Dravet Italia

Associazione Italiana
Sindrome di Dravet

residras

platform residras

dravet-registry.com

RESIDRAS Residras & Platform Residras

What is Residras and Platform Residras?

Residras is a specialized registry focusing on Dravet Syndrome and other syndromes related to mutations in the SCN1A and PCDH19 genes. Initially established in Italy, the registry has expanded to include an international platform known as Platform Residras, making it a crucial resource for both Italian and European medical communities.

The History of the Registry

Associazione Dravet Italia Onlus, in collaboration with a Scientific Medical Committee, created Residras. Initially, the registry included a limited number of Italian centers specialized in the diagnosis and care of Dravet Syndrome. After a successful pilot phase that helped refine the registry's operations, the initiative was expanded to include all Italian centers.

Building on this success, Dravet Italia Onlus launched the international "Platform-RESIDRAS," which shares the same dataset as the Italian registry but operates under a different Coordinating Committee.

Aim of Residras

The Residras registry aims to establish a comprehensive database through the collection of longitudinal, retrospective, and prospective data from both pediatric and adult patients. This data collection supports research into epilepsy syndromes related to SCN1A and PCDH19 mutations and serves several fundamental purposes:

- **Defining the Natural History**: Documenting the disease's progression over time.
- **Genotype-Phenotype Correlation**: Characterizing clinical variability through detailed analysis.
- **Epidemiological Data**: Gathering data on incidence, prevalence, and complications such as SUDEP (Sudden Unexpected Death in Epilepsy).
- **Treatment Efficacy and Safety**: Assessing the long-term impact of various treatments and identifying predictive factors for efficacy and adverse effects.
- **Genetic Factors**: Investigating genetic influences on prognosis.
- **Biomarker Identification**: Searching for biomarkers that can help evaluate the effectiveness of emerging therapies.
- **Promotion of Research**: Encouraging national and international collaborative research .
- **Quality of Life and Pharmacoeconomic Impact**: Analyzing the disease's impact on patients, focusing on the economic aspects, including costs.

This comprehensive data will enhance diagnostic, therapeutic, and care strategies, improving patient outcomes and allowing for large-scale genotype-phenotype correlations. The data is accessible under strict adherence to data protection laws and with the approval of the Management and Coordination Committee.

Why Do We Need a Registry?

A registry like Residras is vital for advancing scientific collaboration on rare diseases and orphan drugs. It provides essential data on the number of affected patients and their geographic distribution, improving the organization of healthcare services and enabling researchers to focus on the pathogenesis of these conditions, potentially leading to the development of new therapies.

A well-maintained registry promotes scientific research by offering a rich dataset to generate new correlations and studies. By fostering collaboration between European and non-European countries, the registry plays a critical role in coordinated international research efforts, particularly important given the rarity of these diseases.

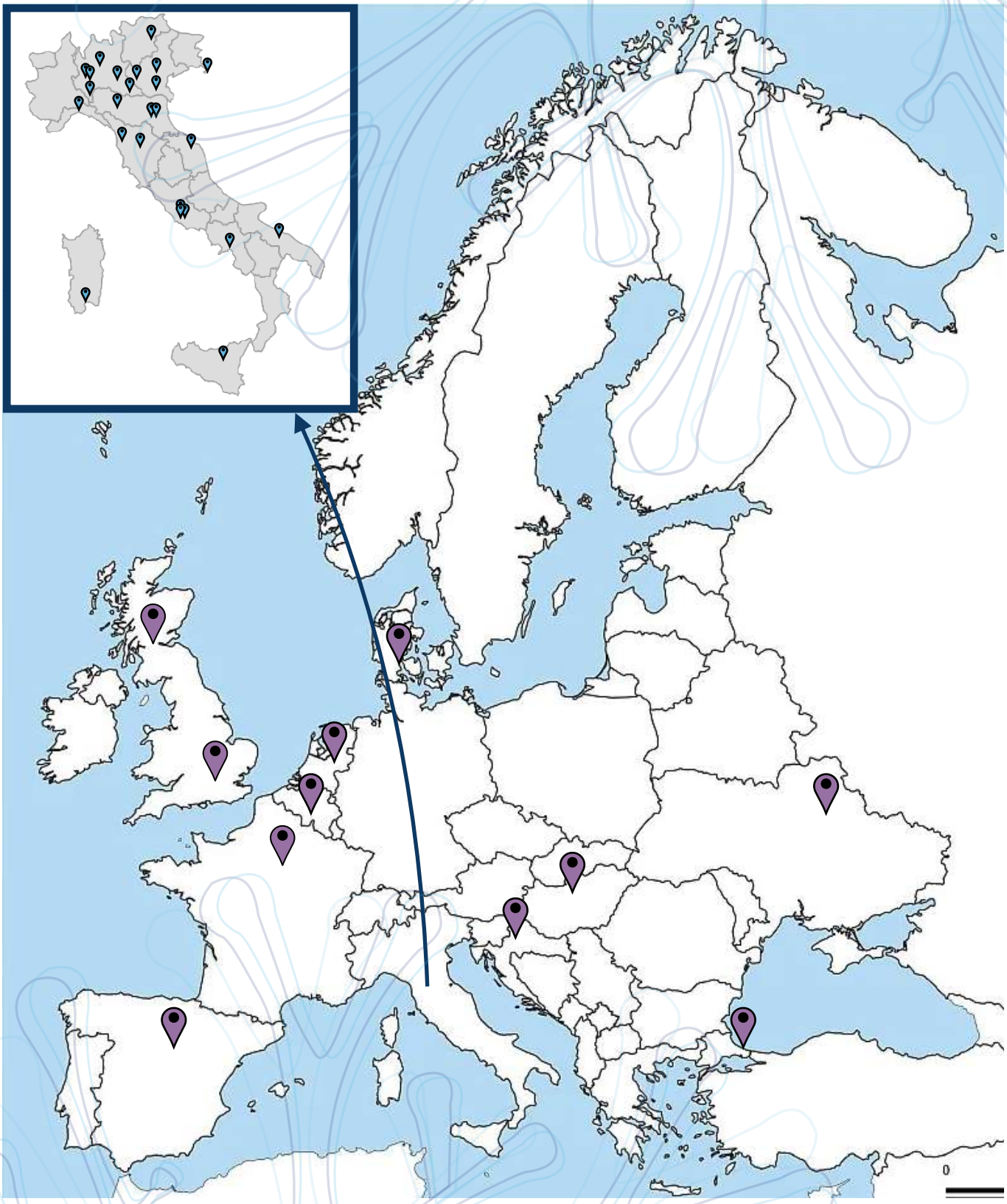
Access to the Data

Each participating center will have access only to its own data, with the possibility to conduct research and analyses on the broader dataset upon request. Data for research projects can be requested through the Coordination Committee via the official website (see below).

Contact Information

- **Website**: www.dravet-registry.com
- **Email**: info@dravet-registry.com

Engaged centers



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Pubblication

- **Istore: a project on innovative statistical methodologies to improve rare diseases clinical trials in limited populations.** Schoenen et al. Orphanet Journal of Rare Diseases (2024) 19:96.

- **A registry for Dravet syndrome: The Italian experience.** Balestrini S et al. Epilepsia Open. 2023;8:517–534.

- **Multicenter prospective longitudinal study in 34 patients with Dravet syndrome: Neuropsychological development in the first six years of life.** Battaglia D et al. Brain & Development 43 (2021) 419–430.

- **Efficacy and safety of Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: A real-world study.** Specchio N et al. Epilepsia. 2020;00:1–10.

- **Dravet syndrome: Early electroclinical findings and long-term outcome in adolescents and adults.** Darra F et al. Epilepsia. 2019;60(S3):S49–S58.

- **Clinical and genetic factors predicting Dravet syndrome in infants with SCN1A mutations.** Cetica V et al.Neurology 2017;88:1–8.

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POSITION STATEMENT

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Istore: a project on innovative statistical methodologies to improve rare diseases clinical trials in limited populations

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Abstract

Background The conduct of rare disease clinical trials is still hampered by methodological problems. The number of patients suffering from a rare condition is variable, but may be very small and unfortunately statistical problems for small and finite populations have received less consideration. This paper describes the outline of the iSTORE project, its ambitions, and its methodological approaches.

Methods In very small populations, methodological challenges exacerbate. iSTORE's ambition is to develop a comprehensive perspective on natural history course modelling through multiple endpoint methodologies, subgroup similarity identification, and improving level of evidence.

Results The methodological approaches cover methods for sound scientific modeling of natural history course data, showing similarity between subgroups, defining, and analyzing multiple endpoints and quantifying the level of evidence in multiple endpoint trials that are often hampered by bias.

Conclusion Through its expected results, iSTORE will contribute to the rare diseases research field by providing an approach to better inform about and thus being able to plan a clinical trial. The methodological derivations can be synchronized and transferability will be outlined.

Keywords Bias assessment with multiple endpoints, Finite populations, Multiple endpoints, Natural history modelling, Rare disease clinical trials, Similarity of subgroups

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Background

Currently, around 30 million people in Europe suffer from one of the around 7000 distinct Rare Diseases (RD). These diseases differ in prevalence, though most of them are very rare. It is therefore necessary to adopt a finite population sampling framework, unlike in non-rare conditions, where it is acceptable to think of a clinical trial as being sampled from an infinitely large population.

The iSTORE project on innovative statistical methodologies to improve rare diseases clinical trials in limited populations starts from acknowledging that there are hurdles for implementing an efficient clinical trial to evaluate new treatments in RD. Key such hurdles encompass insufficient knowledge about the natural disease course, uncertainty has how to compose a suitable primary outcome variable, optimizing the design for sensitivity to treatment effect, for example by linking the selection of a primary outcome measure to bias mitigating tools, and uncertainty as to how to show similarity of treatment effects across subgroups. These problems are general in RDs, although to a different extent from disease to disease. Thus, formulating solutions in terms of adequate statistical tools provides important contributions to RD research and reflects the ambitions of the International Rare Diseases Research Consortium (IRDiRC) [1], as well as the rare disease moonshot initiative [2]. In iSTORE, a toolbox of highly transferable methods will be developed along the use case of the Dravet Syndrome. Dravet is a prototype disease of developmental and epileptic encephalopathies (DEE), addressing the aforementioned challenges. However, iSTORE's developments are not limited to Dravet Syndrome but are aimed to be highly transferable to other diseases as well.

The iSTORE project is divided into four work packages. Work package 1 deals with the administrative and organizational work within the iSTORE project. Work packages 2, 3, and 4 focus on the development of innovative methodological approaches. Figure 1 provides an overview of the objectives, organization, and work flow of these methodological work packages. The paper is organized in six sections that describe the clinical problem, provide an overview of the project's methodological approaches and the challenges following the workstream in Fig. 1.

In "Clinical problem" section, we describe the clinical problem and data source. The data will be used for modeling the natural history of seizures and identifying similarity between subgroup and populations in "Modelling natural history data of DS" and "Identifying similarity between subgroup and the population". In "Statistical analysis of multiple endpoints" section, innovative methods for dealing with multiple endpoints are described. The last section is dedicated to evaluating the level of evidence from RCTs with multiple endpoints. In the discussion we focus on the expected results and impact.

Clinical problem

Challenges in rare diseases and specifically in developmental and epileptic encephalopathies (DEE) research: DEE are rare diseases characterised by their low prevalence [3], clinical, and genetic heterogeneity [4], complexity, and multifaceted features. The patients suffer from refractory epilepsy along with several other neurodevelopmental, psychiatric, and motor comorbidities [5, 6]. Developmental and epileptic Encephalopathies (DEE) are an epitome of rare diseases elucidating various of these clinical trials hurdles. A general challenge in DEEs research include patient heterogeneity, as they do not share a unique homogeneous phenotype, nor genotype [4], making homogeneous subgroups too small and impending choosing uniform endpoints, ending with subgroups with different therapeutic profiles and different product development requirements [7]. Other challenges are encountered while conducting randomized clinical trials in rare diseases following the regulatory requirement of a high level of evidence. These obstacles are predominantly related to the small number of subjects, a hurdle well exemplified by Gallin [8]. In their study, no less than 10 years are required to recruit 39 patients. Similarly, Rees [9] confirmed in December 2014 that 30.2% of RD CTs conducted between January 2010 and December 2012 were discontinued, with the most frequently reported reason being insufficient patient accrual. Other obstacles include the large spectrum of the phenotypes in RDs (although due to a single genotype) [10] and to a big gap in our knowledge of the natural history of the disease and patients reported outcome measures.

Due to the peculiarity of these DEEs, another challenge that emerges is the need to compare different clinically relevant subgroups and/or a subgroup with the whole population, specifically when assessing efficacy, safety, and tolerability of a newly developed treatment or drug. Comparisons and analyses of subgroups is an integral part of the clinical trials, and guidelines have been published in this regard [11]. But, unfortunately, in rare and ultra-rare diseases, given that analysis of subgroups selection within clinical trial datasets might not be informative, there is still this huge need for innovative methodologies of subgroups comparison. Additionally in DEE, there is frequently a requirement to evaluate similarity of profiles (such as, for example, number of seizures). Moreover, in view of the DEE's nature, vigilance is required on specific features of the longitudinal profiles, such as, for example, seizures' variability over a period or in relation to aging and missing data.

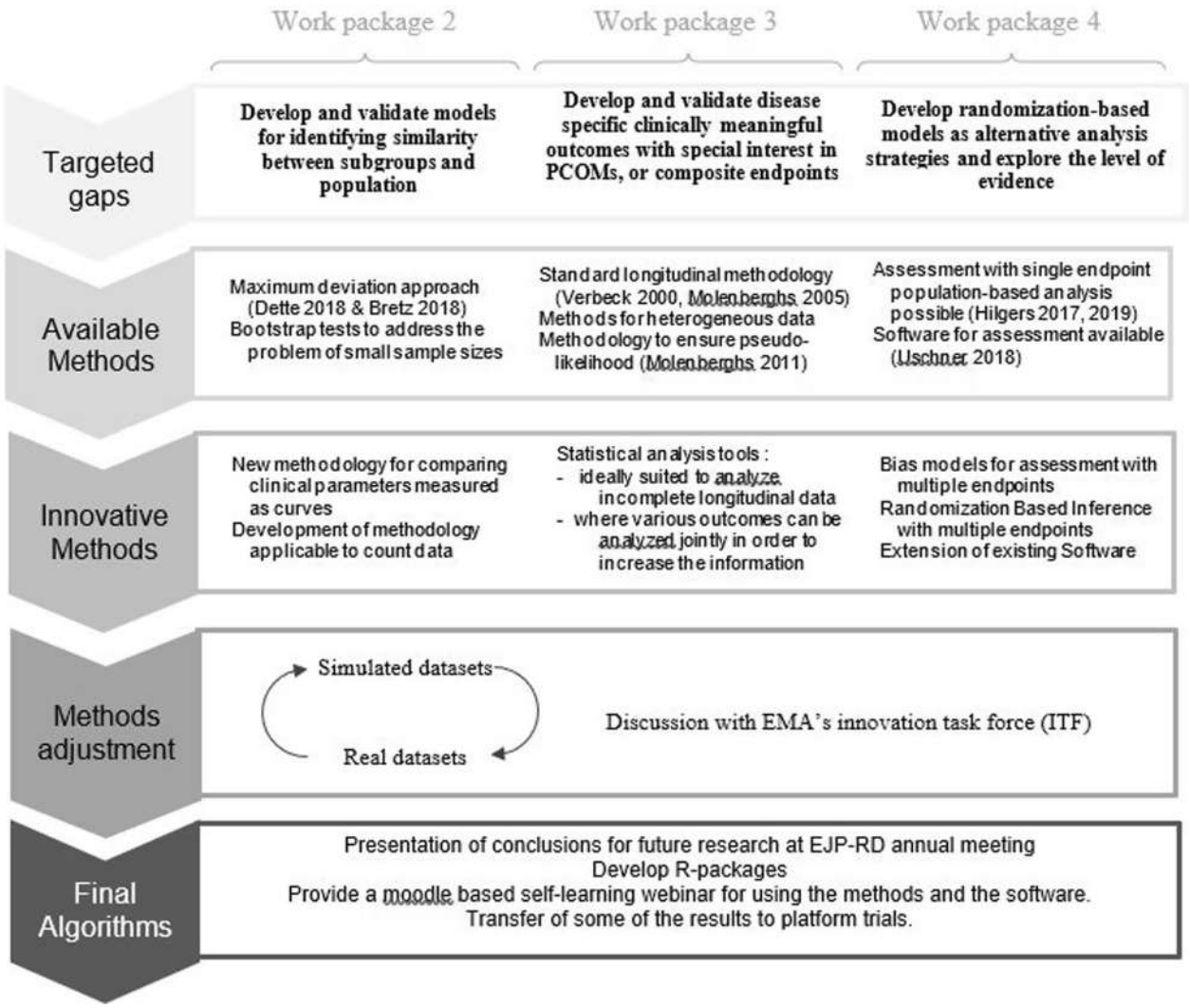

Fig. 1 Objectives, organization, and workflow of the methodological developments in the iStore project: the project is organized in three methodological work packages. Each of them will provide innovative statistical methods suitable for RD

Another challenge in DEE is the endpoints' true representability of different disease aspects. Therapies in DEEs, like for other epilepsies, are based mainly on anti seizure medications and are usually assessed through randomized controlled trials. Typically, the decrease over 50% of the mean seizure frequency compared to baseline is defined as primary endpoint [12–15], which is highly representative of seizure decrease but might be less meaningful for other symptoms of the disease. Families, patients, and physicians agree that the impact of these DEEs go beyond seizures [16–18] and trials for treatment evaluation should take into account other endpoints as well [19]. Consequently, the Food and Drug Administration (FDA) in 2009 [20] and the European Medicines Agency (EMA) in 2016 [21], have encouraged the concept of Patient Reported Outcomes (PROs) as self-assessment of affected individuals. Gradually, the use of PROs in clinical trials has increased significantly since 2005 [22–24]. PROs can be used to determine affected individuals' experience, particularly concerning improvement or aggravation of subjective symptoms, to stratify participants, to refine clinical trial design and to illustrate the risk-benefit balance allowing to choose the personalized best treatment [24]. These seem particularly necessary to effectively evaluate the impact of treatments in the field of rare epilepsy but also, more generally, in the field of rare diseases. And to further support use of endpoints that target what really matters for affected individuals, regulatory agencies recently finished the guidance about the use of composite endpoints and PROs [25].

Nowadays, various perspectives of patient outcome assessment, including the clinical outcome assessment, patient reported outcome, the clinician reported outcome, the observer reported outcome, and performance rated outcome [26], are considered appropriate. Specifically in DEEs, a collection of these outcomes are important to map the manifold responses to a treatment. So, including multiple outcomes in a properly selected combination appears to be a promising solution but requires quantification of the impact of bias on the level of evidence, both on the overall composite endpoint as well as on the individual component endpoints. This potential solution is crucial, especially because proof of efficacy in DEE clinical trials for these pathologies is sometimes difficult to provide. Currently, only four among the more frequent rare epilepsies have been subject to orphan drug development, namely Dravet syndrome (estimated prevalence 1:100,000), Lennox-Gastaut syndrome (10:100,000), infantile spams syndrome (12:100,000), and Tuberous sclerosis complex (3:100,000) [27].

Dravet syndrome (DS), a showcase of DEE: Dravet syndrome is a prototype of DEE and is a perfect showcase for these DEEs as it embodies all the challenges encountered in this epilepsy syndromes group, and thus an ideal candidate to test and apply the different innovative statistical methodologies. The onset of DS is usually during the first year (range 2–20 months) in a previously healthy infant. The seizure types and characteristics vary with age. Initially, they are either hemiclonic febrile and afebrile seizures, often alternating sides from seizure to seizure, or focal to bilateral tonic-clonic and/or generalized clonic seizures, and they are often prolonged. In preschool years, other seizure types often appear (myoclonic, focal impaired awareness, atypical absences, atonic, tonic or tonic-clonic) and by adulthood, brief tonic-clonic seizures, often occurring during sleep are most characteristic [6]. The seizures are commonly triggered by low grade fever, illness, vaccination, fatigue, photic stimulation, and visual patterns, and they are characteristically worsened with sodium channel blockers. Besides different age dependent seizures, patients with Dravet Syndrome will suffer from major non-seizure manifestations that are also age dependent and that include neurodevelopmental manifestations (intellectual disability, language delay, etc.), psychiatric disorders (autism spectrum disorders, attention deficit hyperactivity disorder, etc.) sleep disturbance (insomnia and other sleep disorders) and motor symptoms (crouched gait and acquired orthopedic malformations) [28]. These symptoms affect patients to variable degrees, culminating into a heterogenous group with a wide spectrum of symptoms. Thus, subgroups can be identified with different age of seizure onset, different combination of symptoms and/or comorbidities, different severity level of the manifestations, possible different genetic basis (although the majority have SCN1A mutation > 80%), or different genetic variant types in SCN1A. In each of these subgroups, with eventually a very small number of patients per group, different endpoints are of interest. Moreover, the similarities between subgroups as well with the whole population in terms of efficacy, safety, and tolerability of response to a new treatment needs careful reflection in the assessment. Additionally, it should be noted that Dravet Syndrome is a lifelong disease with evolution of specific comorbidities over time [16]. This requires the collection of longitudinal data on all symptoms.

A common problem with clinical routinely collected longitudinal data is missing data for various reasons. Of course, the problems arising from missing data can be expected to exacerbate with smaller sample as well as population sizes, both of which are strongly related to rare diseases. Missing data may be due to patients' non-compliance with their visit schedule, lost to follow up, incompleteness of information provided by patients and parents (who often provide proxy information), or physician's under-reporting or mis-recording of information previously recorded by the parents in their diary and other notes. Missing data constitute a main complication in relation to the operational domain of the registry (32%) [29]. Assessing response to any treatment in Dravet Syndrome should take into account the improvement, stability, or worsening of all the seizure and non-seizure manifestations evaluated by physicians and technology (devices), but also and more critically reported by patients themselves and their caregivers [30, 31]. This suggests the need to include various disease aspects in a tailored clinical trial endpoint, which may take the form of patient reported outcomes (PRO).

Data source - RESIDRAS register

We now briefly describe the data source, the RESIDRAS registry, that provides patient data on the Dravet Syndrom. The Associazione Dravet Italia Onlus [32], was established in Verona in 2010. The specific aim was to facilitate scientific research in Dravet Syndrome. For this purpose, a scientific committee created the national register "Registro Nazionale della Sindrome di Dravet e altre Sindromi correlate a mutazione dei gene SCN1A e PCDH19" (RESIDRAS). The Registry is an essential instrument to improve knowledge of the disease through the collection and systematic registration of patient information, with a constant flow of clinical data on patients. For every patient, there is at least a follow up of one year included. The RESIDRAS structure is used for the FP7 project "Research to improve diagnosis, prevention, and treatment in children with

difficult to treat Epilepsy" [33]. The aim of the Registry is to acquire epidemiological, clinical, and genetic information and make this available to the scientific community, to national health services, and to patients and their families in order to support an adequate programme in the diagnostic-therapeutic-assistance areas. In fact, the collection of patient data affected by the mutation of the SCN1A and PCDH19 gene could help to evaluate the real dimension of the problem and promote research, with the ultimate objective of offering improved assistance.

The Italian Registry model has been developed by a working group consisting of expert clinicians, members of the Scientific Medical Committee, representatives of patient associations, experts in DS and registries and information technologies useful for their implementation. The working group, after having identified the main aims of the registry, developed its structure and established 11 headings: Anagraphic Data; Genetic Investigations; Family History; Personal History; Onset of Epileptic seizures, Seizures Follow-up; Neurological and Cognitive Follow-up; Therapy; Adverse events; Gait Analysis and Grow and Cardio Parameters sheet. Each of these headings is composed of a number of variables, mandatory and optional. Due to the positive experience, Dravet Italia Onlus set up an international registry called "Platform-RESIDRAS". These two registries [34] have the same data set structure, but separate Coordinating Committees. The Registries have adopted the principles of Fairification (FAIR: Findable Accessible Interoperable Reusable). They are in line with the "Set of common data elements for Rare Diseases Registration". This is the first practical tool released by the EU RD Platform that aims to increase the interoperability of RD registry data, given that they contain 14 out of 16 data elements common to all rare disease registries in Europe, a key asset for further research [35]. The Registries will use the following ontology codes: Unified Medical Language, Human Phenotype Ontology, Orphanet Rare Disease Ontology, HPO ORDO Ontological Module. The Registries have received a monitoring report in order to assess the workflow and GDPR compliance. It is included in the ENCEPP Databases - The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance, a network coordinated by the European Medicines Agency. To date, a total of more than 650 patients have been entered in the registries, 400 in RESIDRAS and the other cases in the RESIDRAS platform. For the research within the iStore project we use an extract from the RESIDRAS registries at a fixed time point to develop and test the innovative methodological approaches. This data extract contains all patient data that has been collected in both registries up to this time point.

Modelling natural history data of DS

In this section, the methodological approaches are described alongside the challenges to tailor these to modeling natural history data in Dravet Syndrome. To flexibly and adequately describe longitudinal outcomes, specifically when they consist of various components, potentially of differing data types, the standard linear and generalized linear mixed models [36, 37] may not be sufficient. One then should consider existing extensions that accommodate overdispersion as well as correlation with sufficient flexibility [38–40]. The joint analysis of several longitudinal sequences of different types was examined by Ivanova, Molenberghs, and Verbeke [41]. In addition, the possibility of excess zeroes in count outcomes (e.g., number of seizures) should be accommodated if needed [42–44]. Some, but not all models yield directly marginally interpretable mean and/or correlation functions. If this is not the case, additional computations are needed [45–47]. Additionally, assessing model fit and the impact of potentially influential subjects is imperative [48–50].

For small datasets or datasets with long sequences of repeated measures, and/or datasets with a large number of different variables measured longitudinally, computational issues may arise, in the sense that the conventional likelihood and Bayesian estimation algorithms may fail to converge or may take an inordinate amount of time to do so. Pseudo-likelihood and related methodology have proven to be very useful in this respect [37, Ch. 9, 12, 21 and 24]. A so-called pairwise fitting approach for high-dimensional longitudinal data was developed by Fieuws and Verbeke [51] and Fieuws et al. [52]; see also Molenberghs and Verbeke [37, Ch. 25]. Further approaches consist of splitting the sample in sub-samples, analysing each of these separately, and appropriately combining the results [53]. These computational tools can be applied simultaneously as well, as was done by Ivanova, Molenberghs, and Verbeke [41]. Splitting samples becomes a bit more involved when cluster sizes are unequal, e.g., because longitudinal sequences are of unequal length. One then needs to carefully consider a weighting scheme to apply [54]. In some cases, and somewhat less well known, one can fall back on closed-form estimators, which of course do not suffer from convergence issues [55].

