FULL-LENGTH ORIGINAL RESEARCH

Epilepsy in ring 14 syndrome: A clinical and EEG study of 22 patients

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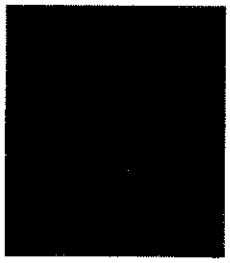
Epilepsia, **(*):1–10, 2013
doi: 10.1111/epi.12393



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Summary

Purpose: To characterize epileptic phenotype, electroencephalography (EEG) features, and epileptic evolution in patients with ring 14 r(14) syndrome.
Methods: Twenty-two patients with ring chromosome 14 were enrolled in the study. We examined age at onset, seizure semiology and frequency at onset and at follow-up, drug responsiveness/resistance, and interictal/ictal EEG data. The degree of severity of the epileptic phenotype negatively influences child cognitive development.
Key Findings: The incidence of epilepsy in patients with r(14) syndrome is virtually 100%, characterized by early onset, polymorphic seizures, and drug-resistant seizures. In addition, we ascertained focal secondarily generalized epilepsy, seizure cluster tendency, frequent status epilepticus, and a rather typical epilepsy evolution. EEG abnormalities consisted of slow background activity with pseudoperiodic bursts of generalized slow waves in the early stage, focal frontotemporal or temporoposterior slow waves with multifocal spikes interposed, and unusual rhythmic fast recruiting posterior spikes followed by secondary generalization. The degree of severity of the epileptic phenotype negatively influences child cognitive development.
Significance: This study provides a more precise definition of seizure types, natural history, and drug responsiveness of r(14) syndrome, a highly epileptogenic chromosomal condition.
KEY WORDS: Ring 14 syndrome, Childhood epilepsy, Electroclinical phenotype, Epileptic evolution.

The ring 14 r(14) syndrome, first described by Gilgenkrantz et al. in 1971, is a rare cytogenetic disorder. It is generally associated with a variable loss of genetic material of the terminal long arm of the chromosome (14q32.31-qter) (Gilgenkrantz et al., 1971; Zelante et al., 1991; Wintle et al., 1995; Zollino et al., 2009). Individuals with r(14) syndrome generally have developmental delay, distinctive facial features, varying degrees of cognitive disability, behavioral disorders, and seizures. Other common features include hypotonia, microcephaly, growth retardation, ocular abnormalities, and mild skeletal abnormalities. Epilepsy is the most common and severe feature encountered in the syndrome, with very few exceptions (Bowser-Riley et al., 1981; Gilgenkrantz et al., 1984; Zelante et al., 1991). Accurate clinical and genetic characterization of this condition has been recently attempted, and putative genes involved in epileptogenesis do not seem to map to the terminal 14q region, usually deleted in formation of the ring (Zollino et al., 2009). In fact, a linear terminal 14q deletion syndrome does not include epilepsy among its component manifestations. Instead, seizures are likely caused by the ring itself, rather than loss of chromosomal material (Van Karnebeek et al., 2002; Zollino et al., 2009). Furthermore, the evidence of other ring chromosomes without epilepsy emphasizes the importance of selected rings in mapping epilepsy susceptibility genes.

Accepted August 27, 2013.
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Epilepsy occurs in virtually all cases of r(14) syndrome, but its electroclinical phenotype is poorly characterized and a detailed description of clinical and electroencephalography (EEG) findings and their natural history is lacking.

The purpose of the present study is to better define the seizure types, natural history, and drug responsiveness of r(14) syndrome, a highly epileptogenic chromosomal condition.

Subjects and Methods

With the support of the Ring 14 International Association, a total of 22 patients with ring chromosome 14, coming from several countries (eight from Italy, six from France, three from United Kingdom, three from the United States, one from Sweden, and one from Brazil) were enrolled in the present study up to December 2009. Sixteen of the patients have been described previously in a detailed genetic study (Zollino et al., 2009). Subjects included were 13 male and 9 female patients, aged 26 months to 22 years.

All data have been obtained from the following: (1) medical records and questionnaires translated in different languages and filled in by general practitioners and neurologists, and (2) systematic assessments of patients seen at participating medical centers or at dedicated clinics during meetings of the Ring 14 family support group. All data were entered and extrapolated from the password-protected Electronic International Database of Ring 14 by two medical doctors (SG and AS). This registry collects selected and linked-anonymized clinical and genetic information of patients with chromosome 14 aberrations (www.ring14.com). Consent forms were signed by parents or legal guardians regarding medical record examination, physical examination, and performance of genetic and instrumental tests.

The following characteristics of epilepsy were examined: age at onset, seizure semiology and frequency at onset and at follow-up, drug responsiveness/resistance, and interictal/ictal EEG data.

Seizures were classified in accordance with the International League Against Epilepsy (ILAE) classification of epilepsy and epileptic syndrome (Engel, 2001). EEG studies were recorded according to the international 10-20 system during wakefulness, spontaneous sleep, and arousal. Long-term video-EEG was performed in a few select cases.

