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Electroclinical pattern in MECP2 duplication syndrome: Eight new reported cases and review of literature

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Summary

Purpose: Duplications encompassing the MECP2 gene on the Xq28 region have been described in male patients with moderate to severe mental retardation, absent speech, neonatal hypotonia, progressive spasticity and/or ataxia, recurrent severe respiratory infections, gastrointestinal problems, mild facial dysmorphic features (midface hypoplasia, depressed nasal bridge, large ears) and epilepsy. Epilepsy can occur in >50% of cases, but the types of seizures and the electroclinical findings in affected male individuals have been poorly investigated up to the present. Herein we describe eight patients with MECP2 duplication syndrome and a specific clinical and electroencephalographic pattern.

Methods: Array CGH of genomic DNA from the probands was performed, and an Xq28 duplication ranging from 209 kb to 6.36 Mb was found in each patient. Electroencephalography studies and clinical and seizure features of all the patients were analyzed.

Key findings: We found that epilepsy tended to occur between late childhood and adolescence. Episodes of loss of tone of the head and/or the trunk were the most represented seizure types. Generalized tonic–clonic seizures were rarely observed. The typical interictal EEG pattern showed abnormal background activity, with generalized slow spike and wave asynchronous discharge with frontotemporal predominance. Sleep electroencephalography studies also demonstrated abnormal background activity; spindles and K complex were often abnormal in morphology and amplitude. Response to therapy was generally poor and drug resistance was a significant feature.

Significance: Although these cases and a review of the literature indicate that epilepsy associated with MECP2 duplication syndrome cannot be considered a useful marker for early diagnosis, epilepsy is present in >90% of adolescent patients and shows a peculiar electroclinical pattern. Consequently, it should be considered a significant sign of the syndrome, and an EEG follow-up of these patients should be encouraged from early childhood. Moreover, the definition of a more specific epileptic phenotype could be useful in order to suspect MECP2 duplication syndrome in older undiagnosed patients.

Key words: MECP2, Chromosomal duplication, Epilepsy, EEG pattern, X-linked mental retardation.

Mutations in the methyl-CpG binding protein 2 (MECP2) gene located in the subchromosomal region Xq28 and highly expressed in the brain are the cause of typical Rett syndrome, affecting almost exclusively females (Amir et al., 1999; Bienvenu et al., 2000). Mouse models have suggested that an overexpression of this dosage-sensitive gene could also be pathologic (Collins et al., 2004), and since 2005 microduplications of Xq28 region encompassing MECP2 gene have been reported in male patients and in mildly symptomatic female patients (Meins et al., 2005). Thanks to the use of multiplex ligation-dependent probe amplification (MLPA) and array-comparative genomic hybridization (array-CGH), additional male patients with
duplications of this region have been identified: up to now, >100 individuals carrying cryptic duplications spanning 0.3–4 Mb have been described (Van Esch et al., 2005; del Gaudio et al., 2006; Carvalho et al., 2009; Clayton-Smith et al., 2009). MECP2 duplication syndrome is now considered a cause of X-linked mental retardation with a well-described phenotype characterized by neonatal or infantile hypotonia, severe to profound mental retardation with poor speech development, lack of deambulation or motor delay with ataxic gait and progressive spasticity, recurrent respiratory infections, gastrointestinal problems, and mild facial dysmorphic such as midface hypoplasia, depressed nasal bridge, and large ears (Ramocki et al., 2010).

Epilepsy is found in about 50% of cases (Echenne et al., 2009; Ramocki et al., 2010) with a different distribution according to age. Although this represents a significant symptom of this syndrome, to date neither the ictal manifestation nor the electroencephalographic pattern have been described thoroughly (Echenne et al., 2009). Herein we report the long-term follow-up of eight patients with MECP2 duplication syndrome with the aim of defining the epilepsy characteristics and the electroencephalography (EEG) pattern. These cases and a review of literature indicate that epilepsy associated with this X-linked mental retardation syndrome has some distinct features.

