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Multicentre prospective study in patients affected by Dravet Syndrome

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1. Introduction and literature review

1.1 Historical introduction, definition and epidemiology

Dravet syndrome is a rare form of epilepsy, occurring in the first year of life in otherwise healthy children, characterized by impaired psychomotor and neurologic development. It was initially described in 1978 (Dravet, 1978), as a “severe myoclonic epilepsy of infancy” (SMEI) and in the following years several authors reported similar cases in Europe and in Japan (Dalla Bernardina et al., 1982; Ogino et al., 1986). It became subsequently obvious that there was some kind of variability between patients, particularly due to the lack of myoclonic features (Dravet et al., 1992; Kanazawa, 1992; Yakoub et al.,1992): patients without myoclonia shared the same course and outcome of other patients and could be included in the same syndrome as borderline or atypical forms (SMEIB). For this reason and because this form of epilepsy was not limited to infancy, the eponym ‘‘Dravet syndrome’’ was proposed (Commission on Classification and Terminology of the International League Against Epilepsy, 1989).

According to the ILAE classification (1989), Dravet syndrome, is defined by:

- Positive family history of epilepsy or febrile convulsions
- No previous personal history of disease
- Seizures beginning in the first year of life in the form of generalized or unilateral febrile clonic seizures
- Secondary appearance of myoclonic jerks and often partial seizures
- EEG showing generalized spike-waves (SW) and polyspike waves (PolySw), early photosensitivity and focal abnormalities
- Retarded psychomotor development from the second year of life
- Presence of neurological signs: ataxia, pyramidal signs, and or interictal myoclonus
- Resistence to all form of treatment
- Intellectual deficiency and personality disorders in all affected children

In 2010 these electro-clinical criteria were slightly modified (Berg 2010):

- Family history of epilepsy or febrile convulsions is not constant but variable, according to the authors
- The initial seizures are not always generalized or unilateral clonic but may be focal or myclonic; they are not always febrile and the clonic seizures often evolve to status epilepticus
- Not only myoclonic jerks and focal seizures, but also atypical absences and obtundation statuses appear secondarily
- Photosensitivity may be associated to pattern-sensitivity
- Neurological signs are not always present but are frequently observed
- The MRI is normal at onset
- Cognitive deficiency and personality disorders are present in all affected children during the course of the disease, but they are of variable degree, from slight to severe

Since first being described, DS has been increasingly recognized worldwide; yet it remains a rare disorder with an incidence of 1 in 15,700 to 1 in 40,900 (Hurst, 1990; Wu et al, 2015; Bayat et al. 2015; Brunklaus et al, 2012). Its prevalence in children with seizure onset in the first year of life varies between 3% and 8% (Dravet et al, 2005).

1.2 EEG description and clinical features

The initial symptoms appear before the 12th month of age, usually between the 5th and the 8th month in an otherwise healthy infant. The onset is marked by repeated generalized or unilateral clonic (in some cases hemiclonic with alternating side) seizures, usually triggered by fever.

Seizures are often prolonged, recur in clusters in the same day and may evolve into status epilepticus. Factors that raise body temperature, such as vaccinations or hot water immersion, can precipitate seizures (Dravet 2011).

At this stage of the disease EEG is usually normal both while awake and during sleep. EEG recordings may show diffuse or unilateral slowing of the background activity, if recorded after a prolonged seizure. In some patients EEG can show generalized spike waves (SWs), either spontaneous or elicited by intermittent photic stimulation (IPS) (Dalla Bernardina et al., 1982; Dravet et al., 1992). The absence of this feature however does not preclude diagnosis (Ragona et al., 2010).

The first seizure is often considered a febrile seizure; so few investigations are performed and no treatment is given. However, shortly thereafter, usually from 2 weeks to 2 months, other febrile and/or afebrile seizures recur, and from the 2nd year of life, seizures became drug-resistant and polymorphic: patients experience atypical absences, segmental and/or massive myoclonic seizures, focal and, rarely, tonic ones. From the second year of life, EEG reveals a slow background activity during wakefulness, poor organization during sleep and a progressive appearance and increase of epileptic discharges.

Paroxysmal epileptic abnormalities are mainly characterized by generalized spike or polyspike waves, diffuse or involving the frontocentral regions; in some cases discharges are induced by eye closure (Bureau, Dalla Bernardina; 2011)

Developmental stagnation becomes evident from the second year of life. Children start walking at a normal age but an unsteady gait develops for an unusually long period. Language also starts developing at a normal age, but it progresses very slowly and many patients do not reach the stage of constructing elementary sentences. Patients' fine motor abilities do not develop well. They are disturbed by segmental myoclonus and by a poor eye–hand coordination. During the disease course behavioural disorders appear, mainly characterized by attention deficit, hyperactivity and oppositive behavior. In a minority of cases autism spectrum traits have also been reported (Caraballo, Fejerman, 2006; Wolff et al., 2006; Ragona et al., 2010).

In the same period neurological signs appear in most of the patients, as well as hypotonia, ataxia (60%), pyramidal signs (20%), uncoordinated movements, and interictal myoclonus (Dravet 2011). During the disease course neurological signs change, comorbidities appear and cognitive deficits become highly disabling. In adulthood seizures are less frequent than during childhood and occur, in most cases, only during sleep (Oguni et al., 2001; Akiyama et al., 2010); by contrast the neurological signs are prominent and

disabling. Some authors reported cerebellar, pyramidal and extrapyramidal signs, in variable percentage of adult patients (Martin et al, 2010; Fasano et al, 2014). Only Genton and Dravet reported in their series myoclonus, in 55% of patients (Genton et al., 2011). Cognitive abilities are variably impaired, ranging from mild to severe deficits (Akiyama et al, 2010; Genton et al 2011; Catarino et al, 2011) In these studies the severity of cognitive impairment has been assessed only through clinical observation, there are no data about cognitive profiles or specific impaired functions. Furthermore several authors reported behavioral disorders such as autistic features, obsessive traits and externalizing behavioural disorders, while hyperactivity and attention deficits become less frequent than in childhood (Catarino et al, 2011; Berkvens et al, 2015). Adult patients present several comorbidities: orthopedic disorders, dental problems and endocrine dysfunctions are often described; cardiovascular signs, mortality and SUDEP are reported in a minority of patients, more often in childhood and adolescence (Skluzacek et al, 2011; Genton et al 2011). According to the severity and the complexity of the clinical picture in adulthood, patients require a comprehensive care: families, specialized caregivers and doctors should cooperate in order to offer patients the best possible quality of life (Granata 2011).

A study in a sample of patients affected by Dravet Syndrome was performed during my Phd, in the Department of Pediatric Neuroscience of the Neurological Institute C. Besta, in order to assess, through standardized tools, the long-term evolution of Dravet syndrome. In this session, the main data have been reported. These data have not been published.

The study included 13 adult patients (8 females and 5 males), who received a clinical diagnosis of Dravet Syndrome during childhood. The patients have been followed during childhood in the Department of Pediatric Neuroscience of the Neurological Institute C. Besta, Milan (8 cases) and in the Department of Child Neurology and Psychiatry of the Neurological Institute C. Mondino, Pavia (5 cases). Mutation analysis of the SCN1A gene was performed in every patient, using the Sanger method, denaturing high performance liquid chromatography (DHPLC) and, whenever necessary, multiplex ligation probe amplification (MLPA). A comprehensive assessment was organized for all the patients. During the interview we collected information about family history of epilepsy and / or febrile seizures, age at onset and type of initial seizures, subsequent seizure semeiology and pharmacological history. Detailed anamnesis even included some questions aimed at revealing the presence of comorbidities. The assessment included a neurological examination, paying particular attention to behavioral disorders, language deficits and cardiological evaluation. ECG and cardiological evaluation were performed in eight patients. Cognitive functioning was evaluated through the following tests: Severe Impairment Battery (SIB), Mini Mental State Examination (MMSE), Raven's Colored Progressive Matrices (RCPM) and Wechsler Adult Intelligence Scale - Revised (WAIS-R). We grouped the cognitive domains according to the SIB subdivisions, as follows: (Domain A) Memory and Attention (10 items), (Domain B) Social Interaction, Orientation and Orienting, to Name (7 items), (Domain C) Language (24 items), (Domain D) Praxis, Visuospatial Ability and Construction (10 items).

Behavioural and psychiatric disorders were investigated using the Neuropsychiatric Inventory (NPI), while adaptive functioning with the Barthel modified Index (BMI) and the Vineland Adaptive Behavioural Scale.

The analysis of the collected data led to the following results:

The patients were aged between 19 and 48 (mean age 28.31 ± 7:52). The genetic analysis of the SCN1A gene detected a truncanting mutation in nine patients and a missense mutation in four patients. Family history was positive for epilepsy in five cases and for febrile seizures in two cases.

Epilepsy features during disease course: The age at first seizure ranged between 3 and 8 months (mean age 5,38 months ± 1,52); in ten patients the onset was within six months of life. In the following years, all patients presented several types of seizures: generalized tonic-clonic seizures (thirteen patients), focal seizures (twelve patients) atypical absences (ten patients), and myoclonic seizures (ten patients). All patients except one, experienced, at least, one status epilepticus. During the course of the disease, each patient tried several drugs in different combinations, ranging from six to thirteen drugs (on average eight antiepileptic drugs). Six patients took Stiripentol and two patients tried the ketogenic diet.

Current epilepsy features: Seizures persisted in adulthood in twelve patients; only one patient, who is now forty-eight years old, is seizure free. Nine patients suffered from monomorphic seizures, mainly tonic-clonic; the remaining three patients presented polymorphic seizures. In nine cases seizures mainly occurred during deep sleep or falling asleep or during awakening and were self-limiting. The frequency of the seizures varied widely among patients: daily in one, weekly in one, monthly in seven and sporadic in three cases. Precipitating factors, in adulthood, were mainly represented by perimestrual period (five out of eight females), body temperature alterations (four cases) and emotional distress (four cases). Only three patients reported fever and infections as triggering factors. Currently, all of the patients are still being administered a polytherapy, they take from two to four antiepileptic drugs. Valproic Acid, Benzodiazepine and Topiramate are the most administered antiepileptic drugs; three patients are still taking Stiripentol.

Neurological signs and gait pattern: Neurological examination revealed extrapyramidal signs in all of the cases: bradykinesia, oligomimia, plastic hypertonus were variably associated in each patient. Eight patients presented pyramidal signs, characterized by mild distal hypertonus of the lower limbs and hyperreflexia. Two patients showed cerebellar signs, but nobody presented ataxic gait. Myoclonus was evident in all of the patients. From a functional point of view, in our case series, ten patients were capable of walking alone even for medium-long distances. In five of them, the stride appeared to be slightly bradykinetic and commuter movements were absent. The gait pattern presented mainly anti-flexion of the back, flexed knees and flat-valgus feet.Two other patients maintained the ability to walk only for very short distances, presenting severe bradykinesia and uncertainty in directional changes. They had valgus knees and extra-rotated feet.

The last patient could walk for a few steps only when leaning on a bilateral support; he showed valgus knees and flexion of the lower limbs. For all transfers he needed a wheelchair.

Comorbidities: Nine patients showed orthopedic disorders (flat foot, valgus knees, kyphosis and scoliosis), eight patients presented dental problems, characterized by dental malposition and gengival hypertrophy. In five females we detected endocrinological problems: one patients suffered from hypothyroidism, two patients presented osteoporosis and two patient presented eating disorders. Finally, one patient had irregular menstrual cycle. None of them present cardiac problems, one patient presented incomplete right bundle branch block.

Language: all the patients have a language impairment, of variable degree. We divided them into 4 groups according to their expressive language, that was characterized by:

- a simple conversation (group 1)

– short senteces (group 2)
– isolated words (group 3)
– no words (group 4).

“simple conversation”: three patients were able to sustain a conversation, the speech was characterized by an appropriate verbal fluency and they used simple sentences. They presented conform lexical skills and their verbal language didn’t clinically present phonological deficits. The information content was poor, babyish, however all of the patients of the first group were able to communicate their daily life experiences, showing good spatiotemporal organization. One of them showed planning abilities, eg. about his future job. Two of them appeared capable of complying to the rules of the conversation, they took turns during the conversation and knew how to use verbal and nonverbal signals to regulate interaction. The third patient of this group showed poor pragmatic competences, she was often off-topic or/and talking irrelevantly about things the listener showed no interest in. She presented a verbose and prolix speech, with perseverations and intrusive thoughts.

- “short sentences”: Four patients used very basic sentences; they disclosed: poor lexical skills, impairment of naming, use of passepartout words and tendency to perseveration. They mainly communicated real needs and everyday simple experiences, suggesting simplified abstraction skills. Receptive language competences of three of patients belonging to the second group appeared limited to receiving simple orders.

-“isolated words”: The third group was made of five patients, their speech productions were limited to isolated words, without any grammatical construction. These patients spoke slowly, the showed a high response latency and perseverative reiteration. Their language often appeared slurred. Communicative intentionality was not always clear and these patients often used echolalic and stereotyped words. They were unable to understand simple orders not even when simplified by a visual or gestural support.

-“ no words” :The last one was not capable of speaking: he communicated through facial expressions, smile, crying and screaming.

Cognitive profile: The overall cognitive functions were evaluated using “widespread” tests, such as the RCPM and the WAIS-R. In order to assess the most severe cognitive disabilities, we applied the MMSE and the SIB, two neuropsychological scales used in patients with Alzheimer`s disease. Eight patients were only able to perform the SIB, seven of them also performed the MMSE, four analyzed the RCPM and three patients were administered the WAIS-R. The remaining five cases could not perform any standardized assessment. The SIB pointed out the following results: the patients showed best performance in Domain B (Orientation, Orienting to Name and Social Interaction) and in Domain D (Praxis, Visuospatial Ability and Construction), mainly displaying their cognitive spared skills in visual and spatial orientation and in the visuoperceptual areas. Domain A (Memory and Attention) was, on average, the most compromised area. The impairment of Domain C (Language) was very variable within the sample, but all of the patients presented language disorders. Overall, the sub-item “attention” represented the most compromised area.

The MMSE showed mild cognitive impairment in one patient, moderate in another and severe in two other patients. Three cases of this sample responded adequately to more than 24 items out of 30, showing borderline cognitive competence or no cognitive impairment.

Three patients performed the WAIS-R, in a case highlighting the presence of a mild mental retardation (TQI 66), in other cases moderate mental retardation (TQI<45). The patient with the most preserved cognitive skills did not present discrepancy between verbal (VIQ 67) and performance skills (PIQ 74). The others not only did not show any differences, but they also presented overlapping cognitive profiles (patient 4 VIQ 45, PIQ 45; patient 11 VIQ 45, PIQ 45). The analysis of individual sub items allowed us to grasp the most significant differences between these two patients, highlighting the most preserved abilities in subject 3 (sub-items: Comprehension, Similarities, Object Assembly and Picture Arrangement).

Behavioural disorders: In our sample, NPI revealed behavioural disorders in all of the patients, such as agitation (eight patients), irritability (seven patients), aberrant motor behavior (six patients) and eating disorders (five patients). Disinhibition and sleep disorders were less frequent. Anxiety and mood disorders were shown by only two of the patients. NPI highlighted the absence of psychotic symptoms such as delusions and hallucinations. During this assessment, we were able to notice some autistic-like features in one patient such as poor ability to express emotions, abnormal eye contact and semantic perseveration of the language.

Adaptive functioning: in all the patients examined using the Vineland scale, the adaptive and behavioural developmental quotient appeared significantly impaired: seven patients presented a mental age below the age of three and five patients had a mental age between 8 and 14. The autonomy skills represented the most affected area in half of the patients. Only one patient presented more appropriate socialization skills (mental age >16). The Barthel modified Index confirmed that they all needed to depend on somebody and this was a constant feature of our patients: four of them were totally dependent on their caregiver, three of them needed a considerable support. Nobody lived alone or in supervised community accommodation: eleven patients still lived with their parents, ten of them attended a day center. Two patients were institutionalized.