One reason why sequences may be of unequal length is the occurrence of incomplete data [56]. Whereas full likelihood methods are broadly valid when data are incomplete, i.e., they can be applied when missing data are missing at random (MAR), meaning that missingness, given covariates and observed outcomes, does not further depend on unobserved outcomes, this is no longer true when pseudo-likelihood or other semi-parametric methods are used [57], in which case weighting

procedures have to be applied, or alternatively the analysis has to be pre-processed using multiple imputation [58]. The trade-off between both approaches was investigated by Beunckens, Sotto, and Molenberghs [59]. An important advantage of multiple imputation is its efficiency and the fact that it helps stabilize computations. To examine the stability of the results, it is useful to apply multiple imputation on the one hand, and an ignorable analysis (for likelihood and Bayesian methods) or an inverse probability weighting based analysis (for other approaches). It is also possible to consider more than one imputation mechanisms, to investigate the robustness of the conclusions to imputation assumptions made.

Given that MAR cannot be established unambiguously based on the observed data, and hence that missing not at random (MNAR) (meaning that missingness, even given covariates and observed outcomes, still depends on unobserved outcomes), sensitivity analysis is called for as the capstone of any analysis of incomplete data [60]. Fortunately, a number of sensitivity analysis tools have been integrated with multiple imputation and are available, so that a set of sensitivity analyses under MNAR can be integrated seamlessly and compared with primary analyses under MAR.

Another frequently encountered issue regarding longitudinally observed count outcomes (e.g., seizure counts) might be the presence of a few very large counts and, generally, the presence of (extremely) skewed distributions. As a remedy, one may consider using a rank-based nonparametric approach (e.g., Burchett et al. [61], Dobler et al. [62]). In particular, a promising line of action would be the extension of these longitudinal non-parametric methods to also allow for missing data (e.g., Rubarth et al. [63]). Moreover, a closer examination of similar non-parametric approaches (e.g., generalized pairwise comparisons) in the context of the analysis of (multivariate) outcomes with (heavily) skewed distributions would be worthwhile. The research in this workstream can be based on previous work that has been conducted in the EBStatMax demonstration project, and on the substantial extensions for censored data [64] and suggestions for missing data [65].

Thus, our approach will investigate statistical analysis tools ideally suited to analyze incomplete longitudinal data, where various outcomes can be analyzed jointly, in order to increase the information extracted from the data.

Identifying similarity between subgroup and the population

A very particular challenge in Dravet syndrome is that disease progression is specific to age. To identify disease progression age specific parameters, the natural disease course of Dravet subjects in the RESIDRAS registry will be modeled with highly flexible models. iSTORE will develop tools for improving treatment evaluation starting with clinical outcome formulation, identification of subgroups, and improving the design and analysis of clinical trials. Extending the results of Dette et al. [66] we will develop bootstrap tests for validating the similarity of response profiles (for example, a parameter measured over time) between rare diseases subgroups and the entire population. From a theoretical point of view, we will show that our approach provides a statistically valid procedure and we will empirically verify, via simulations and data analysis, that it is particularly suited for small sample sizes. We also expect that the new procedures will be more powerful than tests based on common statistical principles such as the union-intersection principle [67, 68]. Consequently, our methods are particularly well suited for studying rare diseases such as the Dravet syndrome. The techniques are quite general and thus applicable to varying notions of similarity, which makes our approach useful for a broad range of applications. We illustrate the method in the context of drug development, where we develop tests for the similarity between dose-response curves of a subgroup and the overall cohort of patients in a clinical trial with continuous or discrete responses. As another application, we will consider testing the similarity of class proportions, where the classes could, for instance, represent disability scores. Testing for similarities could serve as an effective approach to merging the international RESIDRAS platform registry with the Italian national RESIDRAS registry. This is relevant as both registries may be influenced by geographical heterogeneities. Overall, showing similarity (of any kind) between the overall population of patients and a particular subgroup can lead to a better understanding of the disease under consideration.

Thus our approach will investigate new methodology for comparing clinical parameters measured as curves between subgroups. Extension of the methods to count data is the next step of our development.

Statistical analysis of multiple endpoints

Multiple clinically relevant endpoints can be tested for treatment effect between two groups, using different statistical analysis methods. In general, we distinguish between two approaches. In the first, each outcome is analyzed by separate statistical tests and the results are subsequently combined. In the second, the outcomes are first combined and subsequently analyzed in a single test. In either of these, it is very common to evaluate the treatment effect in each component of the multiple endpoint. This is sometimes even required by regulatory guidance.

The first approach encompasses the strategies of multiple primary endpoints, co-primary endpoints, and hierarchical testing. Testing multiple primary endpoints requires appropriate measures, such as Bonferroni corrections for multiple testing, to control the nominal type I error probability [69]. In contrast, the co-primary endpoint relies on an "all or none" decision rule, meaning that the treatment effect should be shown in all components of the endpoint simultaneously. The advantage is that then no type I error correction is required [25, 70]. Similarly, hierarchical testing, where the multiple components are tested in a prespecified sequence according to clinical relevance, until the first non-significant test result, does not require type I error corrections [25, 70]. Major disadvantages of these first approach strategies are that a single combined treatment effect measure is not available and that the correlation between the endpoints is rarely considered, although some advancement has been made in this direction [71, 72].

The second approach comprises the concept of composite endpoints and multi-component endpoints. While in composite endpoints dimensionality is reduced by considering the occurrence of any of the components in the endpoint (for example, the first occurrence), in a multi-component endpoint the components are combined within a subject to a single score or rating [25]. In both cases, the examination of a treatment effect regarding a specific component is challenging. Examples of the latter include, but are not limited to, clinical indices and joint modelling.

A special case that links the hierarchical idea with combining the outcomes first, is the generalized pairwise comparisons (GPC) methodology [73–76], which results in what can be called a prioritized endpoint. GPC is an extension of the pairwise comparison version of the Mann–Whitney [77] or Gehan-Wilcoxon [78, 79] tests to multiple outcomes. The most frequently used GPC test compares the outcomes prioritized by clinical severity, in all possible pairs of subjects, with one subject from each treatment arm. If in a pair a difference is established on an outcome, the subsequent outcomes are not further considered. This results in an analysis that gives more weight to more severe outcomes. This contrasts the commonly applied time-to-first event analysis of a composite endpoint, where the time of the event is weighted rather than the severity of the event.

Importantly, in GPC any number and any type of outcome can be combined. Moreover, the correlation between these outcomes is captured, without explicitly modelling it [76]. Although it has been applied mainly in large sample trials, the exact permutation test of a GPC endpoint, has good small sample properties [76, 80, 81].

Interestingly, several extensions of the prioritized GPC exist. The non-prioritized GPC evaluates each of the outcomes in all possible pairs [76, 82], following the idea of the non-parametric O'Brien test [83]. Additionally, extensions to longitudinal outcomes [84] and for N-of-1 trials [85] are available. Covariate adjustment in GPC is feasible through stratification [86], although in small samples the stratum size needs careful attention. Interestingly, both regulatory agencies FDA and EMA, have endorsed the GPC analysis of a prioritized endpoint as primary analysis for the approval of the drug tafamidis in the rare disease amyloid cardiomyopathy [87].

Another technique, which was recently introduced is the multidomain responder index [88], which sums the scores of responses, defined by a clinically meaningful change across all components.

Traditional techniques for comparing two groups on multiple endpoints and showing an overall positive treatment effect on all components of the multiple endpoint are the ordinary least squares (OLS) and generalized least squares (GLS) tests of O'Brien [83]. Applying both test statistics to the standardised components of the multiple endpoint results in a weighted sum of individual t-statistics of the endpoint components. Here, the OLS test uses equal weights whereas the GLS test uses unequal weights utilizing the estimation of the correlation matrix between the endpoint components. According to Logan and Tamhane [89], the OLS test is the more preferable one because it converges faster to a limiting distribution than the GLS test statistic. It should be noted that these test procedures, in contrast to multiple test procedures such as the Bonferroni procedure, take the correlation structure of the multiple endpoint into account.

Since the distributions of the OLS and GLS test statistics under the null hypothesis are only approximate, they can lead to an inflation of the type I error, especially in clinical trials with small sample sizes. Läuter [90] improved both test procedures and developed a sum statistic that takes the factorial structure between the components of the endpoints into account, which follows an exact t-distribution under the null hypothesis. Thereby, methods of elliptically contoured distributions were used [91].

Our investigation is focused on comparing statistical methodologies suited for the analysis of multiple outcomes, potentially with longitudinal profiles, on their small sample behavior and sensitivity to discriminate treatment effects on individual or joint endpoints.

Evaluation of level of evidence from RCTs with multiple endpoints

The fact that populations in rare diseases are limited in size suggest that tailored approaches are needed when conducting clinical trials, in particular with multiple

endpoints. As rare diseases show a large spectrum of different symptoms, the use of multiple endpoints is considered advantageous. However, it is unclear whether the inclusion of multiple endpoints will result in a gain of level of evidence and how to measure and quantify the impact of bias on the level of evidence in this setting. In particular in a randomized two-arm parallel group design with multiple endpoints regulators recognized that clinical trials "may be subject to unanticipated, undetected, systematic biases. These biases may operate despite the best intentions of sponsors and investigators, and may lead to flawed conclusions. In addition, some investigators may bring conscious biases to evaluations" [25]. The impact of (allocation) bias on the trial can be quantified by comparing the actual biased type I error rate with the nominal significance level. Adopting this approach, we aim to implement a model to quantify the allocation bias effect on the result of a randomized clinical trial with multiple endpoints based on the convergence strategy of Blackwell and Hodges [92] and Proschan's biasing policy [93].

The selection of the randomization procedure to mitigate bias and thus to increase the level of evidence is unknown in RD trials with multiple endpoints. We will consider a randomized single-center clinical trial in a two-arm parallel group design with a single time point, without interim assessment, and adopting analyses for different types of continuous multiple endpoints. We focus on different types of multiple endpoints as multiple primary endpoints and multi-component endpoints. The aim is to extend the bias model and the results for a single endpoint [94] to multiple endpoints and follow the recommendation in [95] with respect to the test statistics for multiple endpoints using population based inference. Firstly, we will derive an analytical model for the analysis procedures: Bonferroni, GLS-, OLS-Test and Läuter Test. This will be followed by a simulation study for the assessment of the level of evidence for multiple endpoints with population-based modelling in single center trials. Since rare disease clinical trials are often multi-center and international, the simulations will be extended to multi-center clinical trials in the next step. Therefore, a center effect term needs to be added to the model.

As the amount of allocation bias will vary between the "quality" of the endpoint components, our approach will allow the assessment of the level of evidence regarding different bias effects of the endpoint components. Thereby, we aim to provide a recommendation for the balance between the number of components in the multiple endpoints and the impact on the level of evidence under bias due to population-based modeling. When using sum statistics, as in the OLS and GLS tests, the number of endpoints influences the impact of allocation bias on test decisions. This is due to each endpoint introducing an endpoint-specific bias effect term to the test statistic [96]. Additionally, the approach will provide recommendations on the choice of randomization procedure based on the level of evidence. Our model approach will be embedded in the R-package randomizeR [97] to enable future analogous evaluations in similar disease areas. Overall, the derivations will aim to provide recommendations for the design of clinical trials with multiple endpoints in the field of rare diseases that increase the validity of the clinical trial by raising the level of evidence. Note that our approach can also be viewed as a basic concept that is transferable to platform trials as well.

In a second step, we will investigate a methodological approach for evaluating the evidence level of clinical trials with multiple endpoints in finite populations obtained by randomization-based inferences. The randomization-based inferences are particularly linked to the randomization procedure. Randomization-based models are not yet developed or embedded in a population based model approach. With multiple endpoints this becomes even more challenging.

Our investigation is focused on the development and implementation of a multi-component biasing policy enabling us to quantify the impact of bias on the test decision in a clinical trial with multiple endpoints.

Discussion

The following discussion will elaborate on the summary provided in Table 1. The developments in iSTORE can be viewed as a Comprehensive Toolbox necessary to understand the course of a disease, to identify important subgroups, to assess multi-dimensional outcomes including patient-centered outcome measures (PCOMs), and to optimize bias mitigating trial design in rare diseases. It should be pointed out, that in general the tools may be used separately, and depending on the disease, modification might be necessary. However, the consortium is convinced of the high level of impact of the tools or implemented roadmap.

Expected results

The methodologies developed by this consortium will fill some important gaps commonly identified in trials on rare diseases with limited populations. The Dravet Syndrome serves as a use case for the methodological development, but—as frequently encountered in statistical methodologies—the tools provide a high level of transferability on a case by case basis. First, the methodologies will contribute to ameliorating the evaluation of efficacy of novel treatment regimes, targeting more precisely what matters for patients, and taking into account comparative evaluations in patient sub-populations. Secondly,

Table 1 Comprehensive toolbox for understanding and improvement of developmental and epileptic encephalopathies

What is known	What is expected	Impact
Methods for testing similarity between distinct groups of patients	Methods for testing similarity between subgroups and overall population	Powerful methods for improved understanding of rare diseases and allowing extrapolation of information between groups
Linear and nonlinear mixed effect modeling	Tailored approaches for modelling count data in limited populations to model longitudinal data of Dravet patients	Understanding of flexible modelling of natural history course data
Several statistical methodologies are capable of evaluating multiple endpoints in a single analysis	Recommendations of statistical methodologies to analyzing multiple endpoints of potential different data type in small sample trials	More efficient analysis of randomized trials with multiple endpoints in small sample trials
Quantification of impact of bias on the level of evidence in two arm parallel group design with single endpoint	Extension of the bias model to multiple endpoints corresponding to the analysis	More efficient randomized trials with multiple endpoints: Optimize trial designs with respect to level of evidence in case of multiple endpoints

the novel approaches will address a problem common to many longitudinal studies, namely the occurrence of incomplete data. This will allow for a valid statistical analysis of numerous cohorts studies whose conclusions are affected by large amounts of missing data. Therefore, the project team will propose solutions to methodological challenges that have not been satisfactorily addressed so far. On the other hand, in addition to the considerable impact in the field of biostatistical research, these new methodologies will also lead to improved analyses of concrete datasets from studies on rare epilepsies (e.g., a further prospective trial based on the RESIDRAS registry). Thus, the data of the RESIDRAS registry are essential to define i.e. meaningful endpoints and subgroups of similar disease characteristics for a future prospective clinical trial Moreover, through the involvement of some consortium partners in the EpiCare network, we will foster the idea of designing more efficient and patient-friendly therapeutic trials for rare epilepsies in the near future. The effectiveness of these therapeutic trials will focus on what matters most to patients and optimize design and analysis, thereby increasing the level of evidence. To facilitate the application of the proposed methods, open access software will be provided, along with corresponding instructional videos.

Transferability

The new statistical methodologies developed in this project, although adapted to rare epilepsy trial approaches, can be easily transferred to almost all rare diseases. For example, counts are frequently used as primary outcomes not only in epilepsy, but also in epidermolysis bullosa (i.e., the reduction of blister numbers compared to baseline, see [98]). Moreover, heterogeneity of patients is often present (for example, due to different underlying genotypes). Therefore, it is valuable to have methods at hand that account for this heterogeneity by, for example, allowing for comparisons of subgroups to the overall population with a specific RD. Indeed, such approaches will be developed in the proposed collaborative project. Furthermore, in RD research in general, outcomes are often assessed longitudinally, in order to increase power in genuinely small populations, and to obtain conclusions about the natural history of the disease. However, the burden for patients when participating in a study is usually substantial, especially in rare diseases. Therefore, the amount of missing data is expected to be considerable, and merely excluding subjects with missing data might seriously affect the statistical analyses, given that the sample sizes are already low. Therefore, the new methodologies developed in the corresponding work packages on longitudinal data analysis in presence of missing data would not only resolve these issues with respect to rare epilepsies but serves as a solution to a problem that stems from the very nature of RD data. Last but not least, developing multi-component endpoints that truly capture what really matters to patients and their families is highly needed by patient representatives in any rare disease.

Operationally, the transfer of the project findings to other RD areas will be facilitated by the fact that several partners involved in the present project are already participating in the activities of various European networks on rare diseases (ERN), in particular ERN skin for rare skin diseases (G Zimmermann and G Molenberghs), the ERN EpiCare for rare and complex epilepsies (R Nabbout, I Brambilla, G Zimmermann), and EJP-RD (R Nabbout, RD Hilgers, G Molenberghs, G Zimmermann) and ERICA, the European Rare Disease Research Coordination and Support Action consortium (RD Hilgers). Moreover, as already mentioned above, the highly interdisciplinary composition of the project team (international partners from academia, clinical research, industry, and patient networks) and existing links to EMA and FDA will foster transferability and visibility of the project outcomes beyond the scope of rare epilepsies. Providing open-access and open-source software implementations along with manuals and tutorial videos will further enhance the use by various stakeholder groups within the rare diseases community. Of course, the project outcomes will also be circulated in the scientific community by publishing papers on the most important findings and presenting the novel methodological development at international conferences. This might also increase awareness of the methodological challenges related to rare diseases among biostatisticians, thereby attracting more researchers to dedicate their workforce to improving statistical techniques for analyzing RD data.

Abbreviations

DEE	Disease of developmental and epileptic encephalopathies
EMA	European Medicines Agency
ERICA	European rare disease research coordination and support action
ERN	European reference network
FDA	Food and Drug Administration
GDPR	General data protection regulation
GLS	Generalized least squares
GPC	Generalized pairwise comparisons
IRDiRC	International rare diseases research consortium
iSTORE	Innovative statistical methodologies to improve rare diseases clinical trials in limited populations
MAR	Missing at random
MNAR	Missing not at random
OLS	Ordinary least squares
PROs	Patient reported outcomes
RCT	Randomized controlled trial
RD	Rare Diseases
RESIDRAS	Registro Nazionale della Sindrome di Dravet e altre Sindromi correlate a mutazione dei gene SCN1A e PCDH19

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Availability of data and materials
The data that support the findings in this paper are available on request. RESIDRAS registry is not public available. Please contact IB.

Deklaration

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Not applicable.

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The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

A registry for Dravet syndrome: The Italian experience

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Abstract

Objectives: We describe the Residras registry, dedicated to Dravet syndrome (DS) and to other phenotypes related to *SCN1A* mutations, as a paradigm of registry for rare and complex epilepsies. Our primary objectives are to present the tools and framework of the integrative platform, the main characteristics emerging from the patient cohort included in the registry, with emphasis on demographic, clinical outcome, and mortality.

Methods: Standardized data of enrolled pediatric and adult patients were collected in 24 Italian expert centers and regularly updated at least on a yearly basis. Patients were prospectively enrolled, at registry starting, but historical retrospective data were also included.

Results: At present, 281 individuals with DS and a confirmed *SCN1A* mutation are included. Most patients have data available on epilepsy (n = 263) and their overall neurological condition (n = 255), based on at least one follow-up update. Median age at first clinical assessment was 2 years (IQR 0–9) while at last follow-up was 11 years (IQR 5–18.5). During the 7-year activity of the registry, five patients died resulting in a mortality rate of 1.84 per 1000-person-years. When analyzing clinical changes over the first 5-year follow-up, we observed a significant difference in cognitive function ($P < 0.001$), an increased prevalence of behavioral disorders including attention deficit ($P < 0.001$), a significant worsening of language ($P = 0.001$), and intellectual disability ($P < 0.001$).

Significance: The Residras registry represents a large collection of standardized national data for the DS population. The registry platform relies on a shareable and interoperable framework, which promotes multicenter high-quality data collection. In the future, such integrated platform may represent an invaluable asset for easing access to cohorts of patients that may benefit from clinical trials with emerging novel therapies, for drug safety monitoring, and for delineating natural history. Its framework makes it improvable based on growing experience with its use and easily adaptable to other rare and complex epilepsy syndromes.

KEYWORDS
epilepsy syndrome, natural history, rare disease, registry, SCN1A

1 | INTRODUCTION

Dravet syndrome (DS) is one of the most common developmental and epileptic encephalopathies (DEEs) with an incidence of 6.5 per 100 000 live births (95% CI 3.2–10.00).[1] Its hallmark clinical presentation is with prolonged, febrile and afebrile, generalized clonic or hemiclonic seizures with onset within the first year of life. Other seizure types with later onset include myoclonic, atypical absence and focal seizures. Episodes of seizure clusters and status epilepticus are frequent. In addition to seizures, most children, often during late infancy or early childhood, manifest cognitive, motor, and behavioral impairment.[2] DS is caused by loss-of-function pathogenic variants in the gene coding for the α1 subunit of the sodium channel (SCN1A), which is

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important for action potential initiation in inhibitory GABAergic interneurons.[3] There is a wide spectrum of clinical entities caused by *SCN1A* mutations ranging from genetic epilepsy with febrile seizure plus (GEFS+) to DS, with variable disease course even within the core DS phenotype.[4]

Epilepsy is treatment-resistant although the seizure burden is highly variable. As in all severe DEEs, the most acceptable clinical poise should be individually established based on a balance between reduction in seizure severity and frequency and minimisation of treatment-related adverse effects.[5] Controlled trials have demonstrated antiseizure efficacy of stiripentol in association with clobazam and of add-on cannabidiol and fenfluramine.[6-9] However, there are no current treatments that address the overall disease, in addition to seizures. Precision medicine approaches such as antisense oligonucleotides (ASO) and adeno-associated virus (AAV)-delivered gene modulation are potential treatment options for DS, which are currently being investigated on a research basis, with some now transitioning to clinical trials.[10]

Disease course is variable and not fully characterized, and the extent to which prompt treatment with the most effective medications can alter prognosis is unclear. The few available studies of outcomes in adulthood show that epilepsy severity progressively decreases from childhood to adolescence and throughout adulthood, and reduced frequency of convulsive status epilepticus is associated with better seizure outcome.[11] On the other hand, persistence of seizures in adolescents and adults correlates with cognitive and neurologic impairment, and there is ongoing cognitive dysfunction in adulthood, independent of seizure control. Early onset of seizures, especially myoclonic, correlates with the severity of intellectual disability and language impairment.[12,13] Most adults with DS require a considerable amount of support and are unable to live independently.[14-16] Also, the incidence of premature mortality, including sudden unexpected death in epilepsy (SUDEP), is elevated in childhood, but data on this ominous outcome are lacking in adulthood.[17,18]

Although DS is likely the most studied genetic DEE and is the target of regulatory trials for orphan drug designation, there is still limited knowledge on several clinical aspects including natural history, risk of comorbidities, mortality, treatment response, and safety. Such knowledge becomes crucial for developing novel treatment strategies especially in view of identifying the potential for disease modifying therapeutic interventions in the precision medicine framework. In this perspective, disease registries represent an ideal tool to move forward in research and improve knowledge in the field of rare diseases.[19]

Key Points

- The Residras registry aims to gather a large collection of standardized national data of patients with Dravet Syndrome.
- The registry platform relies on a shareable and interoperable framework, which promotes multicentre high-quality data collection.
- Such integrated platform could be easily adaptable to other rare and complex epilepsy syndromes.

The Italian Registry of Dravet Syndrome and Other Syndromes correlated with *SCN1A* and *PCDH19* mutation (Residras) was established to provide an integrative infrastructure for collection of standardized molecular and clinical data of patients with DS from national centers[20] where they are diagnosed and regularly followed up.

The primary purposes of this study are to present: (a) the tools and framework of the integrative platform; (b) the main characteristics of the patient cohort ie, demographic, clinical outcome, and mortality; (c) the concept of the Residras registry, which we progressively adapted to the emerging diagnostic and therapeutic challenges of an epilepsy syndrome under intense clinical investigation; and (d) to set the basis for a wider international reach of the registry, which is now being adopted in additional EU countries.

2 | METHODS

2.1 | History of the registry

The "Residras" project started in 2010 under the auspices of the Scientific Committee of Dravet Italia Onlus,[21] an association of patients and physicians, focusing on research on DS and related syndromes. The Scientific Committee identified a minimum of mandatory items to be included in the dataset. Members of the Scientific Committee participated with their centers in a pilot phase. Meyer Children's Hospital, in Florence, was selected as the coordinating center. Fondazione Monasterio, a public institution for healthcare research[22] with a particular experience in rare diseases registries, was identified as a partner for the platform creation, data storage, confidentiality, and management of privacy. By June 2013, the dataset for the pilot phase was ready, and

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the first patients were enrolled to test the process of data inclusion in the platform. Once the process was improved in terms of consistency, reliability, and ease, pilot centers started to enroll all their patients, either new or in follow-up, and in April 2015, Residras was opened to all Italian centers upon their specific request and ethics committee approval.

2.2 | Current format of the registry—coverage, interface for clinicians, data use, and research applications

Registry coordination is entrusted by a Scientific Advisory Committee (10 expert clinicians on DS), an external Steering Committee for management (Fondazione Monasterio), an external Advisor on Rare Disease (National Centre on Rare Diseases), a patient association (Dravet Italia Onlus), and three patients' representatives.[20]

Twenty-four centers are currently participating to data input (Figure 1). Written informed consent or assent was provided by adult patients with capacity to consent or by families or legal guardians for children and adults lacking capacity.

The Residras registry contains clinical and epidemiological data that are compiled on a standardized online template during regular clinical visits. The information is schematically divided into the following sections: demographic features (date of birth, gender, parental details, place of birth, place of residence, willingness to be contacted on a regular basis to update the registry with longitudinal data and participate in a future clinical study), medical history (including pregnancy, delivery, neonatal period, neurodevelopment, neurological examination), clinical and genetic diagnosis, family

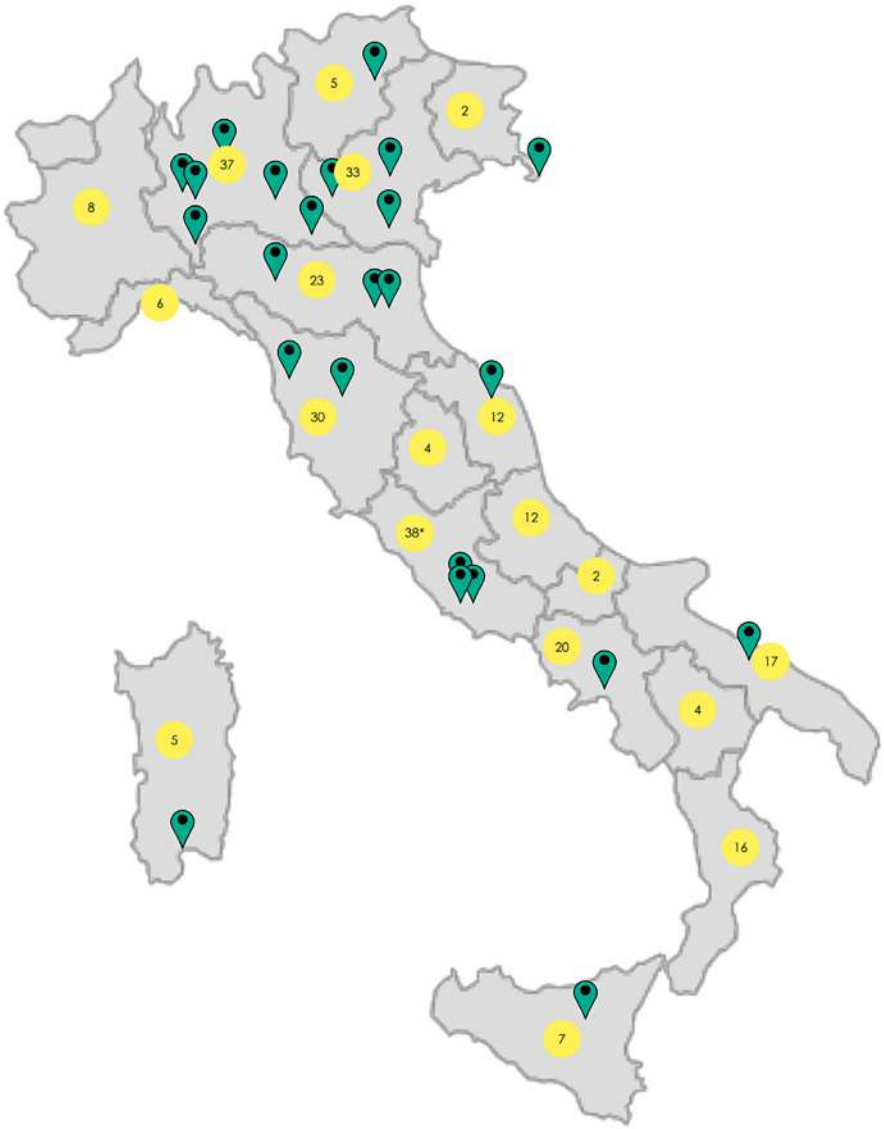

FIGURE 1 Coverage of Residras with illustration of the participating centre distribution (green pins) and number of enrolled patients who are resident in each italian region (yellow circles). *Patients resident abroad (n = 2).