**Case Reports**

We report on eight patients referred to epilepsy centers and to child neuropsychiatry departments in Northern Italy. The referring clinician of each patient enrolled in the study was asked to collect an assessment protocol including detailed medical history, paediatric and neurological examinations, and instrumental tests. Epileptic seizures and types of epilepsy were classified according to International League Against Epilepsy criteria (Berg et al., 2010). All patients had repeated prolonged video-EEG recordings, according to the International 10–20 System, and electromyographic (EMG) activity was recorded by surface electrodes. The EEG recordings of all patients were reviewed: all of the recordings were obtained during wakefulness; in five cases EEGs were performed during sleep. For each case, age at onset of epilepsy, seizure type, and EEG abnormalities and outcome were analyzed.

The characteristics of the series are summarized in Table 1. Facial phenotypes are shown in Fig. 1.

### Patient 1

Patient 1 (Fig. 1A) was the second child of unrelated parents, with a healthy older brother. He was born at term by normal delivery. Developmental psychomotor milestones were delayed: control of the trunk was achieved at 16 months, standing position only with support was reached at 24 months, and speech was absent.

At the time of the last evaluation (age 24 months) he showed minor facial anomalies, and neurologic examination revealed diffuse hypotonia and mental retardation with absent speech. He had never had seizures.

| Table 1. Epileptologic and EEG features in patients with MECP2 duplications |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Case number | Age (years) | Seizure type | Slow background activity | Unusual fast/theta rhythmic activity | Unusual activity during sleep: high voltage spindles | Slow and spike waves complexes with frontal/ temporal predominance | Erratic myoclonic jerks | AED | Response to treatment |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1/2 | No seizures | – | + | + | – | – | Wakefulness | – |
| 2/3 | No seizures | – | + | + | + | – | Wakefulness | – |
| 3/10 | 9 | Atonic head drop | + | – | NA | + | Wakefulness | VPA, ETS, LEV, LTG, Resistant |
| 4/13 | 12 | Atonic head drop | + | + | + | + | Wakefulness | VPA, Resistant |
| 5/16 | 13 | Drop attacks | + | – | + | – | VPA, CLB, LTG, Resistant |
| 6/18 | 11 | Head nodding in series | + | – | + | – | VPA, LTG, TPM, CLB, ETS, LEV, Resistant |
| 7/19 | 11 | Drop attacks | + | + | NA | + | – | VPA, CBZ, LTG, Resistant |
| 8/25 | 24 | Atonic head and trunk drop | + | + | NA | – | VPA, LTG, Resistant |

NA, nonavailable; AED, antiepileptic drug; VPA, valproic acid; ETS, ethosuximide; LTG, lamotrigine; LEV, levetiracetam; CLB, clobazam; TPM, topiramate; CBZ, carbamazepine.
obtained in wakefulness showed slightly slow background activity and high voltage abnormal fast activity over the anterior regions; abnormal 12-Hz, high voltage, asynchronous spindles were recorded during sleep. Erratic myoclonic jerks were present both in wakefulness and in sleep.

Patient 2

Patient 2 (Fig. 1B) was the only child of unrelated healthy parents. He was born at term by cesarean section after a pregnancy characterized by intrauterine growth retardation. Psychomotor development was delayed: head control was achieved at 6 months of age, control of the trunk at 11 months, and upright position at 20 months; language was absent. At the time of the last evaluation (age 3 years and 8 months) he had never presented seizures. He showed minor facial anomalies and was able to walk with an ataxic gait; language was absent. EEG showed irregular background activity and unusual fast activity over the central and posterior regions in wakefulness; during sleep abnormal high-amplitude asynchronous spindles were recorded, as well as slow sharp waves (Fig. 2A).

Patient 3

Patient 3 (Fig. 1C) was adopted at 2 months of age, so data about his parents and the pregnancy are not available. He was hypotonic and showed psychomotor delay: independent walking was achieved at 2 years and language was absent. He presented minor facial anomalies, and during infancy he had recurrent respiratory infections.