Clinical history was collected in terms of family history of epilepsy, intrauterine development, delivery (Apgar scores), and developmental milestones (motor, language, and social behavior). Clinical evaluation was also completed including physical, neurologic, and neuropsychological data. Assessment of patient characteristics and resources included an evaluation of the child's cognitive function through direct norm-referenced tests when possible, and adaptive functioning including behavioral concerns and evaluation of the ecologic context. All patients underwent neuroimaging studies, most commonly by magnetic resonance imaging (MRI).

In an attempt to construct a deletion map and to establish genotype–phenotype correlation, we previously performed a detailed genetic study, as reported separately, to establish the mosaic status of the ring and the size of a cryptic terminal deletion in the ring (Zollino et al., 2009). Subsequently, genetic findings were compared with clinical features to establish a potential correlation between the severity of cognitive, epileptic, and neuroradiologic phenotypes and loss of chromosomal material.

Results

Epileptic phenotype

Table 1 reports clinical features of patients presenting with epilepsy.

In our series of 22 patients with ring 14 chromosome, all subjects had epilepsy (22/22). The median age at the last available neurologic follow-up (December 2009) was 9 years and 8 months, with a range of 26 months to 22 years. Subjects included were 13 males and 9 females, aged 26 months to 22 years. Mean age at the first seizure was 1 year and 2 months (range 1 month–4 years). Onset of epilepsy was before the age of 1 year in 14 patients (14/22); among them, 11 had seizures before the age of 6 months.

Epileptic seizure at onset

Seizure type at onset was generalized, tonic–clonic, myoclonic–tonic, and clonic in 9 of 22 patients (cases 1, 6, 7, 11, 13, 16, 17, 19, and 20). Both focal hemiclonic and generalized seizures were also present in two subjects (cases 4 and 14). In the remaining 11 cases (2, 3, 5, 8, 9, 10, 12, 15, 18, 21, and 22), clinical and EEG data supported a diagnosis of focal seizures with secondary generalization, mainly originating from the midtemporal and frontal lobes. All cases except one presented with afebrile seizures. In subjects 15 and 18, the occurrence of afebrile seizures was concomitant with a viral gastrointestinal infection and shortly after vaccination, respectively. Soon after onset, epilepsy showed a particularly severe phenotype; in fact, 8 of 22 patients experienced prolonged seizures or clusters of repetitive seizures leading to generalized tonic–clonic or unilateral clonic status epilepticus (SE) (cases 2, 3, 6, 7, 8, 9, 12, and 13); in 9 patients, seizures were prolonged or occurred in daily clusters (cases 1, 4, 5, 14, 16, 18, 19, 21, and 22). Only five patients presented at onset with a single-episode epileptic event. In more than half of the patients the seizures occurred during sleep or falling asleep (12 cases; 1, 3, 4, 5, 6, 9, 12, 13, 16, 17, 19, and 20). This circadian pattern was not clearly reported in the remaining patients. In more than half of the patients, phenobarbital (PB) was the most effective and commonly used drug against seizures (14 of 22 cases; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 22). In three subjects the therapeutic choice was carbamazepine(CBZ) (cases 1, 17, and 21). In two children (cases 2 and 19), inaugural seizures were treated with intrarectal and intravenous

Table 1. Epilepsy features

Case no. Patient sex	Age/semiology, at onset	AEDs, at onset	Age/semiology, at last follow-up	AEDs, at last follow-up	Drug response
1. M	25 m/generalized clusters	CBZ, BZD, VPA	7 y 2 m/generalized, focal, atypical absences, CSE	PHT, CBZ, LTG, PB, LEV, TPM, CLB, oral midazolam	DR
2. F	10 m/focal + SG SE	Diazepam, i.v. PHT, midazolam	3 y 5 m/CPS clusters, myoclonic	CBZ, VPA, TPM, CZP, CLB, VPA + CZP, midazolam	Sporadic
3. M	15 m/focal SE + TCG clusters	Diazepam, lorazepam, CZP + PB	9 y/CPS clusters, CSE, NCSE	PB, CZP, CBZ, OXC, CLB, TPM, LTG, VPA, FLB, pregabalin, PHT, NTZ, deflazacort	DR
4. M	2 m/generalized clusters + focal	PB	20 y/generalized, focal, CSE, NCSE?	ACTH, CZP, VPA, CBZ, GVG, ETS, CLB, PB, FLB, LTG, Ig, NTZ, FLB	Control
5. M	2 m/focal + SG clusters	PB	2 y 10 m/CPS clusters	PB + CLB + VPA, lorazepam	DR
6. M	10 m/generalized >CSE	PB	15 y 10 m/generalized, focal + SG clusters	CBZ, TPM, CLB, BDZ	Sporadic
7. F	15 m/generalized cluster >CSE	PB, VPA + BDZ	20 y/generalized, CPS clusters	CZP, VPA, PB, PB + CBZ	Control
8. M	5 m/focal SE + SG	B6, PB + BZD, CBZ	11 y/focal SG, SE, NCSE?	VPA, GVG, CLB + VPA, TPM + VPA, OXC, VPA + LEV + TPM	DR
9. F	9 m/focal SG >CSE	PB, VGB, PB + BZD	12 y 11 m/focal SG, myoclonic absences	ACTH, VPA, CZP, CBZ, PB, LTG, OXC, ETS	Control
10. F	6 m/focal SG	PB, PB + VPA	20 y/focal + SG CSE	PB, VPA, TPM	DR
11. F	1 m/febrile GTCS	PB	17 y/CPS clusters	Several; VPA + LTG + CLB	Sporadic
12. M	6 m/focal + SG >SE	PB	3 y/CPS clusters, SE focal + SG	PB, CZP, VPA, TPM, LEV	DR
13. M	18 m/generalized clusters >SE	PB, VPA	4 y 10 m/CPS clusters	VPA, CZP	DR
14. F	3 m/generalized clusters/focal + SG	PB + TPM	3 y 5 m/CPS isolated or clusters	TPM + CZP, LTG, LEV	DR
15. F	3 m/focal + SG	PB, CLB midazolam	2 y 1 m/CPS clusters	PB, CLB, midazolam	Sporadic
16. M	3 m/generalized clusters	?	12 y/generalized, atypical absences	LTG, TPM, VPA	DR
17. M	3 m/generalized	CBZ	6 y 10 m/focal + SG clusters >CSE	GVG, LEV, CZP, CBZ	DR
18. F	3 m/CPS clusters	?	2 y 10 m/CPS clusters	VPA, CLB, CZP, vit B6, folic acid, biotin	Sporadic
19. M	5 m/generalized clusters	Diazepam e.r.	5 y/CPS clusters, CSE	PB, VPA, GVG, lorazepam i.v., CZP i.v., PB i.v., CLB, OXC, LTG, PHT, hydrocortisone, LEV + VPA	DR
20. M	4 y/generalized	?	9 y 7 m/generalized, focal + SG	?; LTG	Sporadic
21. M	2 m/focal SG cluster	CBZ	3 y 4 m/CPS clusters	TPM + LTG	DR
22. F	2 m/CPS cluster	PB	22 y/focal SG clusters	VPA, PB, CZP, NTZ, TPM, FLB, CBZ, GVG, LTG, VPA + CLB + LTM	Transient control (15 y, 18 y); sporadic

