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Case Reports

Simultaneous Onset of Infantile Spasms in Monozygotic Twins

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The clinical, electroencephalographic, and genetic findings are reported for three pairs of monozygotic twins who developed infantile spasms in their first year. In all three pairs, the spasms started on the same day in each member of the pair. Neither sequencing of the *ARX* and *CDKL5* (alias *STK9*) genes nor array comparative genomic hybridization assessment revealed any abnormalities. The long-term outcome was poor in all twins, although with different severity in individual pairs. These findings suggest that genes other than those currently known likely play a role in predisposition to infantile spasms, and that genetic susceptibility is linked to a variable phenotypic expression, ranging from quite benign to very severe, in monozygotic twins with no other apparent risk factors.

Coppola G, Grosso S, Verrotti A, D'Aniello A, Pascotto A. Simultaneous onset of infantile spasms in monozygotic twins. Pediatr Neurol 2010;43:127-130.

Introduction

The concept of a genetic involvement in infantile spasms is based on a number of findings: (1) idiopathic infantile spasms recur in approximately 5-10% of cases; (2) family history of epilepsy or febrile convulsions is present in 7-17% of cases [1], and in 40% of cryptogenic cases [2]; (3) recurrence of infantile spasms in siblings [3] or in monozygotic twins [3-6]; and (4) high incidence of infantile spasms in autosomal dominant genetic syndromes such as tuberous sclerosis [7], neurofibromatosis [8], CHARGE syndrome [9], X-linked syndromes as incontinentia pigmenti and double cortex-lissencephaly syndrome [10], or other conditions due to chromosomal translocations (e.g., Down syndrome or Williams syndrome). Recently, some monogenic conditions have been recognized, with various abnormalities of the aristaless related homeobox gene, *ARX* [11,12], the X-linked cyclin-dependent kinase-like 5 gene, *CDKL5* (alias *STK9*) [6,13], or the ATPase-sensitive potassium channel Kir6.2 gene, *KCNJ11* [14].

The clinical and electroencephalographic (EEG) pattern and genetic involvement were studied in three pairs of monozygotic twins who had simultaneous onset of infantile spasms (i.e., on the same day, within each twin pair) before the age of 1 year.

Case Report

Twin Pair 1

Monozygotic male twins, age 4 years 7 months old at presentation, were born to healthy, nonconsanguineous parents. They were born at term (38th gestational week) by caesarean section after an uneventful pregnancy. Birth weight was 2650 g and 2450 g, body length was 50 cm and 49 cm, and occipital-frontal circumference was 34.5 cm and 35 cm for twin A and B, respectively. In both, the Apgar scores were 8 at 1 minute and 10 at 5 minutes. Monozygosity of the twins, who were born of one placenta, was confirmed by typing to four highly polymorphic microsatellite markers, which yielded a probability index of approximately 99.97%.

Developmental milestones were reported as normal until the age of 8 months, when daily episodes of flexor spasms were noted in both twins on the same day (often within an interval of few minutes). Fifteen days later, when daily clusters of spasms recurred, the infants were admitted to the authors' clinic and West syndrome was diagnosed. Ictal EEG indicated bouts of spasms quite similar in number and duration in both children (Fig 1). A hypsarrhythmic pattern was observed, almost overlapping in both twins (Fig 2). At admission, both twins exhibited moderate generalized hypotonia, absence of grasping reflex, and poor visual pursuit. Neurometabolic stepwise investigations (which included assays of serum amino acids, very long chain fatty acids, lysosomal enzymes, lactate, pyruvate, serum ammonia, and urine organic acids) and cytogenetic karyotyping were performed.

To exclude a mitochondrial disorder, stepwise investigations were performed, including assays of serum and cerebrospinal fluid lactate and pyruvate, plasma acylcarnitine profiles, urine organic acids, and plasma amino

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Received November 28, 2009; accepted April 14, 2010.

