DRAVET ITALIA Onlus

Associazione Italiana Sindrome di Dravet Onlus

4° HORIZONS FOR DRAVET SYNDROME

INTERNATIONAL SYMPOSIUM "DRAVET SYNDROME AND OTHER SODIUM CHANNEL RELATED ENCEPHALOPATHIES"

15-16 MARCH 2018, VERONA | PALAZZO DELLA GRAN GUARDIA

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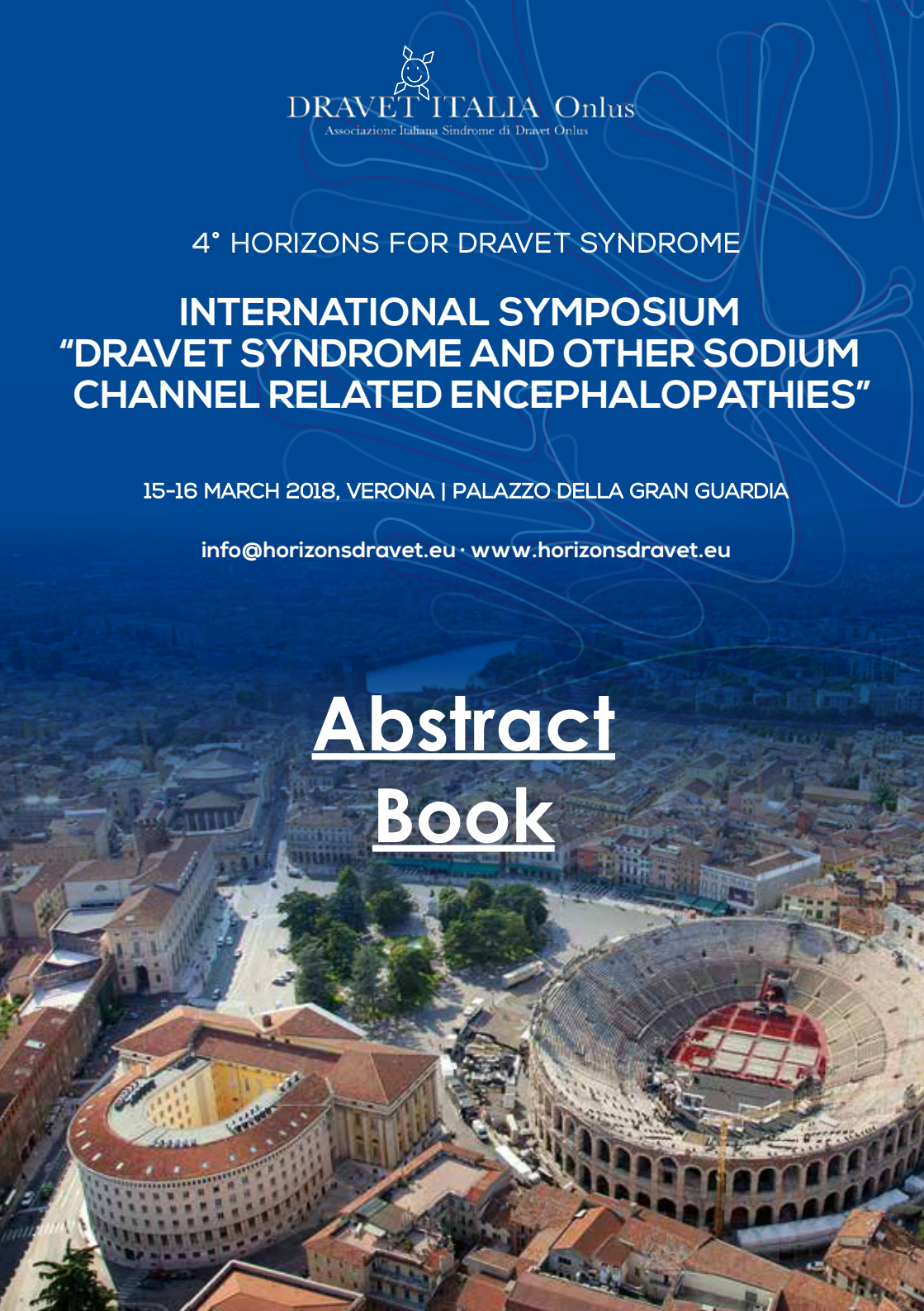
Abstract Book



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Dear friends and colleagues,

On the occasion of 40 years since Dravet Syndrome was first defined, and 8 years after organizing the first Workshop in Verona, we are very pleased to invite you once again to this magnificent city for the **"Dravet Syndrome and Other Sodium Channel Related Encephalopathies" International Symposium**.

The Symposium consists of two days focusing on scientific research relating to genes SCN1A, SCN2A and SCN8A.

The study of epilepsy and the care of children have changed remarkably in recent years, after the identification of the genetic causes of some epilepsy syndromes. The main epilepsy gene- the sodium channel alpha 1 (SCN1A)- has been linked to Dravet Syndrome, to a number of less severe forms of epilepsy, and to febrile convulsions. However, more than 15 years after the causative role of this gene was identified in these forms, and in spite of the large number of patients identified, the spectrum of clinical manifestations associated with SCN1A mutations continues to be enriched by new phenotypes and only recently has enough evidence been collected to foresee to what extent early clinical and genetic predictors seem to influence prognosis. Thanks to the advent of next-generation sequencing, the process that will eventually lead to fully highlight the phenotypical spectrum, long-term outcome, and role of genetic variation in the epilepsies associated with mutations of the other two main sodium channel genes associated with epilepsy- SCN2A and SACN8A- will hopefully be quicker but is until now nonetheless proving relatively slow.

Even slower, and particularly complex, is the process that has led to the gathering of evidence on the sensitivity of these conditions to medication. It has taken more than 16 years since the first controlled trial demonstrated the efficacy of add-on stiripentol in Dravet syndrome, before new trials to test the efficacy of two different molecules, fenfluramine and canabidiol, were launched in this same syndrome, and none seems to be on the horizon for the conditions associated with SCN2A- and SCN8A-related epilepsies. In order to address the main clinical, genetic and treatment issues that concern families, the specialists, and basic researchers alike; to explore to what extent disorders arising from mutations in this gene family overlap and differ; to better define the specific burden of comorbidities; and to explore the bases for rational treatment approaches, we have organized a thematic workshop to gather world-leading specialists in Verona to discuss available evidence and perspectives for future developments.

It will be a pleasure to share these two days of scientific research with you.

On behalf of scientific committee

Bernardo Dalla Bernardina, Renzo Guerrini

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March 15th, 2018

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Poster

From Dravet Syndrome to Sodium Channel Encephalopathies

Renzo Guerrini

In the human genome more than 400 genes encode ion channels, which are transmembrane proteins mediating ion fluxes across membranes. Being expressed in all cell types, ion channels are involved in many physiological processes. Mutations in genes encoding ion channel subunits, or their interacting proteins, cause inherited ion channelopathies whose frequency ranges from relatively common to very rare disorders and whose severity can be mild to severely disabling. Different classes of ion channels play crucial roles in maintaining the balance between excitatory and inhibitory inputs in the brain. Gain or loss of function mutations in their genes are associated to epilepsy and, often, to other neurological manifestations. The best known clinical conditions in the epilepsy domain are associated with mutations in voltage gated sodium channel subtypes, ensuring action potential generation and propagation in the brain (SCN1A, SCN2A, SCN3A, SCN8A and SCN1B). More than 700 mutations in *SCN1A* ($Na_V1.1$) have been associated with genetic epilepsy syndromes ranging in severity from simple febrile seizures to Generalized Epilepsy with Febrile Seizures (GEFS+), an autosomal dominant epilepsy disorder associated to missense mutations, to Dravet syndrome, the most severe form. Dominant mutations in *SCN2A* ($Na_V1.2$) channels also cause a wide spectrum of epilepsy phenotypes, ranging from Benign Familial Neonatal-Infantile Seizures, to more severe phenotypes including developmental delay and intractable seizures. Other epilepsy phenotypes have been associated with mutations in *SCN3A* (Na_V 1.3), *SCN8A* (Na_V 1.6), and in *SCN1B* ($Na_V1.1$ b subunits). For most of these sodium channelopathies the therapy is mainly empirical and symptomatic, often limited by lack of efficacy and tolerability for a significant number of patients. Developing new and more specific therapeutic approaches is therefore required.

Sodium Channel and Human Disease

Jeffrey Noebels MD, PhD - Baylor College of Medicine

A steady drumroll of genetic discoveries linking mutations in voltage-gated sodium ion channels to human neurological disease highlights the urgent need for a deeper understanding of how each leads to epilepsy, and how, where, and when the altered mutant sodium channel function can be therapeutically reversed. There is also increasing need to learn why certain variants in the same gene lead to a more complex syndrome with a far broader spectrum of comorbidities, including cognitive delay, neuropsychiatric, and central autonomic dysfunction. The significance of each variant, which may spell the difference between lifelong disability or sudden death, requires accurate functional interpretation in order to stimulate drug discovery and guide the use of mutation-specific therapies.

For nearly two decades, Dravet Syndrome research has played a critical role in launching the era of precision medicine in epilepsy, bringing molecular diagnosis to many affected children. However the results of clinical exomes point to recurring mysteries that are no longer possible to ignore and may provide the key to monogenic comorbidity. For example, seemingly slight changes in current gating kinetics that lead to devastating disease, implicating the involvement of unknown non-pore functions; the selective impairment of some neurons and not others; and alterations in brainstem network excitability linked to premature mortality. And finally, the coexistence of mutations in other channels leading to blended syndromes. While these mechanisms are slowly becoming clear, more research is required to uncover, and model their meaning, either singly or in complex combinations.