M, male; F, female; y, year/years; m, month/months; SE, status epilepticus; CSE, convulsive status epilepticus; NCSE, nonconvulsive status epilepticus; SG, secondary generalization; TCGS, tonic–clonic generalized seizures; CPS, complex partial seizures; CBZ, carbamazepine; BDZ, benzodiazepine; VPA, valproate; PHT, phenytoin; PB, phenobarbital; CZP, clonazepam; GVG, vigabatrin; TPM, topiramate; CLB, clobazam; LTG, lamotrigine; LEV, levetiracetam; OXC, oxcarbazepine; FLB, felbamate; NTZ, nitrazepam; ESM, ethosuximide; Ig, immunoglobulins; i.v., intravenous; DR, drug resistant; e.r., endorectal.

drugs, but a chronic regimen was not initialized. In three patients, the first antiepileptic drug was unknown.

Epileptic seizures during evolution

In all patients, with few exceptions, the following features were noted at follow-up: (1) a better definition of a focal onset of seizures, (2) clustering of complex partial episodes with longer seizure-free periods, (3) frequent SE, and (4) no patient with only one type of seizure.

In fact, patients experienced various seizure types, often easily induced by febrile or nonfebrile illness. In patients 1, 3, 4, 8, 11, and 16, clusters of generalized (tonic and myoclonic) and/or unilateral clonic seizures, complex partial seizures with or without secondary generalization (preceded

by eye deviation and oral automatisms), and atypical absences coexisted. Patient 3 showed typical hypermotor frontal seizures during the evolution, as described previously (Giovannini et al., 2010). Seizures could be nocturnal and diurnal with predominant occurrence during sleep. Refractory tonic and myoclonic–tonic SE occurred in three patients (cases 1, 4, and 8). In cases 6, 7, 10, and 13, clusters of complex or simple partial seizures with a secondary generalization led to convulsive SE. In three children, nonconvulsive status epilepticus (NCSE) and "minor motor seizures" appeared after cessation of prolonged single or few repetitive seizures (cases 3, 4, and 8). In cases 2, 5, 12, 14, 15, 17, 18, 19, 21, and 22, clusters of complex partial seizures characterized by hypotonia, oral automatisms, or slow eyelid myoclonias were the only epileptic manifestation during awakening, but a few myoclonic–tonic seizures occurred during sleep. Patient 9 deserves consideration because of the appearance, after her first 5 years of life characterized by clusters of complex partial and tonic-adversive seizures, of typical absence seizures characterized by slight impairment of consciousness with palpebral myoclonias as the only feature of epilepsy. In case 20, with the latest onset of epilepsy (4 years), nocturnal tonic–adversive seizures with decreasing frequency coexisted with daily episodes of staring.

However, during follow-up, we observed an increased frequency of partial complex seizure clusters, with a less severe clinical motor semiology and more complex automatisms.