doi:10.1016/j.pediatrneurol.2010.04.010 • 0887-8994/$—see front matter

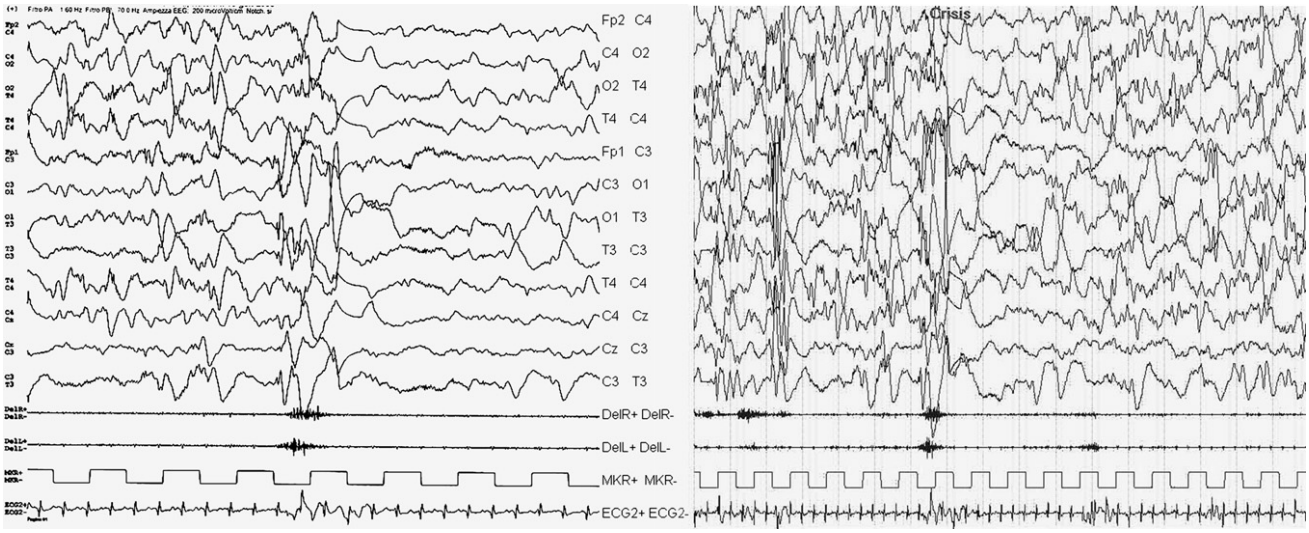

Figure 1. Twin pair 1: At age 8 months, ictal electroencephalograms of two monozygotic twins exhibit recurrent brief generalized spasms (left, twin A; right, twin B).

acids, as well as muscle histology and ultrastructure studies. The diagnosis of a respiratory chain complex defect was made based on a reduction in respiratory chain complex enzyme activity of 80% or more, relative to control tissue samples. GLUT-1 deficiency was also excluded. Sequencing of the *ARX* and *CDKL5* genes, together with array comparative genomic hybridization assessment, disclosed no abnormal findings. Magnetic resonance imaging findings at 8 months were normal, except for a mild excess of prefrontal space. Visual and auditory evoked potentials were normal.

Spasms were poorly responsive to the following treatments, either singly or in various combinations: vigabatrin, adrenocorticotropic hormone (as the synthetic analog tetracosactide depot, 1 mg/mL), valproate, topiramate, lamotrigine, clonazepam, and carbamazepine. At the age of 11 months, the almost subcontinuous tonic seizures in both children (Fig 3) were partially controlled by the following the combination valproate-clonazepam-phenobarbital and phenytoin. At the age of 2 years and 5 months, ketogenic diet in combination with valproic acid, phenobarbital, and clonazepam led to seizure control in both children. Nevertheless, psychomotor development was severely delayed. The twins were unable to sit unaided or to grasp objects with either hand, and they exhibited stereotypic movements, involving mainly the head and upper limbs. At the age of 2 years and 6 months, EEG recordings indicated multifocal spike and wave discharges and frequent diffuse fast rhythms at 10 Hz, which were interpreted as unusual interictal activity.

At the age of 4 years, seizures were well controlled by a combination therapy including phenobarbital, valproate, zonisamide, and clonazepam. At the age of 5 years and 7 months, both children exhibited severe mental delay and autistic-like behavior. Both were aphasic, and only one of the pair could sit unaided or grasp objects.