We are now in the second wave, the era of precision physiology. Here the goal is to explore not only the cellular but the network impact of the mutations and define the cascade of developmental remodeling that occurs in a brain born with a critical defect in sodium channel function. Fortunately, we have an extraordinary new generation of tools for this task, and advanced scientific and clinical collaborative approaches. Focused studies on the functional biology of human sodium channel mutations will be essential to the success of precision treatments for Dravet Syndrome.

SCN1A related phenotypes

Ingrid E Scheffer
University of Melbourne, Austin Health and Royal Children's Hospital, and Florey Institute
Melbourne, Australia

SCN1A, encoding the alpha 1 subunit of the sodium channel, is the most important epilepsy gene. It is implicated in a wide range of epilepsies as both the causative gene and also as contributing to complex inheritance. It is best known as the gene responsible for more than 80% of patients with Dravet syndrome, but has also been implicated for other developmental and epileptic encephalopathies including rare patients with epilepsy with myoclonic-atonic seizures. Recently a recurrent missense mutation has been associated with a profound early onset developmental and epileptic encephalopathy with a prominent movement disorder. SCN1A was first associated with the self-limited epilepsy syndrome of genetic epilepsy with febrile seizures plus, with about 10% of families harbouring an SCN1A mutation. Understanding the pathophysiology of mutations and phenotype-genotype correlation will inform the development of targeted precision medicine approaches.

Beyond the epilepsies in SCN1A diseases

Rima Nabbout

SCN1A gene, first reported in GEFS+ (genetic epilepsies with febrile seizures plus) is the major gene in Dravet syndrome. Although DS was described as a severe myoclonic epilepsy in infancy, it becomes more and more evident that patients with mutations in this gene present many other symptoms partly related to the epilepsy phenotype and outcome might be mostly related to the gene anomaly impact beyond seizures.

Beside seizures, patients with DS present a spectrum of neurological and non-neurological symptoms. Intellectual disability, Autism spectrum disorder, behaviour and sleep disorders are frequently reported. Some characteristic and specific phenotypes are emerging emphasizing the complexity of these diseases and the need for early and longitudinal follow-up and for specific rehabilitation and educational programs.

Non neurological symptoms as cardiac rhythm abnormalities, gait disorders are also frequent. The expression of the SCN1A gene in heart and initial axonal segment can explain these symptoms. Further human studies but also further input from engineered animal models of SCN1A mutations will enable to better understand these symptoms.

Considering DS as developmental disease with epilepsy due to SCN1A dysfunction permits to draw a better care path and to target therapies that have an efficacy that goes beyond seizures to the other symptoms of the disease.

Mechanisms and models of SCN1A mutations

Massimo Mantegazza

Université Cote d'Azur and Institute of Molecular and Cellular Pharmacology (IPMC) - CNRS UMR7275, Valbonne-Sophia Antipolis, France.

Pathological mechanisms of *SCN1A* mutations have been studied with in vitro and in vivo experimental models. Mutations inserted into cDNA constructs and expressed in transfected cultured cells in vitro have allowed the screen of numerous mutations and the identification of their effect on the functions of the $Na_V1.1$ sodium channel. This is the initial dysfunction that in vivo can modify the properties of neuronal networks leading to pathologic phenotypes. Gene targeted mouse models have been generated for some specific mutations and have provided information on pathologic features of different types of neuron, on modifications induced in neuronal networks, and on associated phenotypes. Neurons generated from induced pluripotent stem cells (iPSCs) provide a window for observing functions of human neurons in vitro, but studies of *SCN1A* mutations have generated controversial results. I will present the mechanisms that these experimental models have allowed to disclose, highlighting their pros and cons.

Scn1a epigenome editing by the CRISPR/Cas9 system restores excitability and firing activity in Dravet inhibitory interneurons.

Vania Broccoli
Gaia Colasante, Gabriele Lignani, Raffaele Ricci, Edoardo Fraviga,San Raffaele Scientific Institute, Milan.

Dravet syndrome (DS) is a severe epileptic encephalopathy caused by heterozygous loss-of-function mutations in the SCN1A gene, indicating that a haploinsufficient mechanism underlies the onset of the pathology. Hence, elevating the expression levels of the wild-type SCN1A allele would increase Nav1.1 protein levels possibly ameliorating the pathophysiological defects in this disease. The CRISPR/Cas9 system is a flexible molecular platform which can be adapted for multiple function, including to activate gene expression by linking the defective Cas9 (dCas9) to a strong gene activation domain. In fact, the dCas9-VP64 system as well as subsequent variants, have been shown to significantly raise the transcriptional activity of genes in many different conditions and cell types with high specificity. We, then, tested whether the Cas9-based epigenome editing could rescue haploinsufficiency of Scn1a restoring physiological levels of the voltage-gated sodium channel Nav1.1. We screened sgRNAs targeting the Scn1a gene promoters and identified one sgRNA able to potentiate basal transcription of Scn1a gene in cell lines as well as in primary neurons, with high specificity. Accordingly, levels of Nav1.1 protein were increased and were sufficient to potentiate firing ability of wild-type immature GABAergic interneurons. The same effect of Scn1a transcriptional activation was elicited in Dravet GABAergic interneurons, with a rescue of their functional properties in terms of excitability and firing. Our results pave the way for exploiting epigenome editing as an effective and targeted approach to rescue Nav1.1 protein levels in Dravet mutant inhibitory interneurons and, thereby, ameliorating disease neurological symptoms.

Where are we with treatment options?

Helen Cross ▪ *Renzo Guerrini* ▪ *Lieven Lagae*

Cannabinoids in the treatment of Dravet Syndrome

Cannabis related products have long been used in day to day life, as well as recreational drugs. There have also long been merits claimed in the treatment of various medical conditions. Use has been limited however due to the tetrahydrocannabidiol component, responsible for the psychoactive effects. Attention was brought to the possible utilisation of such products in the treatment of epilepsy through an internet report of a mother in Colarado, who had researched the possible benefits and worked with a Colarado based medical marijuana group to extract a cannbidiol product that could be utilised for her daughter with Dravet syndrome. At the time of the trial she was experiencing nearly 50 convulsive seizures/day – with utilisation of the cannabidiol (CBD) rich product this reduced to 2-3 nocturnal convulsions/month. Subsequent social media and internet reports led to the belief that this much be a miracle treatment, specifically for Dravet syndrome.

GW pharma have been researching utilisation of cannabidiol (<0.1% THC) in epilepsy for >10 years. An initial open label study across 11 sites in the USA showed possible benefit and safety in a group of individuals with complex epilepsy; 32/137 evaluated for efficacy had Dravet syndrome 49% of whom reported >50% improvement in seizures. However further study also demonstrated CBD to raise levels of the clobazam metabolite norclobazam, related to some of the side effects seen eg somnolence. Within the open label study on multiple logistic regression clobazam use was the only independent predictor of reduction >50% in motor seizures overall. An RCT subsequently conducted by GW Pharma across 23 sites in Europe and the US has shown a significant reduction in convulsive seizures in children with Dravet syndrome on CBD compared to placebo. Further analysis suggests additional benefit from combination with clobazam, but also an independent effect from CBD.

Data from the Dravet studies, that from further studies in Lennox Gastaut syndrome, have been submitted to the FDA and EMA for consideration of licence.

Where are we with treatment options? -

Helen Cross ▪ ***Renzo Guerrini*** ▪ *Lieven Lagae*

Stiripentol

Stiripentol has been approved as adjunctive therapy with clobazam and valproate for Dravet syndrome in Europe, Canada, and Japan. Dravet syndrome is often associated with cognitive impairment and high pharmacoresistance. A randomized placebo-controlled trial in patients with Dravet syndrome inadequately controlled by clobazam and valproate showed significantly higher responder rates (71% versus 5%; *P*,0.0001) and decrease in seizure frequency (–69% versus +7%; *P*,0.002) on stiripentol than on placebo. A second, independently performed, randomized controlled trial confirmed these results and both trials were plotted in a meta-analysis. Efficacy was supported by three subsequent observational studies, with 46 (France), 23 (Japan), and 82 (USA) children with Dravet syndrome treated with stiripentol for up to 5 years. Based on the experiences of more than 2,000 patients with Dravet syndrome who were exposed to stiripentol, drowsiness, loss of appetite, and weight loss are the most frequent adverse events and may be reduced by decreasing the dosage of co-medication. The inhibition of stiripentol by the cytochrome P450 complex (CYP2C19, and CYP3A4) leads to clinically significant interactions. Experimental data, both in vitro and in vivo, have established that stiripentol is a GABAergic anticonvulsant and acts on different sites than benzodiazepines. The pharmacodynamic interactions also enhance the anticonvulsant effect of the stiripentol–clobazam combination in patients with Dravet syndrome, irrespective of the GABAergic effect.

Where are we with treatment options? -

Helen Cross ▪ Renzo Guerrini ▪ ***Lieven Lagae***

Fenfluramine in Dravet Syndrome: Results of a Phase 3 Randomized, Double-blind, Placebo-controlled Trial

(abstract based on data presented at AES, Washington 2017)

Dravet syndrome (DS) is a rare, severe, treatment-resistant epileptic encephalopathy. Fenfluramine (FFA) has been reported to have sustained anti-convulsive activity in a small cohort of patients with DS. Here we describe the results of a Phase 3 clinical trial comparing two doses of fenfluramine and placebo on the change in mean convulsive seizure frequency (CSF) in DS subjects. Methods: Subjects aged 2 to 18 years with a diagnosis of DS and in whom convulsive seizures were not completely controlled by their current anti-epileptic drug regimen were enrolled in the study. Subjects who had ≥6 convulsive seizures during the 6-week baseline period were randomized in a 1:1:1 ratio to placebo, FFA 0.2 or 0.8 mg/kg/day (maximum 30mg/day). After a 2-week titration period patients were maintained on their randomized dose for an additional 12 weeks. The primary efficacy endpoint was the change in mean convulsive seizure frequency between FFA 0.8 mg/kg/day and placebo during the 14-week treatment period compared with the 6-week baseline observation period.

