingers, mild facial dysmorphism	N/A	Bilateral pes talus	Postaxial polydactyly of toes

+, presence of a characteristic; –, absence of a characteristic; Abn, abnormal; DD, developmental delay; MR, mental retardation; N/A, data were not available; NI, normal; PH, periventricular heterotopia; yo, years old; mo, months old.

result from abnormal synchronized neuronal discharges present in human brain [Meisler et al., 2001; Kim et al., 2007]. Linkage analysis by Nakayama et al. [2000] identified an autosomal dominant febrile seizure locus (*GPR98*) on chromosome 5q14-q15. Various types of epilepsy, from well-controlled generalized seizures to early refractory tonic–clonic or myoclonic epilepsy, were observed in 3/5 of patients described by Le Meur et al. [2010]. Interestingly, patient 4 with deletion of only the first exon of *MEF2C* reported by Le Meur et al. [2010] has never experienced seizures and his EEG was normal. Seizure variability is often observed in periventricular heterotopia (PH), characterized by nodular masses of gray matter containing neurons and glia with disorganized or rudimentary lamination. PH can occur in isolation or with other congenital abnormalities [Andrade, 2009]. Mutations in some genes, for example, *FLNA1* [Fox et al., 1998] and *ARFGEF2* [Sheen et al., 2004], and CNVs have been shown to cause PH [Andrade, 2009]. PH was present in all three patients described by Cardoso et al. [2009] in contrast to cases reported by Le Meur [2010], in whom none presented with PH. These data, in aggregate with our results, further suggest that PH is caused by mutations in genes located distal to *MEF2C* (Fig. 3).

Previous studies have also demonstrated an important role for *MEF2C* in heart development [Lin et al., 1997; Vincentz et al., 2008; Ghosh et al., 2009]; however, no structural heart defects were observed in our patients and no heart defects have been mentioned in reports of other children with similar 5q14 deletions [Cardoso et al., 2009; Engels et al., 2009; Le Meur et al., 2010]. In mice homozygous for a null mutation of *Mef2c*, the heart tube did not undergo looping morphogenesis, the future right ventricle did not form and a subset of cardiac muscle genes was not expressed. Thus, murine *Mef2c* was proposed as an essential regulator of cardiac myogenesis and right ventricular development [Lin et al., 1997]. Overexpression of *Mef2C* in mouse cardiomyocytes has been observed to cause cardiomyopathy [Xu et al., 2006]. Interestingly, one patient with deletion of *MEF2C* presented concentric myocardial hypertrophy [Engels et al., 2009]; however, no heart abnormality was reported in a patient with duplication of *MEF2C* [Le Meur et al., 2010].

We propose that the minor cranial and facial dysmorphic features, present in all described patients, are also caused by *MEF2C* haploinsufficiency [Arnold et al., 2007; Verzi et al., 2007]. Mice lacking *mef2c* function appear to have a similar set of defects in the oral cavity, including a small lower jaw, cleft palate, and displaced position of the tongue [Verzi et al., 2007]. In addition, a critical role of *Mef2c* in mature B-cell proliferation, survival, and homeostasis has been demonstrated recently [Wilker et al., 2008].

In conclusion, all patients with deletions encompassing *MEF2C* demonstrated severe mental retardation, seizures, and hypotonia. Clinical variability in this group may be explained, at least in part, by differences in deletion size and location. We believe that in most reported cases with 5q14 deletion, haploinsufficiency of *MEF2C* is responsible for the majority of observed clinical features, as suggested by Le Meur et al. [2010]. In cases where the deleted region did not contain *MEF2C*, the chromosomal breakpoints were located close to the *MEF2C*. Therefore, a position effect cannot be excluded.

In patient 1, we identified a small 140 kb deletion of three exons of *MEF2C* with the aid of intragenic oligonucleotide probes on the clinical-targeted microarray. This implies that even single exon