These data confirm that cognitive impairment, behavioural problems and the lack of autonomy of the patients, represent the prevailing problems in adulthood. The severe cognitive decline associated with the progression of the disease, hampers the application of the standardized neuropsychological tests, because of the “floor effect”. These patients are considered “non testable”, because their neuropsychological abilities are much below the lower limit, indicating that there is a severe cognitive impairment. Nevertheless, the evaluation of cognitive abilities in these patients could allow us to identify the least affected cognitive areas, as a starting point to develop compensatory strategies. They could also be used when assessing the effect of the rehabilitation treatment, for example of a metacognitive treatment. We have therefore developed a potentially accessible protocol for patients with severe cognitive disabilities, including SIB and MMSE, used for the evaluation of patients with severe Alzheimer’s disease. Notwithstanding, in five patients we could not administer the full version of the SIB due to the extreme severity of the clinical picture. Analysing the results we have thus defined that these patients suffer from a profound cognitive disability. We classified patients based on their ability to perform only the SIB and suffering from severe cognitive delay, those capable of sustaining the SIB and the MMSE with moderate cognitive disability. The SIB seems to be a sensitive test, suitable to assess adult patients suffering from Dravet Syndrome. Therefore this appears to be the right tool to discriminate between different abilities in the low score and avoid the floor effect.

1.3 Genetics

In 1997 Scheffer and Berkovic described in a very large family (2000 individuals) a familial epilepsy syndrome, that encompassed a range of phenotypes from simple febrile seizures to mild generalized epilepsies and, less commonly, severe epileptic encephalopathies named GEFS + (Generalized Epilepsy with Febrile Seizure Plus).

In 2000 Escayg and colleagues identified, in two GEFS+ families, mutations in the SCN1A gene, encoding for the alfa-subunit of the neuronal voltage-gated sodium ion channel, type1 (NaV 1.1). The prominence of febrile seizures in GEFS+ and in Dravet Syndrome, induced Claes and colleagues to look for these mutations in SMEI patients leading to the discovery of the SCN1A mutations in 7 patients affected by Dravet Syndrome (Claes et al, 2001).

The NaV 1.1 channel is a heteromeric complex consisting of the alfa-subunit, which forms the larger central pore of the channel, and two smaller auxiliary beta-subunits. The alfa-subunit regulates the sodium ion selectivity and can function as a channel on its own, whereas the beta-subunits modulate the voltage dependence and the cell-cell interactions, interacting with the extracellular matrix, with other adhesion molecules and with the cytoskeleton (Catterall et al, 2000).

NaV1.1 is expressed in the central and peripheral nervous systems and in cardiac myocyte; within the CNS, it shows higher expression in dendrites and cell bodies (Duflocq et al., 2008; Ogiwara et al., 2007).

Catterall and colleagues demonstrated that the haploinsufficiency of a NaV 1.1. channel leads to epilepsy, disinhibiting neural circuits throughout the brain. They generated a mouse genetic model of DS and discovered that the GABAergic inhibitory interneurons in the hippocampus of DS mice have a substantial defect in sodium currents and action potential firing, whereas excitatory pyramidal neurons are unaffected. Therefore the disinhibition of neural circuits leads to neuronal hyperexcitability (Catterall et al, 2000; Yu et al., 2006).

After the first description, several studies confirmed the presence of mutations in the SCN1A gene in DS patients, including the borderline ones. The frequency of mutations is detected in as many as 85% of patients (Wirrell et al, 2017; Brunklaus et al, 2012). Truncating mutations account for nearly 50% of the abnormalities, while the remaining ones include splice site and missense mutations. Intragenic deletions and whole gene deletions, including SCN1A and contiguous genes, account for 2-3% of all cases. About 12.5% of all cases exhibit no point mutations. (Guerrini, 2012; Hattori et al, 2008; Marini et al, 2009). In 95% of patients, mutations are de novo, but familial SCN1A mutations may also occur (Sugawara et al, 2002; Meisler et al, 2010). Somatic mosaic mutations have been reported in some patients and should be taken into account when estimating the recurrence and for genetic counselling (Depienne et al, 2006; Marini et al, 2006); furthermore mosaic SCN1A mutations might contribute to explain the phenotypic variability within the same family.

Truncating, nonsense, frame shift mutations and partial or whole gene deletions have been correlated with a severe phenotype and they appear to be significantly correlated with an earlier age of seizure onset (Sugawara et al, 2002; Marini et al, 2007; Brunklaus et al, 2014). Moreover, the severity of the phenotype has been correlated with the SCN1A missense mutations falling into the pore forming region of the sodium channel, while missense changes associated with the GEFS plus spectrum are nearly always localized outside the pore forming region (Kanai et al, 2004; Meisler et al, 2005). However, in spite of these

observations nowadays there is no clear evidence of a correlation between genotype and phenotype, as reported in a recent series by Cetica and colleagues (Cetica et al, 2017). Moreover the fact that the same inherited mutation could show variable phenotypes among family members, suggests that possible modifier-genes can play a crucial role in determining the final phenotype (Oguni, 2005; Parihar et al.,2013).

1.4 Psychomotor development

Since the initial description of the disease, psychometric data have been reported in literature, but, in many cases, cognitive impairment has only been evaluated through a clinical medical assessment and reported in retrospective studies.

In 1991 Giovanardi-Rossi and colleagues reported a series of 15 patients, with a mean age of 14.5 years. They detected a cognitive impairment in all of the cases, severe in 11 (IQ lower than 50) and moderate in 4 cases (IQ between 50 and 75). The authors pointed out the presence of language impairment in all of the patients and of behavioural and/or affective disorders in 11 cases(Giovanardi-Rossi et al, 1991). The group of Yakoub reported a sample of 17 patients with SMEI, followed until a mean age of 6 years and 2 months: all patients presented speech delay and severe hyperkinesia. In this series, 12 patients underwent psychometric evaluation with the Brunet Lezine test at a mean age of 5 years. The developmental quotient (DQ) was ±50 in 5 cases, ±60 in 3, and ±70 in 4. In this study, 2 patients repeated the evaluation longitudinally, showing a cognitive impairment (Yakoub et al,1992).

Wang and colleagues described 10 patients, aged from 2 to 11 at the last evaluation. All of them presented a developmental speech delay, clumsiness and behavioural disorders. Cognitive abilities were assessed in 5 of them, older than 4 and they all revealed IQ scores between 42 and 76. This study highlighted that the neuropsychological impairment was not indefinitely progressive, on the contrary the cognitive decline began with a steep fall during the first stage of the disease, up to 4–5 years, reaching a plateau later, without further regression. As a matter of fact, the authors highlighted the need for an early intervention and special educational support for all of the patients (Wang et al, 1996).

In 2006 Caraballo and Fejerman, reported a large series of 53 patients, followed until a mean age of 11 years. They revealed a mild mental delay in 18 children (34%), moderate in 21 (40%) and severe in 14 (26%). The age range in this sample was large (4-14 years) and the age at evaluation was not specified.

Buoni reported the only Dravet patient, aged 13, with a normal cognitive outcome. In this case his intellectual quotient, evaluated by WISC-R, was 125 (WISC-R). The authors attributed this uncommon favourable cognitive outcome to the progressive reduction of seizures after the age of four. (Buoni et al, 2006). Several retrospective longitudinal studies have been carried out focusing on early age, whereas, nowadays, only few prospective longitudinal studies have been reported (table 1, Battaglia et al 2016 modified)

Table 1 General data of neuropsychological studies

Authors	Case number	Age range	Study type	Assessment techniques	Longitudinal data	Overall DQ/IQ outcome
Wolf et al. (2006)	20	0.11–16 years	Retrospective, partially longitudinal	observation, Brunet–Lézine	***In 14 cases (0.11-13 years)*** ***1-3 y*** **normal→60** ***4–6 y*** lower ***Over 6 y*** less than 40	***over 6 y*** less than 40
Ragona et al. (2010)	37	0.6–28 years	Retrospective, partially longitudinal	observation, Griffiths, Wechsler	***In 8 cases (0.6–10 years)*** Developmental steep falling curve in the first 4 years	***0.6–6 y*** normal 5, MMR 5, MoMR 3, sevMR 2 ***7–10 y*** MMR 3, MoMR 2, SevMR 1 ***Over10*** MMR 2, MoMR 2, SevMR 12
Ragona et al. (2011)	26	0.4 m–8 yeas	Retrospective, partially longitudinal	Griffiths, Brunet-Lézine	***Study at 1 and five years*** Group 1 (19 cases): steep falling (mean, 39 points) Group 2 (7 cas) mild falling (mean 12 points)	***At first examin. (mean 11 m)***: all but two normal ***Slowing from the second year of life***
Nabbout et al. (2013)	67	Last follow up 1.1–23.9 (mean 6.4)	Prospective, partially longitudinal	Brunet–Lézine, Wechsler, observation	First evaluation: at a mean age of 34 months (SD = 22, range 9–91), significantly higher than at second evaluation Second evaluation: at a mean age of 66 months (SD = 43, range 15–175), severe cognitive decline (around or below 40) except 2 cases.	Significant lower DQ or IQ with increasing age, stronger after 3 y
Battaglia et al. (2013)	9	4.6–13	Retrospective	Griffiths, Wechsler		MMR 7 Mo MR 2
Chieffo et al. (2011a)	5	0.6–4 years	Prospective longitudinal	Visual function, Griffiths	***At onset***: normal ***Outcome***: normal 2, borderline 2, MMR 1	
Chieffo et al. (2011b)	12	0.9–10 years	Retrospective, partially longitudinal	Griffiths, Wechsler	***Decline from the third year of life*** ***Milder falling from the fourth year***	***At first assessment*** (6–84 m): normal 6, mildly delayed 8 ***At outcome (4-10 y)***: normal 1, borderline 6, MMR 5
Ricci et al. (2015)	5	3–6/8 years	Prospective longitudinal	Visual function, Griffiths, Wechsler, Specific skills	***At first assessment***: MMR 4, MoMR 1 ***At outcome***: MMR 1, MoMR 4	
Chieffo et al. (2016)	13	3-4/7 years	Prospective longitudinal	Griffiths WISC III Language assesment	DQ at onset: normal 8 borderline 1, MMR 3 nt 1 Language at onset: normal 2, good word comprehension 7 impaired comprehension 3 word production impaired 6 out 11	DQ/IQ at Outcome: normal 2, borderline 3 MMR 4,MoMr 3 nt 1 Comprehension Abilities: normal 6, borderline 4 nt 2 Productive skills: impaired 5 borderline 4, nt 3

MMR: mild mental retardation; MoMR: moderate mental retardation; SevMR: severe mental retardation.

The first accurate neuropsychological study was carried out in Marseille and collected data of a series of 20 patients ranging from 11 months to 16 years, with a follow-up of over three years in ten cases (Cassé-Perrot et al., 2001; Wolf et al., 2006). In 12 cases a correlation analysis between the neuropsychological profile and the clinical features was performed; the clinical variables analysed were the frequency and the semeiology of seizures, the frequency of the status epilepticus, EEG data and antiepileptic treatment. The sample was divided in three groups according to age at testing: in the first group there were 4 children aged between 11 months and 2 years, in the second group 12 children aged between 2 and 6 years; in the last group there were 11 children aged between 6 and 16 years. The age of the 20 children tested at the first neuropsychological assessment varied between 11 months and 12 years. In all of the children aged from 1

to 4 years, a decline of the developmental quotient (DQ) was observed, indicating a stagnation in their development. In most children aged between 4 and 13 years, developmental quotients remained at a low level ranging from 20 to 40, revealing a cognitive stabilization after the age of 4 years. The authors pointed out that diffuse neuropsychological deficits were found in all of the patients and they became evident at the end of the first year of life. Motor abilities, linguistic and visual competence were strikingly affected**.** In children with a favorable outcome, language skills tended to be better preserved than visuomotor functions. Behavioral disturbance with hyperactivity and autistic traits were frequent.

In 2010 Ragona et al. described the neuropsychological evolution in a series of 37 patients (21 cases with a truncanting mutation, 10 with a missense mutation).

All of the patients underwent neurological examination, cognitive assessment (by Griffiths or Wechsler Scale), behavioural observation and EEG. Twenty three patients of the series have been followed-up longitudinally for a mean period of 6.3 years (6 months to 18 years).

Authors divided their patients into three groups, according to the age at the last assessment (mean age of 16 ± 6.9 years). Analysis of the results demonstrated that the developmental delay became evident from the second year of life, at various degrees and the percentage of patients with severe cognitive impairment rose with age. In the first group (16 patients older than 10 years), ten patients presented a severe cognitive impairment, in the second group (6 patients evaluated between 7 and 10 years of age), cognitive delay was severe in one patient, moderate in two and mild in three. In the third group (15 patients evaluated between six months and six years), psychomotor development appeared heterogeneous, ranging from normal cognitive abilities to severe cognitive impairment.

The authors suggested that the decline in the first years of life did not correspond to an actual cognitive deterioration but only to a stagnation. Moreover, in many patients the clinical picture was worsened by behavioural problems, such as attention deficit, hyperactivity and opposition, whereas autistic disorders were less frequent (Ragona et al., 2010).

In 2011 Chieffo and colleagues reported results of a prospective investigation: the authors enrolled infants with early severe febrile seizures and followed longitudinally for the possible emergence of DS during the first 4 years of life (Chieffo et al, 2011a). The aim of this study was to identify the onset of neurodevelopmental abnormalities in children with DS studying the earliest stages of neurodevelopment, period in which psychomotor development was “apparently normal”. They performed a neurodevelopmental assessment including the visual function in a sample of five cases (two typical DS and three borderline DS). Only one patient carried a mutation in the SCN1A gene. In all cases but one visual function was impaired, including cerebral visual processing; in particular fixation shift abilities were defective far beyond the time of normal maturation. The parallel neurodevelopmental assessment using the Griffiths Scales revealed the emergence of cognitive decline after the onset of the visual disorder, according to literature data (Wolff et al, 2006, Nabbout et al, 2013). In the following years the same group reported data of the follow up until school age (6-8 years) of the above-mentioned cases, especially focusing on the development of the cortical visual function. They revealed an impairment of visuo-motor items demonstrating a continuity in the deterioration of visual function. For this reason they hypothesized a possible ‘‘vulnerability’’ of the visual dorsal pathway linked to sensory-motor areas, underpinning the process of spatial information and visual-control of actions. The authors speculated that there was a genetic component in determining the cognitive impairment in DS,

other than epilepsy activity, because the early visual function impairment became evident before the manifestation of severe seizures (Ricci et al, 2015).

In 2011 Chieffo and colleagues analyzed a larger series of DS patients (12 cases) confirming that visual motor integration, visual perception as well as the executive functions were the most impaired abilities; by contrast language abilities appeared to be less impaired. Moreover language qualitative analysis showed frequent phonological disorders in spite of a relatively good comprehension ability suggesting a possible dissociation between expressive and receptive language functions. They stated that the predominance of phonological defects in verbal auditory processing might suggest an impairment of the sensory motor interface in the auditory cortical dorsal stream. From a pathophysiologic perspective, they speculated that the language disorders associated with the impairment of other abilities such as visual attention, visuo-spatial organization, working memory and executive functions might be consistent with a cerebellar dysfunction, possibly present in DS (Chieffo et al., 2011b).

In the following years, the same group described the first prospective longitudinal study focused on early language development in 13 Dravet patients (5 with typical DS, 8 with a borderline DS). In this series only 8 patients carried a mutation in SCN1A gene. Specific assessments of detailed language features (pragmatic, receptive, and productive) were performed during the first years of life. Full clinical observation including neurological examination, long term monitoring EEG and developmental/cognitive and language assessments were serially performed at the third year of life and during the last assessment (at a mean of 6 years of age: ranging from 4 years to 7 years and 8 months). This study confirmed a worsening of cognitive abilities and a characteristic language impairment with a relative preservation of receptive abilities and a strong impairment of productive skills (Chieffo et al, 2016).

1.5 Epileptic Encephalopathy versus Channelopathy

In 1987 Dalla Bernardina and colleagues studied 29 patients, followed until a mean age of 11 years and 8 months, and they categorised them into three clinical groups (poor, poorer and the poorest prognosis) according to school performance and language. The authors concluded that the presence of myoclonus (myoclonus status, massive jerks and interictal myoclonus) and the severity of the seizures were the factors that seemed to negatively influence this cognitive evolution (Dalla Bernardina et al, 1987).

In 2006 Wolf et al, reporting data of the first neuropsychological study, correlated the appearance of the cognitive impairment to the severity of epilepsy during the first two years of life (Wolf et al. 2006). They hypothesized a specific relationship between the number of convulsive seizures (>5 per months), their duration and the degree of cognitive impairment. Emphasizing these correlations, the authors confirmed that SMEI can be considered as a true epileptic encephalopathy, as defined by the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE) in 2001 (Engel, 2001) and as subsequently reaffirmed (Berg et al, 2010).