Results: A total of 119 subjects with DS enrolled in the study and were randomized to treatment (n=39, 0.2mg/kg/day; n=40, 0.8mg/kg/day; n=40, placebo). The average age of the subjects was 8 years (range: 2 to 18 years). 110 (92%) patients completed the study (100% 0.2mg/kg/day; 85% 0.8mg/kg/day; 93% placebo). The baseline mean CSF across treatment groups was approximately 40 seizures/month. For the primary endpoint, FFA 0.8 mg/kg/day achieved a 63.9% reduction in mean monthly CSF compared to placebo ($p<0.001$). The median percent reduction in monthly CSF was 72.4% among FFA 0.8 mg/kg/day patients compared to 17.4% in placebo patients ($p<0.001$). A significantly greater proportion of subjects in FFA groups achieved a ≥50% or ≥75% reduction in mean CSF and longer median seizure free-interval compared to placebo, with 0.8mg/kg/day superior to 0.2mg/kg/day on all endpoints. Collectively these data suggest a dose response relationship. The incidence of serious adverse events was similar in all three groups with 12.5% (n=5) of patients in the 0.8 mg/kg/day group and 10.3% (n=4) of patients in the 0.2 mg/kg/day group experiencing at least one treatment emergent serious adverse event compared to 10.0% (n=4) of patients in the placebo group. Prospective cardiac safety monitoring throughout the study demonstrated no clinical or echocardiographic evidence of cardiac valvulopathy or pulmonary hypertension. Conclusions: Patients treated with FFA experienced statistically significant, clinically meaningful reductions in mean CSF compared with patients treated with placebo, with the 0.8mg/kg/day dose superior to 0.2mg/kg/day. FFA was generally well tolerated with no clinical and/or echocardiographic signs of valvulopathy or pulmonary hypertension. FFA may represent an important and effective new treatment option for patients with DS.

Management of prolonged seizure from prehospital treatment to intensive care unit

Federico Vigevano

Federico Vigevano – Paola De Liso
Neurology Unit - Neuroscience Department, Bambino Gesù Children's Hospital – Rome

Nearly 90% of patients with Dravet Syndrome (DS) present prolonged seizures or Status Epilepticus (SE). Xu et al (2014) in a case series of 138 pts reported that 71% of seizures last more than 15' and 46% more than 30'. Moreover, in one third of DS patients SE represents the first seizure (Ragona et al. 2010). The incidence of this syndrome among patients with SE occurring during the first 2 years of life is just 17%, but if we consider recurrent SE episodes it increases up to 56% (Le Gal, 2014).
Prolonged seizures may have different semiology. Unilateral or bilateral clonic seizures begin during the first year of life, reaching a peak around the 6th month; seizures precipitants can be almost always identified: fever, cold, hot water baths, stress, physical exercise. Prolonged focal seizures, mainly those with an occipital localization, can be triggered by ILS.
Unusual SE sequelae, i.e. developmental regression and brain atrophy, has been reported by Chipaux et al (2010), while Myers et al (2017) described 5 cases of fatal cerebral edema due to intractable SE. The SE frequency is lower in DS patients on Stiripentol (40%).
The data regarding the established SE treatment are very few and weak; Tanabe et al (2008) reported an excellent efficacy of intravenous barbiturates and midazolam in terminating ongoing SE.
Oromucosal and nasal midazolam are the most cost-effective nonintravenous prehospital rescue medication. Compared to rectal diazepam, oromucosal midazolam is more likely to be accepted and easier to be administered by parents and caregivers.

Long-term outcome of Dravet Syndrome

Francesca Darra

The long-term outcome of the Dravet Syndrome is unanimously considered globally poor by various Authors (Jansen et al. 2006, Dravet et al. 2009, Akiyama et al. 2010, Genton et al. 2011, Catarino et al. 2011, Takayama et al 2014). After adolescence seizure frequency and polymorphism progressively decrease in adulthood. Myoclonic seizures, atypical absences and photo-pattern sensitivity markedly reduce. However in the majority of the patients Generalized or Focal to Generalized TC seizures mainly nocturnal and often in cluster persist. Only a few patients reach a long-lasting seizures freedom and none can stop pharmacological treatment. Despite the decrease of the seizures severity with age, cognitive outcome is poor, most patients having moderate to severe intellectual disability with severe language impairment. With age DS turns out to be more and more a multisystem disorder. Myoclonus, pyramidal and extrapyramidal involvement induces an increase of the neurological disorder leading to a severe gait impairment. Behavioral disorders such as obsessive attitudes, aggressiveness and agitation are very frequent. Autistic like features can be observed in cases with severe Intellectual disability. The majority of the patients are highly disabled and none is living independently. In order to illustrate their long-term outcome we report the clinical features of 48 adults (27 M. 21 F.) diagnosed with DS (before the age of 7 years in 77%), aged between 18-50 years, followed at the Child Neuropsychiatry Unit of Verona University and Besta Institute of Milan. At the last visit 41/48 subjects are still having seizures; 7 only (15%) are seizures-free from at least 2 years. The seizures are generalized or focal to generalized in 93% of the cases. The cases having polymorphic seizures are rare and Convulsive Status Epilepticus is very rare. The seizures occur yearly or monthly, mostly at night. All patients are taking AEDs. 9/48 (19%) have a normal IQ (2) or mild cognitive impairment (7); 18/48 (37%) have a moderate cognitive impairment and 21/48 (44%) a severe cognitive impairment. 80% of the patients show myoclonus associated in 60% of the cases with extrapyramidal signs. 42% of the patients have a gait impairment of variable severity; 15% are unable to walk. Language is absent in 33% and poor in 48% of the patients. Nearly half of the patients have severe behavior disorders and 26% show autistic like features .Only very few patients have good personal autonomy. The described disorders and disabilities correlate with intellectual disability. Looking at the clinical features at onset and during the early course of the disease, the elements having an unfavorable prognostic meaning appear to be the earlier age at onset (before 6 months) and the association of Myoclonic manifestations, Photo-pattern sensitivity and self-induced seizures. The high recurrence of long lasting convulsive status Epilepticus during the first years also has an unfavorable meaning.

Teenagers - Clinical cases

Domenica Battaglia, Ida Turrini, Giorgia Olivieri, Daniela Chieffo, Valentina Arcangeli, Francesco Guzzetta, Charlotte Dravet.
Child Neuropsichiatry Unit. Fondazione Policlinico Universitario A. Gemelli - Catholic University-Roma

Epileptic features in 34 teenagers with Dravet syndrome.

Aim of the study: To describe the natural history of epilepsy in a cohort of Dravet Syndrome (DS) patients aged 10-20 years, attempting a correlation between epilepsy severity during the course of the disease and cognitive functions and adaptive behavior at the outcome.

Patients and methods: We report the epileptic outcome of 34 DS patients (20 F; median 16 yrs 2 mths) followed at the Neuropsychiatry Unit of Catholic University in Rome between 2000 and 2017. Patients underwent full clinical examinations - including seizure semeiology and frequency, neuropsychological (Wechsler Intelligence Scales for Children-revised (WISC-R) or Raven Cognitive Progressive Matrices (RCPM) and adaptive behaviour assessments (Vineland Adaptive Behaviour Scale) - longitudinal video-EEG recordings, neuroimaging and genetic tests. On the basis of seizure type and frequency, we determined three severity levels of epileptic outcome: mild (A), moderate (B), high (C).

Results: Our series included 22 patients with complete form of DS, and 12 with the incomplete one. During childhood we observed only one patient in group A, 13 in group B, and 20 in the group C. During adolescence there were: 14 patients in group A (mean age15 yrs3mths), among them 9 patients have been seizure-free during the last year, 11 patients in group B (mean age 15 yrs 3 mths) and 9 patients in group C (mean age 19 yrs). According to cognitive abilities, during adolescence 9/34 patients had a mild intellectual disability (ID); 14/34 had a moderate ID and 10/34 had a severe ID, only 1 patient did not present ID. The analysis of cognitive course from school-age to adolescence was possible in 24 children: we observed - a stability of cognitive level in 13 adolescents, among them 12 presenting a lowering of epilepsy severity, while a reduction of cognitive level was observed in 11 adolescents, among them 5 presenting a lowering epilepsy severity. Adaptive functions showed better performances in communication and socialization areas, independently of the group the patients belong to, and worse results in daily living and motor abilities.

Conclusion: Three different outcomes of epilepsy are identifiable in adolescence in this cohort. But the relationships between the respective outcomes of epilepsy and cognitive functions were heterogeneous. During adolescence we observed an improvement of epilepsy course in the majority of the patients (20/34), while only 9 patients preserved a mild cognitive disability. The cognitive functions were more impaired in the group of patients with the most severe epilepsy. However the improvement of epilepsy during adolescence was not accompanied by an improvement of intellectual abilities, rather by a stability of cognitive level, even though it was correlated with an improvement of the adaptive functions and daily living skills. Further multicentric studies on larger and prospective cohorts are needed to clarify the reciprocal role played by different co-factors such as genotype, epileptic features and treatments in determining the epileptic, cognitive/behavioral outcome of DS during adolescence.