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history, age at seizure onset, and mortality. Entries for longitudinal follow-up include seizure assessment (ie, type, frequency, episodes of status epilepticus, hospital admissions), neurological assessment (ie, examination, cognition, behavior, formal neuropsychological testing), treatment (ie, type, max dosage, duration, response, adverse events), EEG (ie, date, background activity, epileptiform abnormalities, photosensitivity, recorded seizures), and other investigations (eg, neuroimaging). Seizure assessment was based on clinical criteria, and seizures were classified as hemiclonic, generalized motor (including tonic-clonic and clonic), absence, focal-onset, reflex, massive (ie, generalized) myoclonus, and action myoclonus (ie, triggered by voluntary movement).[23] Status epilepticus was defined as per the latest International League Against Epilepsy (ILAE) definition.[24] A descriptive assessment of cognition and behavior was based on indicators of everyday functioning, informal cognitive tasks (eg, biographical info, remembering objects, making a judgment, playing with toys), contextual information (eg, language and education level), and presenting mental state (behavior, orientation, speech, mood, and perception). On this basis, cognitive function was classified by the clinician, into the following categories: normal, borderline, mildly impaired, moderately impaired, and severely impaired. Schooling progress was established based on the reported educational level and classified as normal, mildly reduced, moderately reduced, severely reduced, or absent, based on the level expected for the patient's age. Assessment of intellectual disability and language function was based on formal cognitive testing and classified as normal cognitive function, borderline, mild, moderate, severe, or profound. Formal cognitive assessment was performed according to age and verbal function, using one or more of the following neuropsychological tests: Brunet-Lezine, Griffiths mental development scale, Bayley Scales of Infant and Toddler Development (Bayley-III), Leiter International Performance Scale-Revised (Leiter-R), Raven's Coloured Progressive Matrices, Stanford-Binet test, Wechsler Preschool and Primary Intelligence test (WPPSI), Wechsler Intelligence Scale for Children (WISC), Wechsler Adult Intelligence Scale | Fourth Edition (WAIS-IV), and Mini-Mental State Examination (MMSE). At the first visit, a total of 178 patients (70%) were assessed by neuropsychological tests, while the number of patients with available formal testing decreased at subsequent follow-up, as shown in Table S1. Autism spectrum disorder was formally diagnosed through administration of the Autism Diagnostic Observation Schedule (ADOS);[25] autistic spectrum symptoms were defined when only some of the diagnostic criteria listed in the Diagnostic and Statistical Manual of Mental disorders, 5th edition (DSM-5), were present;[26] Defiant, disobedient, or disruptive behavior was classified as "behavioral abnormalities." Diagnoses of attention deficit, with or without hyperactivity, or obsessive-compulsive disorder, were defined according to the International Classification of Diseases 10th Revision (ICD-10). Unavoidably, data were not systematically collected at the same time points for all patients, and not using the same psychometric tools and scales in all centers, given the intrinsic design of the Registry, the different ages at first inclusion in the registry, and the inconstant availability of direct neuropsychological expertise in the various sites.

As a quality control step, a set of mandatory fields need to be filled to save information provided in each section. Additional quality control elements include a centralized system to check for duplicate case enrolment. Residras applies the principles of Findability, Accessibility, Interoperability, and Reusability (FAIR) for humans and computers,[27] thus enabling efficient analysis of data across multiple sources and making data "as open as possible and as closed as necessary."[28] The platform has been customized for data collection specific to DS and includes 14 out of 16 Common Data Elements (CDEs) for Rare Diseases Registration released by the Joint Research Centre of the European Commission.[29] The two elements not included are 6.3 (undiagnosed cases are not included) and 7 (there is no collection of biological samples). The ontology codes used include Unified Medical Language System (UMLS),[30] Human Phenotype Ontology (HPO),[31] and Orphanet Rare Disease Ontology (ORDO).[32]

To access the system, each user is assigned a personal username and password. The online input and access to the data are restricted to healthcare practitioners from each center. The access codes are generated by administrators once the user has signed a written agreement. Healthcare practitioners have an online and secure access to patients' data. Only fully anonymized data are available to researchers and for analysis—all enrolled participants have a registry identification code, which is automatically generated.

Investigators from expert centres who wish to access data for clinical research purposes are required to submit a research protocol to the internal Scientific Committee. When unanimous approval is obtained, the proposing team can access a subset of anonymized data depending on the study requirements.

The interface layout has been designed ad hoc to facilitate navigation and allows the use of various tools integrated into the system. The Residras home page describes *SCN1A*-related conditions, the Residras platform (including aims, participating centres, statistical data, instruction

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to accredit a new center), and news in the field of *SCN1A*-related disease.[20] Healthcare practitioners can access with their personal login and complete or create a new patient follow-up. Patient health is summarized in a dashboard that helps physicians to have a detailed overview of the collected longitudinal data and to edit synopsizes or medical reports.

A diagnosis of DS is made based on the clinical definition and is distinct by other SCN1A-related epilepsies.[2] Although inclusion of patients in Residras is based on clinical criteria, for the purpose of this study, we only considered DS individuals with a proven *SCN1A* mutation, classified as pathogenic or likely pathogenic according to the international guidelines of the American College of Medical Genetics and Genomics (ACMG).[33]

2.3 | Statistical analysis

We present data as absolute number and percentage for categorical variable and median and interquartile range for continuous variables. We assessed clinical differences between first visit and after 5-year follow-up (Tables 1 and 2), and between first and last visit (Tables S2–S5), using the Chi-square test for independent data. We analyzed patterns of changes in neurological condition and epilepsy features between first visit and after 5-year follow-up, after stratifying patients by age at first visit (Tables 3 and 4). When sensitivity analyses were performed using McNemar test for paired data, for subjects reporting both first and last visit data, results did not change. The mortality rate was calculated as number of observed events (death) divided by the person time at risk. Alluvial diagrams were used to illustrate the flows of variation in clinical conditions, from the first visit (time 0), every 6 months until the fifth year of follow-up (time 60) (Tables 1 and 2), and after stratification by age at first visit (Figures S1–S22); each bar shows the frequency distribution of the variable over the corresponding time, change in the condition over time is represented by the flow stream direction going through different colors.

3 | RESULTS

Although Residras has a nationwide coverage (Figure 1), patients' geographic distribution remained non-homogeneous with a lower proportional representation from the South of Italy. This is mainly related to the habit of families of seriously ill patients residing in the South to reach hospitals of the northern regions, seeking for a second opinion, and does not have any geographic epidemiological implication. To date, 281 individuals with DS with a confirmed *SCN1A* mutation have been enrolled. There are available data on epilepsy (n = 263) and on the overall neurological status (n = 255), with at least one follow-up for most patients (Figure S23). Historical retrospective data were also included, when enrolment date was during a follow-up visit and the patient had not been previously included in the registry or when a diagnosis of DS was made at a later time after the first clinical evaluation. The time at the first visit was defined as the time at the inclusion in the registry for all patients, including the ones with enrolment date after first clinical assessment.

Median follow-up from inclusion in the Registry was 5.5. years (2.8–11.3). Median age at the inclusion in the Registry was 2 years (IQR 0–9) while the median age at last follow-up was 11 years (IQR 5.5–17). During the 7-year activity of the registry, five patients with a diagnosis of DS had died, with a mortality rate of 1.84 (95% CI 0.77–4.42) per 1000-person-years (median age at death 6.3 years, range 2.5–23.4). The causes of death were diverse and included status epilepticus, cerebral hemorrhage, SUDEP, acute encephalopathy of unknown cause (with onset 40 days before death), and brain tumor.

There was a significant difference in cognitive function over the first 5 years of follow-up ($P < 0.001$) with evidence of a lower cognitive level after 5 years in the majority (median time to first deterioration in cognitive level 1.5 years, IQR 0.9–2.4). There was a significant change in the schooling progress over time; in particular, we observed increased prevalence of poor schooling progress (from 17.6% at first visit to 45.5% after 5 years) ($P < 0.001$). There was an increased prevalence of behavioral disorders over time including attention deficit ($P < 0.001$) and autism spectrum symptoms ($P = 0.04$). Prevalence of neurological examination abnormalities increased at last visit ($P < 0.001$) in most patients (median time to first deterioration 1.6 years, IQR 0.9–2.4), including ataxia ($P < 0.001$) and gait abnormality ($P = 0.01$).There was also a significant worsening of language ($P = 0.001$) (median time to first decreased level 1.4 years, IQR 0.8–2.3) and intellectual disability ($P < 0.001$) (median time to first decreased level 1.5 years, IQR 1.0–2.3), where these were formally assessable.

Significant differences related to the epilepsy features over the first 5-year follow-up included an increased prevalence of generalized seizures ($P = 0.03$) and reduction of hemiclonic seizures ($P = 0.001$). The main clinical variables included in the analysis and their variation over the first 5-year follow-up are illustrated in Tables 1 and 2. The pattern of improvement, stability, or deterioration related to each clinical variable is summarized in Tables 3 and 4, and Tables S4 and S5. The main clinical variables stratified by age group and clinical differences between first and last follow-up are illustrated in the Figures S1–S22. Different antiseizure treatments were used over time, with the longest treatment duration observed for valproate, clobazam,

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TABLE 1 Neurological condition at first visit and after 5-year follow-up with alluvial diagrams showing variation over time.

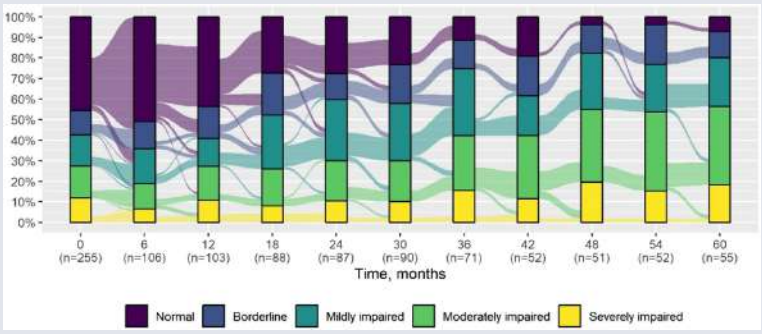
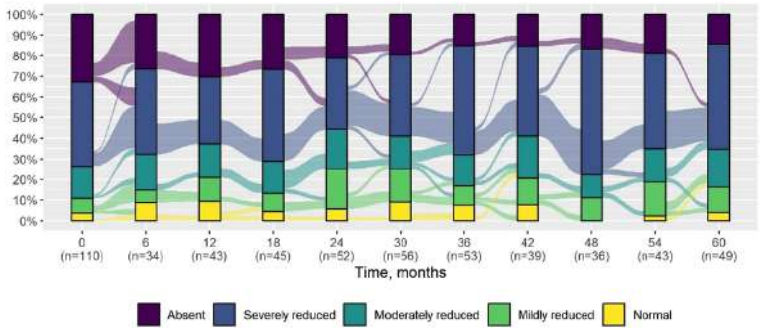
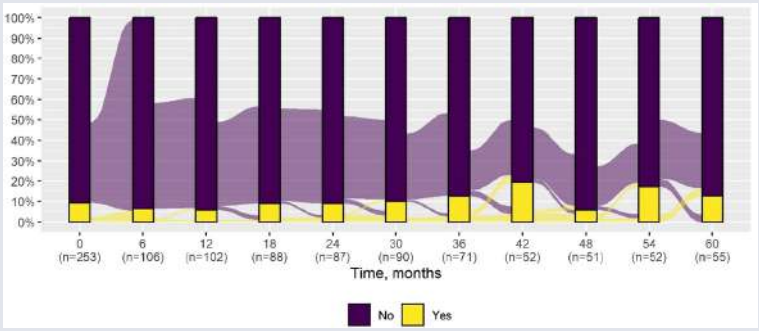
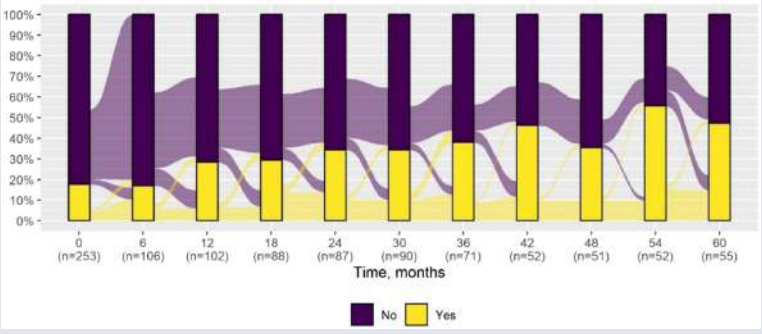
	First visit	Last visit at 5th year	
N	255	55	
Gender			
Females	132 (51.8)	27 (49.1)	
Age			
Median (IQR)	2.0 (0–9.0)	6.0 (5.0–9.5)	
Cognitive function*			
Normal	116 (45.5)	4 (7.3)	
Borderline	30 (11.8)	7 (12.7)	
Mildly impaired	39 (15.3)	13 (23.6)	
Moderately impaired	40 (15.7)	21 (38.2)	
Severely impaired	30 (11.8)	10 (18.2)	
Schooling progress*			
Normal	4 (3.6)	2 (4.1)	
Mildly reduced	8 (7.3)	6 (12.2)	
Moderately reduced	17 (15.5)	9 (18.4)	
Severely reduced	45 (40.9)	25 (51.0)	
Absent	36 (32.7)	7 (14.3)	
Not applicable	145	6	
Behavior*			
Oppositional defiant disorder	24 (9.5)	7 (12.7)	
Attention deficit*	45 (17.8)	26 (47.3)	

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TABLE 1 (Continued)

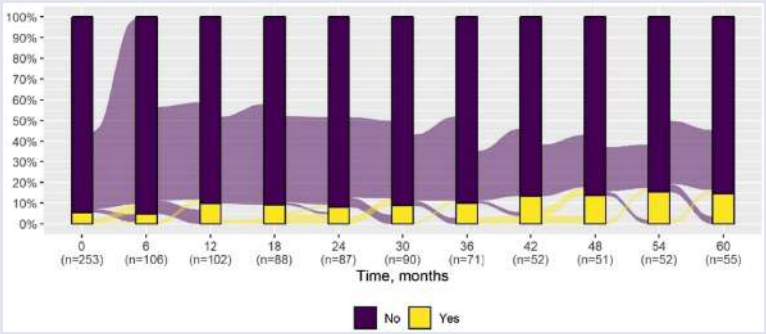
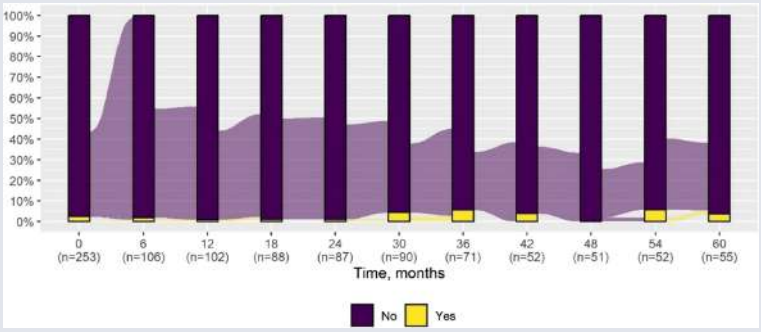
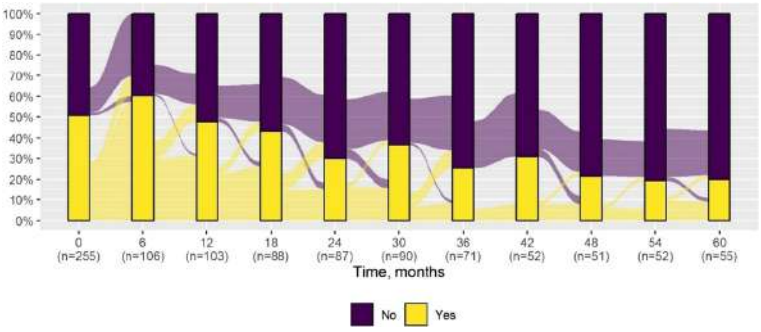
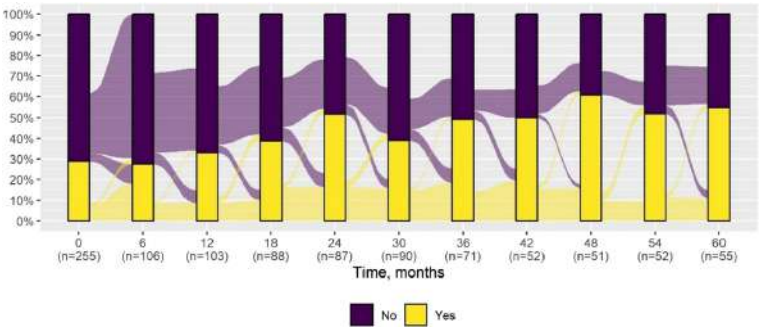
	First visit	Last visit at 5th year	
Autism spectrum symptoms*	14 (5.5)	8 (14.5)	
Obsessive compulsive disorder	6 (2.4)	2 (3.6)	
Neurological examination			
Normal*	129 (50.6)	11 (20.0)	
Ataxia*	73 (28.6)	30 (54.5)	

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TABLE 1 (Continued)

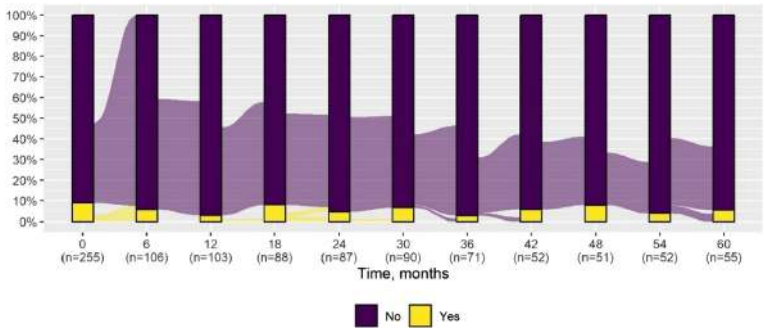
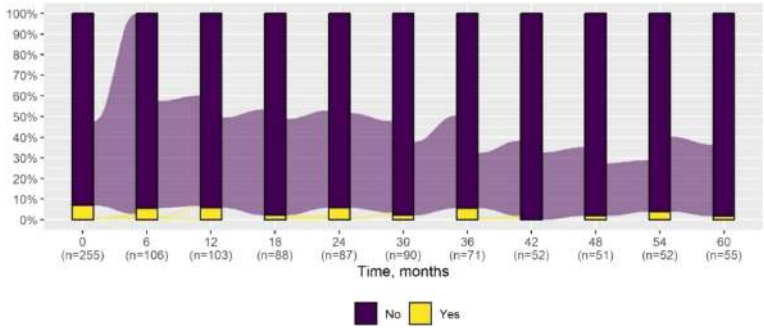
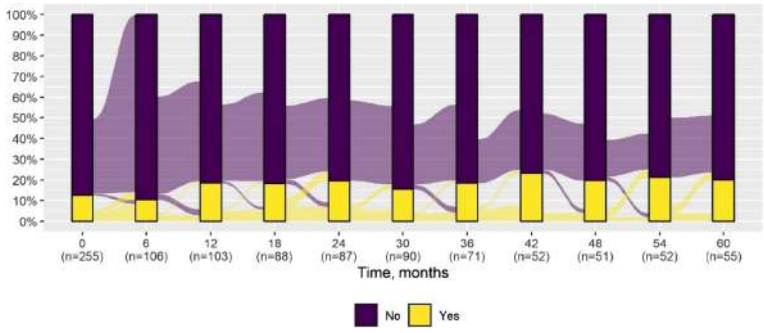
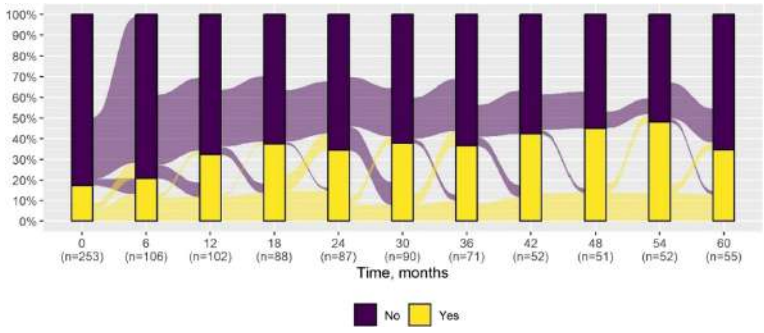
	First visit	Last visit at 5th year	
Pyramidal signs	23 (9.0)	3 (5.5)	
Extrapyramidal signs	18 (7.1)	1 (1.8)	
Action myoclonus	32 (12.5)	11 (20.0)	
Gait abnormality*	44 (17.4)	19 (34.5)	

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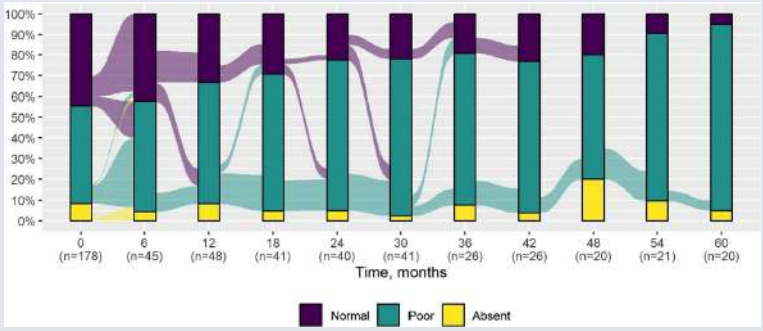
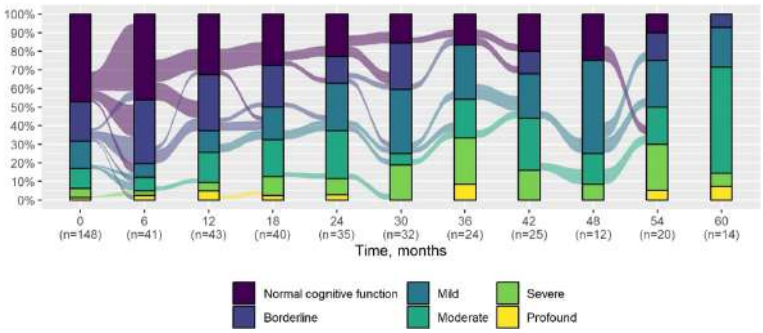
TABLE 1 (Continued)

	First visit	Last visit at 5th year	
Neuropsychological assessment			
Language*			
Normal	79 (44.4)	1 (5.0)	
Poor	84 (47.2)	18 (90.0)	
Absent	15 (8.4)	1 (5.0)	
Not assessable	32	2	
Intellectual disability*			
Normal cognitive function	70 (47.3)	—	
Borderline	31 (20.9)	1 (7.1)	
Mild	22 (14.9)	3 (21.4)	
Moderate	16 (10.8)	8 (57.1)	
Severe	7 (4.7)	1 (7.1)	
Profound	2 (1.4)	1 (7.1)	
Not assessable	32	2	

*Statistically significant difference between first and last visit (p-value <0.05).

stiripentol, and topiramate (Table 5). Precise information on treatment duration is negatively affected by the repeated alternations of drugs many patients experienced during seizure exacerbation periods, especially when they were followed at more than one site.

4 | DISCUSSION

Residras is based on a user-friendly platform that facilitates data collection and analysis of patients with *SCN1A*-related epilepsies, and on a network of 24 expert centers across Italy with specific expertise in rare epilepsy syndromes, with a scope to include further national and international centers and promote a longitudinal standardized data collection. The Residras initiative might represent a paradigmatic example to homogenize data collection and improve research in rare and complex epilepsy syndromes. During the registry setup, several control steps were put in place to ensure high data quality such as centralized control for duplicates, set of mandatory fields, use of standardized ontology codes, and a robust security infrastructure. Although Residras has ample potential for expansion at both national and international level so to constitute a unified source of longitudinal phenotypic data for DS and other *SCN1A*-related epilepsies, feeding the registry for a complex epilepsy is time demanding, which may in part explain why there are no similar registries in place.

We adopted two different approaches to analyze clinical variation over time. We focussed on the first 5-year follow-up to increase specificity of the disease evolution from onset, although clinical assessment at the 5-year time point was not available for most patients (Tables 1 and 2); we then analyzed variation from first to last visit but noting that follow-up length was variable, and therefore, results are less specific to interpret disease course (Tables S2 and S3).

Our data confirm worsening of cognitive ability over time in DS, as already reported in cross-sectional and retrospective studies.[12,34] A minority of patients exhibited normal cognitive skills at last follow-up. Although we excluded febrile convulsions and GEFS+ phenotypes from the analysis, borderline DS phenotypes may explain this finding. Since the longer-term cognitive outcome is often unpredictable early after onset, excluding patients with less severe outcomes in the aftermath as they do not fit

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TABLE 2 Epilepsy features at first visit and after 5-year follow-up with alluvial diagrams showing variation over time.