Differently from what is described above, in a report of 2009, Riva and colleagues illustrated the cases of two DS patients, both carrying a truncating mutation in the SCN1A gene. They were followed from early childhood (11 and 23 months), until 7 and 8 years, with repeated assessments using the Griffiths Mental Development Scale. At the last assessment patients revealed a similar progressive decline of the DQ, despite presenting a different course of epilepsy. Although the sample was not representative, the authors pointed out the role of the mutation in SCN1A per se, in the cognitive impairment observed in these patients (Riva et al 2009).

In 2011 Ragona and colleagues performed a retrospective, multicenter study, to clarify the role of epilepsy and genetic background in determining the cognitive outcome of patients with Dravet syndrome. The sample was made of 26 patients who had been followed with standardized evaluations since seizure onset and followed until at least the age of four. Molecular analysis for SCN1A was obtained for all of the patients, detecting truncating mutations in 17 patients and missense mutations in 3 cases. Authors examined epileptic history paying particular attention to the occurrence of status epilepticus and myoclonic seizures and investigated the cognitive profile course, determining a differential general quotient (dGQ) between the 12^{th} and 60^{th} month of age. Statistical analysis correlated the dGQ with genotype and epilepsy course; the analysis suggested that the epileptic phenotype played a role in determining cognitive impairment and that the early appearance of myoclonus and/or atypical absences might have a negative prognostic impact. By contrast, the frequency of convulsive seizures and of convulsive status did not represent, per se, a bad prognostic factor for cognitive outcome. Moreover, the statistical analysis failed to reveal a meaningful correlation between the presence and type of mutation in the SCN1A gene and cognitive outcome. However, authors hypothesized that several other factors, rather than only epilepsy, might concur to determining cognitive development and its impairment, including antiepileptic drug treatment, rehabilitation, and familial environment. Moreover, authors hypothesized that the channelopathy itself is probably crucial in determining the phenotype.

Two independent Japanese studies of adult patients, confirmed the role of epilepsy in determining the final outcome. In 2010, Akiyama and colleagues described a long term follow-up in 31 patients with DS, followed from childhood to at least 18 years of age. Mutation of SCN1A was identified in 25 out of 29 patients

examined. Their seizures, EEG and cognitive abilities were investigated and statistically analyzed. The authors demonstrated that the seizures' free outcome was significantly correlated to the experience of <3 episodes of convulsive status epilepticus and with the disappearance of spikes on follow-up EEGs. They failed to detect any particular correlation with the SCN1A mutation type, gender, or type of DS (typical or borderline), or with the presence of generalized spike-waves on EEG in early childhood.

Furthermore, the authors pointed out that the less severe intellectual disability was correlated to the presence of occipital alpha rhythms on the background activity of the follow-up EEGs, as if the slow EEG background could represent a brain dysfunction. Eventually, patients with cognitive disabilities invariably presented severe epilepsy with the persistence of frequent seizures and slow EEG background at follow-up, during the long-term clinical course (Akyiama et al, 2010).

Takayama and colleagues described a series of 64 adult patients; they confirmed that the presence of occipital alpha rhythm in the background activity was associated with a milder intellectual disability. Moreover they stated that a period of seizure freedom was related to the appearance of occipital alpha rhythms and disappearance of epileptic discharges. The authors concluded that the epilepsy phenotype might influence the long-term outcome of DS (Takayama et al, 2014)

Other authors disconfirmed this hypothesis and highlighted the role of the SCN1A gene, per se, in psychomotor delay, affecting structures/ pathways not directly involved in epilepsy.

In 2013 Nabbout and colleagues reported data from a prospective study: the sample was made of 67 patients, of which 58 carried a mutation in SCN1A. They performed neuropsychological evaluations using the Wechsler or Brunet-Lezine Scales and the authors studied the correlation between developmental/intelligence quotient (DQ/IQ) and age, epilepsy features and the presence of the SCN1A mutation. Among all evaluations, DQ/IQ significantly decreased with age, from normal before 2 years, to low after 3 years, with hyperactivity and attention disorders hampering learning abilities especially up to 6 years.

The authors failed to find significant correlation between the DQ/IQ of the last evaluation and epilepsy data, i.e. first seizure (age, type, duration, fever), seizures during the disease course (type, fever sensitivity), status epilepticus (age at onset, number, fever), photosensitivity, and treatment. The only significant statistical prognostic factor for a lower QD/IQ after 3 years was the presence of myoclonus and focal seizures.

Moreover, the analysis of the cognitive evaluations showed that the mutated and non-mutated groups exhibited a different psychomotor development: no patient had severe delay in the non-mutated group whereas all patients showing a severe delay (26%) were in the mutated group. Nabbout stated that epilepsy did not account for the whole cognitive picture and that mutation in the SCN1A gene could therefore be a key factor for the cognitive delay, in addition to epilepsy. To this end the authors claimed that DS did not correspond to the usual definition of epileptic encephalopathy, i.e. worsening of functions as a consequence of the epileptic activity itself. In fact, the disease was not a pure consequence of epilepsy, but it seemed that the SCN1A mutation per se played a direct role in the psychomotor delay.

Brunklaus and Zuberi in a review of 2014, taking into account the different arguments of the encephalopathy/channelopathy debate, highlighted the emergence of some key aspects: there was overwhelming evidence that Dravet syndrome is a channelopathy causing widespread Nav1.1 dysfunction throughout the brain and this channel dysfunction contributes to the encephalopathy. It seemed plausible that this already-vulnerable system may be susceptible to secondary aggravating events such as status

epilepticus. Furthermore, pharmacologic treatment and the restoration of impaired GABAergic neurotransmission might not only help prevent seizures but might also recover wider neurologic functioning. With this in mind the authors highlighted the concept of Epileptic Encephalopathy, revised in the 2010, emphasizing the original International League Against Epilepsy (ILAE) comment: “We must, however, recognize that the source of an apparent encephalopathy may be the product of the underlying cause, the result of an epileptic process, or a combination of both.”

2. Aims of the study

1) Multicentre prospective evaluation of a group of Italian Dravet patients, carrying a SCN1A mutation, followed from the first year of life, in order to:

- Describe the epileptological onset and evolution (semeiology and age at seizure's onset, presence of convulsive status, semeiology and frequency of seizures)
- Describe neurological features
- Identify the onset of a neurodevelopmental impairment and describe its clinical features
- Evaluate the presence of associated behavioural disorders
- Analyze the pharmacologic treatment
- Perform a correlation analysis between genetic determinants and cognitive evolution
- Perform a correlation analysis between the cognitive evolution and epileptological variables, paying particular attention to: age at onset, presence of myoclonic seizures, occurrence of epileptic status

2) Prospective evaluation of a sample of Dravet patients and a control group followed in a tertiary neurological centre, followed from seizure onset in order to:

- Compare clinical (epileptological, neurological and neuropsychological) and electrophysiological features during the first years of life
- Define etiologic determinants in the control group to reach an early diagnosis and to give targeted treatment

This study was funded by the EU 7th Framework Programme (FP7) under the project DESIRE grant N602531 (to R.G.) and RESIDRAS.

3. DESIRE

In 2013 the European Union's Research and Innovation funded DESIRE (Development and Epilepsy - Strategies for Innovative Research to improve diagnosis, prevention and treatment in children with difficult to treat Epilepsy), in order to investigate about the epileptogenic developmental disorders.
Specific objectives of DESIRE were to advance the state of the art with respect to:

- the genetic and epigenetic causes of epileptogenic developmental disorders, particularly epileptogenic malformations of cortical development, to elucidate molecular networks and disrupted protein complexes and search for common bases for these apparently heterogeneous disorders.
- the diagnostic tools (biomarkers) and protocols through the study of a unique and well-characterized cohort of children to provide standardized diagnosis for patient stratification and research across Europe.
- treatment of EDD using randomized, multidisciplinary clinical protocols and testing preclinical strategies in experimental models to also address novel preventative strategies.

This Project includes the Workpackage 2 (WP2), focused on identifying genetic causes and pathophysiological mechanisms of Dravet syndrome (DS) and Landau Kleffner Syndrome/Epilepsy with Continuous Spikes and Waves During Slow-Wave Sleep (LKS/CSWS).

4. Patients and methods

1) Multicentre prospective evaluation of a group of Italian Dravet patients

The sample was composed of 17 Dravet patients followed, since January 2012, in 4 Italian tertiary clinical centres with paediatric epilepsy expertise, involved in DESIRE study.

The centres involved in the study were:

- Paediatric Neurology, Neuroscience Department, Meyer Children's Hospital, Florence
- Child Neuropsychiatry, university of Verona, Verona
- Department of Paediatric Neuroscience, Istituto Nazionale Neurologico C. Besta, Milan
- Department of Neuroscience, Bambin Gesù Children's Hospital, Rome

Inclusion criteria were the following:

- Febrile or prolonged afebrile seizure's onset in the first year of life
- Normal psychomotor development before seizure onset
- No significant personal antecedents
- Not more than six months from the first observation

The full assessment included:

- Collection of clinical data through a standardized form including demographic data, family and personal history
- Collection of detailed epileptic history and the pharmacologic treatment
- Neurological examination
- Behaviour assessment, performed by a clinical observation
- Developmental/cognitive assessment, performed using the Griffiths Mental Development Scale or the Wechsler scales (Wechsler Preschool and Primary Scale of Intelligence (WPPSI) according to the age and level of cooperation. According to the DSM-V, patients were classified as follows: borderline (IQ between 70 and 84), mild mental retardation (IQ between 55 and 70), moderate mental retardation (IQ from35 up to 55), and severe mental retardation (IQ lower than 35).

For each patient the ''differential general quotient'' (dGQ) was obtained by comparing the first available GQ with the last available GQ for every patient.

-EEG recordings while awake and sleep with video and polygraphic monitoring

Clinical, EEG and neuropsychological assessments were performed when they were enrolled (T0), at the 12^{th} (T1),18^{th} (T2), 24^{th} (T3), 36^{th} (T4), 48^{th} (T5) and 60^{th} month (T6).

All patients underwent, within the second year of life:

- Mutation analysis of the SCN1A gene including mutational screening by denaturing high performance liquid chromatography (DHPLC) followed, whenever necessary, by multiplex ligation probe amplification (MLPA).
- MRI

2) Prospective evaluation of a sample of Dravet patients and a control group

The sample is composed of 5 Dravet patients and 9 patients of the control group followed, since January 2012, in the Department of Paediatric Neuroscience of Besta. The inclusion criteria and the timing of assessments were the same of the first study.

In the control group all the patients underwent a diagnostic workup that included:

- Karyotype
- Array comparative genomic hybridation (Array-CGH)
- TruSeq Custom Amplicon
- Nextera rapid capture

Data concerning Dravet patients were gathered and analysed, anonymously, referring to the Italian National Registry for Dravet Syndrome and SCN1A-Related Conditions (RESIDRAS; http://www.residras.com).

A informed consent for the involvement into DESIRE study was obtained by parents or tutors for all the patients.

5. Results

5. 1 First part of the study: Multicentre prospective evaluation of a group of Italian Dravet patients

17 patients, followed in 4 clinical Italian centres, met the clinical diagnostic criteria of DS, according to the ILAE classification. The present age of patients ranges between 14 and 60 months of age (mean age 43 ±11.99). During the last evaluation, 1 patient was younger than 18 months, 4 patients were between 24 and 36 months, 4 patients between 36 and 48 months and 8 patients were between 48 and 60 months. The sample was equally distributed by gender (9 male and 8 female). The prospective nature of the study determined a different number of patients for each visit. The neuropsychological assessment was performed at least two times for each patient of the sample. In some cases there were non-feasible evaluations due to the behavioural problems.

Epileptic features, neurological signs and behavioural disorders were collected:

- when they were enrolled, <12 months (T0): 8 patients
- at the 12th month (T1): 14 patients
- at the18th month (T2): 17 patients
- at the 24th month (T3): 16 patients
- at the 36th month (T4): 8 patients
- at the 48th month (T5): 5 patients
- at the 60th month(T6): At the present time, one patient has performed the assessment

Neuropsychological assessment was performed:

- when they were enrolled, <12 months (T0): 4 patients
- at the 12th month (T1): 8 patients
- at the 18th month (T2):13 patients
- at the 24th month (T3): 13 patients
- at the 36th month (T4): 7 patients
- at the 48th month (T5): 4 patients
- at the 60th month (T6): At the present time, no patient has already performed the assessment

5.1.1 Family and personal history

A positive family history for febrile convulsions and/or epilepsy is reported in 10 patients (58%): only in one case there was a positive family history for both epilepsy and CF, in 5 cases there was a history of only CF and in the remaining 4 cases the family history was positive only for epilepsy. Personal risk factors are reported in a single case (prematurity with acute respiratory distress at birth, normal MRI). The psychomotor development was normal before seizure's onset in all infant but one, who had a mild motor developmental delay (case 1).

5.1.2 Genetic analysis

The genetic analysis detected point mutations of SCN1A gene in all of the patients, including truncanting mutations in 7 cases (41%) and missense mutations in 7 cases. in 3 patients (18%) the result was positive but was not clear the effect of the mutation.

5.1.3 Neuroimaging

The exam was completely normal in 14 patients. In one case MRI revealed reduced posterior cranial fossa and outcropping cerebellar tonsils in the foramen magno. In 2 patients the data is unknown.

5.1.4 Epileptic features and pharmacologic treatment (fig 1, tables 2,3,4)

Six patients (35%) of the sample experienced the first seizure during a febrile illness, whereas the onset was marked by afebrile seizure in 10 patients (58%). For the remaining case the data was unknown.

The age at the first seizure ranged between 2 and 8 months (mean age 4,52 months ±1.73).

The majority of patients (15 cases, 88%), presented the first seizure within the 6th month of life: in these cases the onset was marked by epileptic status in 4, by generalized tonic clonic seizure in 8 cases, unilateral seizure in 3. In the remaining 2 patients the first seizure occurred after the 6th month of life and none of them presented a status epilepticus: 1 patient presented a tonic clonic seizure, the other experienced a focal seizure.

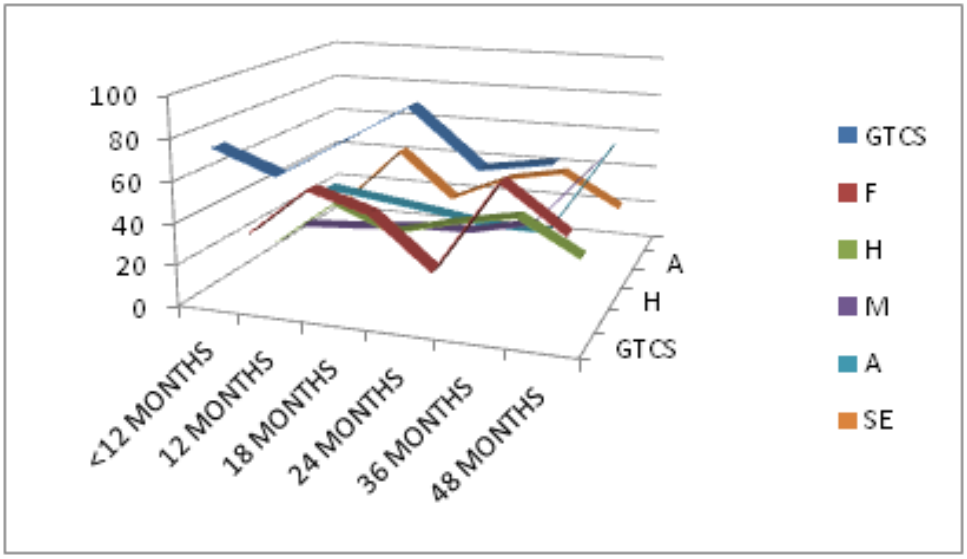
According to the protocol, the first assessment should have been carried out at seizures onset, or, at least, within the following six months. Only 8 patients performed the first follow up within the 12th month of age (mean age 8.37±2.26), the other patients were assessed for the first times in other facilities, closer to their homes and having a paediatric Emergency Room.

At this evaluation, the majority of patients reported monomorphic seizures (6 patients, 75% of cases) and the occurrence of one or more status epilepticus was described in 1 patient. 2 patients did not take antiepileptic drugs, whereas 2 of them were taking 2 drugs. The remaining 4 patients were taking Valpoate in monotherapy. At the 12th month of age, 14 patients were evaluated, 8 patients experienced two or more semeiology of seizures. Tonic clonic or focal with secondary generalization seizures represented the most widespread types, alone or with other seizures (9 patients; 64%). The occurrence of at least a status epilepticus was described in 6 patients (43%). In this sample all of the patients but one (92%) followed a therapy, of which 7 (53%) were taking 2 or more AEDs.