Epileptic seizures at last follow-up

Seizure frequency changed during the clinical course and with patient age. At the last neurologic follow-up, we have identified three groups of patients as follows. (1) *Drug-resistant patients*: 12 patients were drug resistant and several antiepileptic drugs were used with varying degrees of efficacy. Seizure frequency varied from daily to monthly with variable seizure-free periods (up to 3 years in patient 8). In the majority of patients, age at final follow-up was <10 years old. (2) *Patients with sporadic seizures*: cases 6, 11, 20, and 22, ranging in age from 9 to 22 years, presented with nocturnal sporadic and isolated seizures. In case 11, sporadic clusters of daily complex partial seizures (CPS) consisting of atypical absences were present. In case 22, after a seizure-free period of 3 years, rare tonic nocturnal seizures reappeared. Three more patients had ongoing seizures at the last follow-up visit (cases 2, 15, and 18). Patients 2 and 15 had clusters of seizures every 2–3 months that were sensitive only to oral midazolam. Of interest, patient 18 showed some improvement after pyridoxine and folinic acid supplementation, but her follow-up was too short to establish clear efficacy. (3) *Drug-responsive patients*: seizure-free patients (cases 4, 7, and 9) at the last neurologic follow-up were 20 years old (cases 4 and 7) and 12 years and 11 months old (case 9), respectively; all were taking antiepileptic medications. In patient 4, felbamate withdrawal was initialized, but when nocturnal tonic–clonic seizures reappeared, the dosage was increased to achieve control. Patient 7, although seizure-free since the age of 10 years, was still receiving treatment with phenobarbital and carbamazepine. Patient 9 had focal SG at the onset of the epilepsy and then developed myoclonic absences, which disappeared at the age of 10, when she was on ethosuximide. At her last neurologic follow-up, when she was 12 years and 11 months, she had complete seizure control. No patient was going through withdrawal therapy.

Electroencephalographic features

Table 2 reports EEG features of study patients. The following aspects were investigated: background activity, interictal abnormalities with regard to morphology and topography, evolution of EEG abnormalities, and ictal EEG features.

Seventeen of 22 patients had at least one EEG recording during awakening and sleep. In 15 of these subjects, the EEG background activity was moderately slow and poorly organized, with interposed discontinuous rhythmic monomorphous pattern of bifrontal or temporoposterior high voltage slow waves (Fig. 1). Paroxysmal abnormalities (spike and wave complexes, slow spikes, sharp waves, fast rhythms) over the frontocentral or frontotemporal regions, more diffuse during sleep, occurred in subjects 1, 3, 6, 8, 9, 12, and 14 (Fig. S1). In patients 2, 4, 5, 11, and 19, paroxysmal generalized activities were preceded by unusual unilateral or bilateral posterior recruiting spikes/fast rhythms (Fig. 2). The EEG pattern evolution showed the persistence of characteristic bursts of rhythmic high voltage slow waves located in posterior or frontocentral areas. In the few cases with progressive reduction of seizures or seizure control, there was a fluctuation of paroxysmal activities with a predominance of theta activities over the temporal regions up to normal EEG recordings.

Many ictal EEG studies were recorded in our series because of the long duration of seizures and their tendency to occur in prolonged clusters. Both generalized and focal seizures were recorded, but in the latter, the abrupt generalization of the ictal discharge often masqueraded its focal origin, or focal discharges appeared during an apparently generalized seizure. Frequent types of focal seizures recorded were tonic–adversive (cases 2, 5, and 21), complex partial seizures, and focal with a rapid generalization, originating mainly from frontotemporal and mid-posterior regions. Among the generalized seizures were tonic, with a generalized desynchronization on EEG (cases 1, 6, 9, 10, and 12), tonic–clonic (cases 3, 4, 19, and 20), and myoclonic–tonic (case 4). In patient 9, with a successful generalized tonic–clonic seizure control since the age of 4, ictal EEG demonstrated irregular or asymmetric spike-wave discharges of 2.5–3 Hz spike waves, clinically correlated with eyelid myoclonic absences. In patient 3, EEG showed

Table 2. Characteristics of interictal EEG recordings in patients with ring 14 syndrome