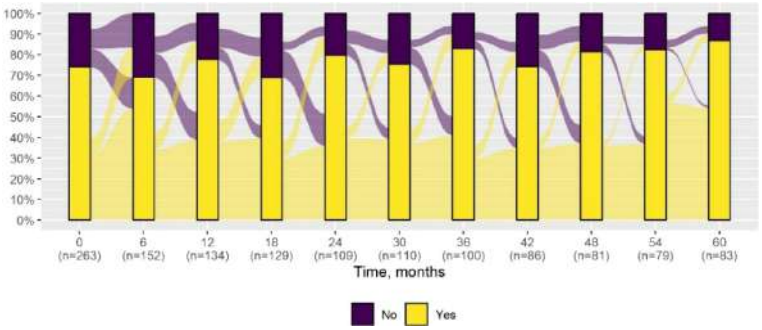
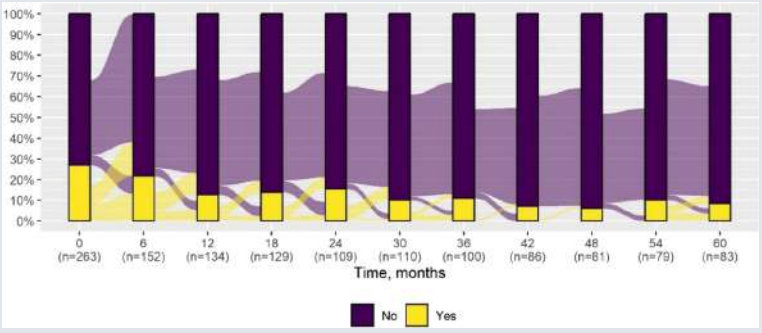
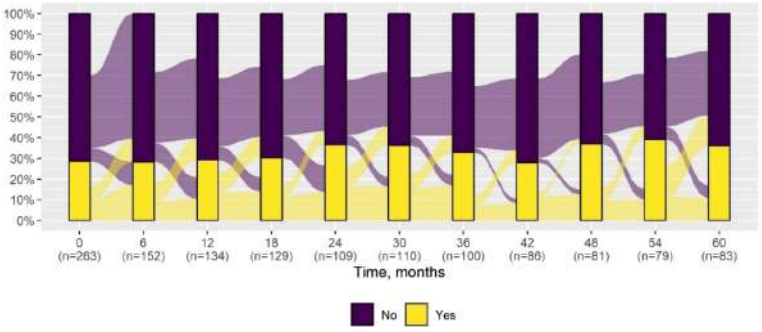
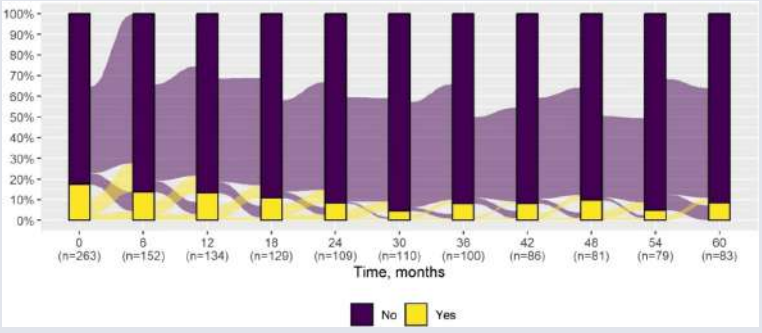
	First visit	Last visit at 5th year	
N	263	83	
Age, years			
Median (IQR)	2.0 (0–7.8)	11.0 (5.0–18.0)	
Generalized seizures*	195 (74.1)	72 (86.7)	
Hemiclonic seizures*	71 (27.0)	7 (8.4)	
Focal-onset seizures	75 (28.5)	30 (36.1)	
Status epilepticus	46 (17.5)	7 (8.4)	

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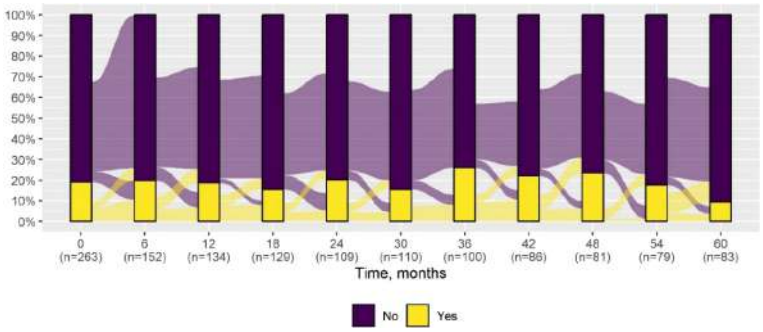
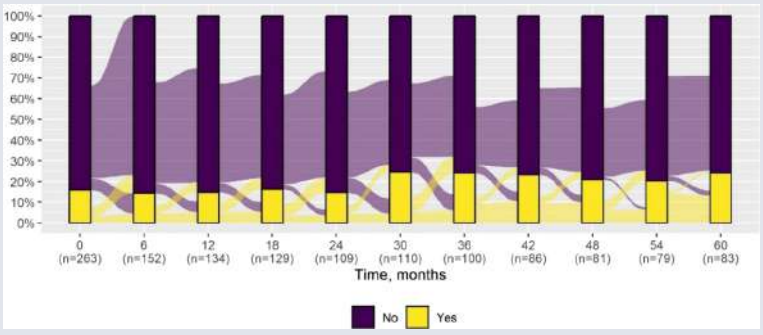
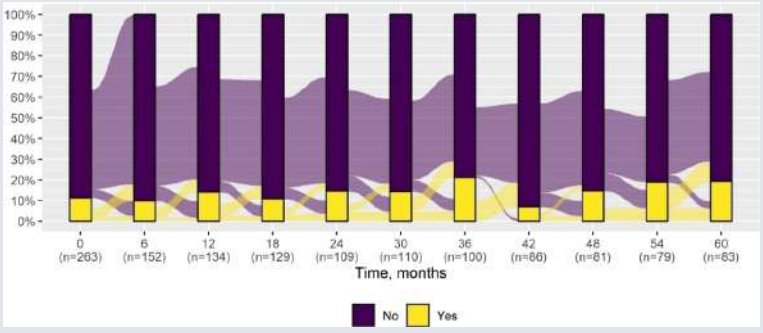
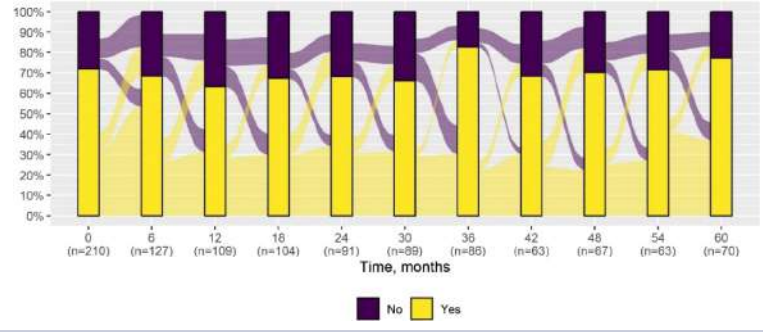
TABLE 2 (Continued)

	First visit	Last visit at 5th year	
Massive myoclonus	50 (19.0)	8 (9.6)	
Absence seizures	42 (16.0)	20 (24.1)	
Reflex seizures	20 (7.6)	5 (6.0)	
Seizure clusters	30 (11.4)	16 (19.3)	
Febrile seizures	151 (71.9)	6 (75.0)	

*Statistically significant difference between first and last visit (p-value <0.05).

the core clinical definition would be artifactual and not reflect the whole spectrum of the syndrome. The items evaluating cognitive, language, and behavioral skills were designed to capture the granularity of the complex neurodevelopmental phenotype, hence to provide in the longer term a robust basis to assess the impact of existing and novel treatment strategies. Although DS diagnosis does not require the mandatory use of specific neuropsychological and behavioral testing, we are now implementing a more systematic and uniform use of a standardized assessment as the data until now collected in the registry derived from a sum of local clinical practices and do not

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TABLE 3 Pattern of changes in neurological condition at 5th year, stratified by age at first visit (0–35 months vs over 35 months).

	Overall	0–35 months	>35 months
N (%)	55	37	18
Deterioration by			
One class	11 (20.0)	8 (21.6)	3 (16.7)
Two classes	14 (25.5)	11 (29.7)	3 (16.7)
Three classes	13 (23.6)	11 (29.7)	2 (11.1)
Four classes	1 (1.8)	1 (2.7)	—
Improvement			
One class	1 (1.8)	1 (2.7)	—
Stability	15 (27.3)	5 (13.5)	10 (55.6)
Autism spectrum disorder			
Deterioration	1 (1.8)	1 (2.7)	—
Improvement	—	—	—
Stability	54 (98.2)	36 (97.3)	18 (100.0)
Behavior			
Oppositional defiant disorder			
Deterioration	7 (12.7)	4 (10.8)	3 (16.7)
Improvement	2 (3.6)	—	2 (11.1)
Stability	46 (83.6)	33 (89.2)	13 (72.2)
Attention deficit			
Deterioration	21 (38.2)	16 (43.2)	5 (27.8)
Improvement	2 (3.6)	2 (5.4)	—
Stability	32 (58.2)	19 (51.4)	13 (72.2)
Autism spectrum symptoms			
Deterioration	7 (12.7)	5 (13.5)	2 (11.1)
Improvement	—	—	—
Stability	48 (87.3)	32 (86.5)	16 (88.9)
Obsessive compulsive disorder			
Deterioration	1 (1.8)	—	1 (5.6)
Improvement	1 (1.8)	—	1 (5.6)
Stability	53 (96.4)	37 (100.0)	16 (88.9)
Neurological examination			
Normal			
Deterioration	26 (47.3)	21 (56.8)	5 (27.8)
Improvement	2 (3.6)	2 (5.4)	-
Stability	27 (49.1)	14 (37.8)	13 (72.2)
Ataxia			
Deterioration	22 (40.0)	20 (54.1)	2 (11.1)
Improvement	2 (3.6)	2 (5.4)	—
Stability	31 (56.4)	15 (40.5)	16 (88.9)
Pyramidal signs			
Deterioration	2 (3.6)	2 (5.4)	—
Improvement	1 (1.8)	1 (2.7)	—
Stability	52 (94.5)	34 (91.9)	18 (100.0)
Extrapyramidal signs			
Deterioration	1 (1.8)	—	1 (5.6)
Improvement	1 (1.8)	1 (2.7)	—
Stability	53 (96.4)	36 (97.3)	17 (94.4)
Action myoclonus			
Deterioration	7 (12.7)	6 (16.2)	1 (5.6)
Improvement	2 (3.6)	1 (2.7)	1 (5.6)
Stability	46 (83.6)	30 (81.1)	16 (88.9)
Gait abnormality			
Deterioration	14 (25.5)	13 (35.1)	1 (5.6)
Improvement	3 (5.5)	2 (5.4)	1 (5.6)
Stability	38 (69.1)	22 (59.5)	16 (88.9)
Neuropsychological assessment			
N (%)	22	15	7
Language			
Deterioration	7 (31.8)	5 (33.3)	2 (28.6)
Improvement	2 (9.1)	1 (6.7)	1 (14.3)
Stability	10 (45.5)	7 (46.7)	3 (42.9)
Not assessable	3 (13.6)	2 (13.3)	1 (14.3)
Intellectual disability			
N (%)	15	11	4
Deterioration by			
One class	1 (6.7)	1 (9.1)	—
Two classes	4 (26.7)	3 (27.3)	1 (25.0)
Three classes	2 (13.3)	2 (18.2)	—
Four classes	1 (6.7)	1 (9.1)	—
Five classes	1 (6.7)	1 (9.1)	—
Improvement			
One class	1 (6.7)	1 (9.1)	—
Stability	2 (16.7)	—	2 (50.0)
Not assessable	3 (20.0)	2 (18.2)	1 (25.0)

inform with sufficient detail the cognitive outcome and how this may be affected by novel treatments. For example, we point out that the number of individuals with autistic symptoms is low, but an increased prevalence is observed during the first 5-year follow-up. A formal diagnosis through ADOS assessment was obtained only in a minority of patients, and this might be related to an underreporting of comorbid autism spectrum symptoms and the limited access to this diagnostic test, only possible in centers with specifically trained staff. The increased prevalence of autism over time might be in part consequent to difficulties in diagnosing autistic features under the age of 3 years. However, since a formal diagnostic assessment for autism spectrum disorder was not regularly available, we cannot draw any definite conclusion on this comorbidity.

Premature mortality is a recognized unfavorable outcome in DS although most available data are from

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TABLE 4 Pattern of changes in epilepsy features at 5th year, stratified by age at first visit (0–35 months vs over 35 months).

	Overall	0–35 months	>35 months
N (%)	83	66	17
Generalized seizures			
Deterioration	14 (16.9)	13 (19.7)	1 (5.9)
Improvement	8 (9.6)	4 (6.1)	4 (23.5)
Stability	61 (73.5)	49 (74.2)	12 (70.6)
Hemiclonic seizures			
Deterioration	6 (7.2)	4 (6.1)	2 (11.8)
Improvement	21 (25.3)	18 (27.3)	3 (17.6)
Stability	56 (67.5)	44 (66.7)	12 (70.6)
Focal onset seizures			
Deterioration	16 (19.3)	14 (21.2)	2 (11.8)
Improvement	11 (13.3)	10 (15.2)	1 (5.9)
Stability	56 (67.5)	42 (63.6)	14 (82.4)
Status epilepticus			
Deterioration	6 (7.2)	6 (9.1)	—
Improvement	18 (21.7)	17 (25.8)	1 (5.9)
Stability	59 (71.1)	43 (65.2)	16 (94.1)
Massive myoclonus			
Deterioration	5 (6.0)	3 (4.5)	2 (11.8)
Improvement	18 (21.7)	15 (22.7)	3 (17.6)
Stability	60 (72.3)	48 (72.7)	12 (70.6)
Absence seizures			
Deterioration	12 (14.5)	11 (16.7)	1 (5.9)
Improvement	6 (7.2)	3 (4.5)	3 (17.6)
Stability	65 (78.3)	52 (78.8)	13 (76.5)
Seizure clusters			
Deterioration	14 (16.9)	13 (19.7)	1 (5.9)
Improvement	10 (12.0)	8 (12.1)	2 (11.8)
Stability	59 (71.1)	45 (68.2)	14 (82.4)
Febrile seizures			
Deterioration	6 (7.2)	6 (9.1)	—
Improvement	10 (12.0)	10 (15.2)	—
Stability	45 (54.2)	32 (48.5)	13 (76.5)
Missing	22 (26.5)	18 (27.3)	4 (23.5)

children,[17,18] and there are no survival analyses in the long term. In a cohort of 100 consecutively recruited DS individuals, mortality rate was 15.84 (98% CI 9.01–27.85) per 1000-person-years while the rate of SUDEP was 9.32/1000-person-years. Living individuals had a median follow-up of 17 years, while the median age at death was 7 years.[18] We observed a mortality rate of 1.84 (95% CI 0.77–4.42) per 1000-person-years, with a median age at death of 6 years. Of the five deaths reported, one was due to SUDEP, and one was epilepsy-related (ie, status). Multiple factors may concur in generating the far lower mortality rates we observed with respect to abovementioned report.[18] Firstly, mortality data emerging from a registry need to be interpreted with caution as death may occur before a diagnosis is made or before a patient is included or after the latest follow-up data entry. Secondly, our results might be affected by immortal bias[35] as our cohort was much younger than Cooper et al.'s cohort.[18] In addition, differences in rates of premature mortality may be partly explained by diverse healthcare provision and treatment strategies. The Cooper et al.[18] DS cohort was more heterogeneous in terms of geographic origin and was established almost 20 years earlier, which implies a longer follow-up and little or no access to more recently introduced drugs with a proven efficacy in DS, particularly stiripentol, cannabidiol, and fenfluramine.[6-9] Finally, improved management strategies have been applied over the years, including earlier diagnosis in larger number of patients and avoidance of inappropriate drugs, such as sodium channel blockers, or exceedingly sedative drug regimens.[36,37] All these factors may contribute to reduced mortality rates in subsequent generations of patients.

Additional highly relevant assets provided by the registry include its reliability in delineating the natural history of DS, the longitudinal comparison of medical practice between DS expert centres, the availability of a wide clinical and genetic spectrum of patients with *SCN1A*-related epilepsies whose characteristics, homogeneously recorded, are available for genotype-phenotype correlations, and comprehensive characterization of comorbidities occurring at different ages.

Accuracy in managing a registry for DS has limitations, as it may be expected for a severe disease starting early in life, associating chronic disability with periods of acute exacerbations and comorbidities that require multiple medical interventions and treatment adjustments. Unavoidably, some data are missing, inclusion of follow-up information might not respect the set deadlines, the various centers may apply different levels of completeness in reporting relevant information, and application of clinical diagnostic criteria for milder DS forms or other SCN1A-related epilepsies is not necessarily uniform. Information on the use of rescue medications might be limited if data are not timely included in the registry. Additional limitations include the lack of systematic data on seizure frequency at each follow-up, as seizure diaries were not regularly used by families or data were not regularly entered by clinicians. Given the likely underreporting of seizure frequency, we omitted them from the analysis. The data on cognitive, language, and behavioral skills gathered in the registry do not yet provide the granularity of the complex neurodevelopmental trajectories. Data on treatment are also limited with lack of systematic information on treatment response but

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TABLE 5 Use and duration of antiseizure treatments.

	N (%)	Cumulative duration, years	Median individual duration (IQR), years
N	251		
Drug			
Sodium valproate	237 (94.4)	3164.6	4.5 (1.0–11.3)
Clobazam	184 (73.3)	1501.6	3.9 (1.0–8.0)
Stiripentol	134 (53.4)	906.3	4.2 (1.5–7.6)
Topiramate	119 (47.4)	1098.0	3.4 (1.0–8.5)
Levetiracetam	92 (36.7)	505.2	1.7 (0.5–5.7)
Phenobarbital	73 (29.1)	632.2	1.3 (0.4–6.9)
Clonazepam	73 (29.1)	594.3	3.2 (1.0–8.0)
Fenfluramine	52 (20.7)	85.3	1.2 (0.2–1.8)
Carbamazepine	48 (19.1)	130.8	0.3 (0.1–2.9)
Lamotrigine	40 (15.9)	200.5	1.1 (0.2–5.7)
Ethosuximide	37 (14.7)	228.5	1.6 (0.5–5.2)
ACTH	21 (8.4)	16.2	0.1 (0.0–0.3)
Nitrazepam	16 (6.4)	182.2	4.7 (1.2–14.6)
Magnesium valproate	16 (6.4)	209.8	12.2 (3.7–22.3)
Zonisamide	16 (6.4)	69.0	1.9 (0.5–3.6)
Acetazolamide	13 (5.2)	52.5	2.3 (0.6–5.4)
Ketogenic diet	13 (5.2)	36.3	0.9 (0.5–3.5)
Nervus Vagus Stimolation	13 (5.2)	138.8	8.7 (5.4–11.3)
Vigabatrin	12 (4.8)	26.5	0.8 (0.3–3.7)
Cannabidiol	10 (4.0)	24.2	2.0 (0.3–2.3)
Phenitoin	10 (4.0)	32.6	1.1 (0.5–5.8)
Clinical Trial ZX008	9 (3.6)	15.3	0.7 (0.4–3.1)
Felbamate	8 (3.2)	15.4	1.2 (0.5–1.8)
Primidone	6 (2.4)	80.4	13.9 (5.5–19.3)
Lacosamide	5 (2.0)	5.6	0.5 (0.3–1.0)
Benzodiazepine	4 (1.6)	30.0	2.6 (0.8–9.3)
Diazepam	4 (1.6)	4.2	0.5 (0.3–1.2)
Oxcarbazepine	4 (1.6)	7.7	0.7 (0.5–0.8)
Perampanel	4 (1.6)	5.3	1.2 (1.0–1.5)
Gabapentin	3 (1.2)	2.4	0.1 (0.1–1.2)
Midazolam	2 (0.8)	7.8	3.9 (2.0–5.8)
Rufinamide	2 (0.8)	1.4	0.5 (0.4–0.5)
Tiagabine	2 (0.8)	1.0	0.5 (0.3–0.7)

only providing a snapshot on clinicians' prescription habits over follow-up. However, the aim of this study was not to add novel findings to the existing literature on DS but to provide a registry model for a rare epilepsy syndrome highlighting its strengths and limitations and discussing how this can be further implemented to serve as a basis to collect data on natural history, novel disease-modifying treatments, and genotype-phenotype correlation.

A further complication in registry curation for a chronic disease that certainly exists across all health systems is related to transition to adult care. Unless there is continuity of care, for example in a specialized institution, patients may transition to adult neurology centers with limited expertise in DS and propensity to adhere to a registry conceived for an infantile onset disorder. Despite 7 years of Registry activity, we could not obtain regular systematic follow-up information for the majority of patients, with increasing patients' loss to follow-up over time, eg, longitudinal data on cognitive function are currently available only for a minority of patients. This is due to a combination of factors including limited personnel resources and geographical bias due to patients residing

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in the South often traveling or moving to other regions for medical care.

Registry curation is time-consuming and might be particularly challenging for those centers with high number of patients if no specifically dedicated personnel is available. Multiple associations for specific genetic disorders are being founded with often the aim to establish dedicated registries, but it may become challenging for the treating physicians to fill different registries with variable formats and become familiar with them. The registry inception and initial activities were supported by limited funding raised by a no-profit patient association (Dravet Italia Onlus). These multiple challenges and initial pitfalls we illustrated should not discourage from establishing registries for specific rare and complex epilepsies as they may represent a basis for funding support within the framework of rare diseases initiatives and private funding. Residras has now been funded by the Italian Ministry of Health (project code PNRR-MR1-2022-12376642, https://www.pnrr.salute.gov.it/portale/pnrrsalute/dettaglioBandiPNRRSalute.jsp?lingua=italiano&id=295), based on a project to promote the registry activities, increase its coverage and curation, and limit the number who may be lost to follow-up.

There are also initiatives by clinicians and scientists to collect information on rare epilepsies such as the Network for Therapy in Rare Epilepsies (NETRE),[38] and there are examples of registries for rare diseases which include epilepsy among their clinical manifestations such as Tuberous Sclerosis Complex.[39] There is a recently started project of a registry for rare and complex epilepsy syndromes, the EpiCARE Registry Project.[40]

Applying the Residras model to other rare and complex genetic epilepsies would help gathering up for each of them a critical mass of homogeneously stored information on epidemiology, disease course, attract dedicated funding and easing access to cohorts of patients that may benefit from clinical trials and drug safety monitoring. Therapy development for rare diseases faces several specific challenges, including small populations for clinical studies, difficulty in determining relevant outcome measures and endpoints, and poorly understood natural history.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

ETHICS STATEMENT

We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

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Original article

Multicenter prospective longitudinal study in 34 patients with Dravet syndrome: Neuropsychological development in the first six years of life

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Abstract

The objective of this study was to identify developmental trajectories of developmental/behavioral phenotypes and possibly their relationship to epilepsy and genotype by analyzing developmental and behavioral features collected prospectively and longitudinally in a cohort of patients with Dravet syndrome (DS).

Thirty-four patients from seven Italian tertiary pediatric neurology centers were enrolled in the study. All patients were examined for the *SCN1A* gene mutation and prospectively assessed from the first years of life with repeated full clinical observations including neurological and developmental examinations. Subjects were found to follow three neurodevelopmental trajectories. In the first group (16 patients), an initial and usually mild decline was observed between the second and the third year of life, specifically concerning visuomotor abilities, later progressing towards global involvement of all abilities. The second group (12 patients) showed an earlier onset of global developmental impairment, progressing towards a generally worse outcome. The third group of only two patients ended up with a normal neurodevelopmental quotient, but with behavioral and linguistic problems. The remaining four patients were not classifiable due to a lack of critical assessments just before developmental decline.

The neurodevelopmental trajectories described in this study suggest a differential contribution of neurobiological and genetic factors. The profile of the first group, which included the largest fraction of patients, suggests that in the initial phase of the disease, visuomotor defects might play a major role in determining developmental decline.

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Early diagnosis of milder cases with initial visuomotor impairment may therefore provide new tools for a more accurate habilitation strategy.
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Keywords: Dravet syndrome; *SCN1A* mutation; Developmental decline; Visuomotor defects; Neuropsychological longitudinal study; Neurodevelopmental trajectory

1. Introduction

Developmental decline and behavioral disorders have typically been described in patients with Dravet syndrome (DS) since the initial reports [1–4]. After apparently normal early development, they become evident during the second year of life following the onset of seizures, although studies on precognitive abilities [5] have confirmed that mild developmental impairment may be "easily overlooked in small children" [6].

Several studies have focused on the developmental features of patients with DS in the first decade of life. However, these studies are mostly retrospective [4,7–9] or with partial follow-up [10,11]. To date, the only longitudinal prospective analysis with serial assessments was carried out in a small cohort [5,12] specifically examining visual and visuocognitive development from the first year of life until school age. The objective of this prospective study, which concerns the same age range, was to analyze developmental and behavioral data collected in a larger cohort of DS patients in order to identify trajectories of developmental/behavioral phenotypes over time, and possibly to understand their relationship to epilepsy and genotype.

2. Patients and methods

Thirty-four patients (18 male patients) from seven Italian tertiary pediatric neurology centers with a diagnosis of DS were enrolled in the study. The diagnosis was based on the occurrence of febrile and afebrile, generalized and unilateral, often prolonged in status epilepticus, clonic or tonic-clonic seizures in the first year of life in an otherwise normal infant, later associated with other types of seizures and developmental decline. The cohort included complete forms with all different types of seizures observed in DS and incomplete forms without myoclonic and atypical absence seizures, as proposed by Guerrini and Oguni [13]. Molecular analysis (direct sequencing and MLPA) of the *SCN1A* gene was performed at the neurogenetics laboratory of Meyer Children's Hospital (Florence, Italy) as previously described [14]. Female patients who were negative for *SCN1A* gene mutations were also screened for *PCDH19* gene mutations and were excluded from the study if mutations were found. None of the patients had *PCDH19* mutations.

Written informed consent was obtained from the parents or guardians of all patients.

The study design, which took into account health limitations and compliance aspects, consisted of a prospective assessment from the first to the sixth year of life with repeated full clinical observations including neurological and developmental examination and video EEG recordings.

Examinations were planned for eight subsequent periods starting in the first year of life (T0), followed by six consecutive half-year periods (T1-T6), up to the final two assessments performed yearly (T7-T8). Neurodevelopmental assessments were performed using the Griffiths scales (age range 0–8 years) [15,16]. According to the ICD10 ranges of intellectual disability (ID), Griffiths scale score band equivalents were classified as follows [17]: borderline development with a Global Developmental Quotient (GDQ) between 75 and 85, mild ID (GDQ level between 57 and 74), moderate ID (GDQ level from 45 up to 56) and severe ID (GDQ level from 32 to 44). We defined developmental decline as GDQ values dropping from a higher to a lower level, as this is a more definite way of establishing a real regression than a simple fluctuation within the same level range. To compare the Griffiths subscale scores, a corrective formula was used as per the Griffiths Scales of Child Development, Third Edition.

To complete developmental assessments, we monitored language development with a focus on the descriptions of parents and our own observations of the primary critical points of early language acquisition. The language evaluation assessment was performed using the Primary Language Test (TPL Axia, Organizzazioni Speciali Eds 1995) in children between 12 and 36 months of age and the Language Evaluation Test (TVL Cianchetti and Fancello, Erickson Eds 2003) for those older than 36 months. More specifically, we analyzed five specific functions: a) first word production, b) phonetic and phonological maturation, c) lexical accuracy, d) morpho-syntactic growth and e) how production develops compared to comprehension. Both tests provide age-specific normative data that make it possible to classify results as normal if the z score value is lower than −1.5, mildly impaired if between −1.6 and −2 or severely impaired if higher than −2.