During the following visits, several patients experienced polymorphic seizures and at the 48th visit all of the patients presented more semeiology of seizures. During the fourth year of life the percentage of the patients that experienced generalized tonic clonic seizures was still high (80%), but the occurrence of status epilepticus decreased (20%).

Fig 1 percentage of epileptic seizures reported in Dravet patients during the follow up

GTCS generalized tonic clonic seizures F focal H hemyclonic M myoclonic A atypical absences SE status epilepticus



5.1.5 EEG data

We analyzed data of Dravet patients followed in Neurological Department of Besta, Milan. These data have been described in the second part of the study.

5.1.6 Neurological features

The sequential assessments revealed the appearance of neurological signs starting from the 12th month of age, with a progressive increase in time and with the association of more signs in the same patient. Segmental myoclonus and ataxia were the most frequent neurological signs.

All of the patients presented a normal neurological examination when they were enrolled, whereas at the 12th month of age 2 out of 14 patients (14%) presented segmental myoclonus

At the 18th-month-visit 18% of cases presented two or more neurological signs (myoclonus, ataxia and/or hypotonia). In the following years the occurrence of ataxia and myoclonus increased either alone, for both, or associated with other signs: ataxia was described in 44% at the 24th month (7 patients out of 16) and in 50% of the patients at the 36th month (4 patients out of 8). The presence of myoclonus raised from 18% at the 24th month (3 patients out of 16) to 50% at the 36th month. At the 48th month only one patient presented a normal neurological examination and ataxia was the most common sign (40%).

5.1.7 Developmental/cognitive evolution and behavioural disorders (table 5 and fig 2)

The differential general quotient (DGQ) obtained between the first and the last evaluation of each patient, showed a variable trend of psychomotor development. On the basis of an arbitrary cut-off of 20 points of dGQ, we divided the patients in 3 group:

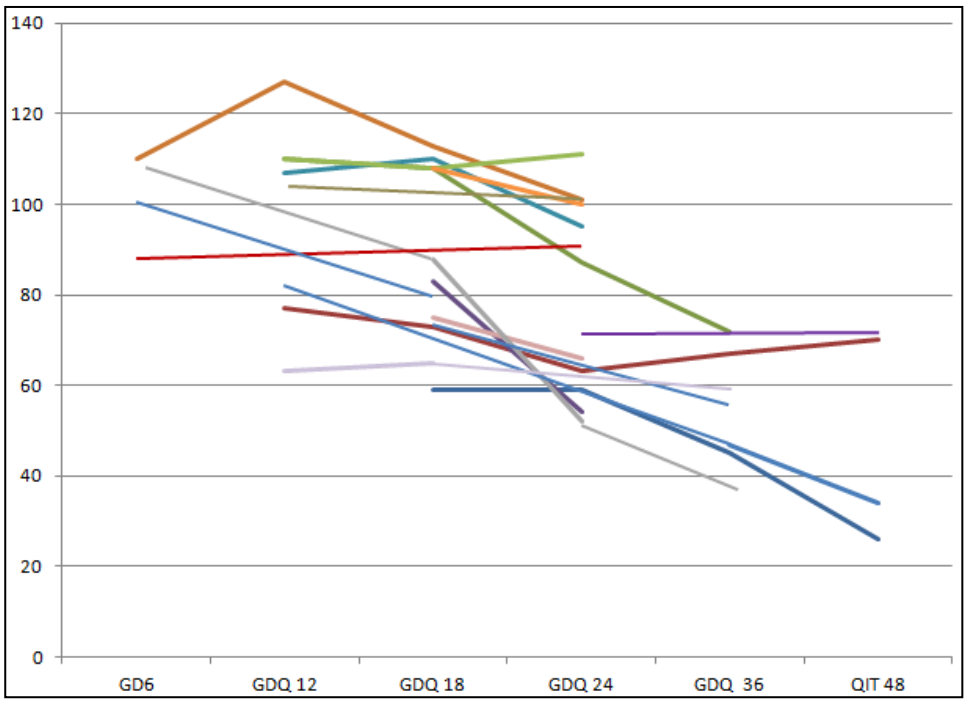
- first group, composited of 6 patients (cases 1, 3, 4, 7, 15, 17), exhibited a steep DGQ higher than 20, ranging from 20 to 72 points – mean 39 points. At the last evaluation 2 patients presented a borderline GQ, 1

patient presented a mild cognitive impairment, 1 patient presented a moderate cognitive delay and 2 showed a severe cognitive delay.

- second group, composited of 8 patients (cases 2, 5, 6, 11, 12, 13, 14, 16), had a DGQ lower than 20 (ranging from 2 to 18, mean was 8 points) At the last evaluation 4 patients presented a normal GQ, 1 patient presented a borderline GQ and 3 patient presented a mild cognitive impairment.

- third group: in the remaining 3 cases (8, 9, 10) there was not a decalage in GQ showing a stable development profile between the first and last evaluation. At the last evaluation 2 patients presented a normal GQ and 1 patient presented a borderline GQ.

Fig 2 Cognitive profiles in the sample of 17 Dravet patients longitudinally evaluated



The children's behavioural clinical observation detected a struggle starting from the 18th month: 3 patients out of 17 (17%) presented attention deficit while one showed signs of hyperactivity.

The behavioural disorders increased during the follow up and during the time the association of 2 or more of them often impaired the child's development: at the 24th month 6 patients out of 16 (37%), at the 36th month 3 patients out 8 (37%) and at the 48th month 2 patients out 5 (40%) presented more associated behavioural disorders. The attention deficit was the behavioral disorder more represented at the 24th control (7 patients out 16, 43%), at the 36th month (4 patients out 8) and at the 48th month (50% of the patients).

Hyperactivity was observed in 38% of the sample at the 24th month (6 patients out 16) and its occurrence remained high: 37% at the 36th month (3 patients out 8) and 40% at the 48th month (2 patients out 5). Autistic features were observed from the 24th month only in one patient and in 2 patients in the following visits.

5.1.8 Analysis: epilepsy features and cognitive development (fig 3,4,5 table 6)

A correlation analysis was performed to find a relationship between the age of seizure's onset and the psychomotor development. Both the patients who had their first seizure after the 6th month, showed at the last control, a normal psychomotor development (cases 6, 12).

Patients with an onset of seizures within the 6th month were correlated to the group of the patients who presented a larger DGQ (I group: cases 1, 3, 4, 7, 15, 17). This analysis showed that all of the patients belonging to the first group experienced their first seizure within the 6th month, in fact 5 of them (1, 3, 7, 15, 17) experienced it within the 4th month.

We correlated the occurrence of one or more convulsive status to the patient's development profile, in order to investigate possible connections. To do so we analyzed the group of patients of group1 (DGQ >20) revealing the presence of one or more status epilepticus or cluster's seizures in each patient. Only 3 patients (cases 2,8,11) of the other groups presented at least a status epilepticus.

We correlated the occurrence of myoclonic seizures and the development profile. 4 patients out of 6 (66%) experienced these seizures in the first group, whereas only 3 cases out of the 11 patients from the group 2 and 3 (27%) experienced them.

Fig 3 developmental evolution and age at seizure's onset

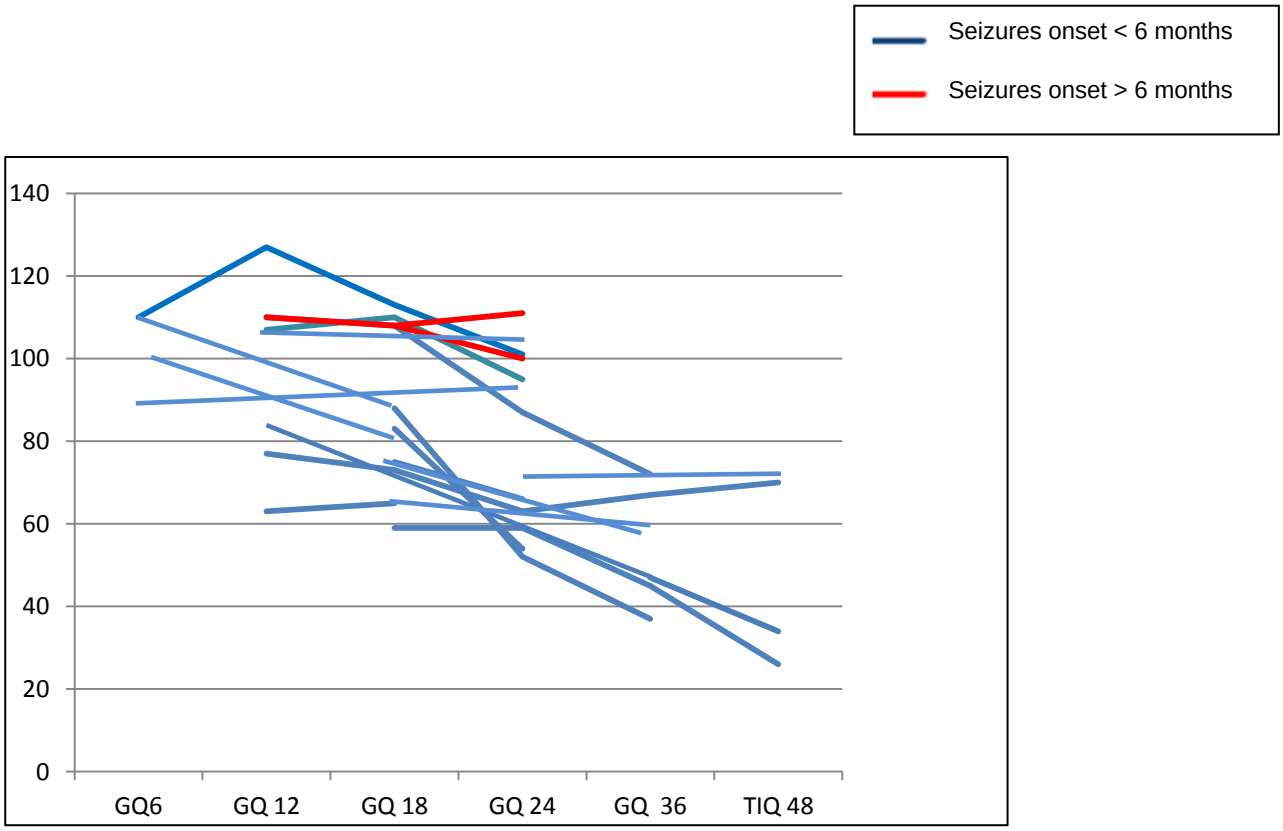


Fig 4 Correlation between convulsive status and cognitive outcome

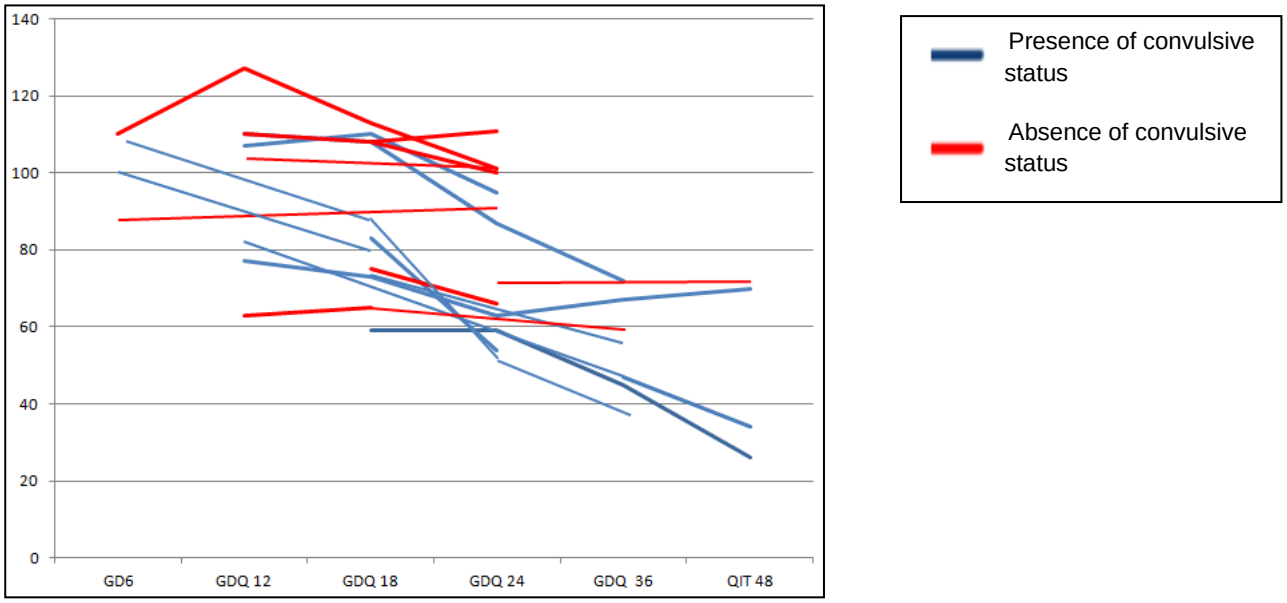
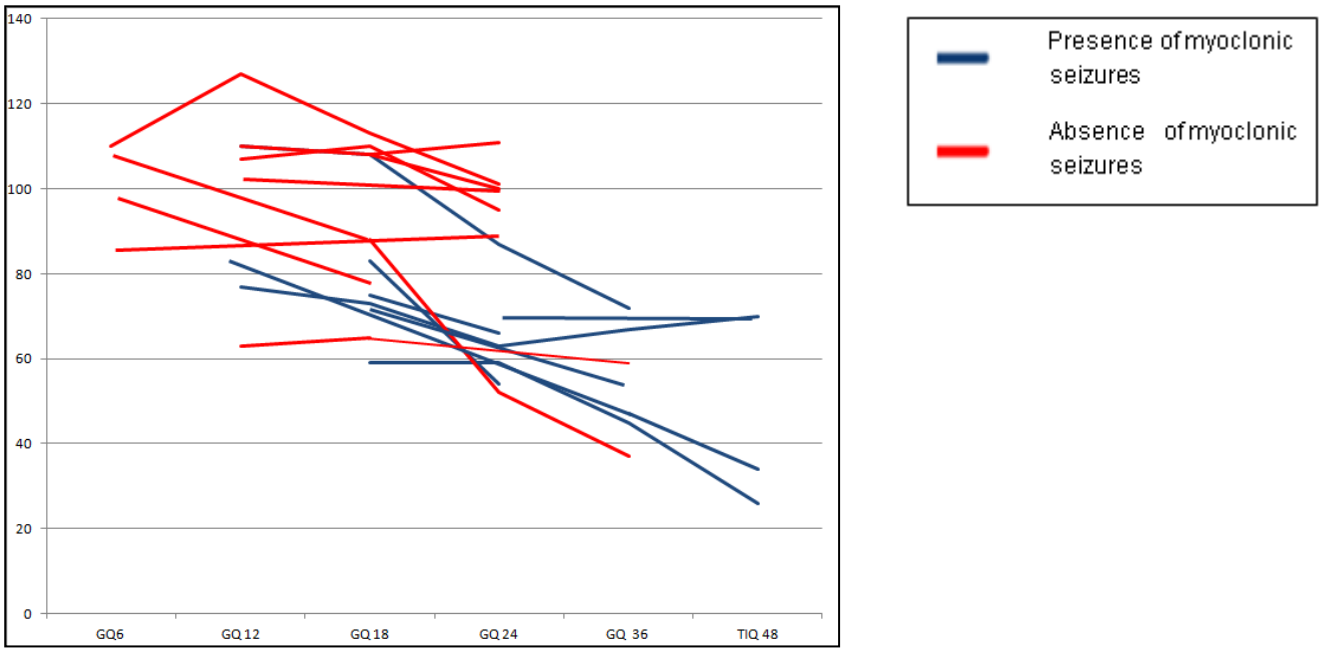


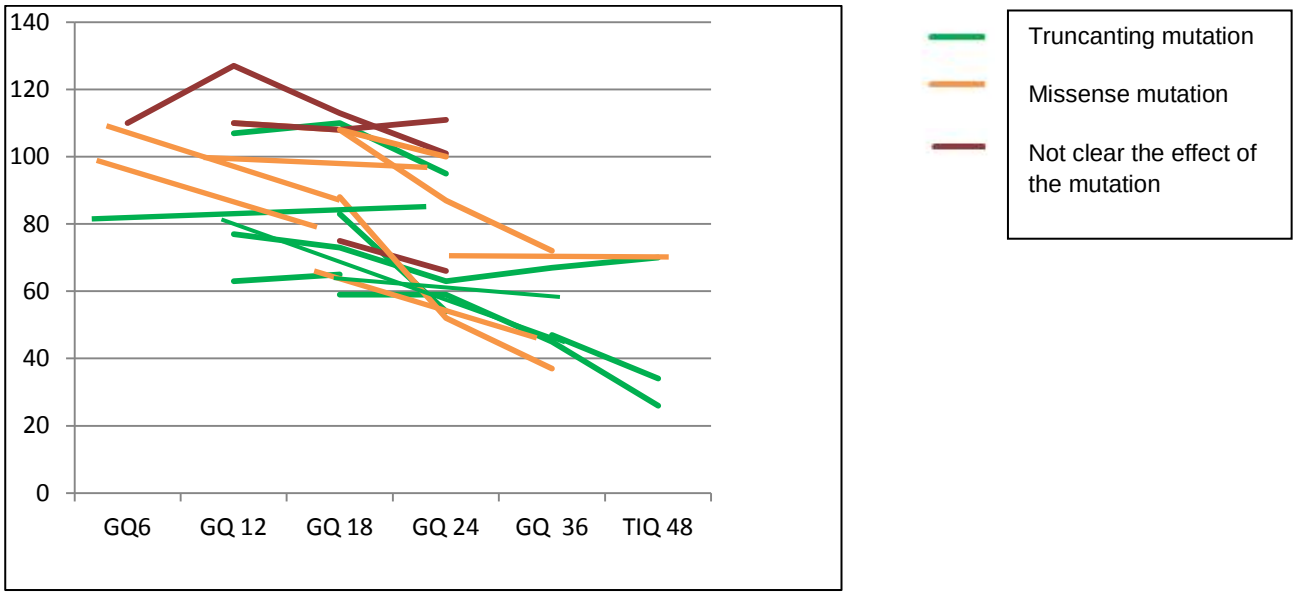
Fig 5 Correlation between myoclonic seizures and cognitive outcome



5.1.9 Analysis: genotype and cognitive development (fig 6)

The statistical analysis failed to reveal significant differences between the type of mutation in SCN1A gene and the psychomotor development: missense and truncanting mutations were equally distributed in the first group, significant distribution differences were not manifested even in the remaining patients. One patient presented also a missense mutation in SCN2A inherited from her mother.

