Inv dup (15): Is the electroclinical phenotype helpful for this challenging clinical diagnosis?

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Abstract

Objective: To study the electroclinical phenotype in 5 patients with large supernumerary marker chromosome referred as inv dup (15), in an attempt to analyze the electroclinical spectrum in order to determine if the binomial epilepsy-EEG is stereotyped enough to corroborate this challenging diagnosis.

Methods: Five patients with large inv dup (15) were submitted to EEG and/or V-EEG, with a minimum duration of 2 h. Two certified neurophysiologists analyzed all EEG tracings simultaneously, blinded to clinical and molecular data. Epilepsy was characterized by detailed history and a standard questionnaire according to International League Against Epilepsy guidelines and corroborated by V-EEG findings.

Results: Epilepsy started during infancy in 4 patients, in 3 with spasms. Spasms were easily controlled in one but not in others. Epilepsy evolved with generalized seizures in two patients and, generalized and focal in one. Currently, 3 patients present refractory epilepsy and two are seizure-free. In one patient, only one isolated episode suggestive of a secondary generalized tonic–clonic event occurred at the age of 12 years without recurrence. Regarding the EEG, patients had distinct features, except for two patients with very high amplitude fast activity, resembling recruiting rhythm. Despite good seizure outcome in 3 patients, EEGs remained remarkably abnormal with frequent epileptiform discharges over poorly organized background.

Conclusions: Our data showed a heterogeneous electroclinical phenotype with generalized and partial epilepsy, presenting distinct degrees of severity and refractoriness.

Significance: Our findings suggest that it is not possible to delineate an electroclinical phenotype in this neurogenetic syndrome. Therefore, inv dup (15) remains as a diagnostic challenge and epilepsy and EEG features are valuable only when inserted in the proper clinical context.

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1. Introduction

The proximal region of chromosome 15q is predisposed to a wide range of structural rearrangements. Deletions of this region may result in either Prader–Willi syndrome (PWS) or Angelman syndrome (AS), respectively, paternally or maternally derived (Knoll et al., 1989). Additional copies of the Prader–Willi/Angelman syndrome critical region (PWACR) may occur as supernumerary marker chromosomes, referred as inv dup (15) (Browne et al., 1997; Crolla et al., 1995; Roberts et al., 2002; Schinzel et al., 1994).

Inv dup (15) can be classified into two major groups according to size, determined by presence or absence of
PWACR. Small inv dup (15), not containing PWACR, seems to have no phenotypic effect (Leana-Cox et al., 1994; Long et al., 1998). Large inv dup (15) usually contain two maternally derived extra copies of the q11–13 region and are associated with abnormal phenotypes (Crolla et al., 1995; Robinson et al., 1993, 1998). Although some rare variants of large inv dup (15) do not follow the common pattern (Cockwell et al., 2001; Mignon et al., 1996; Shim et al., 2001), most cases are characterized by mental/growth retardation, epilepsy, behavioral problems, and dysmorphisms (Crolla et al., 1995; Robinson et al., 1993, 1998).

Because suggestive facial traits are mild, especially at early ages, proper delineation of epilepsy and behavior may represent important features for diagnosis (Battaglia et al., 1997). There are few series, with small samples or case reports, that attempted to clarify these findings, especially considering the electroclinical picture.

Herein, we aimed to study the electroclinical phenotype in 5 patients with large inv dup (15), in an attempt to analyze the electroclinical spectrum of this syndrome and its possible contribution to diagnosis.

2. Methods

2.1. Subjects

All patients included in this study had mild dysmorphic features (downslanting palpebral fissures; epicanthal folds and low-set ears), severe mental retardation and a behavioral profile featured by poor social interaction, stereotyped movements and use of objects without a purpose, characterizing a pervasive developmental disorder not otherwise specified. Echolalic and meaningless speech was outstanding and peculiar in all patients. Neuroimaging and metabolic studies, performed earlier, were normal.

All patients included in this study provided informed consent according to the Declaration of Helsinki. The local Ethics Committee permitted this study.

2.2. Methods

Patients were referred for genetic analysis based on their clinical profile analyzed by two geneticists. After genetic confirmation, they were sent to electroclinical evaluation at the Project for the Study of Epilepsy in Chromosomal Abnormalities.