The onset of seizures occurred at 9 years of age and consisted of daily episodes featuring a sudden head drop followed by somnolence. EEGs featured diffuse sharp and slow waves, more evident over the frontal and temporal areas of both hemispheres, with a mild right prevalence. Erratic myoclonic jerks with no clear correlation with the paroxysmal activity were recorded in

Figure 1.
Frontal view at different ages of some of our patients. (A) Patient 1 at the age of 24 months; (B) Patient 2 at the age of 3 years and 8 months; (C) Patient 3 at the age of 10 years; (D) Patient 4 at the age of 13 years; (E) Patient 8 at age 24 years. Note typical and similar minor facial anomalies in these individuals with MECP2 duplication syndrome: long face more evident in older patients, depressed nasal bridge in infants, midface hypoplasia, large ears, and full lips. Parental consent for the publication was obtained.

Epilepsia © ILAE

Figure 2.
Unusual sleep pattern of patients with MECP2 duplication syndrome. (A) In Patient 2 abnormal high amplitude asynchronous spindles; (B) in Patient 5 long-lasting high amplitude synchronous phasic sleep activities.

Epilepsia © ILAE
wakefulness (Fig. 3A). Initial treatment was with valproic acid (VPA), then ethosuximide (ETS), lamotrigine (LTG), and levetiracetam (LEV) were tried without efficacy. At the age of 10 years the patient showed profound mental retardation and absent speech; he was able to walk with an ataxic-spastic gait, but spent most of the time in a wheelchair. Scoliosis was evident.

**Patient 4**

Patient 4 (Fig. 1D) was the first child of unrelated parents. Family history revealed a maternal cousin with a similar phenotype (Patient 7 of this series). He was born at term after an uneventful pregnancy; the neonatal period was unremarkable. He had minor facial anomalies and showed psychomotor delay: he achieved control of the trunk at 9 months of age and independent walking at 18 months; language was absent. Frequent respiratory infections during early infancy were reported.

Epilepsy started at 12 years of age with seizures featuring loss of head tone, followed after 2 weeks by two generalized tonic–clonic seizures during fever. Therapy with VPA was started with complete seizure control; after 22 months the patient is still seizure-free.

An EEG performed when the patient was 4 years and 8 months old showed abundant fast rhythms prevalent on posterior regions, and slow anomalies on the central-anterior regions during somnolence. High voltage, 9–10 Hz, synchronous spindles as well as abnormal fast activity were recorded. Erratic nonepileptic myoclonus was rarely present in wakefulness (Fig. 3B). When the patient started having seizures, EEG showed irregular background activity, with bilateral abnormalities over the anterior regions, with left predominance (Fig. 4A).

Even after seizure control the same EEG anomalies are still present.

At the age of 12 years and 8 months, he showed severe mental retardation with absent speech and diffuse muscular hypotonia, and was able to walk a few steps with an ataxic gait.

**Patient 5**

Patient 5 was the second child of unrelated healthy parents; a male cousin from the maternal branch was reported to have absent speech and ataxic gait. The patient was born after a normal delivery from an uneventful pregnancy. Psychomotor development was delayed; sitting position was achieved at 10 months, independent walking after 2 years of age, and he showed delayed speech. Frequent respiratory infections were reported.

The first seizures occurred at 13 years of age, in the form of drop attacks. They were controlled only by polytherapy for a few years (VPA, clobazam [CLB], and ETS) and then reappeared. LTG was added and ETS stopped with benefit and he is now seizure-free. The EEG studies showed rhythmic theta activity over the frontal areas and paroxysmal spike and slow waves with fronto-temporal predominance (Fig. 4B). Sleep EEG studies demonstrated the appearance of unusual phasic activities, consisting of long-lasting high-voltage synchronous spindles (Fig. 2B). During sleep, an increase was recorded of spike and slow waves, which tended to become more diffuse.

At the age of 16 years the patient presented mild facial dysmorphisms and had severe mental retardation with poor speech (only a few single words). He could walk independently with an ataxic-spastic gait and severe scoliosis was present.
Patient 6

Patient 6 was born to unrelated healthy parents at the 39th gestational week from cesarean section by the mother’s request. At birth he was cyanotic and bradycardic, and he showed hypoventilation and delayed meconium emission. Psychomotor development was delayed: independent walking was achieved after 2 years of age; language was absent. In infancy the patient showed generalized hypotonia and failure to thrive; he also showed gaze dyspraxia. From infancy until the present he has had frequent severe respiratory infections that have required hospitalizations. Minor facial anomalies were observed.