Fig 6 correlation between genotype and developmental evolution



5.2. Second part of the study: Prospective evaluation of a sample of Dravet patients and a control group

The sample was made of 14 patients which were followed in the Department of Paediatric Neuroscience of the Neurological Institute "C Besta", Milan. 5 patients (3 female and 2 male), met the clinical diagnostic criteria of DS, according to the ILAE classification. In 9 patients (6 female and 3 male) the clinical evolution did not suit the criteria for DS, and represent the control group. During the last assessment the mean age of Dravet patients was of 45 months±10.13 (ranging from 35 to 60 months); the mean age of patients of the control group was of 39.7 months±13.06 (ranging from 21 months to 59 months).

Epileptic features, neurological signs and behavioural disorders were collected in Dravet cases:

- when they were enrolled, <12 months (T0): 1 patient
- at the 12th month (T1): 4 patients
- at the18th month (T2): 5 patients
- at the 24th month (T3): 5 patients
- at the 36th month (T4): 3 patients
- at the 48th month (T5): 2 patients

Neuropsychological assessment was performed:

- at the 12th month (T1): 3 patients
- at the 18th month (T2): 5 patients
- at the 24th month (T3): 5 patients
- at the 36th month (T4): 3 patients
- at the 48th month (T5): 2 patients

Epileptic features, neurological signs and behavioural disorders were collected in control group:

- when they were enrolled, <12 months (T0): 1 patient
- at the 12th month (T1): 4 patients
- at the18th month (T2): 9 patients
- at the 24th month (T3): 8 patients
- at the 36th month (T4): 6 patients
- at the 48th month (T5): 2 patients

Neuropsychological assessment was performed:

- at the 12th month (T1): 2 patients
- at the 18th month (T2): 9 patients
- at the 24th month (T3): 7 patients
- at the 36th month (T4): 6 patients
- at the 48th month (T5): 2 patients

5.2.1.Family and personal history

A positive family history for febrile convulsions or seizures was reported in 3 Dravet patients and in 6 patients of the control group. 2 Dravet patients presented positive family history for epilepsy and 1 for febrile convulsions. 4 patients of the control group presented positive family history for epilepsy and 1 for febrile convulsions. 1 patients of the control group presented positive family history for both. None of the Dravet patients nor of the control group presented personal risk factors. All the patients of the control group showed a normal psychomotor development before the onset of seizures. In the Dravet sample only only patient presented a mild motor developmental delay before the first seizure (case 1, head control at 6 months, first steps at 19 months).

5.2.2 Neuroimaging

MRI was performed in both samples: all of the Dravet patients showed a normal exam. 5 patients of the control group presented a normal exam, in two patients neuroradiological abnormalities included minor malformations (arachnoid cysts in the posterior cranial fossa). The remaining one did not perform MRI.

5.2.3 Genetic analysis (fig 7)

The genetic analysis detected point mutations of the SCN1A gene in all of the Dravet patients, showing truncanting mutations in 80% of the cases and missense mutations in the remaining 20%. All of the patients of the control group performed the diagnostic workup for seizures starting within the first year of life. In 6 patients single gene mutation was found and in particular:

Case 6: she presented a missense mutation in PCDH 19 gene (c.2873 G>A, p. Arg958 Gln), inherited from mother

Case 7: she presented a chromosomal syndrome (del 7q31.1-q31.31)

Case 9: she presented a missense mutation in SCN1A gene, exon n°13, (c.1811G>A, p.Arg 604 His), inherited from father

Case 10: he presented a novel missense mutation in DEPD5 gene, exon n°3, C.133 A>T p.Asn 45 Tyr, inherited from father

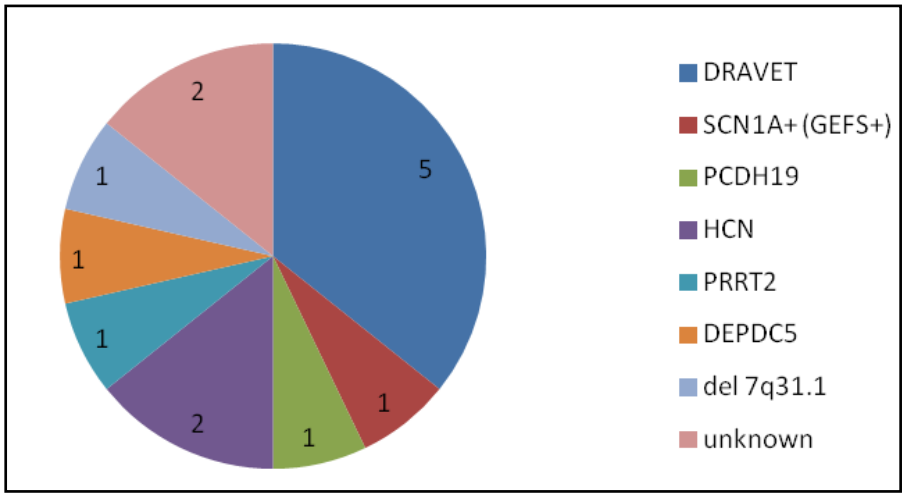
Case 11 he presented a missense mutation in HCN4 gene, exon n°8, C.2648 C>G p.Pro883Arg, inherited from father

Case 13, she presented a frameshift mutation in PRRT2 gene, exon n°2, C.771 delG p.Gly259 ValfsTer54. Novel mutation, inherited from father

Case 14, he presented a novel missense mutation in HCN2 gene, exon 8, c.2167G>A, p. Val723lle, parents have not been analysed yet

Cases 8 and 12 have not been diagnosed yet.

Fig 7 Clinical and genetic diagnosis of the Besta series



5.2.4 Epileptic features and pharmacologic treatment (fig 8, 9)

All of the Dravet patients experienced the first seizure within the 6th month of age, for 7 patients of the control group (77%), the onset was later. Three Dravet patients presented generalized tonic clonic seizures and 2 patients experienced a status epilepticus. The patients of the control group experienced a wider semeiology of seizures, at onset: 4 patients presented generalized tonic clonic seizures, 2 patients presented a status epilepticus, 2 patients experienced focal seizures and 1 patient presented a myoclonic seizure.

Both samples did not show a clear connection between the first seizure and the fever: 40% of the Dravet patients and 44% of the control group experienced the first seizure during a febrile illness.

All of the patients included in both samples underwent a 18thmonth-visit. Within the 18th month of age, all of the Dravet patients presented weekly or monthly polymorphic seizures, whereas 6 patients of the control group experienced sporadic, monomorphic seizures and 3 of them reported 2 types of seizures. All of the Dravet patients reported at least a convulsive status within the 18th month, whereas no patient of the control group showed any convulsive status after the first seizure. In the following year the epileptic data detected the persistence of polymorphic seizures in Dravet patients and the occurrence of status epilepticus. Most of the patients of the control group experienced sporadic, monomorphic seizures and did not reported status epilepticus.

Fig 8 Percentage of patients that experienced seizures during the follow up: A (Dravet patients) B (control group)

GTCS: generalized tonic clonic seizures H: hemyclonic F: focal M myoclonic A atypical absences SE: status epilepticus

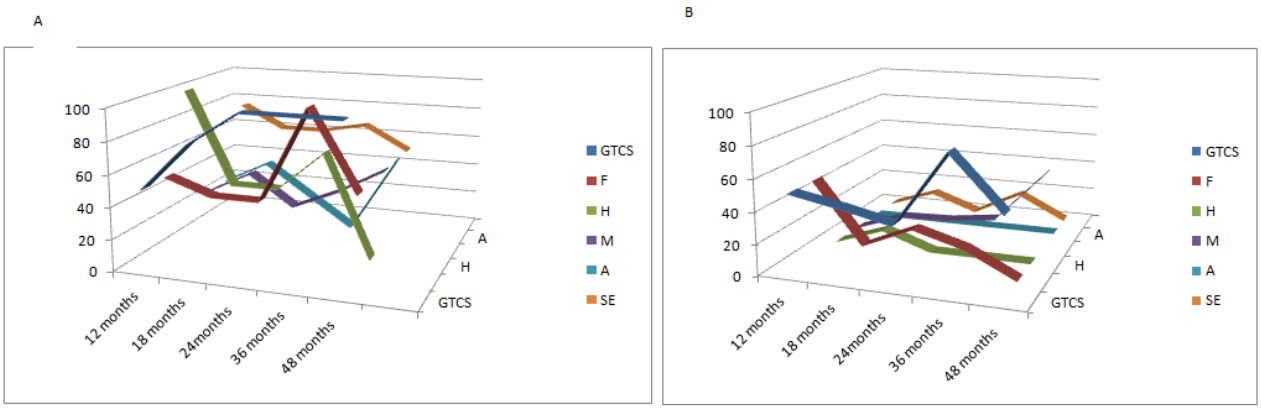
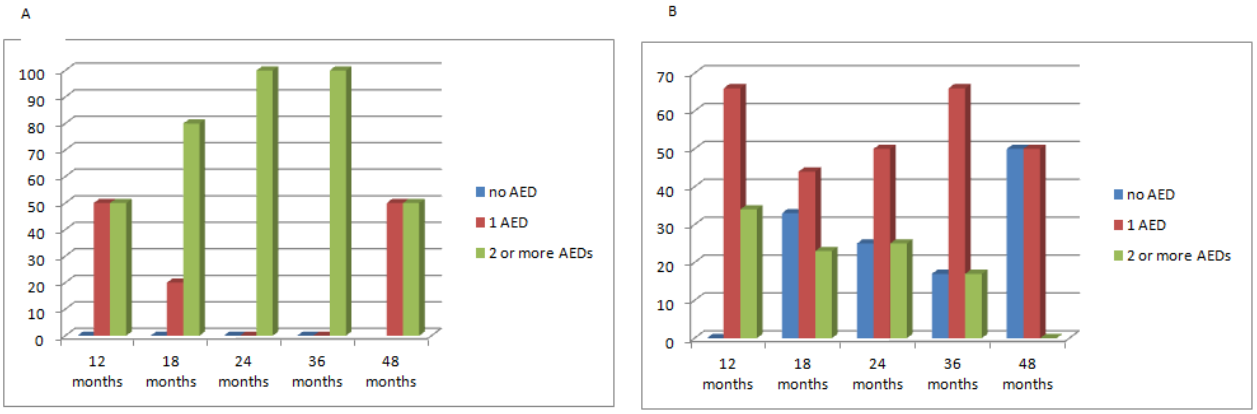


Fig 9 Percentage of patients that followed a therapy during the follow up: A (Dravet patients), B (control group)
AED: antiepileptic drug



5.2.5 Electroencephalografic findings (fig 10,11; tables 7,8)

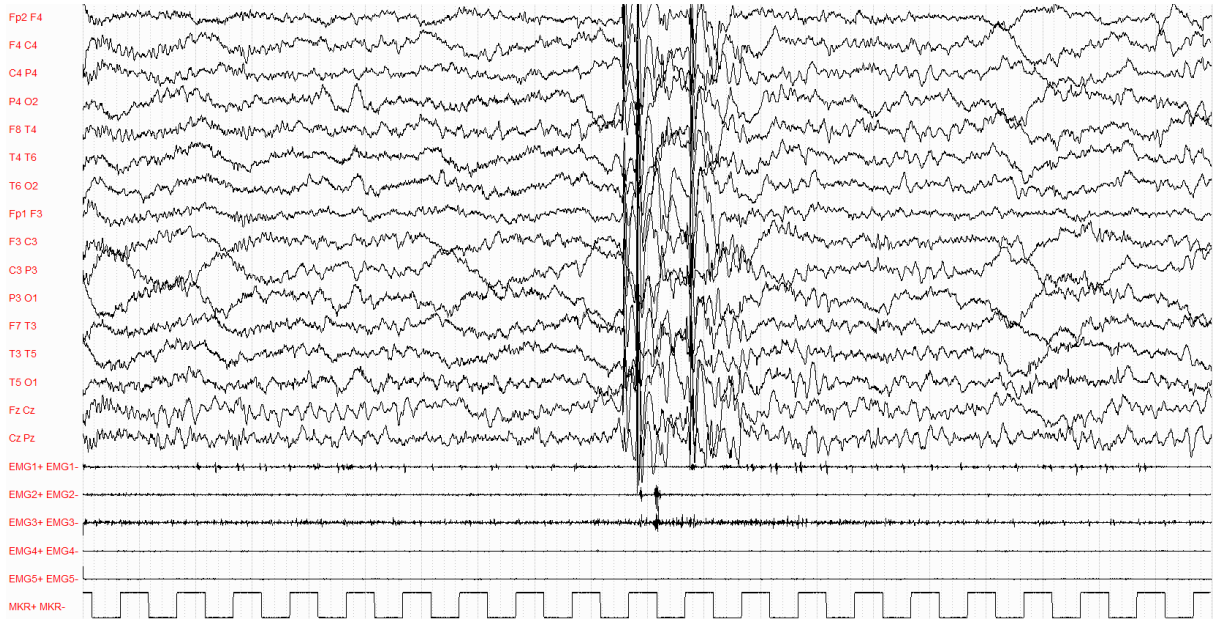
All Dravet patients of the series performed an EEG recording while awake and asleep at the 18th month: 60% presented a normal organization during wakefulness, the remaining 40% showed a slowing of the background activity (3-4HZ). During sleep 40% of the patients presented a poor organization, with few spindles, the remaining 60% of patients had normal EEG during sleep.

During the disease course slow rhythms enhanced: at the 48th month, the 2 patients that performed an EEG presented a slow and poorly organized background activity during wakefulness and sleep appeared poorly organized and with few spindles.

No patients presented interictal epileptiform abnormalities (focal, multifocal, or generalized spike waves or polispike waves) at the onset of the disease; during time the frequency of interictal abnormalities increased.

All patients presented epileptiform abnormalities, at least in one observation, during the study period. A photoparoxysmal response was observed in only one patient during the EEG recording at 36th month.
In 2 cases we recorded seizures : myoclonic seizure and tonic clonic generalized seizure.