Adults: clinical cases

Francesca Ragona

Dravet Syndrome can be fully considered a multisystem disorder in which patients and their families must face different issues over time; during the disease course the clinical picture changes and adults require targeted and multidisciplinary care. We report data from a personal series of 13 adult patients affected by Dravet Syndrome who underwent a comprehensive evaluation aimed at reporting on epilepsy outcome, as well as on motor, cognitive and behavioral evolution , comorbidities and adaptive functioning. Cognitive functioning was evaluated through the following tests: Severe Impairment Battery (SIB), Mini Mental State Examination (MMSE) and Raven's Colored Progressive Matrices (RCPM). Behavioural and psychiatric disorders have been investigated by Neuropsychiatric Inventory (NPI), while adaptive functioning by Barthel modified Index (BMI) and Vineland Adaptive Behavioural Scale (VABS) The disease's impact on family life has been assessed using an Italian interview on familial problems "Questionario sui problemi familiari" (QPF) and a Multidimensional Scale of Perceived Social Support (MSPSS). In our series all patients but one still experience seizures at low frequency. Despite that, most patients are still given heavy politherapy which might, at least in part, negatively impact the motor and cognitive functions. The neurological examination detects in all cases myoclonus and extrapyramidal signs; these signs, together with orthopedic problems, determine gait disturbances in a subset of patients. The gait is characterized by bradykinesia, postural instability and absence of pendular movements. In line with previously reported data the cognitive outcome is poor in all cases, however we documented that the range of intellectual disability was highly variable among patients, ranging from severe to mild: language, attention and memory are the most impaired areas. Behaviour disorders, mainly agitation, irritability and aggressiveness are constantly reported by parents. All patients but one are dependent on the caregivers who complain difficulties in carrying on their job and taking care of their relatives . Our data confirm that the disability of adult patients is mainly related to the severity of mental disability and underscore the impact of the disease on family life, and the importance of comprehensive caring and cooperation with family association.

Marseille experience

Long-term outcome of patients with Dravet syndrome: the Marseille experience.

Pierre Genton,
Géraldine Daquin, Charlotte Dravet

We present a series of 38 adult patients affected by Dravet syndrome examined at the Centre Saint-Paul-Hôpital Henri Gastaut between 1970 and 2017. They are aged 20-53 years with most patients less than 40 years old. None of them live independently and all present with a more or less severe handicap. We analyze the following features: outcome of epilepsy (seizure type and frequency), cognitive functions, behavior, neurologic signs, motor and orthopedic signs, current treatment, social statute. We try to determine if there were elements in the early course of the disease allowing a prognosis about the outcome in adulthood.

1978-2018: A 40 year retrospective view of Dravet Syndrome

Bernardo Dalla Bernardina ▪ *Charlotte Dravet*

Even if DS is strongly related with the SCN1A gene disorders, from 1978 up to this day the genotype-phenotype and genotype-outcome still remain unknown. Early electroclinical features can have a prognostic meaning and can significantly influence the long term outcome which still remain debatable.

Comparing the outcome of subjects born between 1978 and 2000 with those born between 2000 and 2001 we observe a tendency to a relatively better outcome over the years. This in part is probably due to the more adequate therapeutical strategies and taking charge but mostly it is related to the expanded phenotypic spectrum of DS. An early onset and the electroclinical features of a " Typical SMEI" mantain up to this day a poor prognostic meaning.

1978-2018: A 40 year retrospective view of Dravet Syndrome

Bernardo Dalla Bernardina ▪ ***Charlotte Dravet***

Evolution of knowledge of the Syndrome and diagnostic communication today

In 1978, severe myoclonic epilepsy in infancy was a syndrome among other syndromes with intractable epilepsy and cognitive deficit, of unknown, probably genetic, etiology. In 2001 its genetic etiology was demonstrated and recent studies confirm the elevated predominance of the mutations on the *gene SCN1A*. The name was changed for Dravet syndrome. The development over the years of affected patients has shown that this syndrome is actually a disease with three principal aspects: epileptic, cognitive, motor. Numerous experimental studies on animal models have advanced its understanding. It is likely that the genetic background and the epilepsy are both responsible for the cognitive and behavioral development. The diagnosis is essentially based on clinical presentation. The absence of mutation on the gene *SCN1A* does not allow it to be excluded, or, vice versa, the presence of this mutation is not enough to affirm the diagnosis when the clinical picture is not compatible. In the majority of cases the diagnosis can be made early and so adequate treatment and care are made possible. The spectrum of the disease has been widened and different levels of severity are recognized, some less serious than others. At the onset a long-term prognosis cannot be affirmed because the development depends on complex factors, including not only the genetic and epigenetic aspects and pharmacological treatment, but also education, rehabilitation, and the patient's family and social environment.

Dravet syndrome and its mimics

Carla Marini

Associate Professor; Child Neurology Unit, Meyer Children's Hospital and department of NeuroFarba, University of Florence, Florence Italy

The vast majority of patients with Dravet syndrome (DS) carry abnormalities in the SCN1A gene. Yet, there are patients who, based on clinical manifestations, have been classified as DS or DS-like and do not carry SCN1A mutations nor deletions or chromosomal anomalies involving this gene. This presentation examines current understanding of the role of non-SCN1A genes in DS, and what is known about phenotypic similarities and differences. A PubMed searched showed that there is a list of genes that have been associated to DS-like phenotypes including *SCN2A, SCN8A, SCN9A, SCN1B, PCDH19, GABRA1, GABRG2, STXBP1, HCN1, CHD2, and KCNA2*. Mutations in these genes give rise to epileptic encephalopathies that in few patients might share similarities with DS. Indeed, for most of such DS-like epileptic encephalopathies the resemblance is limited to the occurrence of some seizures during febrile illness. Thus, the classification of DS-like might not be appropriate and might be limiting our understanding of the potentials, both in terms of disease causing but also in therapeutic aspects, of a specific gene. As genetic diagnosis becomes more readily available, clinical assessment remains essential. A diagnosis of a known syndrome should perhaps be made according to genetic and clinical aspects due to its potential to guide pathophysiologic understanding and therapeutic strategy.

Phenotypic spectrum of SCN2A related disorders, treatment options and outcome

Markus Wolff

SCN2A mutations are associated with a spectrum of epilepsies and neurodevelopmental disorders. Main phenotypes include benign neonatal/infantile seizures, encephalopathies with early infantile onset epilepsy (<3 months), including Ohtahara syndrome and epilepsy of infancy with migrating focal seizures, and encephalopathies with infantile/childhood onset epilepsy, including West syndrome, Lennox-Gastaut syndrome, and focal epilepsies with an ESES-like EEG pattern. In addition, some SCN2A mutations cause intellectual disability and autism without seizures.

In children with early infantile epilepsies, sodium channel blockers (SCB) are associated with significant seizure reduction or seizure freedom, whereas SCB were rarely effective in epilepsies with later onset, and sometimes induced seizure worsening.

Truncating mutations were exclusively seen in patients with late onset epilepsies and lack of response to SCB. Missense mutations associated with early infantile epilepsy and a good response to SCB resulted in increased sodium channel activity with gain-of-function (GOF). In contrast, mutations in patients with late-onset forms and an insufficient response to SCB were associated with loss-of-function effects (LOF). The correlation between phenotype, GOF vs. LOF effects and the differential response to antiepileptic drugs offers specific treatment options.

The genetics of SCN2A

Andreas Brunklaus

Voltage-gated sodium channels are vital for maintaining the balance between excitation and inhibition across the nervous system. The voltage-gated sodium channel Nav1.2 encoded by the gene *SCN2A* is widely expressed in the central nervous system. It plays an important role in the initiation and conduction of action potentials and is critical for normal early neuronal development.

New research findings suggest that there is a robust association between genotype and phenotype: missense variants in *SCN2A* are mainly seen in benign (familial) neonatal/infantile seizures and encephalopathy with early infantile epilepsy < 3 months of age, whereas truncating variants are predominantly seen in patients with late onset epilepsies and autism spectrum disorder (ASD). Functional characterization of missense variants suggest that variants associated with early infantile epilepsy result in increased sodium channel activity with gain-of-function. In contrast, variants in patients with late-onset forms and ASD are associated with loss-of-function effects.

Genetic variants affecting four members of the human voltage-gated sodium channel gene family—*SCN1A*, *SCN2A*, *SCN8A*, and recently *SCN3A* — have all been associated with epilepsy and other neurodevelopmental disorders. However, it is currently not well understood how to explain the phenotypic heterogeneity among sodium channel neurodevelopmental disorders. Recent experimental work points towards a model in which genetic variants in the four brain-expressed voltage-gated sodium channels translate to imbalances between inhibition and excitation and subsequently neurodevelopmental disorders. Variant type and gene expression timing appear to have profound effects on the phenotype and may inform therapeutic intervention strategies.

Mechanisms and Models - SCN2A

Holger Lerche, Tübingen

Variants in the *SCN2A* gene cause a broad spectrum of epilepsy syndromes of variable severity including neonatal-infantile seizures and epileptic encephalopathies, but also neuropsychiatric disorders such as autism spectrum disorder and schizophrenia. Interestingly, genetic and functional investigations have revealed that disease-associated variants can cause both gain- and loss-of-function (GoF/LoF) effects of the $Na_V1.2$ Na^+ channel which are well correlated to the clinical phenotype and therapeutic applications. Whereas GoF variants cause early-onset disease with epileptic seizures with a good response to Na^+ channel blockers, LoF variants cause later onset disease (beyond the third month of life), are not always associated with seizures and do usually not respond well or even worsen on therapy with Na^+ channel blockers. Particularly for the GoF effects a wide spectrum of electrophysiological changes have been described (shifts in voltage-dependence, slowing of fast inactivation or acceleration of its recovery, increased persistent Na^+ current and an independent subthreshold Na^+ current) with a correlation between the severity of the electrophysiological channel dysfunction and the clinical phenotype. Many early onset syndromes are transient and do recover later on. Additional symptoms like movement disorders or (episodic) ataxia are common. A mouse model has been generated that recapitulates many of the clinical features observed in patients including response to Na^+ channel blockers.