Epilepsy started at 11 years of age, and was characterized by daily episodes with axial hypotonia and drooling, often bringing about an unusual status. Seizures were more frequently observed after wakening, featuring epileptic head nodding in series. VPA treatment was started with partial seizure control. When tonic vibratory seizures during sleep appeared, topiramate (TPM), CLB, and LTG were added to VPA without efficacy. At the age of 16 years he also experienced recurrent tonic seizures, bringing about epileptic encephalopathy, for which LEV was added. EEGs showed sharp and slow waves with frontotemporal predominance, in clusters, increasing while falling asleep, and often spreading bilaterally. Unusual high-voltage synchronous spindles were recorded during sleep.

Despite different combined antiepileptic drug (AED) therapy, he remained drug-resistant; he, therefore, recently started a ketogenic diet with a transitory good response.

Patient 7

Patient 7 was the first child of nonconsanguineous healthy parents. His sister and brother were healthy. Family history revealed a maternal cousin with a similar phenotype (Patient 4 of this series). Delivery occurred at term after an uneventful pregnancy. Developmental milestones were delayed: the patient sat unsupported at 2 years, he started to walk at 4 years with progressive regression of walking from 12 years, and language was absent.

EEG studies recorded before 11 years revealed slow background activity with epileptiform anomalies and consisted of short discharges of slow spikes with diffuse projection, predominant in the left temporal regions. At 11 years of age, the patient developed recurrent drop attacks barely controlled by VPA. The association of VPA and carbamazepine (CBZ) led to seizure control for about 1 year, but at the age of 13 seizures reappeared with daily frequency. CBZ was withdrawn and LTG was introduced with only partial benefit. EEG showed slow background activity, with abundant theta bursts over both frontal regions.

At the time of the last evaluation (age 19 years) he showed minor facial anomalies and had spasticity of the lower limbs and muscular hypotrophy.

Patient 8

Patient 8 (Fig. 1E) was the only child of unrelated healthy parents, born after an uneventful pregnancy. At birth he was hypotonic; psychomotor delay was evident from the age of 4 months. He started walking at the age of 4 years and was able to walk independently with an ataxic–spastic gait until the age of 19 years, when he was confined to a wheelchair. Language development was delayed; he spoke his first words at the age of 5 years. During infancy, he experienced many respiratory infections that required frequent hospitalization.

At 6 months of age he had some generalized tonic–clonic seizures with fever, treated with phenobarbital (PB) until the age of 5 years, without recurrence. He remained seizure...
free without AEDs until the age of 24 years, when he presented several episodes consisting of a severe loss of tone of the head and trunk. All the EEG studies recorded were characterized by slow background activity, monomorphic theta activity over the frontal regions with diffuse slow spike-and-waves complexes (Fig. 5). Seizures were resistant to treatment with VPA, and LTG was added with a good response.

At the time of the last evaluation (25 years), the patient showed mild facial dysmorphisms and had absent speech, tetraparesis with prevalent hypertonia of the lower limbs, and severe mental retardation.

Molecular Genetics Analysis (Methods and Results)

Array CGH of genomic DNA from the probands was performed using the Human Genome CGH Microarray kit 44K (Patients 2, 5, and 7), the SurePrint G3 Human CGH Microarray kit 8 × 60K (Patients 6 and 8), and the SurePrint G3 Human CGH Microarray kit 4 × 180K (Patients 1, 3, and 4) (Agilent, Santa Clara, CA, U.S.A.).

These platforms allow a genome-wide survey with an average resolution of respectively ~130 (44 and 60 K) and ~40 kb (180 K). The labelling and hybridization of the genomic DNA were performed following the manufacturer’s protocol. Arrays were analyzed using the Agilent Scanner Control (v A.8.4.1), the Feature Extraction software (v 10.7.1.1), and the DNA Analytics software (v 4.0). An in silico analysis of the unbalanced region indicated by the analysis was made using the March 2006 release of the UCSC Genome Browser (http://genome.ucsc.edu/) and the Database of Genomic Variants (http://projects.tcag.ca/variation).