Case no. Patient sex	Background activity	Epileptiform EEG abnormalities/morphologic distribution	EEG evolution
1. M	Diffuse theta activity with high voltage bifrontal theta-delta waves intermixed; midtemporal theta (>left)	Diffuse sharp theta activity; asynchronous frontocentral spikes with left predominance	Unmodified
2. F	Normal awake EEG	Bilateral centrotemporal sleep: bilateral centrotemporal sharp theta activity; right posterior repetitive spikes	Unmodified
3. M	Good organization; short bifrontal theta-delta activity and left discontinuous temporal theta sequences	Synchronous and asynchronous frontotemporal spikes (>left), diffuse spike waves during sleep	Interictal: multifocal with left predominance, more diffuse during sleep Ictal: a. isolated events b. + c. frequent events during follow-up
4. M	Slowing of background activity with midtemporal and diffuse theta activity	Pseudo-periodic slow waves and generalized activity right posterior fast rhythms and bilateral asynchronous temporooccipital focal abnormalities	a. generalized spike waves originating from left temporal posterior region, facilitated by eye closure and IPS b. sporadic left FT spikes
5. M	Background abnormalities with pharmacologic rapid rhythms; bifrontal monomorphous theta-delta sequences; midposterior monomorphous theta, facilitated by eye closure	Diffuse slow abnormalities; bilateral centrotemporal and posterior spikes (>right) recruiting posterior spike waves with a rapid generalization	Unmodified
6. M	Slow background activity; discontinuous midtemporal theta sequences: *theta-delta bifrontal waves*	Multifocal abnormalities: left centro-temporooccipital and right frontocentral spikes	Focal right temporal slow waves, slow spikes
7. F	*Monomorphous diffuse theta activity* (scarce regional differentiation)	No abnormalities	No abnormalities
8. M	Slow background activity with rare alpha rhythms, diffuse theta with right frontotemporal slow activity	Diffuse slow waves (>FT) and TO slow-spikes; long lasting awakening bursts of left frontal spike waves and diffuse during sleep (>left)	Left frontal spike waves during sleep
9. F	Diffuse slow activity; awakening and sleep bifrontal slow abnormalities	Sleep polyrhythmic activity interposed with pseudo-periodic high voltage generalized bouffées of slow waves; awakening diffuse or bifrontal slow waves; generalized spike-polyspike waves followed by slow degradatin wave with a frontal predominance	Sharp theta activity with sharp waves interposed over left centrotemporal regions, facilitated by hyperventilation
10. F	Diffuse monomorphous slow activity; bursts of left hemispheric theta-delta waves; right central theta activity	Slow wave abnormalities (>right temporo-occipital); bilateral frontal subdelta activity	Background activity ameliorated (7 Hz) (>right); persistence of nocturnal tonic seizures
11. F	Background activity slow with poor organization; prevalence over right posterior regions	Left temporooccipital abnormalities with diffusion; multifocal abnormalities with predominance over bioccipital region (>right)	Right temporooccipital abnormalities
12. M	Background activity with reactive posterior theta rhythm; bifrontal delta waves facilitated by sleep	Diffuse slow waves with left frontotemporal slow spikes.	Unmodified
13. M	NA (Not Available)	NA	NA
14. F	Slow background activity	Diffuse slow abnormalities and bilateral frontocentral sharp waves	NA
15. F	NA	NA	NA
16. M	NA	NA	NA
17. M	NA	NA	NA
18. F	NA	NA	NA
19. M	Slow background activity with 2 Hz *bifrontal high voltage delta waves*	Multifocal abnormalities; left temporooccipital spikes and generalized slow waves	Awakening: slow, monomorphous, and high voltage background activity with bifrontal spikes interposed. sleep: scarce organization with sleep figures

Continued

Table 2. Continued.

Case no. Patient sex	Background activity	Epileptiform EEG abnormalities/morphologic distribution	EEG evolution
20. M	Background slowing with bifrontal delta waves sequences	Awakening: occasional synchronous frontal slow spikes; generalizes spike-polyspike waves during sleep	Unmodified
21. M	Slow background activity	No interictal abnormalities	Unmodified
22. F	Background activity poorly organized; discontinuous bifrontal theta-delta pattern	Rare left posterior sharp abnormalities; awakening and sleep diffuse slow spike waves	Unmodified

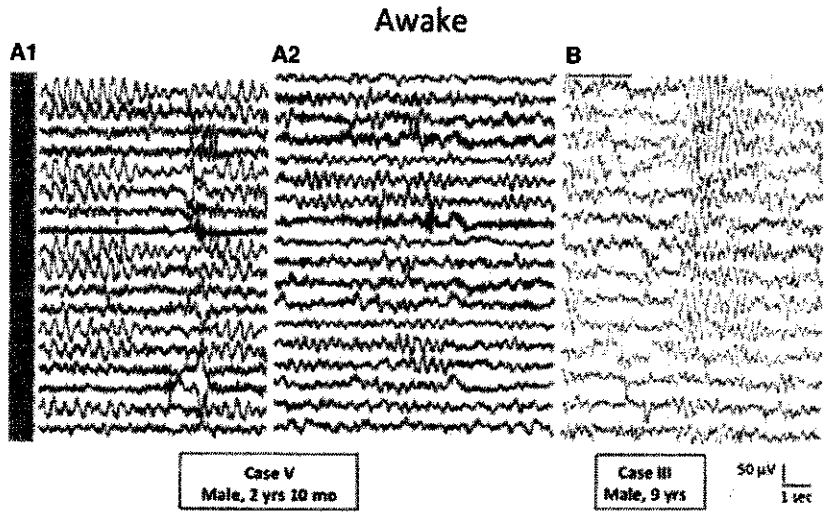

Figure 1.
(**A**) Case 5: Discontinuous rhythmic monomorphous patterns of bifrontal (A1) or anterior temporal right and left (A2) high voltage slow waves. (**B**) Case 3: More diffuse, similarly monomorphous slow activities.
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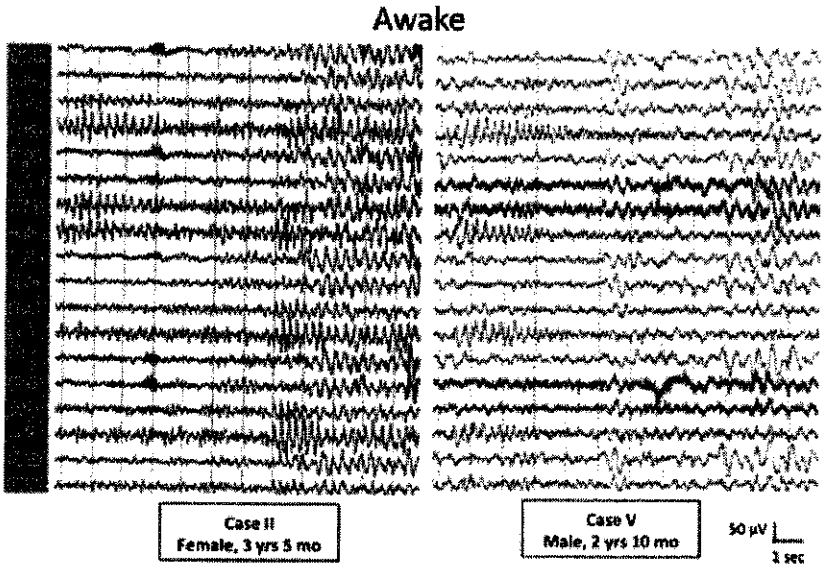