In the same way, behavioral assessments were based on parental reports and direct observations and coded

as normal, mildly impaired or severely impaired. The CBCL [18] also uses reference normative data to create standard scores. Norms account for both age and gender; there are separate norms for girls and boys and separate norms for ages 1 1/2–5, 6–11 and 12–18. The standard scores are scaled so that a score of 50 is average for the youth's age and gender, with a standard deviation of 10 points. Scores above 1 SD (T scores above 60) were defined as mildly abnormal and those above 2 SD (above 70) as severely impaired.

Worsening was defined as any shift from a less severe to a more severe behavioral pattern. This included a reported shift from none to mild, from mild to moderate or from moderate to severe. Improvement was defined as a shift from severe to moderate, from moderate to mild or from mild to none. Finally, we reported epilepsy data including age at first seizure, seizure types, seizure duration, seizure frequency, EEG abnormalities and number of antiepileptic drugs (AEDs) used. Taking variability into account, mean seizure frequency was determined for each different type of seizure: i) we grouped generalized/unilateral clonic or tonic-clonic seizures and focal seizures with and without secondary generalization as "convulsive seizures", and frequency was considered frequent if seizures occurred daily and weekly, moderate if seizures occurred less than once weekly up to once monthly, or rare if seizures occurred less than monthly; ii) for prolonged convulsive seizures (>15 min) and convulsive and non-convulsive status epilepticus (SE) (>30 min), we considered the total number of seizures during each period; iii) myoclonic seizures and atypical absence seizures were considered present if they were observed daily and absent if they were only occasional, for example, in a brief period before a generalized seizure. Furthermore, interictal myoclonic jerks were considered present when they were observed daily and absent when only reported occasionally. EEG abnormalities specifically included slow background activity according to age and generalized spike-waves, as these abnormalities have been reported to be associated with a worse prognosis in the literature [19].

2.1. Statistical analysis

We used absolute and relative frequencies, means and standard deviations to describe the data, then we used statistical hypothesis tests to find statistically significant associations and statistically significant differences in data. We applied the chi-squared test (with William's correction) or Fisher's exact test to identify statistically significant differences in the distribution of categorical data between groups and Student's *t-test,* with regard to quantitative data, to compare the means of variables. We used the Mann-Whitney test to compare visuomotor impairment in "visuomotor" and "global" groups by evaluating the distribution of the mean ratio of the Eye and Hand Coordination Scale (Griffiths D scale) and the Global Developmental Quotient (GDQ) across the two groups at different follow-up times. The statistical significance level was set at 0.05.

3. Results

It was not possible to perform assessments at each planned period in all cases due to health problems in some patients (frequent seizures, severe behavioral disorders) or poor parental compliance (Table 1). However, 22 of 34 patients (64.7%) were assessed at least five times out of nine scheduled examination, and in all patients, the available data enabled an accurate analysis of the developmental trajectory. Follow-up (FU) began at T0 in 73% of cases, with a later start, generally during the second year of life, in the remaining patients. The last assessment was mostly performed at school age (T7 or T8) (22 of 34 patients [64.7%]) and at or after T4 (32 of 34 patients [94.1%]) in order to establish the developmental trajectory. FU stopped at T2 in only two patients (#10 and 20), but in both cases, global developmental impairment was noted at the first assessment during the first year of life.

Table 1 also presents demographic data of our sample along with genetic and neurodevelopmental findings, listed according to developmental level at last FU (from patients with a GDQ in the normal range to those with the most severe developmental impairment). Epilepsy, neurological and behavioral findings are shown in Table 2. An MRI was normal in all but four patients who showed minor nonspecific changes (ventricular enlargement, cortical atrophy).

3.1. Overall analysis

Our cohort included 18 males (53%) and 16 females (47%), and seven subjects (20.6%) were negative for *SCN1A* mutation. The onset of seizures, which were febrile or afebrile, generally clonic, often unilateral and of long duration, occurred between 2 and 10 months of age (mean: 4.8 months). Different seizure types began in the second year of life: myoclonic seizures, atypical absences and focal seizures, with slight myoclonic jerks.

Table 2 shows that the EEGs did not show epileptic discharges in the first period (before the age of 3 years) in 21 of 34 cases (61.7%) and the EEG was normal, i.e. without discharges and without slow background, in 13 cases (38.2%). Of these 13 cases, the EEG remained normal in seven patients in the second period, after the age of three years, and data were not available for two cases in the second period. Six of these seven patients whose EEG remained normal (#1, 2, 3, 4, 12 and 14) had a GDQ between 70 and 95 at the last outcome assessment between five and six years, whereas the GDQ of the seventh case (#21) was lower

Table 1
Overall analysis: general demographic, genetic and developmental data.

Case #	Sex	Assessment number	Mutation	Neurodevelopmental trajectory group	Dravet phenotype	Age of onset of cognitive decline	Age of onset of visuomotor impairment	Age of onset of global impairment	Age of last assessment	GDQ at outcome
GDQ in the normal range at outcome										
1	F	3	c.539 T > G, p.Leu180X (nonsense)	Normal profile	Incomplete	–	–	–	T7 (58 m)	95
2	F	4	c.5531delCAAA (nonsense)	Normal profile	Incomplete	–	–	–	T8 (62 m)	91
3	M	8	c.5018 T > C, p.Ile1673Thr (missense)	Visuomotor group	Incomplete	–	20 m	–	T7 (52 m)	93
4	M	6	c.4305_4308dupGGAT, p. Ile1437Glyfs*8 (frameshift)	Visuomotor group	Incomplete	–	12 m	–	T7 (56 m)	86
Borderline GDQ at outcome										
5	F	7	c.\4476 + 5G > A (splicing)	Visuomotor group	Complete	36 m	36 m	–	T8 (60 m)	81
6	M	8	No mutation	Visuomotor group	Incomplete	68 m	68 m	–	T8 (68 m)	81
7	M	9	c.4557_4562delTCGACC p. Arg1525_Pro1526del (in-frame deletion)	Visuomotor group	Complete	19 m	19 m	53 m	T8 (65 m)	77
8	F	7	c.3725_3726insA, p.Asp1243X (nonsense)	Visuomotor group	Complete	13 m	13 m	60 m	T8 (60 m)	75
9	M	5	c.1837C > T, p.Arg613X (nonsense)	Visuomotor group	Incomplete	25 m	25 m	29 m	T4 (29 m)	75
10	M	2	c.2657C > T, p.Ser886Phe (missense)	Global group	Complete	13 m	–	13 m	T2 (22 m)	76
11	F	5	c.3968C > A, p.Pro1323His (missense)	Global group	Complete	34 m	–	34 m	T4 (34 m)	75
Mild mental retardation at outcome										
12	F	8	~~n~~o mutation	Visuomotor group	Complete	18 m	18 m	42 m	T8 (70 m)	71
13	M	6	c.4756G > A, p.Gly1586Arg (missense)	Visuomotor group	Complete	14 m	14 m	28 m	T7 (49 m)	67
14	M	8	No mutation	Visuomotor group	Complete	39 m	39 m	61 m	T8 (61 m)	70
15	M	7	c.301C > T, p.Arg101Trp (missense)	Visuomotor group	Complete	14 m	14 m	67 m	T8 (67 m)	69
16	M	4	No mutation	Visuomotor group	Incomplete	12 m	12 m	24 m	T8 (72 m)	60
17	F	5	c.783insTGCTCTAATTG (F261ins267X) (frameshift)	Visuomotor group	Complete	51 m	51 m	70 m	T8 (70 m)	59
18	M	2	c.1055_1056dupTG, p.Lys353X (nonsense)	Global group	Incomplete	30 m	–	30 m	T4 (32 m)	68
19	M	8	c.1628delT, p.Leu543X (missense)	Global group	Complete	21 m	–	21 m	T8 (60 m)	71
20	M	2	c.3701delC, p.Ala1234ValfsX36 (frameshift)	Global group	Incomplete	12 m	–	12 m	T2 (17 m)	66
21	M	6	No mutation	Global group	Incomplete	23 m	–	23 m	T5 (39 m)	62
22	F	6	c.3794 T > C, p.Leu1265Pro (missense)	Global group	Complete	18 m	–	18 m	T5 (45 m)	61
23	F	8	c.602 + 1G > A (splicing)	Global group	Complete	35 m	–	35 m	T8 (60 m)	60
24	F	2	c.4415 T > C, p.Phe1472Ser (missense)	Global group	Incomplete	21 m	–	21 m	T5 (36 m)	58

Moderate mental retardation at outcome												
25	F	3	c.3568delA, p.Lys1190SerfsX18 (frameshift)	visuomotor group	Complete	18 *m*	18 *m*	28 *m*	T6 (48 m)	55		
26	M	9	c.4461C > T, p Q1488X (nonsense)	Visuomotor group	Complete	20 m	20 m	40 m	T8 (72 m)	55		
27	F	8	c.5674C > T, p.Arg1892X (nonsense)	Visuomotor group	Complete	19 m	19 m	26 m	T8 (66 m)	55		
28	F	5	c.5347G > A, p.Ala1783Thr (missense)	Global group	Complete	18 m	–	18 m	T7 (61 m)	55		
29	F	7	No mutation	Global group	Complete	32 m	–	24 m	T8 (69 m)	50		
30	F	2	c.5383G > T, p.Glu1795X (nonsense)	Not classifiable	Incomplete	18 m	–	18 m	T4 (31 m)	52		
31	M	4	c.1170 + 1G > T (splicing)	Not classifiable	Complete	25 m	–	25 m	T5 (42 m)	51		
Severe mental retardation at outcome												
32	M	5	No mutation	Global group	Complete	16 m	–	16 m	T8 (61 m)	39		
33	M	4	c.4906C > T, p.Arg1636X (nonsense)	Not classifiable	Complete	29 m	–	29 m	T7 (52 m)	43		
34	F	4	c.4073G > A, p.Trp1358X (nonsense)	Not classifiable	Complete	19 m	–	19 m	T6 (48 m)	42		

M: male, F: female, m: months.
Assessment number: number of assessments performed during the study.

(GDQ = 62). The GDQ of the four patients whose EEGs became abnormal was lower than 70 (#25, 28 and 32), except in one case (#5) in which the GDQ was 81. Generalized spike-waves were present or appeared in 16 patients, and the background was or became slow in 18 patients. Neither background slowness nor spike-waves were observed in the four patients with a GDQ in the normal range at the outcome assessment (#1, 2, 3 and 4).

About two-thirds of cases (22 of 34) showed neurological signs affecting motor coordination and posture (clumsiness, ataxia, crouch gait).

Behavioral disorders were found in all but two patients (#10 and 22), including patients without developmental impairment; ten of them experienced worsening, whereas five patients (#1, 12, 26, 28 and 29) showed improvement over time. During the FU period, attention disorders were observed in 29/32 cases followed by hyperactivity disorders in 20/32. The follow-up of the remaining two cases (#10 and 20) was too short. Oppositional and autistic traits were found less frequently, in 10 and 7 cases respectively.

Table 1 shows that four patients (#1–4) demonstrated no evidence of developmental decline (decrease in GDQ below 85, the upper limit of borderline mental development). In almost all patients, the onset of developmental decline occurred in the second or third year of life, except in three individuals (#6, 14 and 17) who had a later onset of the syndrome. The GDQ at the last FU assessment was within the normal range in four patients (11.8%), borderline in seven (20.6%), mildly disabled in thirteen (38.2%), moderately disabled in seven (20.6%) and severely disabled in three (8.8%).

Language skills were impaired early in most patients. Production of first words was delayed in 25 of 34 patients (73.5%); nine patients (26.5%) had no language delay in the first three years all belonging to the normal or borderline cognitive groups, except for one patient with mild mental retardation. Phonological competence was poor in 30 of 34 patients (88.2%), and expression was worse than comprehension in 27 of 34 patients (79.4%). In subsequent years, while phonological competence improved slightly in 23/30 cases (76.7%), other language difficulties persisted and other types of language abnormalities appeared including in emergent skills such as morpho-syntactic abilities. It is worth noting that seven patients whose language development was previously within the normal range also began exhibiting mild disorders, whereas only one patient (#7) belonging to the borderline group became normal.

As shown in Table S1, there was no statistically significant difference in the frequency of *SCN1A* mutation in the groups based on GDQ at the outcome assessment ($p = 0.666$). Patients with complete forms had a lower GDQ ($p = 0.005$). The worst outcomes were only observed in patients with complete forms (six moderate

Table 2
Epileptic, neurological and behavioral findings.

Case #	Neurodevelopmental trajectory group	Seizure Onset	Seizure Freq.	SE No.	> 15 m seizure No.	Myoclonic seizures	Absences	Interictal myoclonus	EEG discharges	EEG slowness	AEDs	Neurological signs		Behavioral disorder	
												First 3 y	After	First 3 y	After
In the normal GDQ range at outcome															
1	Normal profile	4 m	◐/◐	0/0	0/0	○/○	○/○	○/○	○/○	○/○	2/2	No	No	Severe, HD, AD	Moderate, HD, AD
2	Normal profile	4 m	◐/○	1/0	1/0	○/○	○/○	○/○	○/○	○/○	1/1	No	No	Mild, AD	Mild, AD
3	Visuomotor group	3 m	◐/◐	2/0	4/0	○/○	○/○	○/○	○/○	○/○	2/4	No	No	Mild, AD, HD, OD	Moderate, AD
4	Visuomotor group	3.5 m	○/○	1/0	2/0	○/○	○/○	○/○	○/○	○/○	1/2	No	No	Mild, AD	Mild, AD
Borderline GDQ at outcome															
5	Visuomotor group	8 m	○/○	1/0	1/1	○/○	○/●	○/○	○/○	○/●	4/3	No	No	Mild, HD, OD	Mild, HD, OD
6	Visuomotor group	3 m	○/◐	4/2	1/1	○/○	○/○	○/○	●/○	○/○	4/5	No	No	Mild, HD, AD	Moderate, AD, DT
7	Visuomotor group	5 m	○/○	1/0	0/0	●/○	○/○	○/○	●/●	○/○	1/1	No	No	Moderate, HD, AD	Moderate, HD,AD
8	Visuomotor group	3 m	◐/◐	5/1	3/0	●/●	● /○	○/●	○/○	●/●	3/5	No	Ataxia	Mild, AD	Mild, AD, OD
9	Visuomotor group	5 m	●/	12/	4/	○/	○/	○/	●/	○/	5/	Clums		Mild, AD	
10	Global group	5 m	○/	1/	2/	●/	○/	○/	○/	●/	1/	Mild ataxia		No	
11	Global group	7 m	○/○	1/0	4/0	●/○	● /○	○/○	○/○	●/●	2/3	No	Ataxia	No	Mild, HD, AD
Mild mental retardation at outcome															
12	Visuomotor group	4 m	○/●	7/2	1/0	●/○	○/●	○/○	○/○	○/○	3/4	Clums	Clums	Severe, HD, AD, AS	Moderate, AD, OD
13	Visuomotor group	2.5 m	◐/	0/	0/	●/	● /	○/	○/	●/	3/	Clums		Mild, AD	
14	Visuomotor group	4 m	○/◐	3/1	4/0	●/○	○/○	○/○	○/○	○/○	3/2	No	Clums	Moderate, HD, AD	Moderate, AD, OD
15	Visuomotor group	4 m	●/◐	2/0	2/0	●/●	○/○	○/ ○	●/●	○/●	3/2	Clums	Clums	Mild, AD, HD	Severe, AD, HD, OD
16	Visuomotor group	5 m	◐/○	0/0	0/0	○/○	○/○	○/○	●/●	○/○	4/2	No	No	Mild, OD	Moderate, OD
17	Visuomotor group	5 m	○/◐	0/0	0/0	○/○	● /●	○/●	●/●	○/○	4/3	Clums	Ataxia	Mild, HD, AD	Moderate, AD, DT
18	Global group	7 m	○/	1/	0/	○/	○/	○/	○/	●/	1/	Clums	Ataxia	Mild, HD, AD	Moderate, AD, DT
19	Global group	4 m	◐/◐	1/0	0/0	○/●	○/●	○/●	●/○	○/●	4/3	Ataxia	Ataxia	Severe, DT, AS, AD	Severe, AD, AB
20	Global group	6.5 m	○/	ND/	ND/	○/	○/	○/	○/	○/	2/	No		Mild, AD	
21	Global group	2 m	○/○	1/0	1/0	○/○	○/○	○/●	○/○	○/○	2/2	No	No	Mild, HD	Mild, HD, AD
22	Global group	8 m	○/◐	1/0	0/0	●/○	● /○	○/●	●/○	●/●	2/2	No	No	No	No
23	Global group	4 m	◐/◐	5/1	5/4	●/●	● /●	●/●	●/●	○/○	5/4	Ataxia	Ataxia, Clums	Mild, AD, OD	Moderate, AD, OD
24	Global group	4 m	◐/	>5/	>5/	○/	○/	○/	○/	○/	2/	No		Mild, AD	

Moderate mental retardation at outcome														
25	Visuomotor group	3 m	●/●	1/1	27/3	●/●	○/○	○/○	○/○	○/●	4/2	No	Ataxia	Mild, AD	Moderate, AD
26	Visuomotor group	4.5 m	○/◐	3/1	>3/0	○/●	○/○	●/●	○/○	●/●	3/3	Ataxia	Ataxia	Severe, HD, AD	Moderate, HD, AD
27	Visuomotor group	4 m	◐/◐	3/0	4/0	●/ ●	○/●	●/●	●/●	○/○	4/4	Ataxia, Clums	Ataxia, Clums	Moderate, HD, AD	Mild, AD
28	Global group	5 m	○/●	6/0	3/0	●/●	● /●	○/●	○/●	○/●	3/4	Clums	Ataxia	Severe, HD, AD, AS	Moderate, AD, HD
29	Global group	5 m	○/◐	1/0	3/0	●/○	●/●	○/●	●/○	●/○	3/3	Clums	Ataxia	Severe, HD, AD, AS	Moderate, HD, AD
30	Not classified	10 m	◐/	3/	0/	○/	○/	○/	○/	●/	4/	Ataxia, C. G.		Mild, HD, AD	
31	Not classified	6 m	◐/●	>5/ <5	>5/>5	●/●	○/○	○/○	●/○	●/●	7/3	Ataxia	Ataxia	Mild, AS, AD	Mild, AS, AD
Severe mental retardation at outcome															
32	Global group	6 m	◐/◐	3/1	3/0	○/●	●/●	○/●	○/●	○/●	4/7	Clums	Ataxia	Severe, HD, AD, **AS**	Severe, OD, AD, AS
33	Not classified	6 m	○/◐	1/1	2/0	○/●	○/○	○/●	○/●	●/○	2/3	No	Ataxia	Mild, HD, AD	Moderate, OD, AD
34	Not classified	3 m	●/●	1/0	10/0	●/●	● /●	○/○	●/○	●/●	5/2	Ataxia	Ataxia	Moderate, HD, AD, AS	Moderate, HD, AD, AS

Legend

Slashes separate symbols for the first three years from those relating to subsequent years;
Seizure frequency: ○ rare (if less than monthly); ◐ moderate (if less than once weekly up to once monthly) ● high (if daily or weekly)
SE: status epilepticus
ND: not determinable
Myoclonic seizures, absences and interictal myoclonus; EEG abnormalities: ○ absent; ● present
Clums: clumsiness
C.G.: crouch gait
AB: aggressive behavior; HD: hyperactivity disorder; AD: attention disorders; OD: oppositional disorder, AS: autistic spectrum; DT: depressive traits
AEDs: antiepileptic drugs

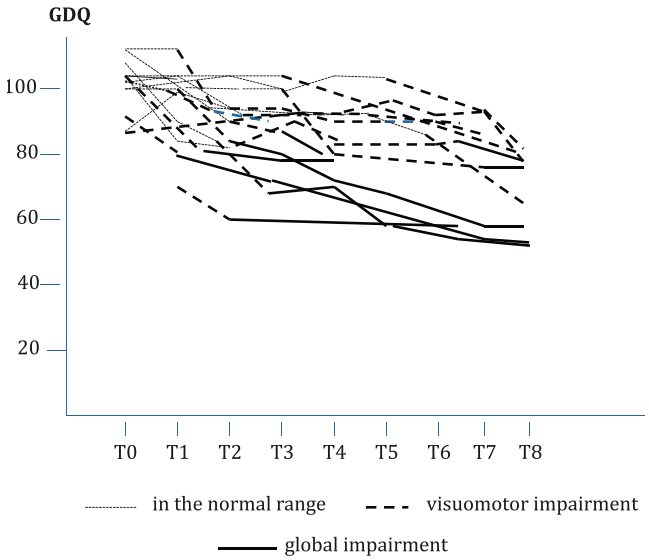

Fig. 1. Developmental profiles of GDQ in the "visuomotor" group.

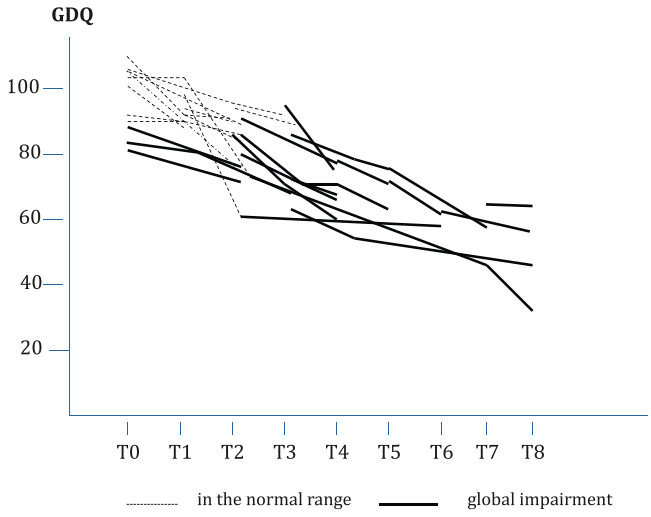

Fig. 2. Developmental profiles of GDQ in the "global" group.

and three severe cases of developmental impairment), whereas normal GDQs were only observed in patients with incomplete forms. Patients exhibited a more severe delay when myoclonic seizures persisted after three years of life ($p = 0.017$). Neurological signs were associated with a lower GDQ in the first three years ($p = 0.008$) as well as after three years ($p = 0.004$) and were absent in patients with normal development. Other items (frequency of convulsive seizures and SE before and after 3 years, EEG background slowness and GDQ at the outcome assessment) showed no significant difference.

3.2. Developmental trajectory analysis

The successive GDQ scores showed a progressive worsening of intellectual ability except in two patients who experienced a brief temporary improvement (#7 and 15). We identified three distinct developmental trajectories. The first, which we call "visuomotor", included 16 patients (#3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 25, 26 and 27) who presented a mild initial (2nd or 3rd year of life) decline after an apparently normal development in all but two patients (#6 and 14), who had a later onset of decline. Impairment specifically concerned visuomotor abilities (corresponding to Eye and Hand Coordination on the D Griffiths scale), with a statistical difference in scores of this subtest between the "visuomotor" group and the "global" group. In order to verify the more specific impairment of visuomotor abilities in the "visuomotor" group in comparison to the "Global" group, we evaluate the mean value of D scale and the mean value of GDQ in both groups ("global" group and "visuomotor" group) for each follow-up times, and we then calculated separately in both groups the ratio "D scale mean/GDQ mean" at different times. A Mann-Whitney test was then performed for each follow-up time to verify the null hypothesis of same distribution of this ratio across the two groups. We found that there was a statistically significant difference in the ratio "D scale mean/GDQ mean" distribution at T4 (1.03 and 0.98 respectively ($p = 0.026$)), at T5 (1.11 and 0.83 respectively ($p = 0.02$)), and at T7 (1.03 and 0.87 ($p = 0.026$)). The test was not performed at T6 due to missing data and it was slightly not significant ($p = 0.053$) at T8. In all three significant follow up times assessment (T4, T5 and T7), the ratio was higher in the "global" group than in the "visuomotor" group, showing that in the "global "group there was only a slight difference between the impairment of visuomotor abilities (D scale mean) and that of the general cognitive competences (GDQ mean), while in the "visuomotor" group the degree of impair-

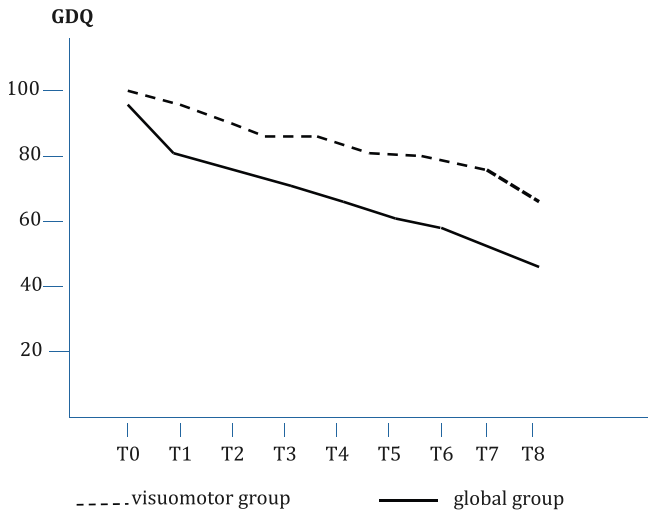

Fig. 3. Mean developmental profiles in the "visuomotor" and "global" groups.

ment of the visuomotor abilities was lower than that of the general cognitive competences. (Table S2)

Impairment in visuomotor abilities was sporadically and temporarily associated with a decline in another scale, particularly the Hearing and Language Scale (5 cases). A decline in the Locomotor Scale concerning gross motor abilities was observed in only two individuals. Four patients (#3, 4, 5 and 6) maintained this partial impairment until the last assessment, whereas the remaining 12 evolved over different periods of time towards an overall deficit by the end of FU (Table 1).

A regular, progressively decreasing developmental curve slope was typical in the "visuomotor" group (Fig. 1). After a variable period, developmental decline generally tended to impact all the abilities assessed by the Griffiths scales (12/16 patients). Of these patients, nine were assessed at a mean value of 65.6 at T8, whereas the remaining three were assessed only at T7, T6 and T4, respectively (Table 1).

The second group of 12 patients (#10, 11, 18, 19, 20, 21, 22, 23, 24, 28, 29 and 32), which we refer to as the "global" group, was characterized by a generally sudden and early (within the two first years of life) onset of global developmental impairment (Table 1). All scales showed a decline; only in three cases was a scale temporarily unimpaired. In this group, developmental curves were characterized by an initially steep slope that became flatter after the fourth year, and showed a mean GDQ of around 55 in those that reached T8 (4 cases) (Fig. 2).

When the two main neuropsychological phenotypes ("visuomotor" and "global") are compared, the mean curves of the two groups clearly show an obvious difference with regard to their evolution (respectively mild and regular in the "visuomotor" group and an initially sharper decline in the "global" group) and their outcomes, with a GDQ of around 70 in the "visuomotor" and 60 in the "global" group (Fig. 3). The two patients (#1 and 2) who still exhibited a fully normal neurodevelopmental profile at the last FU assessment despite behavioral disorders in the first years of life and some mild language problems belong to a third neuropsychological phenotype ("normal"). It should be noted that both patients carried *SCN1A* mutations. The remaining four patients (#30, 31, 33 and 34) were not classifiable because there were no previous assessments before the children showed cognitive decline. This makes it difficult to understand whether there were prior signs of visuomotor impairment before the onset of the more global cognitive impairment.