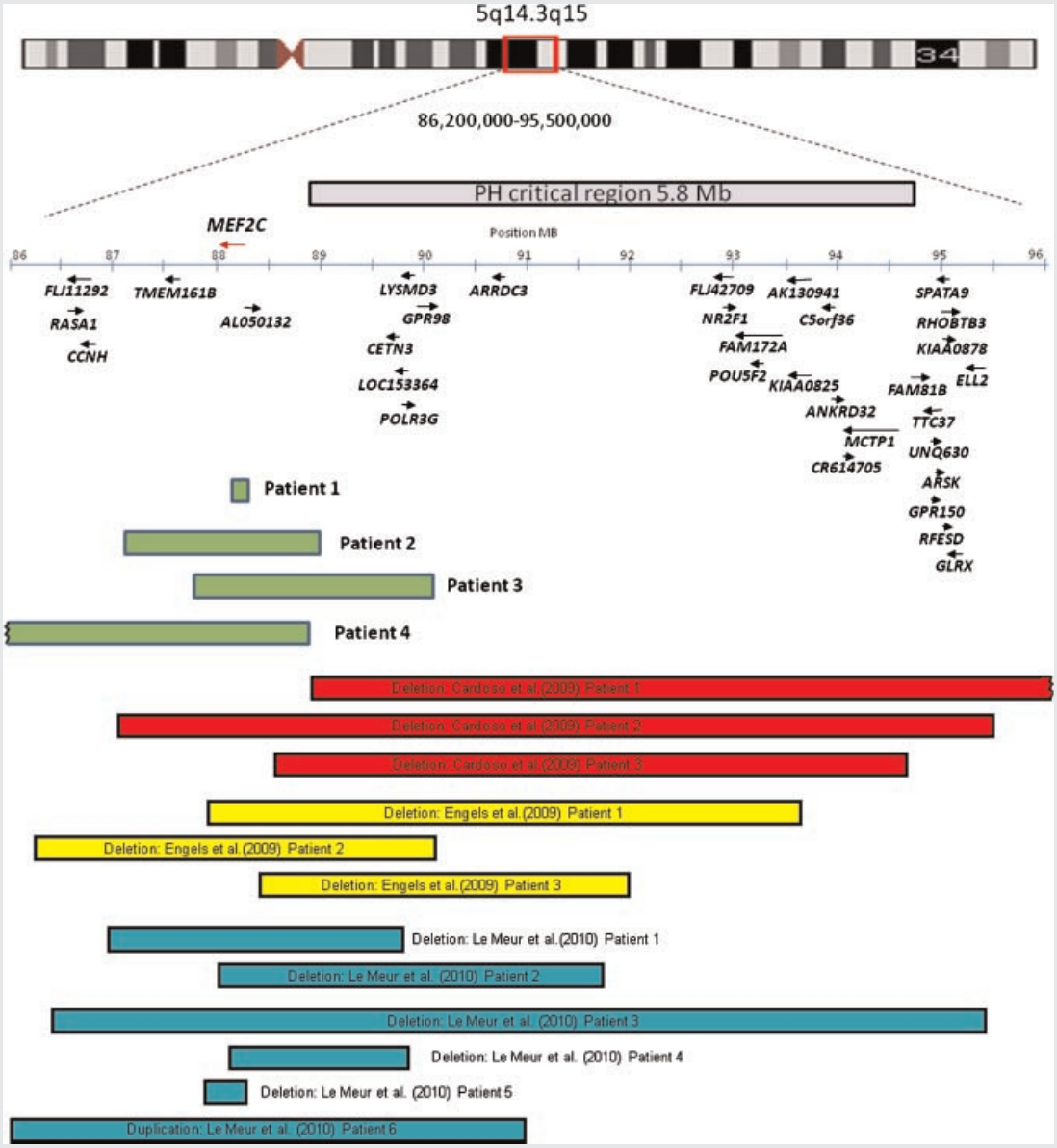

FIG. 3. Schematic representation of the deleted/duplicated regions in chromosome 5q14.3q15 (~10 Mb), showing all known genes contained in this region. The arrows indicate each gene's transcriptional direction. The previously reported pathogenic CNVs [Cardoso et al., 2009; Engels et al., 2009; Le Meur et al., 2010] are placed relative to 5q14.3. Note that deletions in our patient 4 and patient 1 of Cardoso et al., [2009] extend more proximally and distally, respectively. PH region defined by Cardoso et al. [2009] is also shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

deletions or duplications may lead to MEF2C protein truncation and could be pathogenic.

Such events are likely to be undetected using the standard catalogue arrays because the interrogating oligonucleotide probes on the commercially available microarrays are distributed randomly across the genome. It is likely that intragenic abnormalities involving *MEF2C* are not typically identified and, therefore, are under-diagnosed. This shortcoming could be remedied by using an exon-targeted oligo array which would be more likely to identify small, pathogenic exonic CNVs [Bucan et al., 2009; Erez et al., 2009; Wiśniowiecka-Kowalnik et al., 2010].

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