In order to define the de novo or inherited origin of the duplications, the mothers’ genomic DNA was investigated by means of array-CGH or MLPA.

The SALSA MLPA P015C MECP2 kit (MRC Holland, Amsterdam, NL, U.S.A.), containing 13 Xq28-specific probes (spanning SLC6A8, IDH3G, L1CAM, MECP2, and VAMP7) and 16 control probes, was used to verify the presence of a duplication in patients and their mothers following the manufacturer’s protocols.

In each patient, an Xq28 duplication ranging from 209 kb to 6.36 Mb, including the MECP2 gene, was detected. In particular, patients 1, 3, 4, 5, and 7 show duplications similar in size of 465 kb (from 152,746,927 to 153,211,606 bp), 446 kb (from 152,712,273 to 153,158,679 bp), 502 kb (from 152,746,927 to 153,248,667 bp), 475 kb (from 152,712,273 to 153,186,885 bp), and 455 kb (from 152,793,677 to 153,248,722 bp), respectively. Patient 8 carries the smallest duplication of 209 kb, with proximal and distal breakpoints at 152,850,692 and 153,059,427 bp; on the other hand, patients 2 and 6 carry bigger duplications of 3.46 Mb (from 151,032,487 to 154,494,649 bp) and 6.36 Mb (from 148,203,618 to 154,561,665 bp), respectively. The different-sized microduplications of the cousins (patients 4 and 7) are probably due to the different resolution of the array used.

Where it was possible to assess (patients 1, 4, 5, 7, and 8), the duplication was shown to be inherited. The methylation status of the X chromosome in the patients’ mother was assessed using the Humara Androgen Receptor gene methylation assay as previously described (Allen et al., 1992). X inactivation testing on the DNA samples for all the asymptomatic mothers demonstrated a marked skewed inactivation with an XCI ratio >90:10, which explains the absence of clinical features in these patients.

Figure 5.
Unusual monomorphic long-lasting rhythmic theta activity in patient 8. 
Epilepsia @ ILAE
**Discussion**

The aim of the study is to define the epileptic features and the EEG pattern associated with MECP2 duplication syndrome.

Epilepsy is considered one of the main symptoms of Xq28 duplication including the MECP2 locus (Echenne et al., 2009). In a recent review, Ramocki et al. (2010) reported a 52% incidence of epilepsy in 110 patients previously described. The incidence of epilepsy found in our series is higher (6 of 8 patients; 75%) than previously reported, probably because it was generally estimated regardless of the patients’ age.

In fact, a more thorough analysis of several cases published to date reveals a different age of seizure onset during the evolution of the disease, which explains the wide range of variability of incidence of epilepsy: from 33.3% (Smyk et al., 2008) to 65.2% (Friez et al., 2006). It is therefore possible that the variability found in different studies depends on the patients’ age at the time of recruitment. Analyzing not only the incidence of epilepsy in all the patients with MECP2 duplication so far described, but also the age of seizure onset and the duration of follow-up (Table 2), it is evident that seizure onset in early childhood (≤3 years) is a rare event (5/41, 12%), but nonepileptic patients older than 13 years are also very unlikely to be found (3/42, 7.1%).

The six epileptic cases presented here confirm that seizure onset occurs between 9 and 13 years. Only in one case (Patient 8 of this series) generalized seizures associated with fever began at the age of 6 months, as described previously only once (Armfield et al., 1999). As soon as he started therapy he suddenly became seizure-free and remained without epilepsy and AEDs until the age of 24 years. On the other hand, in line with literature data, the two patients younger than 3 years reported here had not yet presented seizures.

Both from literature data and from the present series it is now clear that epilepsy has to be considered a significant symptom in MECP2 duplication syndrome, occurring in >90% of patients with long-term follow-up. The age of epilepsy onset is usually in childhood (prevalent between 4 and 13 years), although in about one of five cases it may occur later (adolescence and young adulthood) (Table 2).