There was no difference in *SCN1A* mutation status (present or absent) or type of mutation (missense or nonsense as well as outside or inside the Na channel pore region) between the "visuomotor" and "global" groups ($p = 1.0$). Similarly, there was no intergroup difference concerning clinical forms (complete and incomplete) ($p = 0.74$) and neurological findings (before 3 years: $p = 0.36$; after 3 years: $p = 0.25$). However, comparison of epilepsy and behavioral findings between "visuomotor" and "global" patients yielded some significant results. As presented in Table 3, "visuomotor" patients had an earlier onset of seizures (<6 months) and less interictal myoclonus after three years than "global" patients ($p = 0.023$ and $p = 0.019$, respectively). Behavioral disorders during the first three years were milder in the "visuomotor" group than in the

Table 3
Epileptic, neurological and behavioral findings: significant results from the comparison between "visuomotor" and "global" groups.

Fisher's exact test			
Phenotype Pattern	"Visuomotor" Group (16 cases)	"Global" Group (12 cases)	*p* values
Seizure Onset			
<6 m	15	6	$p = 0.023$
≥6 m	1	6	
Interictal Myoclonias (After 3 Years)			
Yes	4	8	$p = 0.019$
No	10	2	
Behavioral Disorders (First Three Years)			
Severe	2	4	$p = 0.022$
Moderate	3	0	
Mild	11	5	
No	0	3	
Ab: Aggressive Behavior			
Yes	0	5	$p = 0.008$
No	16	7	
Student's *t*-test			
Mean of Outcome GDQ in Visuomotor Group	Mean of Outcome GDQ in Global Group		*p* value
71	62		$p = 0.0497$

"global" group ($p = 0.023$). Aggressive behavior in particular was absent in the "visuomotor" patients, but present in 41.6% of "global" patients ($p = 0.008$). Finally, the mean GDQ at the outcome assessment was better in the "visuomotor" group (GDQ = 71) than in the "global" group (GDQ = 62) ($p = 0.0497$) (Table 3).

4. Discussion

To our knowledge, our study is the first that offers a large prospective longitudinal observation of the neurodevelopment of patients with DS. The overall analysis of our cohort confirms literature-reported retrospective data on the main clinical findings during the first decade of life: seizure onset in the first year of life, onset of developmental decline in the second to third year, variability of development also including rare individuals with apparently normal neurodevelopment, and no correlation between *SCN1A* mutation and neurodevelopment [1,4,5,7–12,20,21]. In particular, GDQ values at the outcome assessment (up to six years) are similar to those previously reported in the literature [4,5,7–11] although there was possible bias due to the diversity of the FU duration.

As already reported in the literature [2,7,8,10,11,34] developmental impairment was more common in complete clinical forms where there was a high frequency of myoclonic seizures that increased after three years.

With regard to prolonged seizures and SE before three years of age, our study fails to show any statistical correlation with the cognitive outcome. This result is in line with a few earlier publications [7,11,21] but the possible role played by prolonged seizures and SE in developmental decline has been highlighted by other authors [19,22].

Language is another skill whose early impairment has been reported since the first studies of the disease [1–3,23,24]. Delayed word acquisition or specific troubles such as articulation defects (dysarthria) or dysphasia were initially reported. These findings were then confirmed in reports of older patients around the first decade of age [10] and adults [19,25] and were consistent with what has been described in studies on auditory detection and phonological working memory tasks [26]. A possible dissociation between expressive and receptive language has also been suggested recently [27]. Though this is only an empirical study based on observational findings, our sample showed that early language was affected, including expression that was clearly more severely impaired than comprehension in about three quarters of patients. Only in one patient were language abnormalities not observed throughout FU, while all other patients presented with early or late impairment, consisting in particular of frequent phonological errors and lexical poorness that persisted after three years.

Our data also demonstrate the prevalence of neurological signs (consisting of impaired motor coordination and postural disorders) in association with neuropsychological developmental disorders as reported by other authors [7,22]. This is consistent with experimental [28] and clinical [9] studies that emphasize the cerebellar profile of neurodevelopment in DS. Behavioral disorders were also very common, as generally reported in other studies from the early years [4,8–11,21]. Their profiles were widely variable, including attention deficit disorder with hyperactivity, autistic spectrum disorders, aggressive and oppositional behavior to name the most common features. They were multifactorial in origin and it is difficult in each case in the presence of developmental fragility (secondary behavioral disorders) to determine whether the underlying mechanisms are structural (genetic, epileptic or induced by antiepileptic drugs) or the expression of adaptive problems, though the higher severity of behavioral disorders among patients with more impaired developmental competence could point to the latter hypothesis. In our sample, the main behavioral disorders consisted of hyperactivity and attention disorders, with aggressive behavior prevalent in patients with global cognitive impairment.

The original findings of our study concern the developmental trajectories of patients. The course of the neurodevelopmental profile was variable with respect to the type of patterns and the timing of changes. However, the predominant phenotype trajectory concerns patients who generally experienced an onset of developmental decline in the second year of life, with visuomotor involvement in particular. This is consistent with the results of the only prospective longitudinal studies in the literature to our knowledge, carried out on a small sample in the same age range [5,8,12]. In those studies, impairment of visuomotor skills was observed as a cascade involving visual function up to visual higher order abilities. According to the dual stream model [29] these abilities depend on visual dorsal pathways, including extrastriate visual-motor areas that extend from the occipital to the parietal and frontal regions and enable spatial abilities and visual control of actions. The visuomotor impairment in DS patients has already been reported in the literature as part of a "dorsal stream vulnerability" syndrome [12] which is also present in several genetic neurodevelopmental disorders such as Williams syndrome, fragile-X syndrome, Prader-Willi syndrome or other genetic syndromes showing common pathways contributing to defects in visuomotor function [30].

The selective involvement of visuomotor abilities in DS has been widely reported in the literature since the first neuropsychological retrospective studies [4,8,9,11,21]. Our study thus confirms an initial phenotype consisting of a defect in visual sensorimotor integration, or a kind of DS core neuropsychological

phenotype possibly linked to the channelopathy [12,31,32].

The second point that emerges from our sample concerning cases beginning with a visuomotor defect consists of the eventual generalization of developmental impairment over time. In previous retrospective studies, neuropsychological progression of the disease was suggested with an initial predominance of the impairment of visuomotor skills and possibly a further extension of impairment to all fields of abilities [5,9,11,21,33]. It appears plausible that the "already vulnerable system may be susceptible to secondary aggravating events" such as frequency and type of seizure and antiepileptic treatment [22]. Possibly due to other chronic factors (epileptic disorder, antiepileptic drugs), but also to genetic factors (the defect in Nav1.1 channels may become more apparent "as the brain matures with age and adapts towards higher cognitive functioning" [22]), the overall extension of skill impairment could act to mask the original neuropsychological profile. Interestingly, in our sample, the four cases that did not evolve toward a generalization by the end of follow-up had less severe epilepsy.

Two other neuropsychological trajectories could be observed in our study. The second group consisted of 12 patients with early, rapid, apparently sudden developmental decline including all abilities assessed by the Griffiths scales. Their developmental profiles showed an initially steep drop in developmental abilities and a final outcome GDQ mean of about 60, clearly lower than that of the "visuomotor" group" (around 70). Therefore, even though a significant phenotype-genotype correlation was not found in our study (which was limited, however, by the relatively small sample), it is possible that the genetic background [34] may help explain the more severe pattern of the "global" group. On the other hand, this dual developmental profile and outcome of the neuropsychological evolution in DS mimics what has been already found by Ragona *et al.* in a previous retrospective longitudinal study [7] showing an initial steeply falling curve with lower total IQ outcome in one group of cases and a gradual decline with a clearly higher total IQ at outcome in the other group.

Finally, the third neuropsychological trajectory that was identified was that of the "normal" group, which included only two cases. It should be noted however that both patients had language and behavioral disorders. Delayed emergence of developmental impairment cannot be ruled out; on the other hand, we should also consider the possibility of a distinct, mild genetic expressivity as already described [35].

The limitations of our study, particularly with regard to the overall analysis, are the incomplete data from periodical assessments, with bias resulting from different FU durations. The correlation between epilepsy data and developmental impairment could have been underestimated, particularly regarding the role of SE, due to the small number of cases in the different groups. Furthermore, the assessment tools (Griffiths scales, observations) were unable to examine all aspects of early development. Finally, the impact of antiepileptic drugs in contributing to developmental decline was hardly taken into account at all, due to the variability of drug treatments and the relatively small sample. Nevertheless, our study on developmental trajectories helps to improve the knowledge of mechanisms underlying developmental/behavioral disorders in DS. Early diagnosis of milder cases with initial visuomotor impairment may provide new tools for a more accurate habilitation strategy.

5. Disclosure

The authors declare that they have no conflicts of interest.

No copyrighted material from other sources (including the internet) was used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2020.10.004.

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FULL LENGTH ORIGINAL RESEARCH

Efficacy and safety of Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: A real-world study

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Abstract

Objective: Dravet syndrome (DS) is a drug-resistant, infantile onset epilepsy syndrome with multiple seizure types and developmental delay. In recently published randomized controlled trials, fenfluramine (FFA) proved to be safe and effective in DS.

Methods: DS patients were treated with FFA in the Zogenix Early Access Program at four Italian pediatric epilepsy centers. FFA was administered as add-on, twice daily at an initial dose of 0.2 mg/kg/d up to 0.7 mg/kg/d. Seizures were recorded in a diary. Adverse events and cardiac safety (with Doppler echocardiography) were investigated every 3 to 6 months.

Results: Fifty-two patients were enrolled, with a median age of 8.6 years (interquartile range [IQR] = 4.1-13.9). Forty-five (86.5%) patients completed the efficacy analysis. The median follow-up was 9.0 months (IQR = 3.2-9.5). At last follow-up visit, there was a 77.4% median reduction in convulsive seizures. Thirty-two patients (71.1%) had a ≥50% reduction of convulsive seizures, 24 (53.3%) had a ≥75% reduction, and five (11.1%) were seizure-free. The most common adverse event was decreased appetite (n = 7, 13.4%). No echocardiographic signs of cardiac valvulopathy or pulmonary hypertension were observed. There was no correlation between type of genetic variants and response to FFA.

Significance: In this real-world study, FFA provided a clinically meaningful reduction in convulsive seizure frequency in the majority of patients with DS and was well tolerated.

KEYWORDS

childhood epilepsy, convulsive seizures, Dravet syndrome, fenfluramine, SCN1A

Nicola Specchio and Nicola Pietrafusa contributed equally to this paper.

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1 | INTRODUCTION

Dravet syndrome (DS) is a rare, drug-resistant, developmental, and epileptic encephalopathy with onset in infancy,[1] characterized by multiple types of epileptic seizures, developmental delay, cognitive impairment, and crouch gait.[2]

It is estimated that DS incidence ranges from 1 in 15 700 to 1 in 40 000. In >80% of patients, a sodium voltage-gated channel alpha subunit 1 gene (*SCN1A*) genetic variant can be demonstrated, although diagnosis is based on clinical criteria.[25]

Patients with DS have an increased risk of sudden unexpected death in epilepsy, with a mortality rate of 7%-18% under the age of 18 years.[6] A high frequency of generalized tonic-clonic seizures is a major risk factor for this outcome.[7]

Valproate, clobazam, and stiripentol are considered as first-line treatment in DS. Ketogenic diet, topiramate, and cannabidiol (CBD) represent second-line treatment choices. Levetiracetam, bromides, zonisamide, and vagal nerve stimulation can be taken in account as third line.[810]

The drugs most recently approved or nearing US Food and Drug Administration (FDA) and European Medicine Agency (EMA) approval are stiripentol, CBD, and fenfluramine (FFA). Stiripentol waas approved in the USA in 2018, whereas in Europe, Canada, and Japan it has been available since 2007 and 2012.[11,12] In 2018 and 2019, the FDA and EMA, respectively, approved the use of CBD (EMA as add-on with clobazam) for treating seizures in DS, based on a randomized controlled trial (RCT).[13,14] In June 2020, the FDA approved FFA for the treatment of seizures in patients with DS.

FFA was effective for the treatment of convulsive seizures in DS in open-label studies[1517] and in two placebo RCTs.[18,19] It was also reported to be effective for the treatment of nonconvulsive status epilepticus (NCSE).[20]

Although RCTs are required for FDA and EMA approval of an investigational drug, both regulatory agencies can authorize expanded access programs (EAPs), also referred as compassionate use. In the 2019, Zogenix supported an EAP of FFA in patients with a clinical diagnosis of DS, without echocardiographic signs of cardiac valve disfunction and pulmonary arterial hypertension. Here, we evaluate efficacy and safety of add-on FFA in a series of patients with DS consecutively enrolled within the EAPs at four Italian pediatric centers, in a real-world clinical practice context.

Key Points

- DS is a drug-resistant, infantile onset epilepsy syndrome with multiple seizure types and developmental delay
- We administered FFA to 52 DS patients in the context of a recently approved early access program
- The median follow-up was 9.0 months; at last follow-up visit, there was a 77.4% median reduction in convulsive seizures
- Fifty-three percent of patients had a ≥75% reduction, and 11.1% were seizure-free
- The most common adverse event was decreased appetite (13.4%); no signs of cardiac valvulopathy or pulmonary hypertension were observed

2 | MATERIALS AND METHODS

2.1 | Study design and patient population

This is a prospective independent, open-label study conducted at four Italian epilepsy centers prescribing FFA in the context of an EAP granted by Zogenix. All patients with a clinical diagnosis of DS, consecutively seen, whose parents accepted FFA treatment proposal were enrolled if they had no echocardiographic signs of cardiac valve disfunction and pulmonary arterial hypertension and had been on stable doses of antiseizure medications (ASMs) for ≥4 weeks. An institutional review board at each site approved the treatment and study protocols, and parents/caregivers provided written informed consent before any study-related assessments. The study was conducted in accordance with the Good Clinical Practice guidelines and local standard operating procedures.

2.2 | Procedures and study design

Demographic and clinical data, including the *SCN1A* genetic variants, were collected. Genetic variants were stratified into three groups: loss of function, missense in the pore region (S5-S6), and missense outside the pore region.

Primary outcome was efficacy of FFA; secondary outcome was tolerability.

The study was planned with a 28-day baseline period and a titration period, followed by a maintenance period. During the baseline period, parents/caregivers completed written diaries of all countable seizure types. We collected data on convulsive seizures only, defined as hemiclonic, tonic, clonic, generalized tonic-clonic, and focal with clearly observable motor signs. Concomitant ASMs were recorded at baseline. After the baseline observation period, patients received FFA hydrochloride (2.5 mg/mL) in oral solution (Zogenix) at a gradually increasing dose from 0.2 to 0.7 mg/kg/d (twice daily) until tolerated or a maximum dose of 26 mg/d (17 mg/d if in coadministration with stiripentol). Duration of the titration period and maximum dose were at

the physician's discretion, based on clinical response. During the maintenance period, dose changes of FFA and other ASMs were allowed and recorded. Clinical evaluation including seizure count and evaluation of adverse events (AEs) was done every 3 months.

2.3 | Assessment of efficacy

The primary efficacy endpoint was the percentage change in the frequency of convulsive seizures from baseline per 28 days measured through last follow-up visit (titration plus maintenance period). The frequency was calculated as the number of convulsive seizures recorded after FFA initiation, divided by the number of days from titration to last follow-up visit (LFV). The result was multiplied by 28 for a monthly frequency. Both median and mean percentage change from baseline were calculated using the following formula: (seizure frequency through LFV − seizure frequency during baseline) × 100/seizure frequency during baseline.

We noted the proportion of patients who had ≥25%, ≥50%, ≥75%, or 100% reduction in convulsive seizures from baseline; those with seizure reduction ≥ 50% were defined as responders. The proportion of patients with an increase or no change (≥0% and <25%) in seizure frequency was also recorded. These data were calculated as detailed above. The proportions of seizure-free patients and of those with no more than one convulsive seizure for 6 months were also recorded. Finally, to facilitate comparability of findings between this study and previous RCTs, we calculated the monthly median percentage reduction in convulsive seizures and the percentages of responders at 3 months of follow-up.

We evaluated the effect of FFA also on nocturnal and self-induced seizures. To evaluate a difference in efficacy by age, we also compared seizure frequency in patients aged <6 years and those >6 years of age. At last follow-up visit, we administered the Clinical Global Impression (CGI) scale to caregivers to assess the effects of FFA on behavior, autonomy, communication, and motor skills. We applied the chi-square test to determine whether significant differences had emerged in the items explored.

2.4 | Assessment of tolerability

The number and percentage of subjects with AEs were summarized in terms of severity and relationship to study drug. Serious AEs (SAEs) were summarized separately. Cardiovascular safety was assessed via Doppler echocardiogram at baseline and every 6 months (or every 3 months at the physician's discretion).

2.4.1 | Statistical analysis

All demographic, clinical, efficacy, and safety data were analyzed. Continuous data were summarized using descriptive statistics including means, standard deviations, medians, lower and upper quartiles, and ranges. Categorical variables were summarized with frequencies and percentages. A statistical hypothesis testing was planned; for categorical results, a chi-square test or the Fisher exact test was performed, as appropriate. Wilcoxon signed-rank test was used for continuous variables. A P value ≤ .05 was considered statistically significant. Statistical analysis was performed using R version 3.2.3 (R Foundation for Statistical Computing, https://www.r-project.org/).

3 | RESULTS

3.1 | Demographics and baseline characteristics

Fifty-two patients (29 males) with a median age of 8.6 years (interquartile range [IQR] = 4.1-13.9, range = 2.1-28.6), all carrying *SCN1A* genetic variants, were enrolled (Table 1; see Table S1). The median follow-up was 9.0 months (IQR = 3.6-9.5, range = 3.0-14.9). Mean patient weight was 35.0 kg (range ± standard deviation [SD] = 11.0-97.0 ± 21.5). Mean dose of FFA was 0.46 mg/kg/d (range ± SD = 0.2-0.7 ± 0.16). Patients were previously treated with a median of three ASMs (IQR = 2-3). At the beginning of FFA administration, patients were on a median of three ASMs (IQR = 2-3); the most commonly used drugs were valproate (n = 47), clobazam (n = 42), stiripentol (n = 31), topiramate (n = 5), and clonazepam (n = 5). Three patients had previously been treated with an artisanal formulation of CBD. Valproic acid blood levels, measured before starting FFA and at last follow-up, did not differ significantly (77.4 μg/mL vs 71.1 μg/mL, P = .2).

3.2 | Efficacy

Data on seizure frequency were available for 45 patients (86.5%). The remaining seven patients were excluded from the analysis of efficacy because data collection was incomplete (Table 2).

Monthly median convulsive seizure frequency was 6.0 (IQR = 4-14.0) at baseline and 1.9 (IQR = 0.5-4.5) at last follow-up (Figure 1), with a median 77.4% (IQR = 43.6-94.4) percentage reduction in convulsive seizure frequency.

Thirty-two of 45 patients (71.1%) experienced a ≥50% reduction in convulsive seizure frequency with a mean FFA dose of 0.41 mg/kg/d (range ± SD = 0.20-0.80 ± 0.14). Twenty-four patients (53.3%) achieved ≥75% reduction, and five patients (11.1%) became seizure-free (Figure 2).

TABLE 1 Baseline demographics and clinical features (N = 52)

Characteristic	Value[a]
Patients	52
Sex	
Male	29 (54.7)
Female	24 (45.3)
Age at enrollment, y	8.6 (4.1-13.9, range = 2.1-28.6)
Children, age <18 y	46 (88.5)
Adults	6 (11.5)
Weight, kg	35.3 (11.0-97.2 ± 21.5)
Previous ASMs	2 (1-3)
Current ASMs, patients, n (%); dose, mg/kg/d	
VPA	47 (90.4); 20.7 (8.8-40 ± 7.7)
CLB	42 (80.7); 0.4 (0.14-1.0 ± 0.2)
STP	31 (59.6); 30.0 (13.5-52.3 ± 10.5)
TPM	5 (9.6); 4.7 (1.4-8 ± 1.9)
CZP	5 (9.6); 0.07 (0.02-0.1 ± 0.05)
LEV	3 (5.8); 35.5 (31.2-45.4 ± 5.8)
PB	3 (5.8); 2.0 (1.6-2.5 ± 0.4)
ETS	2 (3.8); 27.6 (20.0-35.3 ± 7.6)
ZNS	1 (1.9); 3.5
KD	2 (3.8)
VPA blood levels, μg/mL	
At enrollment	77.4 (35.0-125.0 ± 18.0)
At last follow-up	71.1 (45.0-105.0 ± 13.2)
FFA dose, mg/kg/d	0.46 (0.2-0.7 ± 0.16)
Follow-up, mo	9.0 (3.6-9.5, range = 3.0-14.9)
Convulsive seizures	
GTCS	35 (67.3)
Focal, with observable motor signs	11 (21.1)
Hemiclonic	9 (17.3)
Other type of seizures	
Focal, without clearly observable motor signs	8 (15.4)
Atypical absence	9 (17.3)
Myoclonic	5 (9.6)
Baseline convulsive seizure frequency	
Mean	15.5 (1-100 ± 20.7)
Median	6 (4.0-14.0)
Titration period, d	13.4 (7.0-21.0 ± 3.1)
FFA withdraws	
Inefficacy	1 (1.9)
Increased seizure frequency	2 (3.8)
Refractory SE	1 (1.9)

Abbreviations: ASM, antiseizure medication; CLB, clobazam; CZP, clonazepam; ETS, ethosuximide; FFA, fenfluramine; GTCS, generalized tonic-clonic seizures; KD, ketogenic diet; LEV, levetiracetam; PB, phenobarbital; SE, status epilepticus; STP, stiripentol; TPM, topiramate; VPA, valproic acid; ZNS, zonisamide.

[a]Values are given as n (%), mean (range ± standard deviation), or median (1st quartile-3rd quartile).

Five (11.1%) patients remained seizure-free for at least 6 months, and another four (8.9%) experienced no more than a single convulsive seizure during the same time interval.

Considering 3 months of follow-up, we found a median percentage reduction of convulsive seizures of 73.3 (IQR = 44.5-93.3) and a reduction in convulsive seizures of ≥50% in 68.9% of patients (Table 2).

Monthly median percentage reduction in convulsive seizures in patients with clearly observable focal seizures with motor signs or hemiclonic seizures was 87.1% compared to 74.5% in those with generalized tonic-clonic seizures, with no statistically significant differences ($P = .375$; see also Table 2).

When considering the age at the time of the study, we could not find a significant difference in the monthly median percentage reduction of convulsive seizures ($P = .08$) between patients younger than 6 years and those older than that age. We could only identify a trend suggesting greater efficacy in younger patients (84.3% vs 70.1%; Table 2).

When analyzing the effects of the coadministration of stiripentol on efficacy, we observed that cotreatment with this drug resulted in a monthly median percentage reduction of convulsive seizures of 72.7%, versus 90.6% observed in patients not taking it, and the response rate was, respectively, 65.6% and 81.2%, approaching statistical significance ($P = .085$).

Seven patients (15.6%) had not achieved a significant reduction in seizure frequency, and five of them (71.4%, or 11.1% of the total number) experienced a median percentage worsening of convulsive seizures of 44.8 (IQR = 12.0-86.6).

FFA determined a reduction in the frequency of nocturnal seizures in 56.0% of patients (14 of 25 presenting sleep-related seizures) and a reduction in the frequency of self-induced seizures in 60.0% of patients (three of five).

Titration varied from 7 to 21 days, with an average of 13.4 days. There were no differences in titration duration between different investigators. In patients with loss of appetite, the mean titration period was 13 days, and in the remaining patients it was 13.11 days. We could not find differences in efficacy either.

FFA treatment allowed a simplification of baseline treatment in 24 patients (46.1%) with tapering of other ASMs;

TABLE 2 Efficacy data (n = 45)

Characteristic	Value[a]
Last follow-up convulsive seizure frequency	
Mean	3.9 (0.0-4.8 ± 17.6)
Median	1.9 (IQR = 0.5-4.5)
Percentage reduction in CS frequency from baseline	
Mean	57.1 (54.4)
Median	77.4 (53.6-93.6)
0-<25 reduction in CS frequency	8 (17.8)
≥25% reduction in CS frequency	37 (82.2)
≥50% reduction in CS frequency	34 (75.6)
≥75% reduction in CS frequency	25 (55.6)
100% reduction in CS frequency	5 (11.1)
Seizure-free for 6 mo	5 (11.1)
One seizure in 6 mo	4 (8.9)
Three months of follow-up convulsive seizure frequency	
Percentage reduction in CS frequency from baseline	73.4 (44.5-93.3)
≥50% reduction in CS frequency	31/45 (68.9)
Patients with GTCS	32 (71.1)
Percentage reduction in CS frequency from baseline	74.5 (42.3-93.8)
≥50% reduction in CS frequency	22/32 (68.7)
Patients without GTCS[b]	13 (28.9)
Percentage reduction in CS frequency from baseline	87.1 (61.6-98.1)
≥50% reduction in CS frequency	11/13 (84.6)
Patients younger than 6 y	17/45 (37.8)
Percentage reduction in CS frequency from baseline	84.3 (68.9-98.1)
≥50% reduction in CS frequency	14/17 (82.3)
Patients older than 6 y	28/45 (62.2)
Percentage reduction in CS frequency from baseline	70.1 (41.6-89.4)
≥50% reduction in CS frequency	18/28 (64.3)
Patients with STP	29 (64.6)
Percentage reduction in CS frequency from baseline	72.7 (32.9-87.1)
≥50% reduction in CS frequency	19/29 (65.5)
Patients without STP	16 (35.5)
Percentage reduction in CS frequency from baseline	90.6 (62.3-98.3)
≥50% reduction in CS frequency	13/16 (81.2)

Abbreviations: CS, convulsive seizures; GTCS, generalized tonic-clonic seizures; IQR, interquartile range; STP, stiripentol.

[a]Values are given as n (%), mean (range ± standard deviation), or median (1st quartile-3rd quartile).

[b]Patients with focal (with observable motor signs) and hemiclonic seizures.

in six of 31 patients (19.3%) with concomitant stiripentol, the overall dose of the latter was reduced; moreover, in six patients (11.5%), one of the concomitant ASMs was discontinued. More specifically, in seven patients ASMs were stopped or lowered after 2 consecutive months of seizure freedom; two of them experienced seizure recurrence. In four additional patients, a simplification of concomitant ASMs was performed following 25%-75% reduction of seizure frequency. In three remaining patients who did not benefit from FFA addition, reduction of coadministered drugs, carried out to decrease the drug load, did not influence seizure frequency.

Looking at correlations between clinical/demographic features and outcome (responders vs nonresponders), we could not find a statistically significant difference. A higher number of baseline concomitant and previous ASMs correlated with nonresponders (respectively, $P = .04$ and $P = .02$; see Table S2). Correlation between genetic variant subgroups and response to FFA failed to uncover any association ($P = .2$).

The CGI scale, administered to 49 patients/parents at last follow-up, indicated improvements in different items, including behavior in 21 patients (42.8%, $P = .32$), autonomy in 20 (40.2%, $P = .12$), communication in 28 (57.1%, $P = .003$), and motor skills in 21 (42.8%, $P = .02$). Details are presented in Table S4.