2.2.1. Genetic Studies

2.2.1.1. Cytogenetic analysis. Chromosome studies of the patients and their parents were performed on peripheral blood lymphocyte cultures, after GTG-banding. FISH was performed on patient’s metaphases with SNRPN and GABRB3 probes (Oncor) or D15S21, D15S11 and PML and GABRB3 and PML (Oncor) (Estécio et al., 2002).

In situ hybridization and immunochemical detection were carried out according to manufacturer’s instructions. At least 20 metaphases were analyzed per case.

2.2.1.2. Methylation analysis. Methylation analysis was performed on peripheral blood leukocytes, with the extracted DNA modified by bisulfite treatment, and SNURF-SNRPN exon 1 amplified by PCR (Zeschnigk et al., 1997).

2.2.1.3. Dinucleotide repeat (CA)n polymorphisms. Investigation of the parental origin of inv dup (15) was performed by microsatellite analysis with markers mapped to the segment 15q11–q14 (D15S542, D15S11, D15S113, GABRB3, D15S1002 and D15S1048).

2.2.2. Electroclinical Investigation

Patients with a previous genetic diagnosis of inv dup (15) were referred for evaluation of electroclinical data. In order to avoid oversight of important data undervalued by parents, one of the physicians involved in this project took a detailed history and, additionally, filled in a standard questionnaire for parents and caretakers elaborated with previous knowledge of seizure types described in inv dup (15).

2.2.2.1. EEG Studies. Patients were submitted to EEG and/or V-EEG, with a minimum duration of 2 h. Analysis of previous EEGs obtained with the same technical parameters as those used in our study and a minimum interval of 6 months were taken into account, to allow evaluation of EEG patterns at early ages. Two certified neurophysiologists analyzed all EEG tracings simultaneously, blinded to clinical and molecular data. In one case, due to disagreement, a third staff member reanalyzed EEG tracings. Epileptiform discharges—interictal and ictal—were evaluated for morphology, localization, occurrence, distribution and amplitude. When possible, these features were grouped according to similarities in order to verify if any of them was consistent and constant enough to be considered as a distinct pattern of this syndrome. Background activity was analyzed regarding frequency, amplitude, spatial distribution, inter-hemispheric symmetry and synchrony, during wakefulness, drowsiness and sleep.

2.2.2.2. Epilepsy characterization. Epilepsy was characterized by history obtained from parents and caretakers with a pre-standard questionnaire and V-EEG data, including: 1. frequency of epilepsy; 2. age of onset; 3. seizure type, according to International League Against Epilepsy (ILAE) guidelines (Commission on Classification and Terminology of the International League Against Epilepsy, 1989); 4. occurrence of febrile seizures and/or epilepsy aggravated by fever; 5. severity of epilepsy determined by: (i) daily seizures; (ii) disabling/injurious seizures; (iii) more than three different seizure types; (iv) status epilepticus and;
(iv) history of refractory epilepsy. Two child neurologists analyzed video-EEG monitoring at different moments.

If history was unclear, especially concerning the first seizures, new data was collected from medical records and by personal contact with former physicians.

3. Results

3.1. Genetic Studies

The genotype of these patients was heterogeneous considering markers’ sizes. GTG-banding studies, showed the presence of bissatellited supernumerary marker chromosomes (SMC), except for cases 4 and 5 that showed satellites only at the endings of the long arm or short arm, respectively. All markers had the size of a G-group chromosome. Parental karyotypes were normal. FISH with single-copy sequences that map to 15q11–q13 disclosed two hybridization regions on the SMC, and the expected signals in both normal chromosome 15 homologues. The analysis of the exon 1 SNURF-SNRPN gene methylation pattern showed one paternal and one maternal band, excluding the presence of bissatellited supernumerary marker chromosomes (SMC), except for cases 4 and 5 that showed satellites only at the endings of the long arm or short arm, respectively. All markers had the size of a G-group chromosome. Parental karyotypes were normal. FISH with single-copy sequences that map to 15q11–q13 disclosed two hybridization regions on the SMC, and the expected signals in both normal chromosome 15 homologues. The analysis of the exon 1 SNURF-SNRPN gene methylation pattern showed one paternal and one maternal band, excluding the diagnosis of AS or PWS. Microsatellite analysis showed 3 bands in at least one of the loci studied, indicating the maternal origin of the extra-chromosomes in patients 1, 3 and 5; the markers were uninformative in patients 2 and 4.