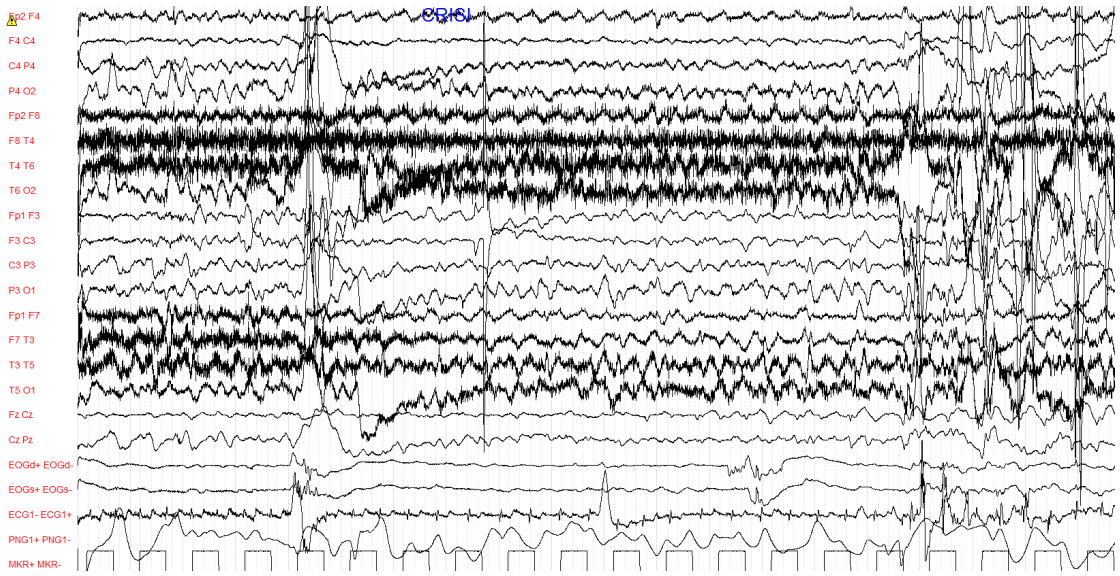
Fig 10 At the 36th month, patient n° 2 presented myoclonic seizures in brief burst. The polygraphic EEG recording showed diffuse Spikes Waves. Note the predominance of polyspike component on the frontocentral regions and vertex



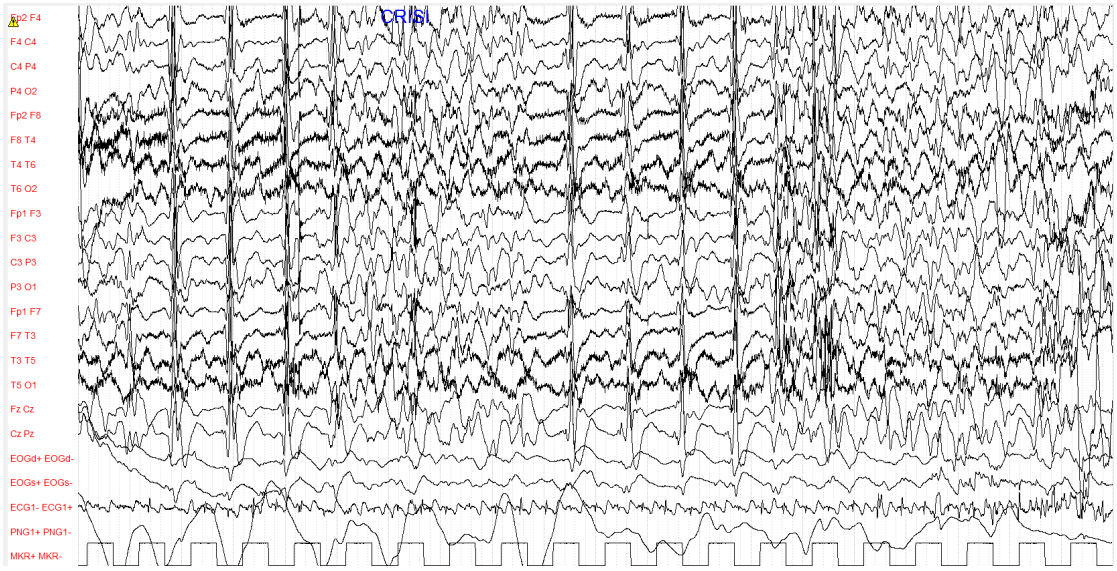
All patients of the control group performed an EEG recording at the 18th month, showing a normal background activity during wakefulness. Sleep was well structured, with physiologic patterns and normal cyclic organization in 7 patients, 2 presented diffuse fast activity and many asynchronous spindles.
EEG showed a gradual organisation, in wakefulness and while asleep during the following visits. 3 patients did not present any interictal epileptiform abnormalities during the EEG recordings within the study period. The other patients presented focal or diffuse interictal epileptiform abnormalities during the second and third year, but the frequency decreased later. Even in this control group we recorded seizures in two cases: myoclonic seizures in case 6 and a prolonged generalized seizure in case 12.
No patients presented a photoparoxysmal response.

Fig 11 (A,B,C): At 36th month patient n°8 presented a seizure characterized by poor responsivity, eye deviation, respiratory changes, hypotonia and distal myoclonic jerks. Midazolam (5 mg) was performed after 5 minutes the seizure's onset. The seizure resolved after 20 minutes from the onset. EEG was characterized by pseudorhythmic, diffuse, irregular spike- slow wave (1Hz). After Midazolam pseudorhythmic spike- slow wave gradually slowed down in frequency, and appeared bilateral temporal spikes

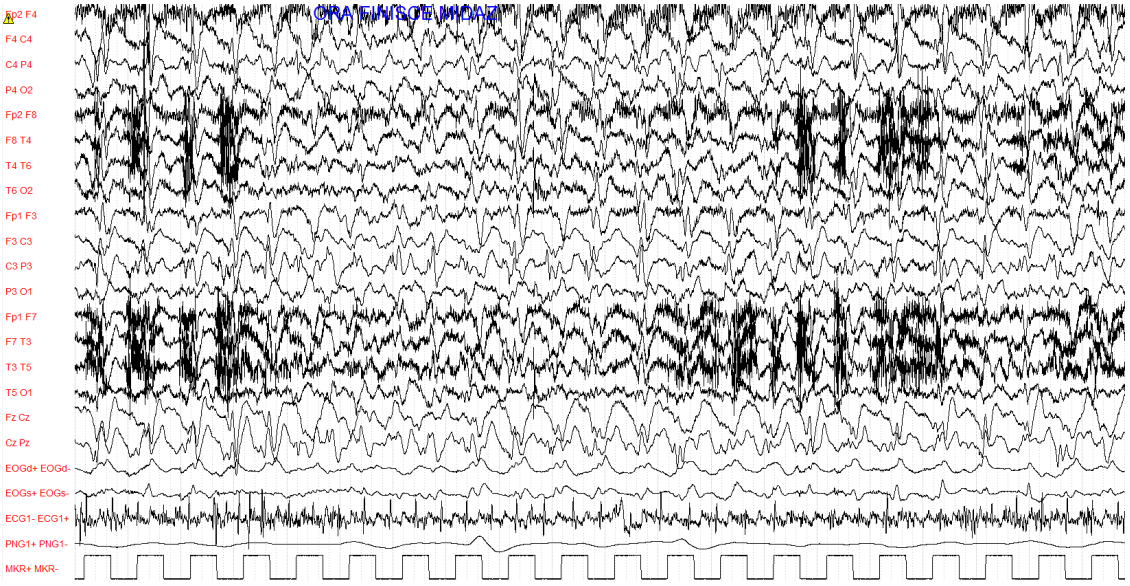
A



B



C



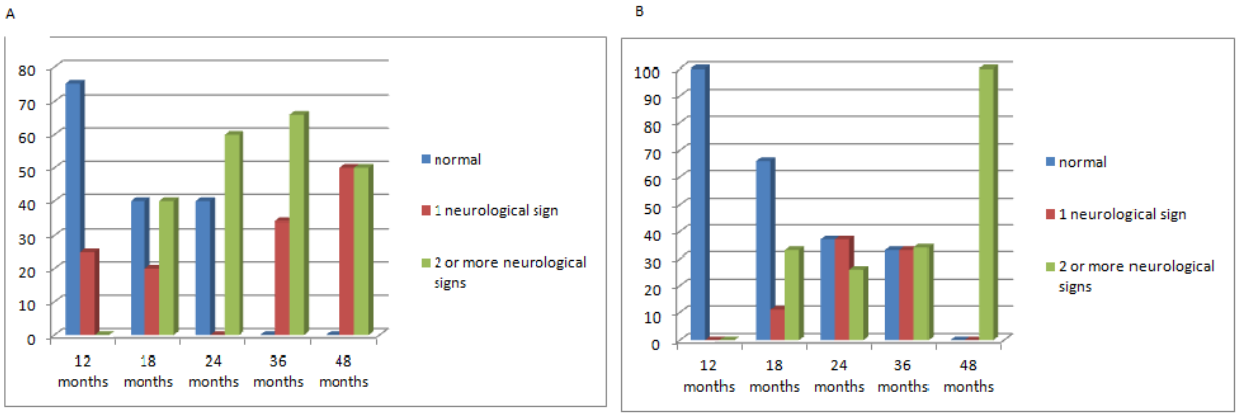
5.2.6 Neurological features (fig 12)

The neurological assessment performed in the Dravet sample, revealed neurological signs starting from the 12th month of age, with a progressive increase in time and with the association of more signs within the same patient. At the 18th and 24th month 2 patients presented a normal neurological signs; ataxia and segmental myoclonus were equally distributed (2 patients out 5, 40%); these signs were isolated or associated with other neurological signs. From the third year, all of the patients presented neurological signs: we found segmental myoclonus in all of the patients and ataxia in 1 patient.

In the control group, the neurological examination showed hypotonia during the 18th month visit in 3 patients, in the following assessment we detected several other neurological signs, often associated: hypotonia, myoclonus, clumsiness, valgus knees and flat feet. In 5 patients we observed hypotonia (cases 6,7,8,10,12), in 4 patients we detected clumsiness (6,7,10,12) and case 2 presented myoclonus.

Cases 2,8 presented dismorphic traits and case 12 presented a posture motor delay (first steps at 19 months)

Fig12 percentage of neurological signs during the follow up: A (Dravet patients), B (control group)



5.2.7 Developmental/cognitive evolution and behavioural disorders (fig 13, 14; tables 9,10,11,12)

At the 18th month all Dravet patients performed a neuropsychological assessment: 40% presented a normal psychomotor development, 40% presented a borderline GQ and 20% showed a mild cognitive delay.

8 patients of the control group performed the same evaluation at the 18th month: 5 patients presented a normal psychomotor development, 2 patients showed a borderline GQ and the remaining case presented a moderate cognitive impairment.

The ensuing follow up revealed a progressive slowing down of the development in all of the Dravet patients: 3 patients exhibited a steep decrease of GQ (cases 1,3,4: mean DGQ 33), the others presented a mild drop (cases 2, 5: mean DGQ 9).

The following assessments of the control group revealed a variable cognitive outcome, at the 36th month 6 patients underwent an evaluation and 3 patients exhibited a normal psychomotor development, 1 patient presented a borderline GQ, 1 patient showed a mild cognitive delay and the remaining presented a moderate cognitive delay. The Differential General Quotient of each patient confirmed the great variability of the cognitive outcome within the sample.

Figures 12, 13 show the cognitive profiles assessed at the 12th month in 3 Dravet patients and at the 18th month in the whole sample. A greater impairment of the language, compared to the other fields examined, was observed during the 12th and 18th month. Only in 1 patient the items “eye and hand coordination” and “performance” were the most impaired abilities.

Tables 12, 13 describe the presence of behavioural problems in both samples:

In the Dravet sample one patient (case 5) did not presented behavioural problems. 4 patients out of 5 presented attention deficit and hyperactivity. One patients presented autistic features.

In the control group, one patient (case 13) did not manifested behavioural problems; attention deficit was present in 7 patient out of 9 and hyperactivity was described in 3 patients. Only patient presented autistic features. 2 patients of the control group showed other behavioural disorders (gratification disorders, withdrawn behaviour).

Fig 13 Cognitive profiles at 12 months in 3 Dravet patients

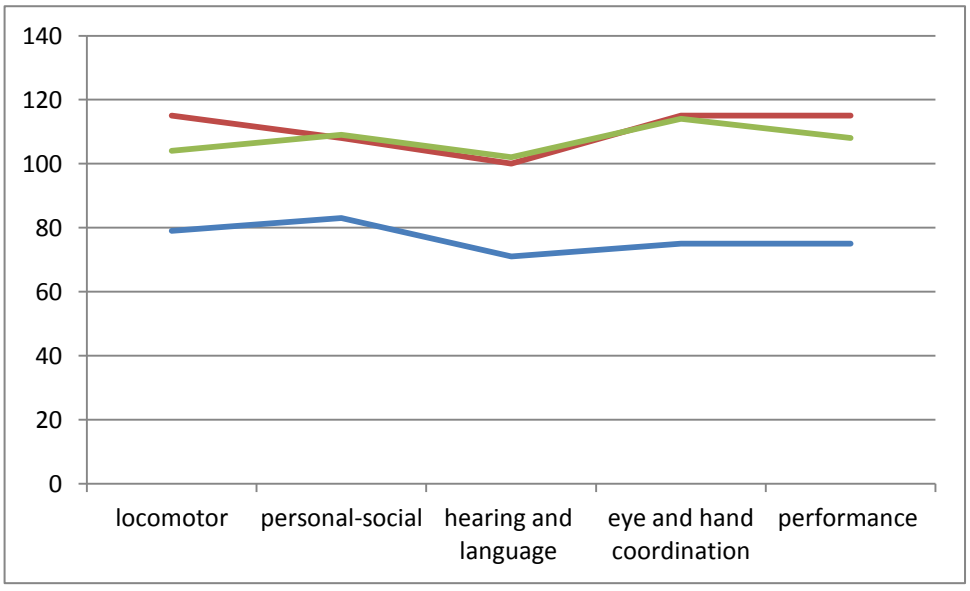
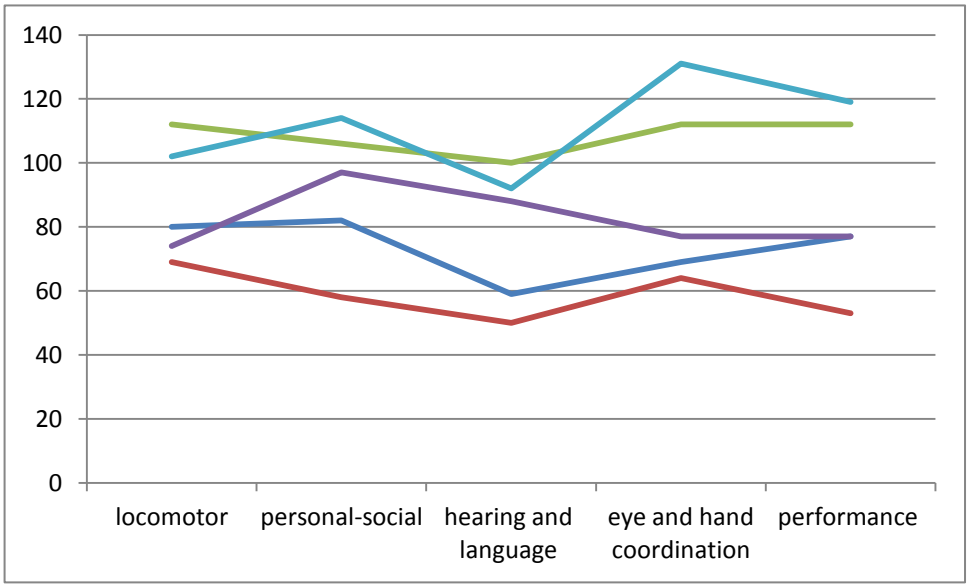


Fig 14 Cognitive profiles at 18 months in 5 Dravet patients



5.2.8 Some clinical history of control group patients (Table 13)

The clinical and electrophysiological features of some patient of the control group, suggestive for diagnostic hypothesis are reported below.

Case n° 6 experienced at the 9th month of age the seizures onset (myoclonic seizures) and successfully presented clusters of focal febrile and afebrile tonic clonic seizures, associated to myoclonic seizures. She had a positive family history for febrile convulsion (mother, maternal aunt and brother). She presented a normal psychomotor development before the seizures, with a progressive cognitive impairment during the follow up. Neuroimaging was normal. EEG presented a normal background organization in awake and asleep during the follow up. The age of the seizures onset, the sensitivity of the fever in an otherwise child leaded the clinicians to perform targeted genetic analyses related to SCN1A spectrum and protocadherin 19 epilepsy. We found a mutation missense mutation in PCDH19 gene, inherited from mother.