3.3 | Safety

Data on safety were available for all patients. The most common AE was decreased appetite (n = 7, 13.4%; Table 3). This AE was apparent at a mean FFA dose of 0.43 mg/kg/d; it was mild in most patients (n = 6) and led to dose reduction in three. Three of seven patients who reported decreased appetite experienced a clinically irrelevant loss of weight, in none of them resulting in FFA discontinuation. One patient subsequently withdrew topiramate, recovering a normal appetite.

Other AEs were observed in six patients (11.5%). One patient had interstitial pneumonia (SAE), which required hospitalization. One patient manifested temporary periungual cyanosis (mild AE). During FFA administration, one patient experienced myoclonic seizures, which had never been observed before (moderate AE); one patient manifested an increase of seizure frequency, and one manifested refractory status epilepticus (SE; SAE with hospitalization); in the latter two patients, FFA was withdrawn.

In two patients, worsening of fever-related seizures was observed; one of them had a prolonged seizure during fever (SAE with hospitalization), and another experienced recurrent febrile seizures that did not respond to endorectal diazepam.

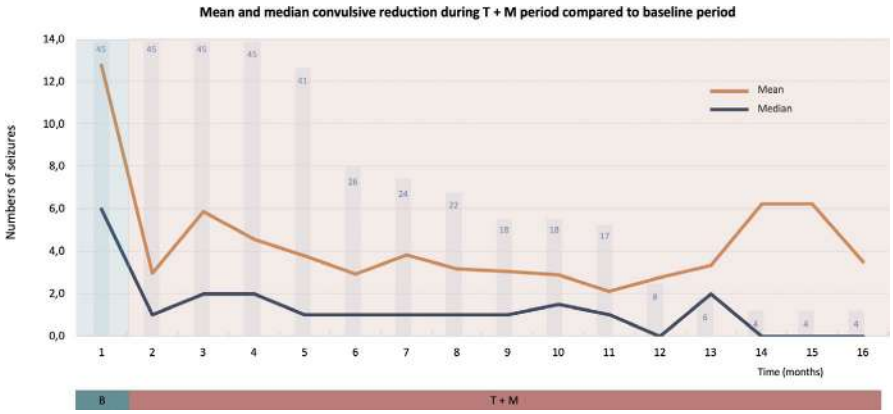

FIGURE 1 The graph shows the mean and median absolute reduction in monthly frequency of convulsive seizures during the titration (T) and maintenance (M) period, compared with the baseline observation period (B). The figure shows, in parallel, the number of patients in follow-up

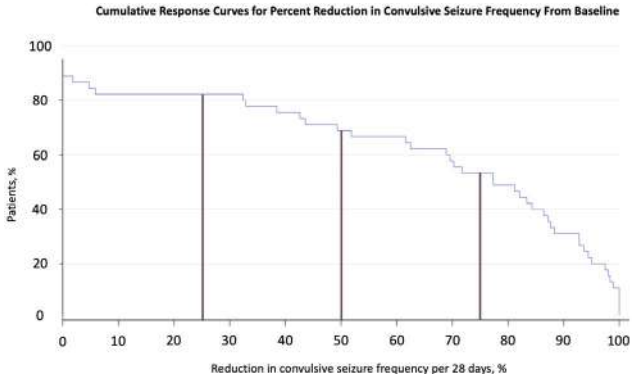

FIGURE 2 Cumulative response curves for percentage reduction in convulsive seizure frequency from baseline. Results are plotted for combined titration and maintenance periods. Vertical lines represent 25%, 50%, and 75% reduction in convulsive seizure frequencies; percentages correspond to the proportion of patients who met or exceeded each response level

TABLE 3 Adverse events reported (N = 52)

Characteristic	Value[a]
Decreased appetite	7 (13.2)
Outcome	
Resolved	2/7 (28.6)
Not resolved/ongoing	5/7 (71.4)
Severity	
Mild	6/7 (85.7)
Moderate	1/7 (14.3)
Decreased weight	3/7 (42.8)
Action taken with FFA	
Reduction of FFA	3/7 (42.8)
None	4/7 (57.2)
Other AEs	6/52 (11.3)[b]
SAE	4/52 (7.5)
Hospitalization	2/52 (3.7)
FFA total daily dose, mg/kg/d	0.48 (0.3-0.8 ± 0.14)
Normal echocardiogram	52/52 (100)

Abbreviations: AE, adverse event; FFA, fenfluramine; SAE, serious AD.
[a]Values are given as n (%) or mean (range ± standard deviation).
[b]See text.

None of the patients experienced clinical or echocardiographic signs of cardiac valvulopathy or pulmonary hypertension.

4 | DISCUSSION

DS is characterized by a high seizure burden, associated with a series of neurological comorbidities such as developmental and motor delay, and behavioral disturbances.[2] Prolonged convulsive seizures in DS often require emergency room admission.[21] The severity and extreme resistance to medications results in an urgent need for developing new and more effective pharmacologic treatments.

In this first open-label, real-world treatment experience with FFA in DS, we documented that add-on FFA administration can provide a durable and clinically significant reduction in convulsive seizure frequency in the majority of patients. Similar results from retrospective and prospective open-label studies in the Belgian DS cohort have been reported in a small group of patients who were treated for over 28 years, based on maintained efficacy, in the absence of signs of valvulopathy.[1517]

We found a median percentage reduction of convulsive seizures of 77.4 and a reduction in convulsive seizures of ≥50% in 71.1% of patients. Even considering differences in study design, results observed in our cohort seem to be slightly better than those reported in the two prospective, double-blind, placebo-controlled trials published earlier (Figure 3).[18,19] When analyzing our data with respect to a 3-month follow-up period, we found a median percentage reduction of convulsive seizures of 73.3, with a ≥50% reduction in 68.9% of patients. These results indicate a slightly better efficacy than observed in recent RCTs.

One factor that may explain the more favorable response we recorded is the possibility of adjusting and personalizing

the dose. One hundred nineteen[18] and 87[22] DS patients were enrolled in two recently published RCTs. In the larger trial (patients without stiripentol), which featured two arms of FFA, 0.2 and 0.7 mg/kg/d, the percentages of ≥50% responders were 38% and 68%, respectively, versus only 5% in the placebo group. In the 0.7-mg/kg/d group, 8% of patients were seizure-free during the 14-week trial duration, and the median reduction in seizure frequency was 74.9%.[18] In the other study, which included patients cotreated with stiripentol, ≥50% responders at 0.4 mg/kg/d were 54% in the FFA arm compared to 5% in the placebo group; the median percentage reduction from baseline in convulsive seizures was 63.1.[19] The preliminary results of an interim analysis of a long-term open-label extension study showed that 64.4% of patients had a ≥50% reduction of convulsive seizures, and 41.2% experienced a ≥75% reduction.[23] Overall, in this extension study, efficacy remained stable over >1-year follow-up, with an average decrease of monthly convulsive seizure frequency of 66.8%.[23] The RCT evaluating the efficacy of stiripentol, added to valproic acid and clobazam in DS,[12] documented a ≥50% reduction in convulsive seizures in 71% of patients, and a significant reduction of episodes of SE.[12]

A recent first description of a patient with DS with NCSE successfully treated with 0.6-mg/kg/d oral load of FFA[20] warrants further investigations (Table 4).

CBD has recently been approved for the treatment of DS based on results of a trial showing that 43% of patients on CBD and 27% of those on placebo experienced at least 50% seizure reduction.[13]

The long-term effects of add-on CBD were reported for patients with DS and Lennox-Gastaut syndrome in an open-label study with a 50% monthly reduction of major motor seizures.[24]

Overall, stiripentol associated with clobazam, FFA, and CBD associated with clobazam are three promising options for patients with DS. Although no comparative trials have been performed, figures emerging from published studies suggest a slight superiority of FFA on convulsive seizures.[25]

In our open-label study, FFA treatment allowed a reduction in the number of associated ASMs, resulting in discontinuation of stiripentol in 13.4% of patients, and of a different drug in 26.9%. Reducing the ASM load is highly warranted in DS, as most patients are on a median of three ASMs, and 38% are still experiencing weekly seizures.[21,26]

Although we did not observe a greater efficacy of FFA in children treated sooner after seizure onset, future studies should specifically address FFA efficacy in the early stages to fully assess its potential as a first-line treatment option.

Assessment obtained by the CGI scale suggested that FFA treatment was accompanied by improved behavior, autonomy, communication, and motor skills. Although assessing behavioral issues in children with severe encephalopathies is always difficult, and providing inferences based on a scale nears oversimplification, we consider this preliminary observation of interest and worth a specific study design in future trials.

A relatively small percentage of our patients did not achieve a significant reduction in seizure frequency, and 11.1% experienced seizure worsening. Among them, one patient experienced, while on FFA, recurrence of convulsive SE that was nonresponsive to common treatments

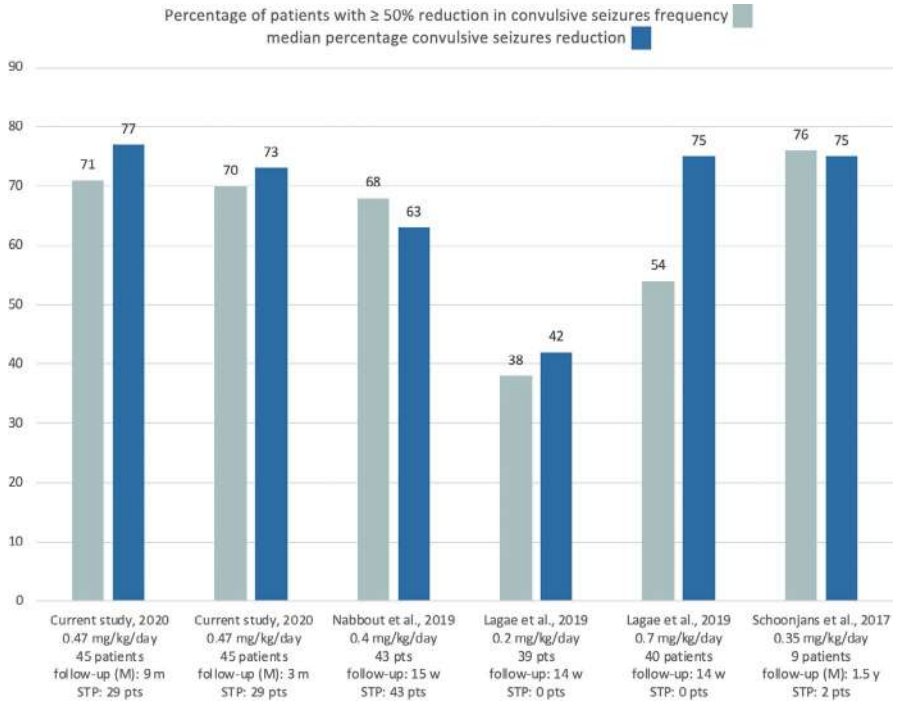

FIGURE 3 Comparison of the percentages of patients with ≥50% reduction in seizure frequency and the median percentage reduction of monthly frequency of seizures in different studies. The figure also shows the mean dose of fenfluramine (mg/kg/d), total number of patients, follow-up, and number of treated with stiripentol in the different studies. M, median; m, months; pts, patients; STP, patients with stiripentol; w, weeks

TABLE 4 Most common concomitant ASM dose adjustments (N = 52)

Characteristic	Value, n (%)
Patients with STP	31 (59.6)
STP reduction	6 (11.5)
STP discontinuation	1 (1.9)
Other ASM reduction	8 (15.4)
Other ASM discontinuation	6 (11.5)

Abbreviations: ASM, antiseizure medication; STP, stiripentol.

(benzodiazepine, phenobarbital), and required anesthesiologic intervention with deep sedation. A previous report mentioned a patient in whom FFA was discontinued due to seizure worsening.[19] Looking at clinical characteristics of patients experiencing worsening of seizures in our series, we could not single out a specific phenotypic profile, or a particular form of DS. Mutation types did not differ either, if compared with the overall sample of patients. Because fluctuations of seizure frequency are frequently seen in DS, future FFA treatment studies will focus on whether its use definitely carries a risk of seizure worsening in some patients.

The AE profile of FFA in our DS cohort appeared to be mild when compared with earlier experiences. We observed anorexic effects, usually mild, early in treatment in 13.4%. None of the patients withdrew FFA due to AEs. In the two previous RCTs, adverse effects on appetite were seen in 38%[18] and 44%[19] of patients; weight decrease was seen in a few patients, and only one experienced weight loss that led to drug discontinuation.[18]

Absence of clinical or echocardiographic signs of cardiac valvulopathy or pulmonary hypertension is consistent with previous studies, where only traces of regurgitation, which is a normal physiologic finding and cannot be considered evidence of valve dysfunction,[27] were observed during a median of 256 days.[18,22,28] Although FFA appears to be safe, further studies are necessary to assess its long-term safety on cardiac valves, even considering that 40 mg/d of FFA carries a 9.2-fold (95% confidence interval = 2.1-40.8) lower risk of severe valvulopathy than 60 mg/d when used as a treatment for adult obesity, most commonly in combination with phentermine.[29]

We found FFA coadministration not to cause significant differences in valproate plasma levels. It was previously reported that FFA does not significantly modify the pharmacokinetics of valproate, clobazam (and nor-clobazam), and stiripentol; however, the association of stiripentol, clobazam, and valproate might have effects on the pharmacokinetics of FFA and norfenfluramine, and therefore, FFA should be adjusted and reduced when added to the previously mentioned triad of drugs.[30] We found no statistical difference in treatment efficacy when comparing patients with versus those without stiripentol ($P = .085$). However, in view of the small sample size and of the pharmacokinetics of these ASMs, this aspect should be investigated further.

We found no evidence that the type *SCN1A* genetic variant may influence sensitivity to FFA; again however, studies with a considerably larger number of patients will clarify this aspect.

Based on studies on zebrafish models of DS, FFA documented its activity on the serotonin receptors and sigma-1 receptors that seem to have a role in mediating seizure activity in DS.[31,32] The selective agonism on 5-HT1D and 5-HT2C and antagonism on sigma-1 receptors might be one of the mechanisms of action of FFA, which seems to be targeted for DS patients. Repurposing of other medications that act on serotonin receptors has been recently hypothesized.[33]

4.1 | Study limitations

This study is limited by the small sample size, the relatively short median follow-up, the open-label design, and lack of a control group.

5 | CONCLUSIONS

Efficacy shown by FFA in reducing convulsive seizures in our cohort, if confirmed by future RCTs, might in the near future support an indication to use FFA as a first-line treatment for DS. We emphasize that, although we have reported that lower doses of FFA were as effective as those used in previous RCTs, based on clinical improvement we reduced the overall load of concomitant medications, and no evidence of interactions with valproate emerged; our observations are preliminary and additional studies are needed to confirm them.

In conclusion, FFA was safe and provided sustained, clinically meaningful convulsive seizure reduction in this real-world study. Other studies are needed to better establish the long-term safety and efficacy, and to clarify the response profile of DS patients to FFA.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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PCDH19 genetic variants). This study was done also through a collaboration of Italian EpiCARE centers.

CONFLICT OF INTEREST

All authors were subinvestigators or principal investigators in the clinical trials ZX008-1502 and ZX008-1503, sponsored by Zogenix. N.S., N.P., M.T., F.V., and R.G. have received consulting fees from Zogenix. None of the other authors has any conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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SUPPLEMENT ARTICLE

Dravet syndrome: Early electroclinical findings and long-term outcome in adolescents and adults

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Abstract

To describe the outcome of Dravet syndrome (DS) in adolescents and adults we conducted a longitudinal retrospective study of two independent cohorts of 34 adolescents (group 1) and 50 adults (group 2). In both cohorts, we collected information about genetic mutation, and semiology of seizures at onset and during disease course. At the last evaluation, we considered the following features: epilepsy (distinguishing myoclonic/complete and nonmyoclonic/incomplete phenotype), neurologic signs, intellectual disability (ID), and behavioral disorders. Moreover, in both cohorts, we performed a correlation analysis between early characteristics of the disease and the outcome of DS with regard to seizure persistence, ID, behavioral disorder, and neurologic impairment at last evaluation. Group 1 includes 22 adolescents with complete form of DS and 12 with incomplete form; group 2 includes 35 adults with complete form and 15 with incomplete form. The seizures persisted in 73.6% of adolescents and in 80% of adults, but epilepsy severity progressively decreased through age. Seizure persistence correlated with the complete phenotype and with the occurrence of reflex seizures. At last evaluation, ID was moderate or severe in 70.5% of adolescents and in 80% of adults. The most severe cognitive and motor impairment was observed in patients with persisting seizures. The severity of cognition, language, and neurologic impairment at last evaluation correlated statistically with the complete phenotype. The study confirms that the global outcome of DS is poor in most cases, albeit epilepsy severity decreases throughout adulthood. The improvement of epilepsy throughout ages is not associated with improvement in intellectual abilities and motor skills; this confirms that the unfavorable outcome is not a pure consequence of epilepsy.

KEYWORDS

adolescents and adults, complete Dravet syndrome, developmental and epileptic encephalopathy, long-term outcome, myoclonic phenotype, reflex seizures

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1 | INTRODUCTION

Dravet syndrome (DS) is a rare disease characterized by drug-resistant epilepsy and by cognitive, neurologic, and behavioral impairment of variable degree.[1] As reported by Scheffer and Dravet,[2] the outcome is heterogeneous: although some patients have a relatively good outcome, others have persisting severe epilepsy complicated by several comorbidities including intellectual disability (ID), language impairment, and behavioral and motor disorders with gait problems.[3,4]

Literature data report persistent seizures, language impairment, and ID in the majority of adolescents[5] and adults.[6–12] The neuropsychological decline usually appears evident in the second/third year of life,[13–16] and becomes more pronounced in adolescence. Cognitive disabilities and behavioral disturbances are among the most important negative prognostic factors of quality of life in adults.[12,17,18] Disabling motor disorders are also frequently reported both in adolescents and adults,[4–6,8–10,18–21] and the presence of a parkinsonian component has been recently outlined.[22,23]

The respective role of genetic mutation, epilepsy, and treatment in determining the final outcome is still largely unknown. The few reported studies aimed at correlating the early electroclinical features and the long-term outcome did not reach consistent conclusions.[3,5,7,16,19,24,25]

We report the results of a retrospective longitudinal study of two cohorts of patients—34 adolescents (group 1) and 50 adults (group 2)—in order to describe the features of DS in two different stages of life, and to contribute to clarify which early characteristics of the disease may predict the outcome.

Key Points

- Epilepsy severity in Dravet syndrome (DS) decreases progressively from childhood to adolescence and throughout adulthood
- Seizure persistence in adolescents and adults correlates strictly with cognitive and neurologic impairment
- The severity of cognitive and neurologic impairment correlates with complete/myoclonic phenotype
- The severity of cognitive and neurologic impairment correlates with the presence of reflex seizures (photo-pattern and self-induced)
- The severity of intellectual disability and language impairment correlates with early onset of seizures

2 | PATIENTS AND METHODS

This longitudinal retrospective study includes two cohorts: Group 1, 34 adolescents longitudinally followed-up at the Child Neurology Unit of Fondazione Policlinico Universitario Gemelli, IRCCS, Roma; and Group 2, 50 adults recruited, longitudinally followed-up, and evaluated with common methods at the Child Neuropsychiatry, University of Verona (31 patients) and at the Department of Paediatric Neuroscience of Neurological Institute C.Besta in Milano (19 patients).

The inclusion criteria for both cohorts were: clinical diagnosis of DS,[1] longitudinal follow-up since infancy in their referral centers, and molecular analysis of the *SCN1A* gene. Mutation analysis of the *SCN1A* gene was performed by the Sanger method, denaturing high-performance liquid chromatography (DHPLC) and, whenever necessary, multiplex ligation probe amplification (MLPA) to detect deletion and duplication. For all the patients, informed consent was obtained from parents or tutors and from the local ethics committees. Further details on the methods are reported separately in the following sections.

2.1 | Group 1—Adolescents

This cohort includes 34 patients (20 female; median age 16 years 2 months) admitted to the Child Neurology Unit of Fondazione Policlinico Universitario Gemelli, IRCCS, between 2000 and 2017, and between 11 and 20 years of age at the last evaluation. The cohort comprises 20 cases reported in Olivieri et al[5] and 7 cases described previously at a younger age.[14,26,27]

Diagnostic work-up included clinical examination, evaluation of seizure semiology, video–electroencephalography (EEG) recordings, magnetic resonance imaging (MRI), and genetic tests. We analyzed epilepsy features during childhood (from onset to 10 years of age) and adolescence (from 11 to 20 years of age), and classified the patients in three groups according to the severity of epilepsy (group A, mild; group B, moderate; group C, severe), as shown in Table 1. In line with literature criteria,[28] we classified DS as "Complete" or "Incomplete DS": the latter term refers to DS without atypical absences (AA) and myoclonic seizures. Intellectual functioning was assessed by Wechsler scales, Raven's Colored Progressive Matrices (RCPMs), or Leiter-R according to the age and to the level of collaboration, and was classified according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) in normal intellectual functioning, mild ID, moderate ID, or severe ID.

To evaluate the course of cognitive abilities from childhood to adolescence (improvement, stabilization, or worsening), we compared the results of the last cognitive assessment available before the age of 10 (24 patients) with the neuropsychological data at the last evaluation. Behavioral disorders

TABLE 1 Group 1, adolescents: Epilepsy severity criteria

Epilepsy	Group	Convulsive sz/ focal sz	Period with absences/ myoclonic sz	Obtundation SE	Convulsive SE	Sz-free period >1 y
Mild	A	Yearly	No	No	No	Yes/no
Moderate	B	Monthly	≤1 mo	No	Yes/no	Yes/no
Severe	C	Daily/weekly	≥1 mo	Yes	Yes	No

Abbreviations: Sz, seizure; SE, status epilepticus.

were assessed by Child Behavior Checklist (CBCL) and by clinical observation.

Neurologic examination evaluated the presence of motor disorders with particular attention to gait disorders and cerebellar and extrapyramidal signs.

We investigated the presence of negative prognostic factors for epilepsy and ID severity at the last evaluation. Analysis of variance (ANOVA) was used to test difference in mean age at follow-up and severity of epilepsy and cognitive outcome in adolescence. Where a difference existed, the age at follow-up was used to adjust association analysis. Ordinal logistic regressions, adjusted for age at follow-up in months, were performed to estimate: (a) the association between relevant explanatory variables (gender, *SCN1A* mutation, DS type, neurologic signs, and behavioral disorders) and severity of epilepsy in adolescence; (b) the association between relevant variables (gender, *SCN1A* mutation, DS type, neurologic signs, behavioral disorders, improvement in epilepsy severity from childhood to adolescence, seizure severity, and at least 1 year of seizure-freedom at the outcome) and cognitive outcome.

2.2 | Group 2—Adults

This cohort includes 50 patients (27 female, mean age 29 years, median 28 years) who were older than 18 years of age at the last evaluation; the patients have been selected within a series of 172 DS patients followed at Child Neuropsychiatry, University of Verona or at the Department of Paediatric Neuroscience of Neurological Institute C.Besta in Milano.

We collected information about family history of epilepsy and/or febrile seizures, age and semiology of seizures at onset, and seizure semiology during the disease course. Seizure semiology has been defined according to the International League Against Epilepsy (ILAE) classification.[29] Photo pattern sensitivity (PPS) and self-induced seizures (SiS), confirmed by EEG polygraphic recordings, were both defined as reflex seizures (RS). During the follow-up, the patients were evaluated comprehensively at regular intervals, by clinical and EEG recordings and by cognitive evaluation (Griffiths and Wechsler scale, according to the level of collaboration and intellectual ability).

At the last evaluation we considered the following features: (a) Epilepsy: we collected information on semiology and frequency of seizures and details on the current antiepileptic treatment. Based on seizure semeiology, we distinguished two phenotypes: (1) "Myoclonic Phenotype" consistent with the "Complete" DS, and (2) "Nonmyoclonic Phenotype" consistent with the "Incomplete" DS. (b) Neurologic examination: we evaluated the presence of pyramidal, extrapyramidal, and cerebellar signs, of gait impairment and action myoclonus. (c) Language abilities: we evaluated the expressive language; according to the level of impairment, we distinguished the following categories: 0, patients with functionally effective language; 1, patients able to sustain a simple conversation; 2, patients using short sentences; 3, patients able to produce only isolated words; 4, patients not capable of speaking. (d) Intellectual disability and behavioral disorders: based on clinical evaluation, we distinguished normal functioning, and mild, moderate, and severe ID. For the statistical analysis, patients with normal cognitive functioning were aggregated with patients with mild ID, patients with moderate ID were aggregated with patients with severe ID. Behavioral disorders were assessed by CBCL, and by the clinical observation. (e) Adaptive functioning was assessed by Vineland Scale in 29 patients of 50.

Statistical analysis has been done by Pearson chi-square test. A P-value <0.05 was regarded as significant.

3 | RESULTS

3.1 | Group 1—Adolescents

The cohort includes 22 patients with the complete form of DS and 12 with the incomplete form. The general demographic, genetic, clinical, and neuroimaging data of the whole series in relation to the severity of epilepsy are summarized in Table 2. During adolescence, epilepsy was mild in 41.1% (14 patients, group A), moderate in 32.4% (11 patients, group B), and severe in 26.5% (9 patients, group C). At the last evaluation, 9 of the 14 patients in group A had been seizure-free for at least 1 year, whereas 5 still experienced either febrile or afebrile generalized tonic-clonic seizures (GTCSs) and/or focal seizures yearly. All the patients of group A were still given antiepileptic drugs (AEDs), with the exception of one patient who was on the ketogenic diet. The 11 patients of group B were all still experiencing seizures, monthly in six cases and weekly in five cases. GTCSs were reported in all

TABLE 2 Group 1, adolescents: general demographic, genetic, clinical, neuroimaging data according to epileptic outcome

	Total	Group A	Group B	Group C
	34	14 (41.1%)	11 (32.4%)	9 (26.5%)
Gender				
Female	20 (59%)	9	7	4
Male	14 (41%)	5	4	5
Mean age	16 y 3 mo	15 y 3 mo	15 y 3 mo	19 y
SCN1A				
No mutation/deletion	4 (12%)	2	2	0
Truncating	11 (32%)	3	3	5
Missense	16 (47%)	8	4	4
Deletion	3 (9%)	0	2	1
Brain MRI				
Thinning corpus callosum	2	1	1	0
Hippocampal atrophy	2	0	2	0
Nodular heterotopia	1	0	0	1
Cerebellar atrophy	1	0	0	1
Cerebral atrophy	1	0	0	1
DS type				
Complete	22 (65%)	6	8	8
Incomplete	12 (35%)	8	3	1
Onset age (mean)	5.75 mo	6.2 mo	5.3 mo	5.6 mo
Childhood severity				
Group A	1 (2.9%)	1	0	0
Group B	14 (41%)	10	4	0
Group C	19 (55.8%)	3	7	9
Seizure-free (last year)	9 (26%)	9	0	0
Cognitive outcome				
No ID	1 (2,9%)	1	0	0
Mild ID	9 (26,4%)	6	3	0
Moderate ID	14 (41,1%)	6	4	4
Severe ID	10 (29,4%)	1	4	5
Behavioral disorders				
No/mild	9 (27%)	6	1	2
Moderate	11 (32%)	4	5	2
Severe	14 (41%)	4	5	5
Neurologic signs				
No	12 (35%)	8	4	0
Cerebellar signs	8 (24%)	3	3	2
Crouch gait	11 (32%)	3	4	4
Extrapyramidal signs	3 (9%)	0	0	3

Abbreviation: ID, intellectual disability.

patients, focal seizures in eight and atypical absences in three. All the nine patients in group C experienced GTCSs, associated with focal seizures in five and with myoclonic seizures in two; seizures clustered mostly during nocturnal sleep and epileptic status was observed in four of nine patients. None of the patients with mild or moderate epilepsy during childhood had a severe epilepsy during adolescence. Twenty of the 33 patients with moderate or severe epilepsy during childhood

improved in adolescence. At the last evaluation, ID was mild in 26.4%, moderate in 41.1%, and severe in 29.4%; a normal IQ was observed in one patient only. Cognitive standardized evaluation performed between 6 and 10 years was available for 24 patients. The degree of ID was unchanged in 13 (only 1 from group C, 6 from group A, and the other 6 from group B); by contrast the IQ level was reduced in 11 patients (5 in group A and 3 each in groups B and C). Epilepsy severity decreased in 10 of the 13 patients with stable IQ (77%), whereas epilepsy severity decreased in 6 of the 11 patients with IQ decline (54.5%).