Figure 2.
Case 2 (left): Unilateral right posterior recruiting spike waves followed by secondary generalization. Case 5 (right): Bilateral recruiting spike waves followed by diffused slow waves.
Epilepsia © ILAE

a pattern of bilateral frontal high voltage continuous rhythmic/pseudo-rhythmic delta activity that was associated with a nonconvulsive status epilepticus (NCSE), as described separately (Giovannini et al., 2010).

Clinical, neuroradiologic, and genetic findings

In Table 3, the primary clinical, neuroradiologic, and genetic data are reported. We focused mainly on the following features: (1) hypotonia, (2) microcephaly, (3) degree of

Table 1. Clinical, neuroradiologic and genetic features

Case no. Patient sex	Hypotonia	Microcephaly	Cognitive disability	Language disturbance	Cerebral MR	Seizure response	Mosaic status and deletion size (Mb)
1. M	+	+ (postnatal)	Moderate	++ (several words)	Normal	Drug resistance	80;15/<0.5
2. F	+	?	Mild	++ (few words)	Meningeal and lateral ventricles dilatation (>right); hypoplastic temporal lobes	Sporadic	(Not Available)
3. M	+	+ (postnatal)	Severe	+++ (few words)	Normal	Drug resistance	80;20/2.5
4. M	+	+ (connatal)	Severe	+++ (absence of language)	Cerebral hypoplasia, small cerebellar hemispheres and big cerebellomedullary cistern	Control	80;20/1.5
5. M	++	+ (connatal)	Severe	+++ (absence of language)	Dandy-Walker variant, hydrocephalus, white matter reduction, thin corpus callosum(CC), parahippocampal verticalization	Drug resistance	83;17/5
6. M	+	+ (postnatal)	Moderate	+ (poor semantic language)	Clivus verticalization, cerebral hypoplasia, pre-pontine cistern dilatation, CC with a posterior incisure	Sporadic	81;19/2
7. F	+	+	Moderate	++ (simple sentences)	Cerebral hypoplasia, thick CC, involution anomaly of CC, lateral ventricle dilatation	Control	80;20/0.65
8. M	+	+ (connatal)	Moderate	++ (poor semantic and mor phosyntactic language)	Inferior vermis hypoplasia	Drug resistance	80;20/<0.5
9. F	+/−	+ (connatal)	Mild	+ (poor semantic language)	Wide frontotemporal perencephalic spaces and occipital horns of lateral ventricles dilation; left hippocampal verticalization, thin CC, hypoplastic encephalic trunk	Control	82;18/<0.5
10. F	+	+ (connatal)	Severe (++)	+++ (absence of language)	Brain CT: normal	Drug resistance	95;5/2
11. F	+	+ (connatal)	Severe	+++ (few words)	Lateral ventricle dilation, thin CC	Sporadic	NA
12. M	+	+ (postnatal)	Moderate	+++ (few words)	Normal	Drug resistance	79;21/<0.5
13. M	+	+ (postnatal)	Severe	+++ (few words)	?	Drug resistance	81;19/del 3.4 dup 2.5
14. F	+	+	Moderate	+++ (few words)	Normal	Drug resistance	79,21/4,3
15. F	+	?	Mild	++	Cystic hypophysis anomaly (Rathke)	Sporadic	90;10/0.65
16. M	+	+ (connatal)	Severe	+++ (absence of language)	?	Drug resistance	80;20/<0.5
17. M	+	+ (postnatal)	Moderate	++ (words combination)	Mega cisterna magna, vermian hypoplasia, clivus verticalization, IVth ventricle dilation	Drug resistant	81;19/2.5
18. F	+	+ (postnatal)	Moderate	++ (single words)	External hydrocephaly	Sporadic	81,19/2.3
19. M	+	+ (postnatal)	Severe	+++ (absence of language)	Cyst of sphenoid wing	Drug resistant	NA
20. M	++	+	Severe	+++ (absence of language)	Normal	Sporadic	NA
21. M	+/−	+ (postnatal)	Moderate	++ (few words)	Normal	Drug resistant	NA
22. F	+	+ (postnatal)	Severe	+++ (absence of language)	Normal	Seizure-free (15–18 a); sporadic	NA

cognitive disability, (4) language delay, (5) neuroradiologic features, and (6) mosaic status and deletion size.