3.2. Electroclinical evaluation

Electroclinical data is summarized in Table 1.

3.2.1. Case 1

This 20-year-old girl was born to unrelated parents, as a second child after fertilization problems and abortion of the first gestation. She was diagnosed with epilepsy at the age of 2 months, when she presented flexor spasms, refractory to anti-epileptic drug treatment, including ACTH. She evolved with frequent and refractory tonic seizures. At 8 years, she had generalized tonic–clonic seizures, only during sleep. Introduction of carbamazein led to satisfactory seizure control. At the age of 13 years, she presented two status epilepticus characterized by loss of contact, without motor phenomena, which lasted for several hours and led to hospitalization. Another prolonged complex partial status occurred at the age of 18 years, with loss of contact, generalized hypertonia and eye-deviation to the left. These events were controlled after association of clobazam to higher doses of carbamazepine, although she maintained sporadic head falls. At the age of 15 years, her EEG showed left temporal epileptiform discharges. V-EEG displayed prominent slowing and trains of spikes and sharp waves over the left hemisphere with temporal region predominance. No seizures were recorded (Fig. 1A).

3.2.2. Case 2

Second child of unrelated and healthy parents, born from an uneventful pregnancy and delivery, except for a cesarean section because of pelvic presentation. Psychomotor development was apparently normal until the age of 5 months when he presented infantile spasms (IS), which led to loss of milestone acquisitions. Seizures were refractory to valproate but were completely controlled after introduction of vigabatrin at the age of 1 year. This 7-year-old boy has been seizure-free for 6 years and without anti-epileptic drugs for 3 years. He evolved with a moderate neurodevelopmental delay, more evident in cognitive than motor skills. His 9-month-old EEG displayed hypsarrhythmia that evolved at the age of 1 year to multifocal discharges and currently to frequent sharp waves over parasagittal and midline regions (Fig. 1B).

3.2.3. Case 3

A girl, born from normal delivery to non-related parents, with an uneventful pregnancy except for decreased fetal movements, presented an evident delay noted from the age of 4 months. At 5 months she had IS, refractory to different drug trials, including high doses of valproate, vigabatrin and ACTH. During her lifetime, she developed different seizure types, which remained daily, despite anti-epileptic drugs. Now at the age of 14 years, she is severely impaired and presents GTC, tonic seizures and drop-attacks (atonic seizures). Atypical absences were neither recorded by V-EEG nor reported by parents. She is now on the co-medication valproate and lamotrigine with control of GTC seizures. EEGs obtained at different ages (6 years, 8 years and 14 years) demonstrated very frequent runs of high amplitude fast activity during sleep, and generalized and irregular spike and polyspike-and-wave complexes during wakefulness (Fig. 1C and D).

3.2.4. Case 4

A 16-year-old girl with severe hyperactivity, the only child of an unrelated healthy couple, with strabismus noticed at 3 months and discrete developmental delay at 9 months, was found at the age of 12 years, having a GTC seizure during sleep. No seizures recurred with low doses of oxcarbazepine (600 mg/day). An EEG performed at 9 years showed left temporal lobe epileptiform activity during sleep. From the age of 10 years, her EEGs have demonstrated frequent bursts of generalized spike and wave complexes and very frequent runs of high amplitude fast activity during sleep. V-EEG confirmed these findings; however no ictal phenomena were recorded during these events (Fig. 1E and F).