Case n°7 presented a febrile convulsion at the seizures onset, followed from febrile and afebrile sporadic tonic clonic seizures during the follow up. The patient presented a borderline General Quotient at the first assessment, with a progressive impairment during the following years and she develop verbal dyspraxia. She presented neurological signs characterized by hypotonia and clumsiness associated to dysmorphic traits. The EEG recording during the controls detected a diffuse fast activity during wakefulness and a poor organization during the sleep. The clinical hypothesis lead the clinical to perform genetic analysis (karyotype and array-CGH) and a deletion of chromosome 7q31 (del 7q31.1-q31.31) was detected.

Case n°13 experienced the first afebrile focal seizure at the 5th month of age and successfully presented clusters of focal and generalized tonic clonic seizures, with a long period seizure free. She following a monotherapy. Parents reported a positive family history for epilepsy and she presented a normal general

quotient during the first assessment and during the follow up. The clinical hypothesis oriented toward a genetic epilepsy and the analysis performed using TruSeq detected a missense mutation in PRRT2.

Case n°10 presented a clinical picture characterized by focal febrile and afebrile seizures, occurring also in clusters. During the first assessment the psychomotor development was borderline (GQ 85) and the EEG showed a pathological slow background activity during wakefulness and sleep. Interictal epileptiform abnormalities did not present. The clinical and electrophysiological features lead the clinical to perform genetic analysis (kariotype and Array CGH) that did revealed any mutation and/or deletion. The patient was successfully analysed using Truseq and Nextera and a mutation in DEPD5 gene was found.

3 patient (cases n° 9, 11,14) presented a suggestive clinical features for GEFS+ spectrum: 2 of them experienced febrile seizures plus, in the remaining case associated to early onset of absences (within the first year of life). Positive family history was described in 2 patients: in case 11, the father presented generalized epilepsy and the brother experienced febrile convulsion, in case 14 a maternal uncle experienced generalized idiopathic epilepsy and a maternal aunt manifested febrile convulsion during childhood. Epileptic seizures were reported also in grandfather's sister. Case 9 and 11 presented a normal psychomotor development at the first assessment and during the follow up, case 14 presented a borderline GQ already at the first visit. Neuroimaging was normal in case 9 and detected aspecific findings in case 11 and 14. EEG presented a normal background activity in awake and sleep in all 3 cases, case 11 presented diffuse interictal discharge during one monitoring.
The clinical and electrophysiological features lead the clinicians to perform genetic analyses. In particular these patients were analysed by TruSeq and Nextera, including genes involving in Generalized Epilepsy with Febrile Convulsions plus. In case 9 we detected a missense mutation in SCN1A gene, inherited from asymthomatic father. Case 11 presented a missense mutation in HCN4 gene, inherited from symthomatic father. Case 9 presented a novel missense mutation in HCN2 gene.

6. Discussion

The aim of this prospective study was to analyse data concerning the cohort of Dravet patients followed in the Paediatric Neuroscience Department of the Neurological Institute C. Besta of Milan and in other Italian centres involved with DESIRE. The early age of enrollment of the patients allowed us to investigate the psychomotor development before the complete manifestation of the disease and to reveal the onset of a developmental stagnation. However, only 8 out of 17 (47%) were examined in one of the 4 centres when they were about 12 months old, being that they were assessed for the first time in other facilities, closer to their homes and having a paediatric emergency room.

The analysis of the data collected, allowed us to highlight the following remarks:

- the onset of febrile and/or prolonged seizures within the 6th month in a previously healthy child represents one of the typical features of Dravet Syndrome: in our series the first seizure occurred within the sixth month in 88% of the infants (15 out of 17 patients). This observation confirms previous literature data (Wirrell et al, 2017; Gataullina &Dulac, 2017; Ragona et al, 2010).
- most of patients of the sample (75%), presented one or more convulsive status and/or prolonged seizures within the 12th month of age, according the previous literature data (Nabbout et al, 2013)
- interictal myoclonus represents a common neurological sign: in our sample this already appeared at the 12th month. This neurological sign, often unacknowledged, must be carefully sought out during neurological examinations. The presence interictal myoclonus has been recently reported by Canafoglia and colleagues (Canafoglia et al, 2017): they described the presence of action myoclonus in all of the sample, but it was not recorded in such an early age (19 patients, mean age 8.5 years, range 2.5–29 years).

The association of these early findings, in an otherwise healthy patient, especially when associated with a normal EEG, can be considered a useful tool to recognize early the disease.

The presence of drug-resistant and polymorphic seizures during the first years of life represents a further peculiarity: in our sample, during the first 18 months of life, half of the patients experienced several kinds of seizures. Generalized tonic clonic seizures were the most frequent, according to literature data (Wirrell et al, 2017), followed by hemiclonic and focal seizures. In our sample, myoclonic seizures already appeared during the first year of life in a minority of cases, but their frequency increased over time (41% of the patients at 18 months of life). 7 patients of the sample were classified as typical SMEI, 10 patients in which the myoclonic component was absent, were classified as borderline form, or SMEIB

We performed neuropsychological assessments, from the first months of the disease, when psychomotor development was still normal, in order to follow its evolution. The neuropsychological assessments performed at the 12th month, revealed a normal psychomotor development in all of the patients, whereas a progressive slowing appeared between the 12th and the 18th month in most of the patients. This period

coincided with the emergence of behavioural disorders, characterized by attention deficit, hyperactivity and in some cases symptoms of the autistic spectrum. These behavioural problems were often associated and hampered child's development.

The Differential General Quotient varied widely in this series showing an high variability of the cognitive evolution: this "spectrum" may also depend on the different time intervals between the first and last follow up for each patient. This bias is linked both to the prospective nature of the study and to a non-feasible evaluation related to the behavioural problems presented by some children.

The correlation analysis between genetic determinants and cognitive evolution did not provide any evidence for a correlation between the type of mutation and the cognitive outcome. Patients with the best cognitive outcome carried both missense and truncating mutations. Conversely, we reported the case of patient n°1, who presented a truncanting mutation in SCN1A and a pathogenic missense mutation in SCN2A. The clinical picture suggested that the patient could be included within the Dravet spectrum, despite showing evidence of a psychomotor delay before seizure onset (head control at 6 months, first steps at 19 months). At the last evaluation she presented the lowest developmental quotient in the series, associated with autistic features. According to recent literature data, mutations in SCN2A have been associated with a spectrum of epilepsies, from benign (familial) neonatal/infantile seizures to early infantile epileptic encephalopathy (EIEE) phenotypes (Wolff et al, 2017), the SCN2A mutation may be seen as a modifier gene, explaining the severity of the clinical picture. These data confirmed what was reported in literature (Gataullina and Dulac, 2017). Therefore, in selected patients with more severe phenotype, it would be useful to perform more extensive genetic analysis, such as TruSeq Custom Amplicon or Nextera rapid capture, in order to verify the presence of a "second mutation"

The correlation between the epilepsy course and the cognitive outcome highlighted that an early onset of seizures (within the 6th month) represents a negative prognostic factor for cognitive evolution.
These data confirm what has recently been reported by Cetica and colleagues. They described the early onset of the first seizure as a predictor factor of DS. In their sample, the risk of Dravet syndrome was as high as 85% in the first group (onset seizures within the 6th month), while the likelyhood of the disease dropped significantly as epilepsy age onset increased (51% in the 6-to 12-month range, and 0% after the 12th month).
Our analysis detected a positive correlation between the occurrence of an epileptic status and the DGQ.
In fact in the first group (dGQ>20) all of the patients presented at least one epileptic status, in the remaining patients (dGQ< 20) only 23% presented at least a convulsive status. 5 out of 8 patients that never experienced a status epilepticus, presented a normal development profile during the last assessment.
Moreover, we verified the correlation between the presence of myoclonic seizures and the cognitive outcome. As described in literature (Ragona et al, 2011; Nabbout et al 2013) myoclonic seizures were a negative prognostic factor.
It's conceivable that epileptic phenotype may play a role in determining the final cognitive outcome, but, it does not seem that a more early and targeted treatment can really modify the natural history of the disease. So other genetic and environmental factors need to be considered, in order to explain this extreme variability. In this study we did not systematically review data on rehabilitation therapy, but, with this on mind,

it would be interesting to gather data, in further studies, about the frequency and the type of the rehabilitation therapy followed by Dravet patients.

In the second part of the study we performed a prospective evaluation of a sample of Dravet patients with a control group, which were examined in the Paediatric Neuroscience Department of the Neurological Institute C. Besta, followed from seizure onset in order to compare clinical and electrophysiological features during the first years of life and to define etiologic determinants in the control group, aiming at reaching an early diagnosis and giving a targeted treatment. The chance to compare these two groups of patients allowed us to detect some particular features, so that we could also perform and limit a differential diagnosis. These observations confirm what we stated above:

- The early onset of the seizures may lead the clinical diagnosis towards Dravet Syndrome, in our control group, only 2 patients out of 9 experienced their first seizure within the 6th month of age. The remaining patients manifested the seizures onset after the 9th month of age.
- The occurrence of the seizures and the status epilepticus during the first years of life can be observed in Dravet Syndrome and in other forms of genetic epilepsy, but the persistence of polymorphic, drugs-resistant seizures is more common in Dravet Syndrome. In this study, 9 patients of the control group experienced monomorphic, drug responsive and sporadic seizures.
- A steep decrease in the General Quotient is usually observed in Dravet Syndrome, whereas patients with other forms of genetic epilepsy show a milder decrease despite often presenting a lower GQ from the beginning.
- Patients with other forms of epilepsy usually present several and peculiar neurological signs and can often be associated to dysmorphic traits and posture-motor delay.

The analysis of clinical and electrophysiological data concerning the patients of the control group, allowed clinicians to follow a diagnostic method, using a flow chart of the epilepsy with an onset within the first year of life. The genetic analyses, performed following sequential steps, allowed us to obtain a diagnostic rate in 77% of the sample (7 patients out of 9). In some cases non-SCN1A genes could present overlapping features with Dravet Syndrome, especially during the first clinical presentation, which could delay the diagnosis. In our sample, case n° 6 presented a PCDH19 related epilepsy.
This syndrome has been associated to Dravet Syndrome for a long time, but it is a different condition. In a recent study Trivisano and colleagues systematically compared PCDH19-related epilepsy and Dravet Syndrome in order to find differences between these two epileptic syndromes (Trivisano et al, 2016). Several differences have been noted, including a later seizure onset compared to Dravet Syndrome: a seizure onset after the 10th month of age or even after the first year of life is significantly suggestive for PCDH19-related epilepsy, while an overlapping window till the 10th month should be considered for both conditions. Early age at onset, within the 6th month, is typically seen in Dravet Syndrome. The increased frequency of seizure clusters is typical of PCDH 19 related epilepsy, whereas in Dravet Syndrome longer seizures are often present. Seizure semiology is one of the most relevant features that should be considered in the differential diagnosis: clonic and hemi-clonic seizures have been exclusively reported in Dravet Syndrome. Other types

of seizures were found in both epilepsies with a prevalence of generalized tonic clonic seizures and atypical absences in Dravet Syndrome and focal motor and hypomotor seizures in PCDH19-related epilepsy. Seizures with affective symptoms have been confirmed to be typical of PCDH19-related epilepsy. In both condition epileptiform interictal EEG features are poor. Various degrees of developmental impairment has been reported in both condition, but a slower cognitive deterioration has been reported in PCDH19-related epilepsy.

In patient n° 13 a mutation in PRRT2 gene was detected. The patient presented a clinical picture seen in for benign familial infantile seizures (BFIS). This data confirmed what is expressed in the literature. PRRT2, encoding the proline-rich transmembrane protein 2 gene was discovered as the main gene associated to paroxysmal kinesigenic dyskinesia (PKD) and infantile convulsions with choreoathetosis (PKD with infantile seizures). Recently mutations in PRRT2 gene have been described in 14 pure BFIS Australian and Asian families (Heron et al., 2012). In a multicentre study, Schubert and colleagues, identified a mutation in the PRRT2 gene in the main of families studied. The authors stated that PRRT2 is the main gene for BFIS (Schubert et al, 2012).

In other patients the clinical features lead the clinical diagnostic hypothesis towards Genetic epilepsy with febrile seizures plus (GEFS+). In these cases we chose to perform the SCN1A sequencing as part of a wider panel, including also other genes related to GEFS+.

In patient n°9 we found a de novo mutation in the SCN1A gene. According to literature, our data confirmed that there are sporadic SCN1A pathogenetic variants in the GEFS+ spectrum. This goes beyond the original concept that GEF+ is a familiar epilepsy syndrome and suggests that a family history is not essential to diagnose a GEFS+ (Myers et al, 2017).

In patient n°11 we detected a mutation in the HCN4 gene and in patient n° 14 we found a mutation in HCN2. The hyperpolarization-actived cyclic nucleotide-gated (HCN) channels include 4 human hisoforms (HCN1-HCN4), presented in the heart and in the central and peripheral nervous system. These channels are contribute to neuronal activity in several ways, including cellular excitability and transmission of synaptic potential. In 2010 Dibbens and colleagues reported that in a sample of children with febrile convulsion and GEFS+, the presence of a specific HCN variant (triple proline deletion)was significantly higher than the general population determining an alteration of current generation. The authors speculated that the alteration of HCN2 could contribute to epilepsy. An other study speculated about the possible role of HCN2 in epilepsy. Zhang and colleagues analysed the phenotypic spectrum in 409 affected individuals in60 families (31 new families) and they compared phenotypic and genetic data to those published in literature in the last 19 years. They detected new phenotypes within the GEFS+ spectrum: focal seizures without preceding febrile seizures (16/409 [4%]), classic genetic generalized epilepsies (22/409 [5%]), and afebrile generalized tonic-clonic seizures (9/409 [2%]). The authors reported that febrile seizures remain the most frequent phenotype in GEFS+ (178/409[44%]),followed by febrile seizures plus(111/409, 27%). At least one individual from all 31 families was screened for 6 GEFS+ genes: SCN1A, SCN2A, SCN1B, SCN9A, GABRG2, and GABRD. Authors reported that the HCN2 gene was analysed in 11 patients. (Zhang et al, 2017). So they thorough about the chance of redefining the spectrum of GEFS plus.

7. Conclusions

The aim of this study was to describe the first, preliminary data, of a prospective, multicentre study in a sample of Dravet patients, carrying a mutation in the SCN1A gene. Nowadays, Dravet Syndrome is considered a genetic epileptic disorder and a model of channellopathy. 40 years have passed since the study of Catterall about the main functions of the sodium channel, which represents Dravet Syndrome's molecular target (Catteral et al, 2017). The genetic murin models showed that the multifaceted pathophysiology and the co-morbidities of Dravet Syndrome arise from a selective loss of electrical excitability and action potential firing in the GABAergic inhibitory neurons, however many aspects still remain unknown, such as the remarkable phenotypic variability within the same family and not much is known about the reasons why the mutation in the SCN1A gene can be so detrimental. It is very likely that other modifying factors, genetic or environmental, may play an important role in the pathogenisis and in the prognosis of the disease. Among these the epileptic features must be considered: according to our data the occurrence of the convulsive status and the presence of the myoclonic seizures play a negative prognostic role at least on the cognitive outcome. The role of antiepileptic therapy should be considered as well. According to recent literature data (Wirrell et al, 2017), the early onset of a targeted therapy does not provide any evidence of benefits on the cognitive outcome, but an earlier diagnosis improves long term outcome for the patients, improving the seizures-control.
Finally the role of the rehabilitation therapy should be considered. An early diagnosis allows an early targeted therapeutic intervention, aimed at enhancing residual resources and reinforcing the most compromised areas, in order to support and improve the quality of life of patients and their families.

The second part of the study and in particular, the analysis of the control group patients, allowed us to make some considerations on the use of targeted sequencing, which including several genes, in clinical practice:

- Collecting of clinical history, paying particular attention to the seizures onset and to the presence of a positive family history, allows to perform a very targeted genetic investigation.
- A team of experts including clinicians and geneticists, should, jointly, discuss the pathogenicity of each variant, being particularly cautios about accepting any identified variant as causative.
- Literature data shows a growing need to broaden and redefine "the disease spectrum". This may also depend on the different phenotypes linked to a given pathogenetic mutation and their discovery has been made easier thanks to the use of large-scale genetic tools.