Regarding the characteristics of seizures, few studies have thoroughly described seizure semiology associated with Xq28 duplication. The reported seizures are frequently polymorphic and include generalized tonic–clonic (Van Esch et al., 2005; Smyk et al., 2008; Echenne et al., 2009; Lugtenberg et al., 2009; Bartsch et al., 2010), atypical absences (Echenne et al., 2009), atonic (Friez et al., 2006; Lugtenberg et al., 2009; Reardon et al., 2010), and myoclonic seizures (Clayton-Smith et al., 2009; Echenne et al., 2009; Lugtenberg et al., 2009). Partial complex

| Table 2. Literature data of epilepsy characteristics in patients with MECP2 duplications |
|-----------------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Authors                                      | Patients (n)       | Epilepsy          | No epilepsy       | Not available data | ≤3 years | 4–12 years | ≥13 years | Not available data | ≤3 years | 4–12 years | ≥13 years |
| Lahn et al. (1994)*                         | 3                  | 3                 | 0                 | 0                 | 0       | 3          | 0         | 0                 | 0       | 0          | 0         |
| Pai et al. (1997)                           | 6                  | 4                 | 2                 | 0                 | 0       | 2          | 0         | 0                 | 0       | 0          | 0         |
| Lubs et al. (1999)                          | 5                  | 2                 | 3                 | 0                 | 0       | 2          | 0         | 0                 | 0       | 0          | 0         |
| Meins et al. (2005)                         | 1                  | 1                 | 0                 | 0                 | 1       | 0          | 0         | 0                 | 0       | 0          | 0         |
| Sanlaville et al. (2005)                    | 2                  | 0                 | 2                 | 0                 | 0       | 0          | 0         | 0                 | 0       | 0          | 0         |
| (functional disomy)                          |                     |                   |                   |                   |         |            |           |                   |         |            |           |
| Van Esch et al. (2005)                      | 13                 | 4                 | 9                 | 1                 | 1       | 1          | 1         | 1                 | 4       | 2          | 2         |
| Friez et al. (2006)                         | 13                 | 9                 | 4                 | 4                 | 2       | 3          | 0         | 2                 | 1       | 1          | 0         |
| del Gaudio et al. (2006)*                   | 0                  | 0                 | 0                 | 0                 | 0       | 0          | 0         | 0                 | 0       | 0          | 0         |
| Smyk et al. (2008)                          | 3                  | 1                 | 2                 | 0                 | 1       | 0          | 0         | 2                 | 0       | 0          | 0         |
| Velinov et al. (2009)                       | 1                  | 0                 | 1                 | 0                 | 0       | 0          | 0         | 0                 | 0       | 0          | 0         |
| Kirk et al. (2009)                          | 3                  | 2                 | 1                 | 1                 | 0       | 0          | 1         | 0                 | 0       | 0          | 1         |
| Lugtenberg et al. (2009)                    | 13                 | 9                 | 4                 | 1                 | 0       | 6          | 2         | 0                 | 0       | 4          | 0         |
| Clayton-Smith et al. (2009)                 | 17                 | 8                 | 9                 | 1                 | 2       | 3          | 2         | 1                 | 5       | 3          | 0         |
| Prescott et al. (2009)                      | 2                  | 1                 | 1                 | 0                 | 0       | 1          | 0         | 0                 | 0       | 1          | 0         |
| Ramocki et al. (2009)                       | 9                  | 4                 | 5                 | 0                 | 0       | 3          | 1         | 0                 | 1       | 3          | 1         |
| Echenne et al. (2009)                       | 5                  | 3                 | 2                 | 0                 | 0       | 0          | 2         | 0                 | 0       | 0          | 0         |
| Budisjeanu et al. (2011)                    | 1                  | 0                 | 1                 | 0                 | 0       | 0          | 0         | 0                 | 0       | 0          | 0         |
| **97**                                      | **51**             | **46**            | **10**            | **5/41**          | **29/41**| **7/41**    | **4**     | **16/42**          | **23/42**| **3/42**    | **0**     |
| (functional disomy)                          |                     |                   |                   |                   |         |            |           |                   |         |            |           |
| Our series                                  | 8                  | 6                 | 2                 | 0                 | 0       | 4          | 2         | 0                 | 0       | 0          | 0         |

*a*Lahn described 10 patients with Xq–Yq interchange. Only three patients present an Xq28 functional disomy.