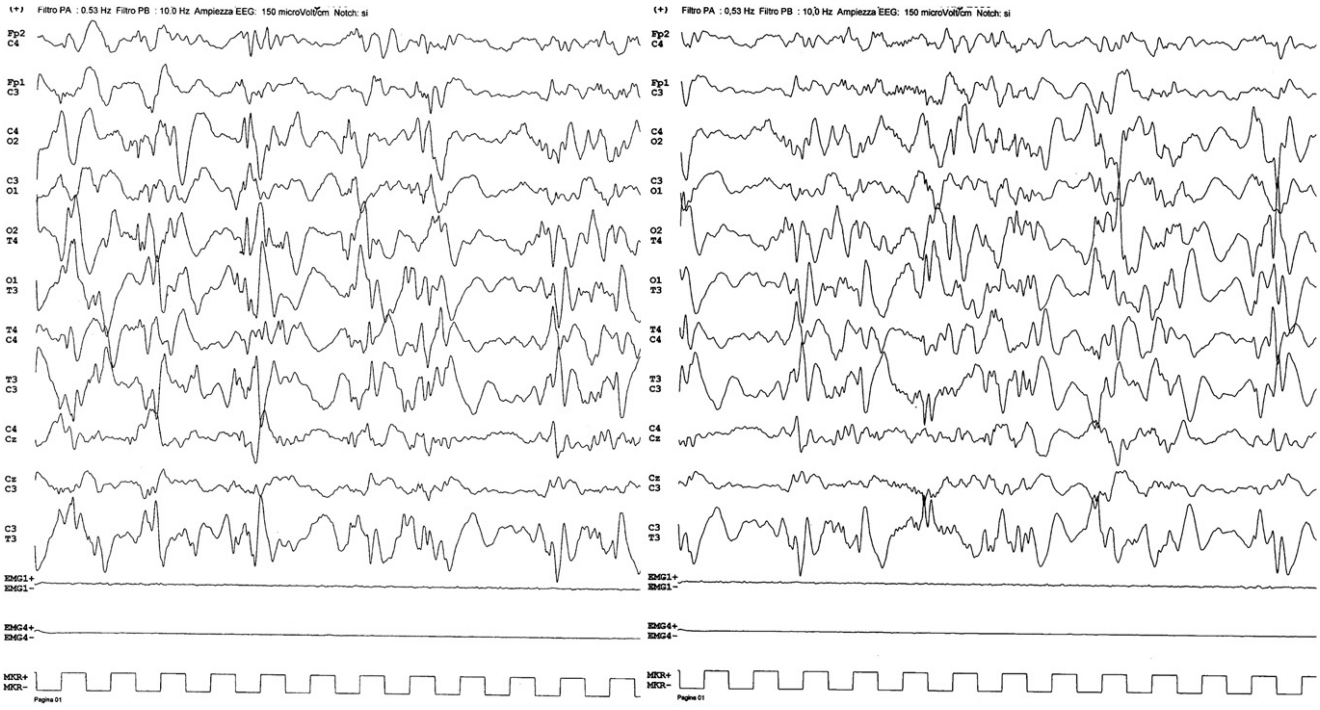

Figure 2. Twin pair 1: At age 8 months, sleep electroencephalograms of the same twins exhibit hypsarrhythmic pattern (left, twin A; right, twin B).