Phenotypic spectrum of SCN8A-related disorders and treatment option outcome

Elena Gardella

SCN8A encodes one of the main voltage-gated sodium channel subunits (Nav1.6) in the brain. Pathogenic variants in *SCN8A*-gene have been originally described in patients with severe developmental and epileptic encephalopathy (DEE), accounting for approximately 1% of DEE. Recently, it came out that *SCN8A* could cause more benign forms of epilepsy with normal cognition and treatable seizures. The amount of *SCN8A* related phenotypes is rapidly increasing, with the inclusion of *SCN8A* in gene panels and the expanded use of whole exome sequencing for diagnostic assessment of patients with epilepsy syndromes.

Based on the electro-clinical features, we identified different phenotypes, either sporadic or unfrequently familial:

1) Early onset severe drug resistant epilepsy, with a mean age at onset of 4 months, cognitive deterioration, pyramidal/extra-pyramidal signs, cortical blindness, severe gastrointestinal symptoms and in some cases death in infancy (ca.5% of patients). Patients had focal prolonged seizures in clusters, with prominent hypomotor and vegetative symptoms, evolving to bilateral tonic and or clonic manifestations. Epileptic spasms-like episodes, epileptic myoclonus, recurrent non convulsive status epilepticus, were also common features. EEG showed progressive background deterioration with epileptic abnormalities and beta/delta activity predominant in the posterior regions. In the majority of cases, epilepsy was extremely drug-resistant; anyway, a positive response to sodium channel blockers has been described.

2) Sporadic patients with mild to moderate intellectual disability, mild or none neurological signs and epilepsy with extended seizure-free periods. EEG showed multifocal epileptiform abnormalities and in some patients, discrete beta activity in the posterior regions, reminiscent of what observed in the severe cases.

3) Families with benign infantile seizures, in some family members associated with paroxysmal dyskinesia later in life, without any cognitive or chronic neurological deficit. Interictal EEG was either normal or showed not specific diffuse abnormalities.

4) Patients with cognitive and/ or behavioral disturbances without epilepsy.

Several degrees of extrapyramidal signs, such as paroxysmal dyskinesia, ataxia and choreoathetosis were common in all groups. Most patients carried a de novo heterozygous missense *SCN8A* pathogenic variant.

All the epilepsy subgroups showed better seizure control with sodium channel blockers. However, milder forms responded better to carbamazepine, often achieving seizure freedom, whereas *SCN8A*-DEE seemed to benefit more valproic acid, oxcarbazepine or phenytoin, usually at supra-therapeutic doses. In *SCN8A*-DEE, ketogenic diet had often a good effect, and levetiracetam had a negative effect, if any.

The Genetics of SCN8A

Rikke Steensbjerre Møller

The mutational landscape of *SCN8A*-related disorders

SCN8A, encoding the voltage-gated sodium channel Nav. 1.6 has been associated with a spectrum of clinical phenotypes including 1) benign infantile seizures and paroxysmal dyskinesia, 2) intellectual disability without seizures, 3) moderate intellectual disability with epilepsy with extended seizure-free periods 4) severe early-infantile epileptic encephalopathies (EIEE).

Since the initial description in 2012, more than 200 cases have been described, and an understanding of the impact of pathogenic *SCN8A* variants is beginning to emerge. *SCN8A*-related disorders are autosomal dominant conditions. In most cases with EIEE, the variant arises *de novo* in the patient. In a few cases, the variant has been inherited from an unaffected mosaic parent. The familial cases all have an autosomal dominant pattern of disease. The pathogenic variants are predominantly located in the transmembrane domains, the inactivation gate, and the C-terminal, and more than 25% of the described patients have recurrent variants in residues Gly1475, Arg1617, Ala1650, Arg1872 and Asn1877.

More than ten EIEE-associated variants have been tested functionally. The majority of these variants are causing gain-of-function features, including hyperpolarizing shifts in the voltage-dependence of activation, impaired inactivation, and elevated persistent current. The gain-of-function causes the sodium channel to be hyperactive, which leads to increased neuronal firing. In comparison, functional studies of *SCN8A* variants found in patients with intellectual disability without seizures, have demonstrated that these variants are causing a loss-of-function of the ion channel, indicating that haploinsufficiency of *SCN8A* is associated with intellectual disability.

SCN8A Mutations: Mechanisms and Models

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More than 100 distinct mutations of *SCN8A* have been identified since the first patient mutation was described in 2012. The majority of known *SCN8A* mutations arose *de novo* in patients with epileptic encephalopathy. Ten of the 12 epileptic encephalopathy mutations that we tested in transfected cells demonstrated gain-of-function alterations of biophysical properties, including impaired channel inactivation (n=8) and hyperpolarized shift in voltage dependence of channel activation (n=2). These gain-of-function mutations of *SCN8A* in epileptic encephalopathy contrast with the loss-of-function mechanism of *SCN1A* mutations in Dravet Syndrome. To investigate the *in vivo* effects of gain-of-function mutations, we generated knock-in mouse models of two patient mutations, the milder *SCN8A*-N1768D and the more severe *SCN8A*-R1872W. Both mutant lines reproduce the spontaneous seizures and sudden death seen in patients. In heterozygous mice with the N1768D mutation there is enhanced spontaneous firing of hippocampal neurons, elevated activity of neurons in the entorhinal cortex, and cardiac arythmias that may contribute to shortened lifespan. The onset of seizures in N1768D mice occurs between 2 and 4 months of age, with additional seizures and progression to death over several days to a week. In contrast, global expression of R1872W results in a single catastrophic seizure on postnatal day 14 or 15, followed by death within a few seconds. Elevated spontaneous neuronal firing was detected in the R1872 mice on postnatal day 13 but not earlier, consistent with the increase in abundance of the *Scn8a* transcript during the first weeks of life. We are currently investigating the effects of conditional activation of the R1872W mutation in different types of neurons.

We have also identified loss-of-function mutations of *SCN8A* in patients with epileptic encephalopathy (n=2) and patients without seizures who have intellectual disability and impaired movement (n=3). In the mouse, spontaneous mutations causing partial- or complete- loss-of-function do not cause seizures but result in behavioral and movement disorders (reviewed in O'Brien and Meisler, Front. Neurol. 2013). There are dramatic effects of strain background on the phenotypes of induced and spontaneous mutations of *Scn8a* in the mouse. Mutations of human *SCN8A* have recently been described in patients with mild disorders. In both human and mouse, the clinical outcome of mutations in *SCN8A* appears to be determined by multiple factors including the biophysical effects on channel function and the genetic influence of variation in modifier genes.

POSTERS

Survey: Falls in Patients with Epilepsy

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Introduction. We promoted a simple questionnaire in order to evaluate the impact of ictal falls in the daily life of epileptic patients.

Methodology. An online survey was open from 12th May to 31st October 2017 and was translated in 9 languages. It consisted of 21 questions mainly related to epilepsy type and falls frequency and semiology. The survey was completed by legal representatives of an unselected cohort of epileptic patients, but only those who reported falls were considered. The attendees were recruited by different methods (mail, social networks, posters).

Results. We collected data from 714 patients across 36 countries affected by Dravet syndrome (DS) (361-50.6%), Lennox-Gastaut syndrome (LGS) (93-13.0%), and other epilepsies (260-36.4%). Falls were reported in 648 (90.7%) surveys. Among patients with sudden and violent falls (335), more frequent in LGS (81.7%), 34% had >50 falls/year. Among patients with falls related to convulsive seizures (488), more frequent in DS (81.4%), 20.4% had >50falls/year.

Conclusions. Although our survey has many methodological limitations, it underlines the burden of ictal falls. The data we collected may be useful to activate initiatives for the search of protection systems aimed at improving the quality of life.

Italian multicentre prospective study in patients with Dravet Syndrome

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Introduction: Dravet patients were enrolled since the first year of life in order to describe the onset and evolution of epilepsy, identify the onset of the neurodevelopmental impairment and its evolution, perform a correlation analysis between genetic findings and cognitive outcome and between epilepsy and cognitive outcome.

Methods:
We evaluated seventeen Dravet patients followed-up in centers involved in the DESIRE project.

Results:
- All patients detected mutations in the SCN1A gene.

- 47% of them presented a psychomotor delay between the 12th and the 18th month.

- earlier seizure onset, recurrence of status epilepticus and of myoclonic seizures can be considered as negative prognostic factors for slowing of psychomotor development.

- The correlation analysis between genetic determinants and cognitive evolution did not provide evidences for a correlation between the mutation and the cognitive outcome.

Discussion:
We failed to identify a clear-cut correlation between the type of mutation and cognitive outcome. Our data suggested that an early seizure onset, the occurrence of status epilepticus and of myoclonic seizures play a negative prognostic role on cognitive outcome.

Conclusions:
It is conceivable that the epileptic phenotype could contribute to determining the cognitive outcome, but other genetic and environmental factors need to be considered.

Homeostatic responses in GABAergic neurons of Nav1.1+/- knock-out mice, animal model of Dravet syndrome.