*b*Patients with complex Xq rearrangement not corresponding with classical Xq28 duplication. Notably one patient with triplication of the MECP2 gene shows a more severe picture and an early onset epilepsy (he died at 3 months of age).
seizures have been also described (Lugtenberg et al., 2009), as well as reflex seizures (Ramocki et al., 2010).

In the present series, patients with epilepsy do not have an absolutely distinct type of seizure, but all patients with epilepsy present a sudden atonic component confined to the head in one (Patient 4) and drop attack in the remainder. Because no seizures were captured with video-EEG recordings and all patients are severely cognitively impaired, the definition of the clear type of seizures is not possible and the cognitive disturbance cannot be determined. Sometimes a myoclonic component is also evident. Only one patient (6) has seizures during sleep. Epilepsy onset could be dramatic, with daily drug-resistant seizures, and the patients’ safety can be seriously impaired when atonic seizures or drop attacks occur. In previously reported cases, drug resistance was found in almost half of the patients (Pai et al., 1997; Lubs et al., 1999; Smyk et al., 2008; Echenne et al., 2009; Lugtenberg et al., 2009; Prescott et al., 2009; Ramocki et al., 2009). Seizures were poorly controlled in most patients in our series (4/6) and in another one (patient 5) seizures were controlled only by a combination of three drugs, namely VPA, LTG, and CLB. Status epilepticus was reported previously (Lahn et al., 1994; Lubs et al., 1999) with fatal outcome in one patient (Smyk et al., 2008), confirming the severity of epilepsy in this syndrome.

EEG features are not fully described in patients with MECP2 duplication syndrome: paroxysmal rhythmic theta activity in the posterior regions (Ramocki et al., 2010) as well as multifocal spike discharges, generalized spike and slow wave activity without polyspike-waves (Echenne et al., 2009) have been observed. In our series, EEG studies obtained during wakefulness show slow, irregular background activity. Unusual monomorphic long-lasting theta and beta rhythmic activity was also present in six patients (Fig. 5). The occurrence of unusual rhythmic EEG activities suggests a genetic background, as with the EEG patterns described in the Angelman syndrome, featuring high-amplitude rhythmic delta activity over the frontal regions (Korff et al., 2005) or prolonged runs of rhythmic theta activity with centrotemporal emphasis (Laan et al., 1997). Interictal epileptiform abnormalities were present in seven of eight patients, consisting of generalized slow spike and wave, presenting asynchronous discharges at 2–2.5 Hz with fronto-temporal predominance (Fig. 4). Erratic myoclonic jerks with no clear correlation to the spike-and-wave complexes were recorded in four patients in our series, both in wakefulness and sleep (Fig. 3). This feature is also considered similar to that described in patients with other genetic syndromes, such as the 4p syndrome (Sgrò et al., 1995) or the Angelman syndrome (Elia, 2009).

Sleep EEG studies also showed abnormal background activity. Spindles and K complexes are often abnormal in morphology and amplitude. Spindle synchronization in some patients was delayed in comparison to the normal stages of evolution of the EEG (Fig. 2). This sleep pattern encountered in younger patients with MECP2 duplication syndrome may recall the so called “extreme spindles,” abnormally high voltage and abnormally continuous sleep spindles, described in children with different types of mental retardation due to brain damage, malformation, and chromosomal defects (Gibbs & Gibbs, 1973).

The occurrence of this EEG pattern was demonstrated in one of our patients (Patient 4) before seizure onset. In particular, this patient’s EEG records presented unusual fast rhythms over a slow background activity and erratic myoclonic jerks between the age of 5 and 7 years; then from the age of 7 and 11 years spike and wave discharges with anterior predominance were clearly recorded, even before seizure onset, which occurred at 12 years. Even after seizure control the same EEG features persisted.

For this reason, in these young male patients we encourage performing EEG longitudinal studies that may reveal a characteristic EEG pattern and provide important prognostic information (Ramocki et al., 2009).