Axial hypotonia was observed in all cases with varying degrees of severity (22/22); its presence since birth influenced feeding problems and predisposition to upper respiratory infections and gastroesophageal reflux. The persistence of hypotonia in some patients determined a motor developmental delay first and gait instability later.

Microcephaly was a distinctive feature of the syndrome and was present in 20 subjects. In cases 2 and 15, head circumference was not reported at birth or during neurologic follow-up. Ten subjects developed microcephaly in the first 6 months of life (cases 1, 3, 6, 12, 13, 17, 18, 19, 21, and 22); in another seven patients, microcephaly was congenital (cases 4, 5, 8, 9, 10, 11, and 16). In three subjects, age at presentation was not specified.

As expected, *intellectual disability* was always present of a moderate (9 patients) to severe (10 patients) degree. In three subjects (cases 2, 9, and 15), despite the young age, cognitive disability was mild. Severe expressive *language delay* was a characteristic feature of the disorder, varying from a few words pronounced to the complete absence of speech. Only two subjects (cases 6 and 9) had a history of mild language development delay, and eventually their language was characterized by phonetic distortions and semantic and morphosyntactic errors.

Magnetic resonance imaging (MRI) findings, available in 19 patients, were normal except for some nonspecific features, such as cystic hypophysis anomaly, mild external hydrocephaly, sphenoid wing cyst, cerebral and white matter hypoplasia, corpus callosum abnormalities, hippocampal dysmorphisms, and cerebellar structural anomalies. Brain computed tomography (CT) was performed in patient 9 and showed normal results.

The *genetic study* has already been described in depth in our genetic survey of 19 patients with ring 14 chromosome, 16 of which are included in the present study: clinical symptoms do not seem to correlate with the size of deletions (Zollino et al., 2009).

Discussion

Epilepsy represents a major complication of r(14) syndrome; it occurs in almost all cases described and does not seem to be related to a structural brain anomaly or to the genetic material loss in the ring (Zollino et al., 2009). To our knowledge, apart from a few recent reports (Ville et al., 2009; Specchio et al., 2012), the electroclinical phenotype of r(14) syndrome is still poorly known and, in the literature, epilepsy is mainly described as nonspecific, generalized, and drug resistant. Moreover, information about epilepsy in chromosomal disorders is predominantly found in genetics journals where descriptions are often very sparse and, as a result, there are only a small number of chromosome abnormalities in which epilepsy represents a consistent feature and its phenotype is well described, such as ring 20 syndrome (Inoue et al., 1997), Angelman syndrome (Laan et al., 1997), deletion 1p36 (Bahi-Buisson et al., 2008), and Wolf-Hirschhorn (Battaglia et al., 2009). In this review, we attempted to better describe the semiology of epileptic seizures, epilepsy evolution, and ictal/interictal EEG patterns in a series of 22 patients with r(14) syndrome.

Epilepsy in r(14) syndrome seems to be a chronic condition with a stormy onset, and repetitive and prolonged seizures that can affect for a long time the evolution of the child's emotional, cognitive, and linguistic development in a critical phase. Based on analysis of our results, it seems possible to recognize a rather typical evolution of epilepsy with few exceptions. As already suggested (Zollino et al., 2012), this study confirms that epilepsy could be empirically divided into three successive age-dependent stages. In the onset phase (stage 1), epilepsy presents a very high activity, sometimes before the development of other recognizable clinical features. According to Morimoto and Ville (Morimoto et al., 2003; Ville et al., 2009), epilepsy is frequently the presenting symptom without any other significant clinical findings (negative familial and personal history, unremarkable pregnancy and delivery, no/subtle dysmorphic features), posing many diagnostic and therapeutic challenges. In fact, if seizure recurrence is in clusters, in young female patients a differential diagnosis with *PCDH19* gene mutations encephalopathy has to be considered (Dibbens et al., 2008). Whether seizures are concomitant with fever, a differential diagnosis with Dravet syndrome is mandatory. Seizures from familial and literature descriptions appear generalized, but, in our series, EEG recordings frequently showed a focal origin, tonic or clonic seizure with a rapid secondary generalization. These seizures are mainly afebrile and occur during nocturnal sleep or upon awakening and often recur in clusters. Noteworthy, half of the cases presented with a generalized and focal convulsive status epilepticus (CSE). The strong susceptibility to clusters of prolonged tonic–clonic seizures and CSE may have important therapeutic implications. In these patients, initial treatment with benzodiazepines often failed to obtain seizure control; high dosage administration of barbiturate and phenytoin were often required. In stage 2 (up to 20 years), follow-up phase, different types of seizures are present in the same subject consisting of both generalized and focal seizures, more often from frontal or posterior temporal regions. Seizures can be nocturnal and diurnal with a tendency to group in clusters but, compared to the onset, intercluster time seems longer with fewer episodes of SE. A kind of NCSE and "minor motor seizures," which appear after cessation of prolonged single or few repetitive seizures, may also occur in the context of partial epilepsy. Nevertheless, seizures are drug-resistant, and if seizure activity persists, really severe and intellectual decline and speech problems become evident. Then, neuronal excitability gradually decreases, perhaps in relation to the cerebral matura-

tion process, achieving a steady state. In fact, entering the stage 3 (above the age of 20) there is a tendency for seizures to decrease, up to cessation in some patients; in a few cases tonic short events can persist during sleep and atypical absences occur during daytime. Further clinical progression does not seem to occur, whereas moderate to severe cognitive disability, severe language impairment, and behavioral concerns persist.