3.2.5. Case 5

This 15-year-old girl presented tonic seizures at the age of 1 year, which soon evolved into hard-to-control epilepsy with daily tonic events until the age of 4 years, when other seizure types became more evident. These included atypical
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1/f/20yrs 0.1 Spasms 1.GTC 1.Sporadic + Valproate, ACTH, Carbamazepine, Clobazam Yes – Sporadic GTC Left temporal lobe epileptiform discharges

2/m/7yrs 0.5 Spasms – – Vigabatrin No Spasms 1 year Seizure-free Epileptiform discharges over parasagittal and midline regions

3/f/14yrs 0.3 Spasms 1.GTC Daily + Valproate, Vigabatrin, ACTH, Valproate and Lamotrigine Yes – Daily Frequent runs of high amplitude fast activity during sleep

4/f/18yrs 12 GTC (?) (isolated episode) – – – Oxcarbazine No GTC Seizure-free Frequent runs of high amplitude fast activity

5/f/8yrs 1 Tonic 1.Tonic Daily + Valproate, Benzodiazepines, Primidone, Topiramate Yes – Daily Frequent runs of high amplitude fast activity

2. AA

3. Astatic

–, Absent; +, Present; AA, atypical absences; ACTH, adrenocorticotropic hormone; CP, complex partial seizures; f, female; GTC, generalized tonic–clonic seizures; GT, generalized tonic seizures; m, male; yrs, years.
Fig. 1. Case 1. A 20-year-old girl, diagnosed with epilepsy at the age of 2 months, with spasms, evolved with refractory tonic seizures. At the age of 13 and 18 years, she presented complex partial status epilepticus. Her current EEG shows sharp waves over the left hemisphere with temporal region predominance (A).

Case 2. This 7-year-old boy had spasms at 5 months, controlled with vigabatrin. He evolved without seizures. His 9-months-old EEG displayed hypsarrhythmia, which evolved at the age of 1 year to multifocal discharges, and currently to frequent sharp waves over parasagital and midline regions as observed here (B).

Cases 3. This 14 year-old girl had spasms at 5 months. Later, she presented refractory epilepsy with different seizure types. Currently, she presents GTC, tonic seizures and drop-attacks (atonic seizures). EEGs obtained at different ages (6 years, 8 years and 14 years) demonstrate very frequent runs of high amplitude fast activity accompanied by tonic seizures, during sleep. Generalized and irregular polyspike-and-wave was observed during wake recordings (C and D).

Case 4. A 16-year-old girl had a GTC during sleep at the age of 12 years. An EEG performed at 9 years showed left temporal lobe epileptiform activity during sleep. From the age of 10 years until now, her EEGs have demonstrated frequent bursts of generalized spike and wave complexes and very frequent runs of high amplitude fast activity followed by slow waves, during sleep (E and F).
absences and atonic seizures, which remained refractory. Myoclonic-astic seizures were also reported in her medical files. An EEG report, performed at the age of 4 years, described recruiting rhythm during sleep and slow-spike and wave complexes, compatible with Lennox–Gastaut syndrome. EEGs remained unaltered until her last evaluation at 8 years. Drug trials included high doses of valproate in different combinations with benzodiazepines, primidone and finally topiramate, always in polytherapy.

4. Discussion

Our patients had a heterogeneous electroclinical phenotype with distinct degrees of severity despite the presence of a large duplication and the clinical and behavioral profiles, previously described by Battaglia et al. (1997). Epilepsy was part of the set of signs and symptoms but drew more attention to diagnosis than did facial traits, which were mild despite the size of inv dup (15).

This is one of the first prospective studies aiming to detail the binomial epilepsy-EEG in patients with inv dup (15). The methodology used to obtain patient information, based on a detailed questionnaire, was applied to avoid omission of data (seizure types or aggravating factors) that might be undervalued by parents. Even with such directed interviews, in some patients it was tricky to determine the nature of the epileptic event. Additionally, V-EEG was performed to determine ictal findings at the moment of the evaluation. Therefore, it is our impression that data on epilepsy and EEG, based solely on retrospective analysis, although contributory, must be carefully evaluated, due to difficulties in the classification of some events in this population with pervasive developmental disorders.

Seizures started in infancy (from 2 months to 1 year) in 4 patients, with IS in 3 and tonic seizures in 1. From the electroclinical point of view, IS was the only similarity observed, since evolution of seizure type and refractoriness was distinct in each case. In addition, satisfactory seizure control was obtained in the first two patients, but not in the latter.