The phenotype of MECP2 gene duplication in male patients covers a large spectrum of neurodevelopmental disorders. Because the extent of MECP2 duplication varies from one patient to another, and because other genes may be involved, possibly leading to different phenotypes, large-scale studies in cohorts are needed to better characterize the range of clinical variability in this genetic disorder (Echenne et al., 2009; Ramocki et al., 2009).

However, in our series the patient (Patient 6) who carries the greatest duplication (6.36 Mb) shows a more severe epileptic phenotype, taking on the characteristics of an epileptic encephalopathy, which could be presumably attributed not only to MECP2 duplication but also to other involved genes. It is possible that the other case (Patient 2) carrying a large duplication (3.43 Mb) has not yet developed a full severe picture because of his younger age.

In fact, although MECP2 is thought to be the most important dosage-sensitive gene contributing to the neurologic phenotypes in patients with Xq28 duplications, the duplications can involve 19 flanking genes, some of which are associated with neurologic disease, including SLC6A8 (non-specific mental retardation), L1CAM (X linked spastic paraplegia), FLNA (neuronal cell migration PVNH), and GDI1 (nonsyndromic XLMR) (Clayton-Smith et al., 2009). It has also been hypothesized that Xq28 rearrangements contribute to or modify clinical phenotype through the disruption of the regulatory regions of nearby genes (Ramocki et al., 2010; Breman et al., 2011). Further studies are needed to
pinpoint dosage-sensitive genes of the Xq28 region and identify the genes responsible for developmental delay/mental retardation and epilepsy and their role in different neuronal populations.

Finally, studies of animal models strongly support the link between the slow progressive worsening of the clinical picture described during life in the MECP2 duplicated patients (expressed by the progressive loss of motor skills, late onset of seizures, and evolution toward drug resistance) and the role of the MECP2 gene. In transgenic mice with MECP2 overexpression, Collins et al. (2004) described a clinical pattern characterized by a progressive neurologic disorder, including seizures and spasticity, and premature death. Of interest, apart from progressive spasticity, the epilepsy subtypes observed in these mice are similar to the absences and tonic–clonic seizures diagnosed in some of the affected men. An abnormal EEG pattern is accompanied by frequent akinetic episodes. The neurologic phenotype in these animals was modulated by the levels of MECP2, since higher protein levels resulted in more severe phenotypes (Van Esch et al., 2005). Regulation of MECP2 is critical for the appropriate development and maintenance of neuronal function; MeCP2 protein has an essential role in fine-tuning dendritic growth and spine maturation (Zhou et al., 2006). Furthermore, recent data suggest that neurologic impairment worsens at seizure appearance, manifesting itself as a loss of previously acquired abilities, such as loss of purposeful hand use, self-help skills, and/or ambulation (Ramocki et al., 2010), just as experienced by our patients.

**Conclusions**

The detailed and prolonged study of eight new cases, the thorough review of the literature concerning epilepsy, and EEG features associated with duplication of MECP2 gene provide some useful observations for clinical practice. First of all, epilepsy has to be considered as a significant symptom of the syndrome experienced in >90% of affected adolescents and rarely observed before 3 years of age. Although there is no absolutely distinct type of seizure, episodes of loss of tone of the head and/or of the trunk are the most represented types. The typical interictal EEG pattern is characterized by abnormal background activity, with generalized slow spike and wave asynchronous discharge with frontotemporal predominance. During sleep, EEG shows abnormal background activity; spindles and K complex are often abnormal in morphology and amplitude. Response to therapy is generally poor and drug resistance is a significant feature.

Although these cases and a review of the literature indicate that epilepsy associated with MECP2 duplication syndrome cannot be considered a useful marker for early diagnosis, we can hypothesize that further EEG and seizure descriptions may help identify a more specific epileptic phenotype.

Because many patients with dysmorphic signs and developmental delay are still left without diagnosis, especially the older ones who may not have had a complete genetic evaluation in the past, we believe that the characterization of a more specific epileptic phenotype could well be used as a suspicion tool to identify patients who were not yet diagnosed.

**Disclosure**

None of the authors have any conflict of interest to disclose.

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

**References**