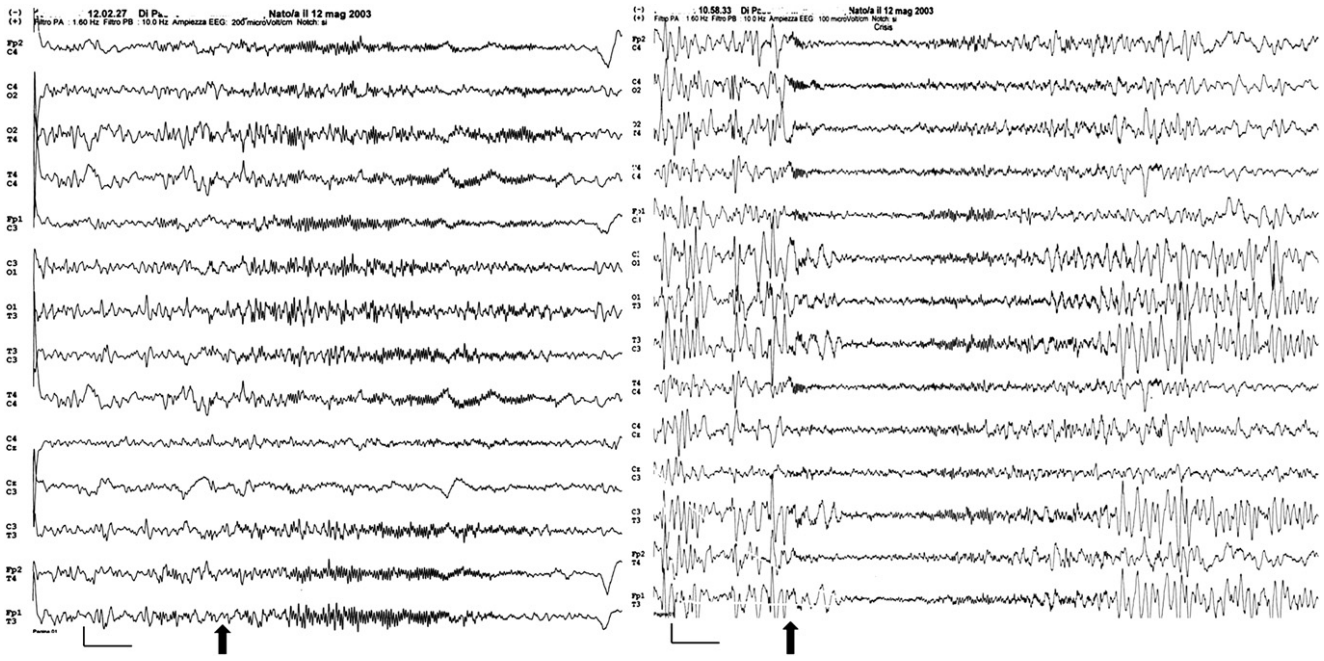

Figure 3. Twin pair 1: At age 11 months, sleep electroencephalograms exhibit almost subcontinuous tonic seizures in both children (left, twin A; right, twin B). Arrows indicate start of tonic spasm.

Twin Pair 2

Monozygotic female twins, age 10 years at presentation, were born to healthy, nonconsanguineous parents. They were born preterm (36th gestational week) after an uneventful pregnancy and delivery. Birth weight was 1950 and 2180 g for twin A and B, respectively. In both, the Apgar score was 9 at 1 minute and 10 at 5 minutes. These twins were born of one placenta and monozygosity was confirmed by typing to four highly polymorphic microsatellite markers, which yielded a probability index of approximately 99.97%. At age 5 months and 20 days, both sisters developed isolated infantile spasms, which shortly became very frequent and were associated with a hypsarrhythmic pattern on EEG. A computed tomography scan excluded brain calcifications, and magnetic resonance imaging did not indicate further abnormalities. Treatment with adrenocorticotropic hormone (as tetracosactide depot, 2 IU/kg in 24 hours) for 2 weeks led to complete disappearance of the spasms, with EEG normalization.

Neurometabolic and mitochondrial disorders were excluded, based on the same tests as in twin pair 1.

At the age of 16 months, almost weekly focal seizures with secondary generalization appeared in both sisters, together with paroxysmal EEG discharges over bitemporal regions. Seizures were partially controlled by anticonvulsant therapy (valproic acid, phenobarbital, benzodiazepines, levetiracetam, topiramate, and lamotrigine) given singly or in combination. The best results were achieved with phenytoin and levetiracetam. As of writing (i.e., patient age 10 years), focal complex seizures recurred every 2 months and were associated with severe mental retardation and behavioral disorder, including aggressivity and wake-sleep cycle disorder. Furthermore, both twins exhibited occipital-frontal circumference of 49 cm (<3rd centile), mild strabismus, malpositioned teeth, and thin hair.

Twin Pair 3

Monozygotic female twins, age 5 years 8 months at presentation, were born at term, by caesarean section, to healthy, nonconsanguineous parents. Birth weight was 2310 and 2420 g, body length was 48 and 50 cm, and occipital-frontal circumference was 34.5 and 33.5 cm for twin A and B, respectively. For both, Apgar scores were 9 at 1 minute and 10 at 5 minutes. They were born of one placenta and monozygosity was confirmed by typing to four highly polymorphic microsatellite markers, which yielded a probability index of approximately 99.97%. First spasms appeared in both twins on the same day, at the age of 7½ months; the spasms were associated with a typical hypsarrhythmic pattern. Clusters of spasms were overall more frequent in the twin B, although adrenocorticotropic hormone therapy (as tetracosactide depot) led to complete seizure control and normalization of prolonged wake and sleep video-EEG recordings in 2 weeks in both children.

Neurometabolic and mitochondrial disorders were excluded, based on the same tests as in twin pairs 1 and 2.