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Several features of Dravet syndrome (DS) patients are replicated in heterozygous Nav1.1 knockout mice. Published results obtained with these mice have shown selective reduction of sodium current and excitability in GABAergic interneurons, reduced inhibition and hyperexcitability in cortical and hippocampal circuits, also in the pre-epileptic period. Surprisingly, no modifications in neocortical dynamics have been observed in vivo in the cortex at physiologic temperature (1), consistent with the presence of homeostatic responses.
We have performed patch-clamp experiments in hippocampal slices of Nav1.1+/- KO mice in the pre-epileptic period (PN14-18) for disclosing homeostatic responses. We investigated the properties of different subtypes of interneurons involved in inhibitory microcircuits. Our results show an impairment of firing and a reduction of the slope of the action potentials in some interneurons, (e.g. paravalbumin positive basket-cells), but an increased excitability in other subtypes. We show that this is a homeostatic response in DS mice able to ameliorate the phenotype of the mouse model. Interestingly, this response may possibly be at play also in other pathologies characterized by hypoexcitability of GABAergic neurons.

Bibliography:
1. *DeStasi et al 2016 Cerebral cortex 26:1778*

Negative dominance of $Na_V1.1$ missense mutants: a novel pathological mechanism in Dravet Syndrome

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Dravet Syndrome (DS) is often caused by truncating or folding/trafficking defective mutations of $Na_V1.1$ sodium channel, which lead to loss-of-function of mutant channels. In numerous pathologies, folding/trafficking impairments frequently associate with negative dominance (reduced membrane trafficking of co-expressed wild-type channels), which can be an additional mechanism possibly implicated in phenotype severity. We already excluded negative dominance for truncating mutations (1). To investigate whether $Na_V1.1$ folding-defective mutants exert negative dominance, we used patch-clamp recordings of transfected tsA-201 cells to study $Na_V1.1$-M1841T, showing a partial recovery in rescuing conditions, and $Na_V1.1$-M1664K, which was not rescued. We compared sodium current density of wild-type $Na_V1.1$ in control conditions or co-expressed with the mutants, separately. $Na_V1.1$-M1841T did not alter current density or biophysical properties, thus excluding negative dominance. $Na_V1.1$-M1664K caused a 64% reduction in current density, thus indicating it exerts a dominant negative effect. Biophysical properties were not affected, except for small changes in the slope of the activation and inactivation curves. Interestingly, the co-expression of β1 accessory subunit did not modify mutants' features, therefore suggesting that it is not involved in the modulation of the negative dominance for these mutants. Thus, missense $Na_V1.1$ DS mutants can show not only loss-of-function, but also negative dominance.

Bibliography

Bechi G., Scalmani P., Schiavon E., Rusconi R., Franceschetti S., Mantegazza M., 2012. Pure haploinsufficiency for Dravet syndrome $Na_V1.1$ (SCN1A) sodium channel truncating mutations. Epilepsia. 53(1):87-100. doi: 10.1111/j.1528-1167.2011.03346.x.

dCas9-based Scn1a gene activation restores inhibitory interneuron excitability and attenuates seizures in Dravet syndrome mice

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Dravet syndrome (DS) is a severe epileptic encephalopathy caused by heterozygous loss-of-function mutations of the *SCN1A* gene, indicating haploinsufficiency as the pathogenic mechanism. Here, we tested whether catalytically dead Cas9 (dCas9)-mediated *Scn1a* gene activation can rescue *Scn1a* haploinsufficiency in a mouse DS model and restore physiological levels of its gene product, the Nav1.1 voltage-gated sodium channel. We screened single guide RNAs (sgRNAs) for their ability to stimulate *Scn1a* transcription in association with the dCas9 activation system. One sgRNA was able to increase *Scn1a* gene expression levels in cell lines and primary neurons with high specificity. Nav1.1 protein levels were augmented, as was the ability of wild-type immature GABAergic interneurons to fire action potentials. A similar enhancement of *Scn1a* transcription was achieved in mature DS interneurons, thus rescuing their ability to fire. To test the therapeutic potential of this approach, we delivered the *Scn1a*-dCas9 activation system to DS pups using adeno-associated viruses. Febrile seizures were significantly attenuated. Our results pave the way for exploiting dCas9-based gene activation as an effective and targeted approach in DS and other disorders resulting from altered gene dosage.

Footprints characterisation in patients with Dravet Syndrome

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Introduction
In Dravet syndrome gait abnormalities have been described only by observational video analysis [1]. Baropodometry might be used to objectify abnormalities of walking patterns [2].

Methodology
Nine Dravet patients and seven control subjects were asked to walk self-paced. A pressure matrix recorded five right and left footprints, which were retained for the analysis, and masked to calculate the contact area, averaged force, contact time, maximum averaged pressure, and other parameters of interest for forefoot, midfoot, rear foot, lateral-foot, medial foot areas, and the whole foot. Differences between the parameters calculated for patients and controls were statistically tested.

Results and Discussion
Our data highlights a reduced force exchanged with the ground at the rearfoot, identifying defective propulsion at the knee and the ankle, compensated by trunk anteposition, that shifts forward the Centre of Pressure and increases the forces applied by fore- and midfoot to the ground. The dominant foot seems to provide more stable support, as previously reported in healthy subjects [3].

Conclusion
According to our results baropodometry can be considered an alternative to identify major gait pattern abnormalities.

Bibliography
[1] Rodda JM, et al. Arch Neurol 2012;69(7):873-8.
[2] Giacomozzi C, Stebbins JA. GaitPosture 2017;53:131-138.
[3] Sadeghi H, et al. GaitPosture 2000;12(1):34-45.

LONG-TERM EFFICACY OF VAGUS NERVE STIMULATION IN DRAVET SYNDROME: AN EXTENDED FOLLOW-UP STUDY.

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Purpose: The management of Dravet syndrome (DS) remain challenging in spite of recent progress. Alongside medical treatment, alternative palliative approaches could be valuable options.
To evaluate the long-term impact of vagus nerve stimulation (VNS) in patients with DS, we performed an extended follow-up study.
Methods: Dravet patients underwent VNS implantation from 2000 to 2014 were enrolled in this single-center retrospective observational cohort study.
The primary efficacy endpoint was to assess changes in seizure frequency from baseline to 6, 12, 24, 36, and 48 months, then yearly until the end of follow-up. Secondary efficacy endpoints were monitored by yearly assessment using Clinical Global Impression of improvement (CGI-I) scores and Vineland Adaptive Behavior Scales (VABS), to longitudinally evaluate the clinical health and functioning skills.
Results: Twelve patients (6 female; median age 23 years, range 8-38) were enrolled in the study.
All patients carried a SCN1A mutation.
The median age at epilepsy onset was 4 months (range: 2-8) and the median time of disease duration prior VNS implantation was of 8 years (range: 2-26). Prior implantation, all patients had daily/weekly polymorphic seizures (generalized convulsive, focal, myoclonic, tonic and atypical absences) variably associated, and 41% experienced episodes of status epilepticus. Nine patients had severe intellectual disability (ID), the remaining had moderate ID (2 patients) and mild ID (1 patient). Prior surgery all had poor adaptive behavior with respect to their cognitive functioning.
After surgery, patients were followed-up for a median time of 7 years (range: 4-18). At 6, 12, 24, 36 and 48 months after surgery, responders (> 50% reduction in baseline seizure frequency) were respectively 41%, 41%, 50%, 33%, and 25%. At last available follow-up, the median seizure rate reduction was 30% and it remained stable over time; at the end of follow-up, 8 patients switch off or removed the device without change in seizure frequency.
During the follow-up period, cognitive function was unchanged, but we recorded a significant improvement in adaptive behavior, communication skills, and in the global clinical health leading to a better quality of life, also in those patients who did not achieve a significant seizure reduction.
Conclusions: Pharmacological therapy remains the main basis for DS treatment, but VSN could be considered an interesting palliative options in selected patients, targeted to seizure frequency reduction and improvement in adaptive behavior.

GENE THERAPY FOR DRAVET SYNDROME: A PROOF OF CONCEPT

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In most cases, the genetic basis of Dravet Syndrome (DS) is a haploinsufficiency caused by mutations in SCN1A. Due to the complex physiopathology of DS, etiological approaches such as gene therapy have unique chances to obtain a global improvement in the life of these patients. We aim to deliver a functional copy of the SCN1A gene to the brain using High-Capacity adenoviral vectors (HC-Ad). To provide proof of concept about the feasibility of this approach we generated a preliminary vector prototype carrying a codon-optimized SCN1A cDNA under the control of a CAG promoter. The expression cassette inserted into the vector genome was stable in E.Coli and gave rise to viable HC-Ad particles following a standard rescue and amplification protocol. The resulting HCA-CAG-SCN1A vector was able to infect neurons and increase the amount of Nav1.1 in a dose-dependent manner. Biodistribution analysis using HC-Ad vectors encoding GFP demonstrated efficient transduction of neurons upon intracerebral administration. Finally, in vitro luciferase reporter assays were performed to select a regulatory sequence with preferential activity in GABAergic/paravalbumin-expressing inhibitory neurons. In summary, the results obtained so far indicate that gene therapy based on HC-Ad vectors is a viable option for the treatment of DS. (introduction, methodology, results, discussion and conclusions).

Late cognitive delay in Dravet Syndrome, after the age of 6 years: report of four cases.

Emma Losito, Nicole Chemaly, Cathrine Chiron, Dorothée Leunen, Rima Nabbout

Objectives Patients with Dravet Syndrome develop a progressive slowing of acquisition from the age of 2 years. Cognitive delay is generally reported after four years of age, without progressive regression.
Some variability has been reported and the slowing of acquisitions with development of intellectual disability may occur later up to the fourth year (Buoni et al. 2006; Nabbout et al. 2013; Guzzetta 2011).

Methodes We report four patients (2 males and 2 females) who preserved a normal or subnormal cognitive level at six years. They were all positive for SCN1A mutation, with mutation de novo in 3/4. Subtypes: one missense, one splicing, one frameshift and one nonsense. They were followed up until a median age of 11 years and 9 months (8 yars 11 months to 13 years 5 mois). Only one patient was seizure free from more than one year at the last follow-up. In the others, the frequency of seizures was variable, from yearly to monthly seizures. All received the tritherapy (VPA+STP+CLB) except one. They had longitudinal neuropsychological testing through standardised psychometric tools. The minimum age at last evaluation was 8 years and 11 months.