Although epilepsy is a common finding in chromosomal disorders, as observed in inv dup (15), IS are unusual and few cases with inv dup (15) and IS have been reported (Bingham et al., 1996; Buoni et al., 2000; Cabrera et al., 1998; Robinson et al., 1993; Webb et al., 1998). Late onset epilepsy was observed in only one patient (case 4) who presented an isolated tonic episode at the age of 12 years. Based on these findings, the authors suggest that future investigation on the importance of genetic studies in early onset epilepsy with cryptogenic epilepsy, even in the absence of suggestive phenotypic traits, may prove helpful for early diagnosis.

In the few case reports which aimed to describe epilepsy in inv dup (15) (Bingham et al., 1996; Buoni et al., 2000; Cabrera et al., 1998; Elia et al., 1998; Singh et al., 2002; Webb et al., 1998), there is a clear predominance of generalized and refractory epilepsy, resembling Lennox–Gastaut, with tonic, atonic, atypical absences, myoclonic, and generalized tonic–clonic seizures (Battaglia et al., 1997; Chifari et al., 2002; Kobayashi and Yoshino, 1999; Takeda et al., 2000). Myoclonic absence-like seizures (Elia et al., 1998), sometimes induced by emotion (Aguglia et al., 1999) were eventually reported. Similarly, most of our patients had generalized epilepsy at onset or during its development. However, focal seizures leading to a non-convulsive status also occurred. Therefore, in our series, we observed a wide range of different seizure types and distinct outcomes.

Two of our patients evolved with an electroclinical picture that resembled a Lennox–Gastaut syndrome. However, in disagreement to Battaglia et al. (1997), none of our patients fulfilled all diagnostic criteria for Lennox Gastaut syndrome (Estéció et al., 2002). Moreover, none of our patients started with these features during childhood, but rather evolved to Lennox Gastaut-like syndrome following a long period of refractory epilepsy with early onset.

Regarding EEG, we were unable to categorize EEGs according to similarities, since they presented distinct features, except for two patients with very high amplitude fast activity, resembling recruiting rhythm. Therefore, it seems inadequate to propose a pattern or an electroclinical profile for this syndrome because of the differences observed not only in our patients but also in those previously published. Buoni et al. (2000) described a concomitant improvement of seizures and EEG features with anti-epileptic drugs. In opposition to this, despite good seizure outcome in 3 of our patients, EEGs remained remarkably abnormal with frequent epileptiform discharges over poorly organized backgrounds, leading to maintenance of anti-epileptic drug therapy in most.

The importance of EEG in helping to delineate the nature of some events in these patients with pervasive developmental disorders could be observed in at least two cases. In patient 4, the presence of focal epileptiform discharges suggested a focal onset, although the child was found during the GTC phase. In patient one, the nature of 3 episodes of status epilepticus could not be determined, due to the lack of proper documentation at the ages in which they occurred. Focal EEG discharges associated with a good response to carbamazepine led us to believe seizures were complex partial and not atypical absences.

In some chromosomal disorders, presence of one specific mechanism, such as deletion in 4p (Minassian et al., 1998; Varela et al., 2004) or size of the deletion in 4p- (Battaglia et al., 2003), factors that determine loss of larger genetic material, are correlated with a severe electroclinical profile. In inv dup (15) the discrepancy between the mild phenotype and the severe chromosomal abnormality detected, further supports the notion that the breakpoint site is contributory to inv dup phenotype rather than the size of chromosomal abnormality (Robinson et al., 1993). At the same token, Chifari et al. (2002) described two patients with large
deletions and mild epilepsy. Therefore, electroclinical heterogeneity in this syndrome may also be associated with the breakpoint and PWACR dosage rather than the difference in the extension of the duplicated segment. Taking into consideration that, in our patients, evolution of epilepsy and EEG was unpredictable, an important reason for defining this genotype–phenotype correlation is to provide better parent counseling as to their children’s follow-up.

5. Conclusion

Significance of a meticulous study of the electroclinical profile in chromosomal disorders has been emphasized, due to its importance for diagnosis, especially in conditions with a mild phenotype such as inv dup (15). However, in inv dup(15) patients, except for the presence of generalized epilepsy, which may start with infantile spasms, the binomial epilepsy–EEG is not stereotyped enough to corroborate and support this difficult diagnosis purely on a clinical basis.

References