At the age of 12 months, both children exhibited mild hypotonia, visual pursuit was well developed, but could not stay seated alone. Findings from cranial magnetic resonance imaging, auditory brainstem responses, and visual evoked potentials were normal in both children. Although twin A stopped taking valproic acid at 18 months of age, her sister developed focal seizures, which consisted of head and eye deviation to the left together with staring, and sometimes with secondary generalization. These seizures in twin B required further therapy with valproic acid and levetiracetam until the age of 4 years; in this patient, prolonged video-EEG recording disclosed recurrent sharp waves over the right parietal-occipital regions. Both children could walk alone at the age of 2½ years. At age 5½ years, both twins exhibited moderate mental retardation. In both cases, sleep and wake EEG recordings indicated no clear paroxysmal epileptic discharges.

Methods

Blood samples were obtained after informed consent. DNA was extracted from peripheral blood using a QIAamp DNA blood kit (Qiagen, Valencia, CA; Hilden, Germany). DNA samples were screened for mutation in *ARX* and *CDKL5* using a Transgenomic (Omaha, NE) WAVE system with denaturing high performance liquid chromatography. The *CDKL5* and *ARX* coding portion were entirely sequenced as well.

Comparative genomic hybridization was performed using the Agilent human genome CGH microarray kit 44B (Agilent, Santa Clara, CA). The array harbors approximately

44,000 60-mer oligonucleotides, covering the entire genome at the average resolution of 75 kb. Competitive hybridization was performed according to the manufacturer's protocols.

Discussion

The three pairs of monozygotic twins reported here share onset of infantile spasms within their first year (simultaneously, within each pair) and an overall poor neurobehavioral outcome in the long term. In none of the twins did family history, pregnancy, or delivery indicate risk factors for metabolic disorders, brain malformations, or acquired brain damage (either prenatal or perinatal).

Consistent with this, cranial magnetic resonance imaging indicated no significant abnormalities in all children. The infantile spasms were diagnosed early and were quickly controlled by hormonal therapy in twin pairs 2 and 3, whereas they were drug-resistant and soon shifted to tonic seizures in twin pair 1. In three of the four children from pairs 2 and 3, spasms were followed by focal onset seizures, after a seizure-free period. In twin pair 2, focal seizures still recurred every 2 months at the age of 10 years and, remarkably, appeared on the same days in both twins.

All children developed a moderate (twin pair 2 and 3) to profound (twin pair 1) mental retardation, associated with behavioral disorders, including autistic-like stereotypic movements (twin pair 1) or hyperactivity and aggressivity (twin pair 2). Acquired microcephaly and mildly dysmorphic body traits were also present in twin pair 2.

The six monozygotic twins reported here contribute their own particular features to the available literature. Factors predisposing to infantile spasms such as prematurity [3,4,15], tuberous sclerosis complex [3], mitochondrial pathology [16], or a *CDKL5* gene mutation leading to early onset variant of Rett syndrome [6], were excluded in the monozygotic twins reported here. Also excluded were other conditions linked to infantile spasms, such as neurofibromatosis type 1, CHARGE syndrome, and cortical migration disorders.

The present twin pairs were born after an uneventful pregnancy and delivery, and no other complications were reported after birth. From this point of view, they are similar to the monozygotic twins reported by Reiter et al. [5], although they had a much less benign outcome. Indeed, all six of the present twins exhibited moderate or severe delay of psychomotor development. These cases suggest that a genetic susceptibility in monozygotic twins with no other apparent risk factors may be linked to a variable phenotypic expression, ranging from quite benign to very severe clinical patterns.

The course of genetic screening including *ARX* and *CDKL5* mutation scanning combined with DNA rearrangement study by array comparative genomic hybridization in the present twin pairs has rarely been performed previously, the exceptions being the monozygotic twins with mt-DNA mutation [16] and those with *CDKL5* mutation and early onset Rett syndrome [6].

Onset of spasms was simultaneous in the present twin pairs, and even their disappearance was almost overlapping, as in most reported cases [3-6], once again pointing to some genetically determined, time-dependent biological factor, apart from any environmental influence. Notably, spasm onset at the same age is also reported in other siblings with West syndrome [3,5].

In conclusion, findings in these six cases indicate that genes other than those currently known likely play a role in genetic predisposition to infantile spasms. A wider genome search in families with recurrence of idiopathic or cryptogenic West syndrome should improve understanding on this topic.

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