Résultats At 6 years they all presented an IQ> 70 (respectively 95,82,78,96). Two of them have presented a clear decrease in their neuropsychological scores (< 70) after 6 years. Two, at around 13 years, still presented a normal IQ and, one of them presented an increase of her scores, probably in link with the behavioural improvement and the achievement of seizure control.

Conclusion The stagnation of acquisitions and the cognitive delay in Dravet Syndrome can occur later than what is classically reported in literature, thus implying a different approach in the communication of the prognostic of the cognitive evolution.

DRAVET SYNDROME: A "NOCTURNAL" PATTERN OF SEIZURES

R. Nabbout. et al.

Objectives: Dravet Syndrome (DS) is a rare epileptic encephalopathy with poor cognitive outcome. Seizures are refractory to AEDs, but they tend to decrease with age. A trend toward a switch from "awake" to "sleep/nocturnal" seizures is reported during the clinical course of DS but literature lacks details about the incidence of nocturnal seizures, the age onset and the proposed change in therapies.

Methods: in a longitudinal cohort of 73 patients with DS followed at our centre, we report 20 patients who developed a pattern of almost exclusive nocturnal seizures. We have retrospectively analyzed the genotype (SCN1A mutation) and the epilpetic phenotype at the onset and the last follow-up (seizure types, treatments, cognitive and behavioural outcome). As for the EEG, we detailed those realised during the phase of "sleep/nocturnal" seizures.

Results: This population presented a strong prevalence of males (14 M and 6 F). All had mutations in SCN1A and with a higher incidence of splicing mutations (35%) . Median age of "noctunal seizures" onset was of 6 y6m(4y-11y). Last follow-up ranged between 5y11m and 24y6m (mean 14y4m, and 19 over 20 patients still presented "nocturnal/sleep" seizures predominantly. All had the tritherapy (VPA, STP, CLB) before the nocturnal seizures onset. Seizures were difficult to control despite AEDs adjustment and many trials. On EEG, 8 showed paroxysmal anomalies, predominant in the frontal region. All had at last follow-up cognitive disability and behavioural disorders.

Conclusions: Despite the limits of our observation related to and the retrospective data and the small sample, we could identify a sub-populations of patients with DS. A better and more systematic prospective collaborative data could help to better understand this group and to guide our treatments choices.

Early electro-clinical features and outcome at 6 years of age of 61 subjects with Dravet syndrome born between 1972 and 2010

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Background: The genotype - phenotype correlations in DS are still unknown and the definition of the early electroclinical features having a prognostic meaning remains debated. In order to evaluate which findings can predict and/or influence the outcome and they have modified over the years, we analyzed comparatively the electroclinical features of 61 DS cases (aged 9 to 46 years) born between 1972-1990 (Group 1: 20), 1991-2000 (group 2: 22), 2001-2010 (Group 3: 19) longitudinally followed at the Child Neuropsychiatric Unit of AOUI, Verona.
Methods: We analyzed demographic and electroclinical findings at onset and during the evolution, age at diagnosis, type of SCN1A mutation, treatment and the epilepsy, neurological and cognitive outcome at the age of 6 years. According to the cognitive outcome the subjects have been divided un two groups: Normal I.Q./Mild ID (18), Moderate/Severe ID (43).
Results: The incidence of cases showing a moderate/severe ID is greater (80%)in groups 1, 2 than in 3 group (53%). The findings statistically related with a worse neurological and cognitive outcome are: age at onset < 6 months, significant and persistent myoclonic manifestations (absences with myoclonias and massive myoclonias), photo-pattern sensitivity, self-induction. No correlations have been found according to type of genetic disorder, first seizure semiology, focal or generalized seizures occurrence and frequency. Diagnosis in the first year is more frequent in group 3 and long-lasting treatment with phenobarbital and phenytoin more frequent in groups 1,2 without reaching statistical significance.
Conclusion: The electroclinical features observing during the first years constitute the only significant prognostic factors, having myoclonic manifestations, photo-pattern sensitivity and self-induction a poorer significance. The relatively lower incidence of subjects with a poorer outcome in group 3 appears mostly related to the expanded phenotypic spectrum of DS. over the years. Only future long-term studies concerning subjects born in the last years will be able to highlight if an earlier diagnosis, different pharmacological treatments and a more precocious and adequate taking charge can significantly modify the prognosis.

Perampanel as add-on in 10 patients with Dravet Syndrome

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Purpose: Dravet Syndrome (DS) is a rare and severe epilepsy syndrome with seizures onset during the first year of life. Perampanel (PER) is a selective non competitive AMPA-receptor antagonist recently approved for the treatment of partial and generalized epilepsies with tonic-clonic seizures as an add-on therapy. The aim of this study was to evaluate efficacy and tollerability of Perampanel (PER) in Dravet patients in daily clinical practice conditions.

Patients and methods: we assessed patients with DS who started add-on PER treatment in 6 epilepsy centers between april 2015 and september 2017. The response to PER was defined as a ≥50% reduction in monthly seizure frequency compared with seizure frequency during the month before PER introduction. Titration schedule of PER was at the discretion of the treating physician according to medical needs.

We report 10 patients, 6 males and 4 females. Age range was 15-43 years (mean age: 25 years). All of them were positive for SCN1A gene mutation and had a moderate-severe intellectual disability. Age of seizure onset ranged from 4 to 24 months (mean 11 months). Before PER, seizures occurred 7 per months (range: 2-14) in average and were mostly brief generalized tonic-clonic. Number of concomitant AEDs were 1-3 and five patients were receiving stiripentol (STP).

Results: Mean add-on PER dose was 4.3 mg per day (2-10 mg). After an average of 16 months follow-up (2-31 months), 8 patients continued on PER: four was seizure free while the remaining 4 patients achieved between ≥90% and 50% seizure frequency reduction. The adverse events were irritability and dizziness. In two cases, adverse events were managed decreasing PER dose. In two patients PER withdrawal was caused by inacceptable behavior disturbances (even though the patient was seizure-free) and inefficacy, respectively. Very recently a patient who achieved seizure freedom two years ago on PER, died during a Status Epilepticus due to a febrile illness.

Conclusions: these findings suggest that PER could be safe, well-tolerated and effective in DS. More studies are needed to assess long-term tollerability and effectiveness of PER in this syndrome.

Movement disorders in SCN8A-related early infantile epileptic encephalopathy

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Background: *SCN8A*-related early infantile epileptic encephalopathy is a clinically heterogeneous disorder characterized by drug-resistant epilepsy and moderate-severe developmental delay, often associated with movement disorders. Aim of this study is to delineate the electro-clinical features of movement disorder in this rare and complex neurologic condition.
Methods: We studied 4 patients (2 female), with a median age of 5.9±4.9 years with *de novo* heterozygous *SCN8A* mutations. All video-EEG recording and all available home-video were reviewed.
Results: Three patients had severe psychomotor delay (DQ=20-45), characterized by tetraparesis and cognitive impairment. These patients showed severe diffuse (axial and limb) hypertonus during the first months of life, followed by axial hypotonia and distal hypertonia during the following period. In one patient a non-epileptic diaphragmatic intermittent myoclonic appeared at the age of 8 years. Distal diskinetic movements were present in one patient. The forth patient, with a better cognitive psychomotor development (DQ 75), showed cerebellar signs such us gait ataxia and upper limbs tremors.
Conclusions: Extra-pyramidal and pyramidal signs were present in all patients with different features depending on the global psychomotor delay. Both extra-pyramidal and pyramidal signs were present in the most affected patients. Further studies are needed to better characterize the movement disorder and address rehabilitation treatment.

Targeted NGS of ion channels in individuals referred with early onset epileptic encephalopathy.

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Epilepsy comprises a wide range of etiologically very heterogeneous clinical conditions and at present, targeted NGS seems to be the elective choice for early and efficient etiological diagnoses.
In our center 155 individuals have been subjected to NGS investigation, with the use of a customized panel including 31 genes, on the Ion PGM™ platform.
The overall diagnostic yield was 17.4%, although, in patients with seizure onset within 12 months of life this resulted to be 22.7% indicating a higher diagnostic specificity for this cohort.
We have identified 21 pathogenic/likely pathogenic variants in the *SCN1A* (8), *SCN2A* (4), *SCN8A* (4), *KCNQ2* (3) and *HCN1* (2) genes. We have detected 7 novel *de novo* variants, 3 known disease-causing variants also resulted *de novo* and 1 familial SCN2A mutation. One novel and one low frequency *SCN1A* variants were transmitted. A maternally inherited highly likely pathogenic *KCNQ2* variant was found in association with a novel *de novo SCN1A* variant. Family segregation analysis in 6 patients is currently ongoing.
Our work further strengthens the importance of a careful phenotype characterization coupled with the power of the NGS technology and indicates in a subset of few genes the major players for epilepsy with very early onset.

Cognitive development in patients with epileptic encephalopathies due to sodium channel mutations (SCN1A, SCN2A and SCN8A).