It's interesting to underscore that cognitive disability is prominent in the context of this chromosomal disorder. On the other hand, at the onset of epilepsy, children are usually reported to be normally developed from a psychomotor point of view. Of interest, case 9, a patient who since the age of 6 years experienced drug-sensitive absences as the only type of seizures at the last psychometric evaluation, showed mild cognitive impairment and adequate linguistic skills at age 12. Moreover, cases 2 and 15, respectively, 3 and 2 years old, had sporadic and not severe seizures, and they showed mild cognitive impairment at the last follow-up. Which will be their cognitive performance at the age of 12 if their seizures will persist to be sporadic?

We believe that it could be possible to speculate that the severity of epilepsy occurring in the first 10 years of life, a critical phase of child psychomotor development, may negatively influence it, according to the recent concept of "encephalopathic effects of the epilepsy," as defined in the "Report of the ILAE Commission on Classification and Terminology," (Berg et al., 2010).

Serial EEG investigations revealed a slow and poorly organized background activity during wakefulness and sleep, frequent bursts of asynchronous rhythmic high amplitude slow waves over the frontal and medioposterior regions, and spike wave complexes over the posterior regions, sometime diffuse and activated by eye closure. These EEG features seem in some way similar to activities seen in Angelman, Wolf Hirschorn, and ring 20 syndromes (Kobayashi et al., 1998; Valente et al., 2003; Uemura et al., 2005), but significant clinical differences are evident among these syndromes, such as the lack of myoclonic epileptic status in r(14) syndrome, the rhythmic cortical myoclonus seen in Angelman syndrome (Guerrini et al., 1996), and the absence of a clear occurrence of non-NCSE typical of ring 20 syndrome.

In our series, MRI findings do not show clear cortical abnormalities that could be strictly correlated to the epileptic phenotype. Nevertheless, functional neuroimaging studies could be useful in the future to better understand pathogenetic mechanisms underlying epilepsy, as demonstrated in ring 20 syndrome in which the data from 18 F-fluoro-L-dopa PET showed reduction in striatal dopamine uptake, leading to the suggestion that the striatal dopaminergic transmission in the basal ganglia plays a key role in seizure interruption (Biraben et al., 2004). Eventually, we tried to establish a correlation between the deletion size in the ring and the severity of epileptic and clinical phenotypes. Unfortunately, as demonstrated in previous studies (Schlade-Bartusiak et al., 2005; Maurin et al., 2006; Zollino et al., 2009, 2012), epilepsy and cognitive disability do not seem to correlate with the deletion size in the ring context and it seems unlikely that a specific deleted locus predisposes to seizures and learning disability. Two hypotheses can be proposed to explain the presence of seizures in r(14) syndrome: (1) instability of the ring, resulting in monosomy 14 in a proportion of cells; and (2) haploinsufficiency of critical genes by position effects, leading to decreased expression of genes contained on the adjacent 14q arm (Kleinjan & van Heyningen, 1998; Baur et al., 2001; Van Karnebeek et al., 2002; Grewal & Moazed, 2003; Lloyd et al., 2003; Schlade-Bartusiak et al., 2005; Zollino et al., 2009, 2012). The latter intriguing hypothesis concerning possible candidate genes involved in neurologic and epileptic phenotype emerged from gene content analysis. In fact, by comparing clinical signs of patients with ring chromosome with those of patients carrying a proximal or a distal deletion, we found that seizures and microcephaly seem to be related to genes residing proximally on 14q11q13 (Zollino et al., 2009, 2012). Contained in the region is the *FOXG1B* gene, expressed in the developing fetal brain. This gene belongs to the forkhead family of transcription factors having a role in the development of the brain and telencephalon (Hanashima et al., 2004). Heterozygous knockout (KO) mice show significant brain anomalies that might be the cause of epilepsy.

In summary, epilepsy occurs in all reported cases. We have not identified characteristic seizure types or a specific ictal/interictal EEG patterns in this syndrome, but in contrast to the prevalence of generalized seizures reported in the previous literature, we would underscore that seizures may be predominately focal, frontal, and temporal in origin. Epilepsy evolves in three successive stages in which seizures over time may become less frequent and severe. During the active phase, epilepsy may be really severe and variably affecting child psychomotor development (encephalopathic effects of the epilepsy). Epilepsy does not seem related to consistent underlying cortical malformations, rather being a functional consequence of the chromosomal abnormalities.

Acknowledgments

We are sincerely grateful to the patients and their families who have contributed to this study with their active participation. We thank the physicians who referred them for study and provided clinical information. We are also grateful to Stefania Azzali, President of the Ring 14 International Association, for the helpful cooperation and encouragement in our research. We would like to thank San Francisco Edit (www.sfedit.net) for editing the manuscript.

Disclosure

The authors declare no relevant conflicts of interest. We confirm that we have read the Journal position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Case 8: Awake EEG shows paroxysmal sharp waves appearing over the right more than the left frontotemporal regions (left), more diffuse during a stage 2 EEG sleep recording (right).