In the control group sample one patient presented a overlapping condition with Dravet Syndrome and an undiagnosed patient presented a clinical picture characterized by drug- resistant seizures, recurrent convulsive status, psychomotor impairment and behavioural disorders. However, none of them showed signs as dramatic as the ones seen in Dravet Syndrome.

8. Supplementary

Table 2:Characteristics of the Dravet patients in the first year of life

FC: febrile convulsion E: epilepsy GTCS: generalized tonic clonic seizures H: hemyclonic F: focal SE: status epilepticus, NK: not known or Not clear the effect of the mutation

Case	Gender	Family hystory for febrile convulsions or seizures	Age at seizure's onset (months)	Semiology of the first seizure	Fever	Number of seizures in the first year	MRI	Genetic analysis: Mutation SCN1A
1	F	NO	4	GTCS	NO	NK	normal	Truncanting+SCN2A
2	F	YES, FC	5	GTCS	YES	10	normal	Truncanting
3	M	NO	4	SE	NO	6	normal	missense
4	M	YES, E	6	SE	NO	NK	pathological	Truncanting
5	F	YES,E	6	GTCS	NK	11	normal	Truncanting
6	M	YES, E	4	SE	YES	4	normal	NK
7	F	YES, FC,E	2	H	YES	31	normal	Truncanting
8	F	NO	5	SE	YES	NK	normal	Truncanting
9	M	NO	8	GTCS	NO	5	normal	NK
10	F	NO	4	H	YES	10	normal	missense
11	M	YES, FC	4	GTCS	NO	15	normal	missense
12	F	NO	8	F	NO	2	normal	missense
13	M	YES, FC	4	H	NO	18	normal	Truncanting
14	M	YES, E	3	GTCS	NO	4	NK	NK
15	M	NO	3	GTCS	NO	NK	normal	missense
16	M	YES, FC	2	GTCS	NO	5	NK	Missense
17	F	YES, FC	4	GTCS	YES	NK	normal	missense

Table 3:epilepsy features of the Dravet patients during the follow up

GTCS: generalized tonic clonic seizures H: hemyclonic F: focal M myoclonic A atypical absences SE: status epilepticus

cases	visit <12 months														
	semeiology of seizures					frequency of myoclonic and absences				frequency of other seizures				SE	
	GTCS	F	H	M	A	sporadic	monthly	weekly	daily	sporadic	monthly	weekly	daily	YES	NO
2		x								x					X
6	X										X				X
7	X		X	X	X				X	X					X
8	X									X				X	
12		X			X	X				X					X
15	X										X				X
16	X										X				X
17	X										X				X

cases	visit 12 months														
	semeiology of seizures					frequency of myoclonic and absences				frequency of other seizures				SE	
	GTCS	F	H	M	A	sporadic	monthly	weekly	daily	sporadic	monthly	weekly	daily	YES	NO
1	x	x	x		x	x						x		x	
2		x	x								x			x	
3			x	x		x					x			x	
5	x		x								x			x	
6	x	x									x				x
7	x	x		x	x	x						x		x	
8	x									x					x
9	x	x									x				x
10	x										x				x
12					x					x					x
13		x	x								x				x
15	x										x				x
16		x								x					x
17	x										x			x	

cases	visit 18 months														
	semeiology of seizures					frequency of myoclonic and absences				frequency of other seizures				SE	
	GTCS	F	H	M	A	sporadic	monthly	weekly	daily	sporadic	monthly	weekly	daily	YES	NO
1	x	x	x	x	x	x							x	x	
2	x										x			x	
3															x
4	x	x	x	x	x			x			x			x	
5	x										x				x
6	x	x									x				x
7	x	x			x				x		x				x
8	x										x				x
9	x	x									x				x
10	x		x		x	x					x				x
11				x		x									x
12		x									x				x
13	x	x	x									x			x
14	x										x				x
15	x										x				x
16	x										x				x
17	x									x					x

Table 3 (continued): epilepsy features of the Dravet patients during the follow up

GTCS: generalized tonic clonic seizures H: hemyclonic F: focal M myoclonic A atypical absences SE: status epilepticus

cases	visit 24 months														
	semeiology of seizures					frequency of myoclonic and absences				frequency of other seizures				SE	
	GTCS	F	H	M	A	sporadic	monthly	weekly	daily	sporadic	monthly	weekly	daily	YES	NO
1	x	x									x				x
2	x	x	x	x	x			x				x		x	
3	x		x								x			x	
4	x		x								x			x	
5	x										x				x
6	x										x				x
7	x	x		x	x	x						x			x
8	x										x				x
9	x	x									x				x
10	x									x					x
11	x			x		x					x			x	
12	x	x										x			x
13	x	x	x									x			x
14	x									x					x
15	x										x			x	x
16	x		x								x				x

cases	visit 36 months														
	semeiology of seizures					frequency of myoclonic and absences				frequency of other seizures				SE	
	GTCS	F	H	M	A	sporadic	monthly	weekly	daily	sporadic	monthly	weekly	daily	YES	NO
1	x	x										x		x	
2	x	x	x	x					x			x		x	
3	x	x	x									x			x
7	x	x			x				x			x		x	
10	x										x				x
11				x		x									x
13		x	x								x				x
15	x										x				x

cases	visit 48 months														
	semeiology of seizures					frequency of myoclonic and absences				frequency of other seizures				SE	
	GTCS	F	H	M	A	sporadic	monthly	weekly	daily	sporadic	monthly	weekly	daily	YES	NO
1	x				x	x				x					x
2	x	x		x				x			x			x	
10	x			x	x	x					x				x
11	x			x		x					x				x
13		x	x		x				x			x			x

Table 4: pharmacological treatment during the follow up

Case	DGQ	AEDs <12 months	AEDs 12 months	AEDs 18 months	AEDs 24 months	AEDs 36 months	AEDs 48 months
1	33		lvt	vpa, lvt	vpa, lvt	vpa, tpm, lvt	vpa, lvt, tpm
2	7	vpa	vpa, clb	vpa, clb, stp	vpa, clb, stp	vpa, cbd	cbd
3	38		vpa	vpa	vpa, clb	vpa, clb,tpm	
4	29			vpa, clb	vpa, clb, stp		
5	12		vpa, lev	vpa, lvt	vpa, lvt		
6	9	no therapy	vpa	vpa, tpm	vpa, tpm		
7	48	vpa, clb	vpa, clb,stp	vpa, clb,stp	vpa, clb, stp	vpa, lvt, czp	
8	-3	no therapy	no	vpa	vpa		
9	-1		vpa	vpa	vpa		
10	0		vpa,etm	vpa,etm	vpa, etm	vpa, clb, stp	vpa, clb,stp
11	18			vpa, clb,stp	vpa, clb,stp	vpa, clb,stp	vpa, clb, stp
12	8	vpa	vpa, clb	vpa, clb	vpa, clb		
13	4		vpa	vpa	vpa	vpa, lvt,czp	vpa, clb
14	9			vpa,lev,clb	vpa,lev, clb		
15	72	vpa	vpa, clb	vpa, clb	vpa, clb	vpa, clb,stp	
16	2	vpa	vpa	vpa	vpa, clb		
17	20	lvt,cbz	vpa, clb	vpa, clb			

Vpa: valproic acid CLB: clobazam STP: stiripentol, TPM: topyramate LEV: levetiracetam CZP: clonazepam ETM: Ethosuccimide CBD: Bedrolite

Table 5: General Quotient (GQ) and Different General Quotient (DGQ) in 3 group of Dravet patients

I group

Case	GQ at 6 months	GQ at 12 months	GQ at 18 months	GQ at 24 months	GQ at 36 months	GQ at 48 months	DGQ
1			59	59	45	26	33
3		110	108	87	72		38
4			83	54			29
7		82	NK	NK	47	34	48
15	109	NK	88	52	37		72
17	100	NK	80				20

II group

Case	GQ at 6 months	GQ at 12 months	GQ at 18 months	GQ at 24 months	GQ at 36 months	GQ at 48 months	DGQ
2		77	73	63	67	70	7
5		107	110	95			12
6	110	127	113	101			9
11			74	NK	56	NK	18
12		NK	108	100			8
13		63	65	NK	59		4
14			75	66			9
16	NK	104	NK	102			2

III group

Case	GQ at 6 months	GQ at 12 months	GQ at 18 months	GQ at 24 months	GQ at 36 months	GQ at 48 months	DGQ
8	88	NK	NK	91			-3
9		110	108	111			-1
10		NK	NK	72	NK	72	0

Table 6: Correlation between epileptic variables and cognitive outcome

	DGQ>20	DGQ<20
onset seizures<4months	83%	54%
At least one status epilepticus	100%	18%
myoclonic seizures	66%	27%

Table 7: EEG in Dravet patients during the follow up

EEG findings and Presence of Photoparoxysmal Responce (PPR) in Dravet group patients												
age	number of patients	backgroung activity awake (%)		backgroung activity sleep (%)		epileptiform abnormalities (%of patients)			seizures recording	PPR (%pts)		
		normal	pathological	normal	pathological	not present	focal	multifocal/diffused		negative	positive	unknown
6 months	1	100%		100%		100%			not present	100%		
12 months	2	100%		100%		100%			not present	100%		
18 months	5	60%	40%	60%	40%	80%	20%	22%	not present	100%		
24 months	5	40%	20%	60%	40%	40%	20%	40%	GTCS (1pt)	100%		
36 months	3		100%	20%	80%	66%	34%	20%	myoclonic (1 pt)	100%		
48 months	2		100%		100%	50%		50%	not present	100%		

table 8: EEG in patients of the control group during the follow up

PS prolonged seizures

EEG findings and Presence of Photoparoxysmal Responce (PPR) in control group patients												
age	number of patients	backgroung activity awake (%)		backgroung activity sleep (%)		epileptiform abnormalities (%)			seizures recording	PPR (%pts)		
		normal	pathological	normal	pathological	not present	focal	multifocal/diffused		negative	positive	unknown
6 months	0											
12 months	3	66%	34%	44%	66%	100%			not present	100%		
18 months	9	100%		77%	23%	56%	22%	22%	myoclonic(1 pt)	1		
24 months	8	71%	19%	25%	75%	62%	25%	13%	not present	1		
36 months	5	80%	20%	60%	40%	60%	20%	20%	PS (1 pt)	100%		
48 months	2	100%		50%	50%	100%			not present	100%		
60 months	1	100%		100%		100%			not present	100%		

Table 9: Cognitive profiles and Different General Quotient in Dravet sample

Dravet case	GQ at 6 months	GQ at 12 months	GQ at 18 months	GQ at 24 months	GQ at 36 months	GQ at 48 months	Differential GQ
1			59	59	45	26	33
2		77	73	63	67	70	7
3		110	108	87	72		38
4			83	54			29
5		107	110	95			12

Table 10: Cognitive profiles and Different General Quotient in control group

Control case	GQ at 6 months	GQ at 12 months	GQ at 18 months	GQ at 24 months	GQ at 36 months	GQ at 48 months	GQ at 60 months	Differential GQ
6			98	91	69	93	78	20
7			83	68	61	60		23
8			108	94	86			22
9		109	94	89				20
10		85	71	75				10
11			101	99	97			4
12			37		36			1
13	100		110					-10
14				80	87			-7

Table 11: Psychomotor development and behavioural disorders in Dravet patients

Psychomotor development and behavioural disorders in Dravet patients											
Age	Griffiths' Scales								Clinical observation		
	locomotor	personal-social	hearing and language	eye and hand coordination	performance	practical reasoning	general quotient	normal	attention deficit	hyperactivity	autism spectrum disorders
Case 1											
6 months											
12 months											
18 months	69	58	50	64	53		59		x		
24 months	59	55	50	67	65		59		x	x	
36 months	47	45	34	43	53		45				x
48 months	46	27	23	28	17		26				x
Case 2											
6 months	60	80	80	70	70		73	x			
12 months	79	83	71	75	75		77	x			
18 months	80	82	59	69	77		73		x		
24 months	74	61	59	61	61		63		x	x	
36 months	62	76	54	76	70		67	x			
48 months	75	71	70	64	63	68	70		x	x	
Case 3											
6 months											
12 months	115	108	100	115	115		110	x			
18 months	112	106	100	112	112		108	x			
24 months	86	97	71	84	95		87		x		
36 months	65	66	46	65	77		64		x	x	
48 months											
Case 4											
6 months											
12 months											
18 months	74	97	88	77	77		83	x			
24 months	52	69	42	58	54		54		x	x	
36 months											
48 months											
Case 5											
6 months											
12 months	104	109	102	114	108		107	x			
18 months	102	114	92	131	119		110	x			
24 months	89	102	92	96	96		95	x			
36 months											
48 months											

Table 12: Psychomotor development and behavioural disorders in control group

Psychomotor development and behavioural disorders in control group patients															
Age	Griffiths' Scales							WPPSI				Clinical observation			
	locomotor	personal-social	hearing and language	eye and hand coordination	performance	practical reasoning	general quotient	TIQ	VIQ	PIQ	normal	attention deficit	hyperactivity	autism spectrum disorders	other
Case 6															
6 months															
12 months															
18 months	100	98	84	107	102		98				x				
24 months	85	96	77	100	96		91				x				
36 months	54	83	58	85	74	72	69				x				
48 months								93	92	97	x				
60 months								86	94	87		x			
Case 7															
6 months															
12 months															
18 months	81	89	76	84	84		83				x				
24 months	77	77	61	61	61		68								x
36 months	72	71	46	61	61	65	61								x
48 months	60	59	51	64	78	59	60					x			x
Case 8															
6 months															
12 months															
18 months	125	96	96	136	90		108				x				
24 months	78	100	91	100	100		94								x
36 months	72	93	90	93	87	80	86				x				
48 months															
Case 9															
6 months															
12 months	114	106	112	105	99		109						x		
18 months	100	104	82	99	98		94						x		
24 months	116	99	63	102	94		89					x	x		
36 months															
48 months															
Case 10															
6 months															
12 months	100	86	59	86	95		85					x			
18 months	107	50	50	85	106		71							x	
24 months	83	72	42	85	94		75							x	
36 months															
48 months															

Table 12 (continued) Psychomotor development and behavioural disorders in control group

Age	Griffiths' Scales							WPPSI				Clinical observation			
	locomotor	personal-social	hearing and language	eye and hand coordination	performance	practical reasoning	general quotient	TIQ	VIQ	PIQ	normal	attention deficit	hyperactivity	autism spectrum disorders	other
Case 11															
6 months															
12 months															
18 months	112	115	88	103	102		101				x				
24 months	110	103	101	93	95		99					x	x		
36 months															
48 months															
Case 12															
6 months															
12 months															
18 months	46	41	37	32	25		37					x			x
24 months															
36 months	46	36	33	28	41		36					x			x
48 months															
Case 13															
6 months	104	94	96	102	103		100				x				
12 months															
18 months	107	120	107	113	102		110				x				
24 months															
36 months															
48 months															
Case 14															
6 months															
12 months															
18 months															
24 months	86	87	68	89	75		80				x				
36 months	89	81	97	87	76	89	87					x	x		
48 months															

Table 13: data of control group patients

Case	Gender	Family hystory for FC and/or epilepsy	MRI	Age at seizure's onset (months)	Semiology of the first seizure	Fever	seizures during the follow up	genetic analysis: single gene	genetic analysis: karyotype and Array-CGH	genetic analysis: TruSeq/ Nextera
6	F	YES, FC	normal	9	M	NO	M, F	PCDH 19 mutation	not performed	not performed
7	F	NO	not performed	11	GTCS	YES	GTCS	not performed	del 7q31.1-q31.31	not performed
8	F	YES,E	normal	9	SE	YS	GTCS	not performed	ongoing	not performed
9	F	NO	normal	9	F	NO	GTCS,A	not performed	not performed	SCN1A mutation
10	M	YES,E	normal	9	F	YES	F, GTCS	not performed	negative	DEPD5 mutation
11	M	YES,CF and E	aspecific findings	10	GTCS	YES	GTCS	not performed	not performed	HCN4 mutation
12	M	no	normal	6	SM	NO	F, GTCS, SM	not performed	negative	negative
13	F	YES,E	normal	5	GTCS	NO	F, GTCS	not performed	negative	PRRT2 mutation
14	M	YES, E	aspecific findings	12	GTCS	NO	GTCS	not performed	not performed	HCN2 mutation

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