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Background: Sodium channel mutations are responsible of genetically and clinically heterogeneous disorders characterized by drug-resistant epilepsy and moderate-severe developmental delay. We aimed to delineate cognitive development in a cohort of patients with *SCN1A*, *SCN2A* and *SCN8A* mutations.
Methods: We studied 13 patients (6 female), aged between 4 months and 10 years with *de novo* heterozygous *SCN1A (n=5)*, *SCN2A (n=4)* and *SCN8A (n=4)* mutations. Eight patients were prospectively analyzed through Griffiths Mental Development Scales and 5 patients were retrospectively analyzed with the same scales. Mean follow-up period was 5 years.
Results: Cognitive impairment was evident before the first year of life in 9 patients and those with *SCN8A* mutations were more severely impaired (developmental quotient -DQ=46). At last evaluation, all patients had moderate-severe and it resulted to be predictable since the first year. On average patients with *SCN1A* mutation showed higher DQ than patients with *SCN2A* mutation (DQ=78 *vs* DQ=66).
Conclusions: All patients showed deterioration or stagnation of milestones development. Patients with *SCN8A* mutations had lower scores in all domains. Despite the limited number of subjects, patients with *SCN1A* mutation showed greater cognitive skills than the others. Further studies needed to better understand developmental evolution and address the rehabilitation treatment.

A relatively mild phenotype associated with mutation of SCN8A

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Mutations in SCN8A gene have been described in relation to infantile onset epilepsy with movement disorders and developmental delay. Recently various authors have reported patients carrying autosomal dominant heterozygous SCN8A mutations and a milder phenotype expression.We discuss the case of a 6-year-old girl with a positive family history for epilepsy, early benign focal epilepsy, well controlled by Carbamazepine, upper limb tremor since birth, ataxia, slight motor delay and normal cognitive development. Neuroradiological study is normal, waking EEGs are normal, while epileptiform abnormalities on the vertex appear during sleep.The girl carries a de novo mutation of the SCN8A gene with nucleotide substitution of c.3943G> A (p.Val 1315 Met), located in the domain III S4 / S5 intracellular linker.In literature two other cases with the same mutation have been reported, both patients have an epileptic encephalopathy. Our patient's milder phenotype could be caused by a modifier effect, possibly a mutation in another gene or a mosaicism.The detailed description of our case should contribute to enlarging the description of the clinical features of SCN8A mutations and to recommending the deepening of genetic investigations to identify other genes that could contribute to modulate its expression.

Correlation between epileptic phenotype and SCN8A gene mutation

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Introduction: missense mutation in SCN8A gene, encoding sodium channel alpha subunit Na(v) 1.6, has commonly associated with infantile epileptic encephalopathy-13 (EIEE13)[1]. It has been found, although less frequently, in other phenotypes such as benign familial infantile seizures and paroxysmal choreoathetosis (BFIS)[2]. This report aimed to broaden the phenotypic-spectrum of SCN8A-related epilepsy.

Methods: we described three patients with different SCN8A mutations, obtained by clinical epilepsy gene panels, leading to distinct phenotypes.

Results: One patient affected by EIEE with a known de novo mutation (p.Arg1872Trp)[1], has showed recurrent absence myoclonic state. Another patient with BFIS has presented recurrent seizures with hemisomal hypotonia, retching, bradycardia; she has never presented movement paroxysmal disorder as reported in her BFIS affected relative. She has carried heterozygous missense mutation (c.4447G>A; p. E1483K)[2]. Interestingly, the third patient showed a de novo mutation not yet described (P. Asn877His), that alters a conserved aminoacid position. This patient had focal epilepsy with refractory seizures and autistic-like features. The background electric activity remained well organized with rare paroxysmal multifocal anomalies.

Discussion and conclusions

There is considerable heterogeneity in the clinical features of patients with SCN8A recurrent mutation[3]. We described three different electroclinic phenotypes, one of which showed a mild phenotype associated to a new pathogenic variant in SCN8A.

Bibliography

1. *Larsen et al., 2015 American academy of neurology: The phenotypic spectrum of SCN8Aencephalopathy*
2. *Gardella et al., 2016 -Annals_of_Neurology: BFIS, PKD, and SCN8A Mutation*
3. *Jacy L. Wagnon et al., 2015: frontiers in Neurology: Recurrent and non-recurrent mutations of SCN8A in epileptic encephalopathy*

SCN2A related epilepsy: longitudinal electroclinical study of 11 cases.

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BACKGROUND: Mutations in SCN2A can be associated with different types of epilepsy and evolution: Ohtahara Syndrome (OS), Epilepsy of infancy with migrating focal seizures (EIMFS), Benign (Familial) Neonatal/Infantile Seizures (B(F)NS, B(F)NIS, B(F)IS, West Syndrome (WS), Lennox-Gastaut Syndrome (LGS), Myoclonic-Atonic Epilepsy (MAE), Genetic Epilepsy with Febrile Seizures Plus (GEFS+), and other encephalopathies with infantile or childhood unclassifiable epilepsies.
AIM: To describe the longitudinal electroclinical phenotypes of 11 patients with SCN2A mutation epilepsy followed in our center.
MATERIALS AND METHODS: They are 11 patients (5 female, 6 male) aged between 2 months and 22 years with epilepsy and SCN2A mutation longitudinally followed in our center. We assessed the following features at onset: familial antecedents, age, neurological exam, interictal EEG, brain MRI, electroclinical semiology, frequency and seizures response to treatment; furthermore, we assessed the type of gene defect and seizure-related, neurological, and neuropsychological outcomes.
RESULTS: Gene variants were missense in 10 cases and truncating in one. The variations were pathogenic in 9 while of "uncertain significance" in 2; de novo in 5 cases, inherited in 5. According to the electroclinical phenotype, 5 patients fit the diagnosis of B(F)NIS, 1 of BIS, 3 patients have a severe developmental encephalopathy (with seizures with neonatal, infantile, and early-childood onset, respectively), 2 patients have GEFS+ with partial seizures. Two cases (one with GEFS+ and one with BFNIS) developed by the age of 5 years a second epilepsy phenotype: an Epileptic Encephalopathy with Status Epilepticus during Sleep (ESES), with atypical absences and inhibitory phenomena in one. At last follow-up all patients are still alive: 5 (aged between 3 and 22 years) are seizure free without treatment, 3 (aged between 2 months and 3 years) have a good pharmacological seizures control; in the last 3 (3, 11 and 16 years old) the seizures persist despite of treatment.
CONCLUSIONS: Our data confirm the great heterogeneity of SCN2A spectrum epilepsy reported by previous studies. However, we documented the relatively frequent recurrence of the GEFS+ phenotype, and confirmed the possible appearance of ESES, as a complication of different initial phenotypes. Furthermore, we demonstrated that ESES in those patients is a true EE (and not a mere EEG pattern) by documenting their long-term neurocognitive evolution.

Myoclonic Status and SCN8A mutations

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SCN8A mutations have been associated with a broad spectrum of epilepsies (Larsen et al. 2015; Gardella et al. 2016). Often they are associated to clinical phenotypes characterized by developmental delay evolving into severe intellectual and neurological disability, early onset intractable seizures leading to an epileptic encephalopathy. Movement disorders are also very frequent such as hyperekplexia, extrapyramidal movement disorders (dystonia, dyskinesia and choreoathetosis) startles and cortical myoclonus. Myoclonic status in non-progressive encephalopathies is a Developmental Epileptic Encephalopathy characterized by the recurrence of long-lasting atypical status epilepticus associated with attention impairment and sub-continuous polymorphous jerks mixed with other complex abnormal movements (Dalla Bernardina et al.2015). We describe three patients (2 F. 1 M.) aged between 16 and 29 years with a SCN8A de novo mutation suffering from intractable focal motor seizures and generalized motor seizures at the age of 2, 3, 11 months. In two cases several a day Atypical Absences with myoclonic component appeared at the age of 7 and 10 months. Long-lasting Myoclonic Status recurring through the evolution appeared from the ages of 15,16 and 19 months. No A.A. have previously described this peculiar Myoclonic Status in carriers of SCN8A mutation, but when analyzing the iconographic data reported by Ohba et al 2014 and Kong et al 2015 we found some cases with features very similar to ours. We stress the importance to look at the possibility that the long-lasting Myoclonic Status, remaining misdiagnosed or misinterpreted as abnormal movements, can strongly contribute to the progressive cognitive and neurological worsening. D., Fontana E., Mastrangelo M., Fiorini E., Russo F., Spaccini L., Dalla Bernardina B., Darra F.

The phenotypic spectrum of SCN2A-related epilepsy

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Introduction
Mutations in *SCN2A* are reported in a broad spectrum of epilepsies from benign familial neonatal-infantile seizures to severe epilepsy syndromes. In order to better define the electroclinical features and associated genotypes, we studied a cohort of mutation-positive patients.

Methods
We identified 22 patients from tertiary epilepsy clinics with mutations in *SCN2A*. Mutations were identified through (i) diagnostic multiple gene panel testing (SureSelectXT custom panel, MiSeq sequencing) and (ii) research whole exome sequencing studies with subsequent Sanger confirmation. Retrospective case note analysis with MRI and EEG review was undertaken.

Results:
Most patients (20/22) had seizure onset in the first week of life, though presentation was as late as 3 years of age (median 2 days of age). Within the cohort, we observed a wide range of electroclinical phenotypes, including Ohtahara syndrome, epilepsy of infancy with migrating focal seizures, familial neonatal-infantile seizures and non-specific early and later onset epileptic encephalopathies. Three patients had a severe movement disorder, and the majority had neurodevelopmental delay. Three patients presented with autistic features. Non-specific features on MR brain imaging included decreased white matter bulk, thin corpus callosum and ventricular enlargement. Most identified *SCN2A* mutations were missense variants, occurring *de novo* and affecting highly conserved amino acid residues.

Conclusions
Mutations of *SCN2A* are emerging as a significant cause of early onset epilepsy. The phenotypic spectrum of the *SCN2A*-epilepsies is broad, and therefore the approach of multiple gene testing via panels or whole exome sequencing is warranted. Further research is required to identify the additional genetic and epigenetic factors that determine severity of clinical manifestations and outcome.

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