Behavioral problems or psychiatric disorders were observed in most patients (25 cases, 73%). The behavioral disorders ranged from mild or moderate—consisting of attention deficit, anxiety, perseveration, rule breaking, aggressiveness—to severe obsessive-compulsive disorder (13 patients), autistic features, and psychosis (5 patients).

We detected motor abnormalities in 22 patients (65% of the whole cohort). The 12 patients without motor deficits belonged to groups A and B. In all cases, motor abnormalities appeared during childhood, usually before the age of 6 years.

The ANOVAs of mean age at follow-up by groups of epilepsy severity revealed a significant difference: patients with severe epilepsy in adolescence were significantly older than those with mild or moderate epilepsy ($P = 0.007$).

Adjusting by age at follow-up, complete form of DS, and SCN1A deletion showed a significantly higher probability for severe epilepsy in adolescence. There were no significant correlations between gender, neurologic signs, behavioral disorders, and severity of epilepsy (Table 3).

The severity of cognitive impairment was significantly associated with the following: complete form of DS, severe epilepsy during childhood, persistence of seizures, and presence of neurologic signs. Improvement in seizure severity from childhood to adolescence reduced the possibility of a severe cognitive impairment (Table 4).

TABLE 3 Group 1, adolescents: association between relevant explanatory variables and severity of epilepsy

	Epilepsy severity in adolescence		
	adjOR[a]	**CI 95%**	***P*-value**
Gender			
Male	1	-	
Female	0.41	0.10-1.62	0.205
SCN1A mutation			
No mutation	1	-	
Missense	3.5	0.26-46.1	0.348
Truncating	14.1	0.96-207.1	0.053
Deletion	**57.6**	**2.0-1656.3**	**0.018**
DS type			
Incomplete	1	-	
Complete	**6.0**	**1.2-28.5**	**0.026**
Neurologic signs			
No	1	-	
Cerebellar signs	3.2	0.5-19.8	0.217
Crouch gait	3.7	0.7-20.2	0.133
Behavioral/psychiatric disorders			
No/mild	1	-	
Moderate	1.8	0.3-11.3	0.511
Severe	3.2	0.6-17.6	0.187

Abbreviations: CI, confidence interval; DS, Dravet syndrome.
Bold values reflect statistical significance.
[a]OR ordinal regression adjusted for the age at follow-up in months.

3.2 | Group 2—Adults

The cohort includes 23 males and 27 females with age at last visit between 18 and 50 years (mean 29 years and median 28 years). In 23 cases (46%), a positive family history for epilepsy and/or febrile seizures was reported. The genetic analysis of the *SCN1A* gene, performed in all 50 cases, detected a truncating mutation in 24 patients and a missense mutation in 24 patients. In two patients, extensive molecular analysis, which also included a Next Generation Sequencing (NGS) multigenic panel, failed to detect mutations or deletions in the *SCN1A* gene, as well in other genes related to childhood epilepsy.

Epilepsy onset ranged from 3 to 12 months (mean 5.6 months and median 5 months). The first seizure occurred within the fifth month of life in half of the patients (26/50). The onset was marked by febrile seizure in 25 subjects. The first seizure was reported as generalized tonic-clonic or clonic (GTCS-GCS) in 24 patients, unilateral seizure (US) in 15, and nonmotor focal-onset seizure (NMFOS) in 7. In four children the onset was characterized by recurrent isolated massive myoclonias (MMs) triggered by fever or bath. In 20 patients, the epilepsy onset was marked by convulsive status epilepticus (CSE) (Figure 1). The clinical diagnosis of DS was made within the age of 3 years in 29 subjects (within the age of 1 year in 14 of 29), between 4 and 10 years in 10, and later in 11. During the disease course, all patients experienced GTCS/GCS. Other types of seizures included the following: US (30 patients) NMFOS (40 patients), AA with or without myoclonias (38 patients), MM and/or absences with myoclonias (35 patients) (Figure 1). Nineteen patients experienced reflex seizures induced by photo pattern stimulation; 14 of them also had self-induced seizures. According to the type of seizures, we distinguished two phenotypes: (a) "Myoclonic Phenotype", 35 patients (70%, 15M, 20F) who

TABLE 4 Group 1, adolescents: association between relevant explanatory variables and cognitive outcome in adolescence

	Cognitive outcome		
	crudeOR	**CI 95%**	***P*-value**
Gender			
Male	1	-	
Female	0.62	0.17-2.24	0.466
SCN1A mutation			
No mutation	1	-	
Missense	3.94	0.49-31.76	0.197
Truncating	6.86	0.76-62.25	0.087
Deletion	1.59	0.11-23.14	0.732
DS type			
Incomplete	**1**	**-**	
Complete	**4.31**	**1.06-17.50**	**0.041**
More than 1 y seizure-free			
Yes	**1**	**-**	
No	**7.22**	**1.32-39.46**	**0.022**
Epilepsy during childhood			
Mild/Moderate	**1**	**-**	
Severe	**6.26**	**1.46-26.87**	**0.014**
Neurological signs			
No	**1**	**-**	
Cerebellar signs	**35.14**	**3.85-320.74**	**0.001**
Crouch gait	**14.33**	**1.96-105.00**	**0.009**
Extrapyramidal signs	**66.28**	**3.33-1317.81**	**0.006**
Behavioral/psychiatric disorders			
No/Mild	1	-	
Moderate	0.61	0.12-3.15	0.551
Severe	0.92	0.19-4.38	0.915
Improvement in seizure severity from childhood to adolescence			
No	**1**	**-**	
Yes	**0.22**	**0.05-0.90**	**0.035**

Abbreviations: CrudeOR, crude ordinal regression; CI, confidence interval; DS, Dravet Syndrome.
Bold values reflect statistical significance.

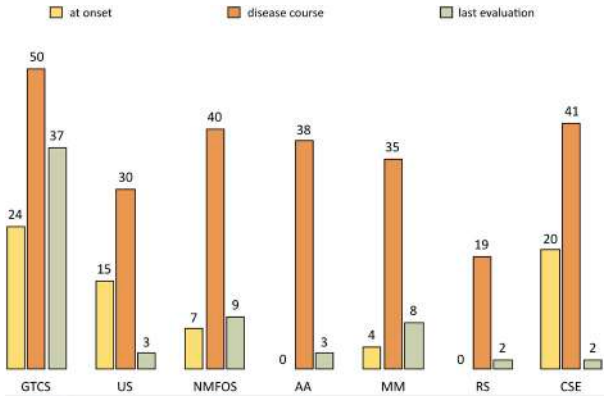

FIGURE 1 Group 2, adults: seizure types at onset, during disease course and at last evaluation. GTCS, generalized tonic clonic seizures; US, unilateral seizures; NMFOS, nonmotor focal seizures; AA, atypical absences; MM, massive myoclonias; RS, reflex seizures; CSE, convulsive status epilepticus [Color figure can be viewed at wileyonlinelibrary.com]

had experienced MM-AM, which were associated with RS in 19 cases; (b) "Nonmyoclonic Phenotype", 15 patients (30%, 8M 7F) who never experienced MM-AM or RS. During the disease course, the majority of patients (41/50, 82%) had more than 3 CSEs. At the last examination, 40 patients of 50 (80%) were still experiencing seizures; the remaining 10 patients, aged between 19 and 50 years (median age 28 years), were seizure-free (seizure-free period ranging between 1 and 10 years, median 3 years). In almost all the patients (37/40, 92.5%), GTCS were reported to occur mostly during sleep, and to recur in cluster in only 9 cases. Frequency of seizures was yearly in 8 subjects, several per-year in 14, several per month in 12, and several per week in 3. Nonmotor focal seizures persisted in 9 of 40 patients (22.5%). Other less common types of seizures were US in three subjects, AA in 3, sporadic MM in 8, MM-AM associated with PPS and SiS in 2. Only two patients still had sporadic CSE. In 12 of 40 patients (30%), the seizures were still triggered by febrile illness. During the disease course, there was a significant reduction of all types of seizures, with the exception of GTCS (Figure 1). At the last examination, all patients were still given AEDs, in polytherapy in the majority of cases (13 patients 2 drugs, 13 patients 3 drugs, 20 patients 4 drugs, and 2 patients 5 drugs). The most used antiepileptic drugs were valproic acid (43/50), clobazam (33/50), topiramate (24/50), and stiripentol (11/50). Only two patients, seizure-free, were given only valproic acid. As expected, cognitive, motor, behavioral, and social impairment of variable severity occurred in the great majority of the subjects through the disease course.

At the last examination, cognitive functioning was normal in three subjects only (6%). ID was mild in 7 patients (14%), moderate in 18 (36%), and severe in 22 (44%).

Neurological examination was normal in six cases only (12%). Motor impairment with pyramidal, extrapyramidal signs, and ataxia was present in 44 patients (88%); motor deficits were worsened by cortical myoclonus in 41 patients (82%). Gait impairment—of variable severity—was present in more than half of the patients (28/50%-56%); 4 patients were bedridden (8%). A gait pattern resembling crouch gait was observed in 13 of 28 cases (26%).

Language skills were normal in 8 cases (16%); 7 patients (14%) were able to sustain a simple conversation, 12 patients (24%) used short sentences, 11 patients (22%) were able to

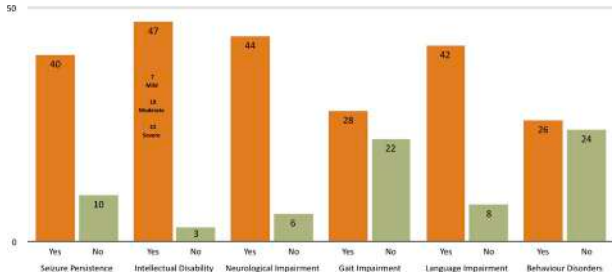

FIGURE 2 Group 2, adults: clinical picture at last evaluation (number of patients) [Color figure can be viewed at wileyonlinelibrary.com]

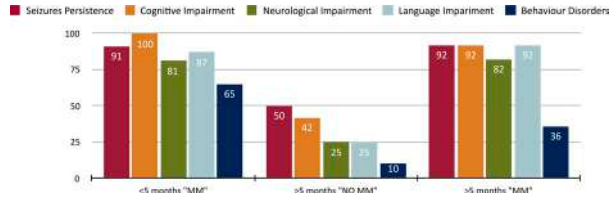

FIGURE 3 Group 2, adults: outcome according to: age at epilepsy onset and myoclonic or no-myoclonic phenotype (in %) [Color figure can be viewed at wileyonlinelibrary.com]

produce only isolated words, and 12 patients were not capable of speaking.

In half of the patients (26/50) the clinical observation and results from CBCL documented severe behavioral disorders: attention deficit, obsessive, and oppositional disorder in 12 patients, marked autistic traits in 11, and psychosis in 2 (Figure 2). The adaptive and behavioral developmental quotient, as evaluated by the Vineland Scale in 29 adults patients, was significantly impaired: the impairment involved communication (median age equivalent: 5.3 years), daily living (median age equivalent: 5.9 years) and socialization (median age equivalent: 4.9 years). Seventeen patients had a mental age below 4 years, and only five patients had a mental age higher than 10 years.

The comparison between the patients who experienced MM (19 cases) and the remaining 10 cases revealed that all the patients with a mental age higher than 10 years belonged to the second group (no MM).

3.3 | Correlation analysis between epilepsy features in adulthood and clinical picture in adulthood

The persistence of seizures in adulthood correlates with the following variables: moderate/severe ID ($P = 0.011$), absent or very poor language ($P = 0.008$), and pyramidal/extrapyramidal signs associated with cortical myoclonus (P = 0.020). Among patients with persisting seizures, the more severe ID ($P = 0.043$) was observed in those still experiencing MM. Seizures persisted in adulthood in only three of the six patients with normal neurologic examination.

3.4 | Correlation analysis between early clinical features and clinical picture in adulthood

Statistical analysis failed to find a correlation between persistence of seizures in adulthood and the following variables: age at onset, age at diagnosis, semiology of the first seizure and its occurrence during fever, and recurrence of status epilepticus. The seizure persistence at the last visit, by contrast, correlates with the following variables: (a) occurrence of MM-AM ($P = 0.002$) even in cases with seizure onset after 5 months of life ($P = 0.025$); (b) occurrence of RS ($P = 0.041$); (c) presence of SiS ($P = 0.041$); and (d) presence of a truncating mutation ($P = 0.026$).

Statistical analysis failed to find a correlation between the long-term outcome (cognitive and language impairment, and behavioral disorders) and the following: age at diagnosis, first seizure semiology, its febrile/afebrile occurrence, recurrence of CSE, and type of *SCN1A* mutation. The severity of intellectual disability correlates with the early onset of seizures (within 5 months) and with the presence of MM-AM and RS. Moderate and severe ID statistically correlates with early onset of seizures ($P = 0.015$). Normal cognitive functioning and mild ID statistically correlates with the lack of MM and/or R ($P < 0.001$) and of RS ($P = 0.013$). The severity of language impairment correlates with the early onset of seizures (6.9746 (2) $P = 0.0305$) and with the presence of MM-AM ($P < 0.001$). The severity of neurologic disorders correlates with the presence of MM-AM ($P < 0.001$). The severity of behavioral disorders correlates with the presence of MM-AM ($P = 0.0019$) and RS ($P = 0.0311$).

We also found a positive correlation between RS and the severity of the clinical picture in adulthood. The appearance of RS statistically correlates with seizures persistence ($P = 0.041$), severe cognitive outcome ($P = 0.013$), and severe behavioral disorder ($P = 0.0211$).

To summarize the results of statistical analysis, seizure persistence and the severity of ID significantly correlate with the occurrence of MM-AM and of RS; moreover, the severity of ID correlates with early onset of seizures (<5 months).

We then analyzed separately the association between the long-term outcome and the two significant variables: MM-AM and early onset (Table S1).

In the group of patients with early onset of seizures (<5 months) there was a higher incidence of MM-AM than in the group of "late onset" ($P = 0.003$), as well as a higher incidence of severe language impairment ($P = 0.030$) and cognitive outcome ($P = 0.0158$). However, the association between MM-AM and the severity of global clinical outcome is confirmed also by the analysis of the "late onset" patients (>5 months). In this group too, the appearance of MM-AM is related to seizure persistence ($P = 0.002$) (>5 months $P = 0.025$), severe cognitive outcome ($P < 0.001$)

(>5 months: $P = 0.0339$), poor language ($P < 0.001$) (>5 months: $P = 0.0033$), severe neurologic impairment ($P < 0.001$) (>5 months $P = 0.0133$), and severe behavioral disorders ($P = 0.019$) (>5 months $P = 0.03524$) (Figure 3).

3.5 | Comparison between adolescents and adults

The severity of epilepsy, behavioral disorders, and motor and intellectual disabilities was different in the two cohorts. The percentage of patients with frequent seizures was 26% among adolescents and 8% among adults; the behavioral disorders were present in 73% of adolescents and in 52% of adults. By contrast, severe ID and motor disorders were more frequently observed in adults than in adolescents.

The statistical analysis demonstrated that the severity of epilepsy, cognition, language, and neurologic impairment, as well as the impairment of adaptive functioning significantly correlated with the complete/myoclonic phenotype, both in adolescents and in adults.

4 | DISCUSSION

The study on a large number of patients followed from the onset of epilepsy confirms that the global outcome of DS in adolescence and adulthood is poor in most cases. The seizures persist in 73.6% of adolescents and in 80% of adults. Nevertheless, as reported previously,[7,8,10,11,30] epilepsy severity progressively decreases from childhood to adolescence in more than half of patients, and further throughout adulthood. Nearly all adolescents and adults with persisting seizures experience GTCS, mostly nocturnal and in cluster. By contrast, only a minority of patients continue to experience atypical absences, myoclonic, and reflex seizures. Moreover, seizure frequency decreases significantly from adolescence to adulthood: seizures are more than weekly in 36% of adolescents but only in 7.5% of adults, and less than monthly in 20% of adolescents but in 60% of adults.

The percentage of seizure-free adolescents (26.4%) during the last year of follow-up was a little higher than that reported in other studies.[30,31] This might suggest that the adolescents of this cohort have benefited from the early diagnosis and the appropriate treatments available in the last two decades. However, another possible explanation is that the spectrum of DS has been expanded and now includes, besides the "complete phenotype" (formerly termed Severe Myoclonic Epilepsy of Infancy, SMEI), the "incomplete or nonmyoclonic" phenotypes. As recently reported by Darra and coauthors,[32] who compared three different groups of patients with DS (born between 1972 and 1990, 1991 and 2000, and 2001 and 2010), the global outcome was more favorable in the younger group, just because of the higher incidence of "incomplete" forms of DS in this group. Actually, in our two series, seizure persistence correlates with the complete/myoclonic phenotype and with the occurrence of reflex seizures as observed originally by Dalla Bernardina[33] and confirmed in following studies.[3,5,11,16,19,30,34,35]

By contrast, we did not find strong correlations between the genetic findings and the evolution of the disease: actually the severity of epilepsy correlated with the presence of *SCN1A* deletion in adolescents and *SCN1A* truncating mutation in adults, but no correlation was found between genetic findings and the severity of comorbidities.

It is acknowledged that, in addition to seizures, many other clinical problems—including cognitive impairment, behavioral disorders, and a number of comorbidities—characterize the disease course. Recent studies have underscored that these comorbidities are the most disabling symptoms in long-term outcome.[3,9,12,15,19,36–38] The analysis of our series confirms that ID and behavioral disorders with the ensuing impairment of adaptive functioning causes a remarkable burden for the families.

In our two cohorts, in line with previous reports,[5–9,11,13,19,21] in the large majority of patients, language is impaired (84% of adults) and ID is moderate or severe (70.5% of adolescents and 80% of adults); normal cognitive functioning, as exceptionally reported[39] was observed in 1 of 24 adolescents and 3 of 50 adults. Also in keeping with previous reports is the relevance of motor disturbances that are present in 65% of adolescents and in 88% of adults, with crouch gait in one-third of cases.[4–6,8–11,18,20,22,23,40–42] The most severe cognitive and motor impairment was observed in patients with persisting seizures.[6,7,10,11,18,19] Whether the severity of these comorbidities is related to the severity of epilepsy or to an intrinsically severe phenotype is still unclear. Behavioral problems are present in 73% of adolescents and 52% of adults and include obsessive-compulsive disorders, rule breaking, aggressiveness, and autistic features. Unlike cognitive and motor deficits, behavioral disorders are not related to the severity of epilepsy.

A second aim of our study was to clarify which early characteristics of the disease may predict the outcome of DS in adolescence and adulthood.

The severity of cognition, language, and neurologic impairment, as well as the impairment of adaptive functioning, statistically correlate, both in adolescence and in adulthood, with the complete/myoclonic phenotype as reported previously.[3,5,11,16,18,19,34] Moreover, among the variables considered in the analysis of the adult cohort, a statistically significant correlation was found between the early onset of seizures, the presence of RS, and the severity of cognitive/language and neurologic impairment. The correlation between the complete/myoclonic phenotype and the worst

clinical outcome might be due to the higher incidence of myoclonic phenotype in the early onset group, but it is confirmed also in the late-onset patients. These data confirm that the epilepsy phenotype actually bears a prognostic value, regardless of the age at onset.

5 | CONCLUSIONS

Our study confirms that DS is a developmental and epileptic encephalopathy characterized by drug-resistant epilepsy and multiple comorbidities including cognitive, neurologic, and behavioral disorders. The global outcome of DS in adolescence and adulthood is poor in most cases, albeit epilepsy severity decreases from childhood to adolescence, and throughout adulthood, and behavioral disorders decrease from adolescence to adulthood. In both the cohorts, the worst outcome is significantly correlated with the "Complete/Myoclonic" phenotype. Further predictors of poor long-term outcome, demonstrated in the cohort of adult patients, are the early appearance of myoclonus, reflex seizures (photo-pattern and self-induced) and the early onset of seizures.

The improvement of epilepsy throughout ages is not associated with improvement in intellectual abilities and motor skills. This confirms that the unfavorable evolution cannot be considered a pure consequence of epilepsy and that other variables are likely to concur in influencing mental outcome. Further multicentric studies on larger and prospective cohorts are needed to clarify the reciprocal role played by different cofactors (such as genotype, epileptic features, and treatments) in determining the epileptic, cognitive/behavioral long-term evolution of DS. Finally, the end points of the next therapeutic trials should include, beside the reduction of convulsive seizures, the effect on myoclonic component and reflex seizures, as well as the effect on cognitive and behavioral disorders.

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CONFLICT OF INTERESTS

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Clinical and genetic factors predicting Dravet syndrome in infants with *SCN1A* mutations

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ABSTRACT

Objective: To explore the prognostic value of initial clinical and mutational findings in infants with *SCN1A* mutations.

Methods: Combining sex, age/fever at first seizure, family history of epilepsy, EEG, and mutation type, we analyzed the accuracy of significant associations in predicting Dravet syndrome vs milder outcomes in 182 mutation carriers ascertained after seizure onset. To assess the diagnostic accuracy of all parameters, we calculated sensitivity, specificity, receiver operating characteristic (ROC) curves, diagnostic odds ratios, and positive and negative predictive values and the accuracy of combined information. We also included in the study demographic and mutational data of the healthy relatives of mutation carrier patients.

Results: Ninety-seven individuals (48.5%) had Dravet syndrome, 49 (23.8%) had generalized/genetic epilepsy with febrile seizures plus, 30 (14.8%) had febrile seizures, 6 (3.5%) had focal epilepsy, and 18 (8.9%) were healthy relatives. The association study indicated that age at first seizure and frameshift mutations were associated with Dravet syndrome. The risk of Dravet syndrome was 85% in the 0- to 6-month group, 51% in the 6- to 12-month range, and 0% after the 12th month. ROC analysis identified onset within the sixth month as the diagnostic cutoff for progression to Dravet syndrome (sensitivity = 83.3%, specificity = 76.6%).

Conclusions: In individuals with *SCN1A* mutations, age at seizure onset appears to predict outcome better than mutation type. Because outcome is not predetermined by genetic factors only, early recognition and treatment that mitigates prolonged/repeated seizures in the first year of life might also limit the progression to epileptic encephalopathy. ***Neurology*® 2017;88:1–8**

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GLOSSARY

CI = confidence interval; **DOR** = diagnostic odds ratio; **FS** = febrile seizures; **GEFS+** = generalized/genetic epilepsy with febrile seizures plus; **OR** = odds ratio; **RESIDRAS** = Italian National Registry for Dravet Syndrome and *SCN1A*-Related Conditions; **ROC** = receiver operating characteristic.

The voltage-gated sodium channel *SCN1A* gene is, among all the known epilepsy genes, the most clinically relevant, with the largest number of epilepsy-related mutations characterized.[1] Epilepsy phenotypes associated with *SCN1A* mutations include familial febrile seizures (FS), GEFS+ (generalized or, as more recently proposed, genetic epilepsy with FS plus), and Dravet syndrome, the last representing by far the most severe phenotype (Online Mendelian Inheritance in Man No. 182389). Observations that in Dravet syndrome, but not in the other *SCN1A*-associated phenotypes, early normal development is followed by severe cognitive impairment and additional neurologic features[2] suggest that early epileptic activity contributes to impaired brain function, resulting in an epileptic encephalopathy.[3] This causal link has not yet been demonstrated, however.

At present, most pediatric epilepsy specialists suggest mutation screening of the *SCN1A* gene soon after an infant experiences prolonged/repeated fever-associated seizures because they

Supplemental data at Neurology.org

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suspect that these seizures may represent the initial manifestations of Dravet syndrome.[4] However, early detection of an *SCN1A* mutation leaves important practical questions unanswered concerning management, prognosis, and counseling in that genotype-phenotype correlations are loose and after a common early clinical presentation the phenotypic spectrum may vary considerably in severity.

We studied 200 individuals with *SCN1A* mutations and explored the prognostic value of mutational data and early clinical findings that may help clinicians to set up management choices adapted to individuals at higher risk of progressing to Dravet syndrome, without delaying them until the epileptic encephalopathy has become obvious.

METHODS We retrospectively analyzed 200 consecutive individuals with mutations in the *SCN1A* gene. Patients were enrolled from 6 Italian tertiary clinical centers with pediatric epilepsy expertise as part of a pilot study we conducted to preliminarily test the accuracy and feasibility of the data entry online form to adopt for the Italian National Registry for Dravet Syndrome and *SCN1A*-Related Conditions (RESIDRAS; http://www.residras.com). We included all patients and healthy carriers with *SCN1A* mutations who were consecutively observed in the participating centers and were >24 months of age when last seen because this is the age at which Dravet syndrome can usually be diagnosed.[5] Clinical data were collected through a standardized form including demographic data, family and personal history, age at/duration of first seizure, presence of fever, and neurologic and neuropsychological outcome (figure e-1 at Neurology.org). The epilepsy phenotype was classified according to the International League Against Epilepsy criteria.[6] However, considering that such criteria predate the identification of *SCN1A* as the causative gene for Dravet syndrome and that some authors have subsequently described mutated patients with Dravet syndrome and seizure onset beyond the first year of life,[7–9] we did not firmly predefine age at first seizure as a cutoff time for diagnosis but relied on the clinical severity. We maintained the distinction between GEFS+ and focal epilepsy because some patients manifested focal seizures only and represented, in our opinion, a distinctive subgroup. We identified the following clinical subgroups: (1) Dravet syndrome (including the so-called borderline forms), (2) GEFS+, (3) focal epilepsy, (4) FS, and (5) *SCN1A* mutation carriers who had never experienced seizures. Definitions of the different clinical subgroups are provided in appendix e-1. To explore the value of early available parameters as prognostic indicators, we focused on patients' characteristics at seizure onset. Because Dravet syndrome is the most severe *SCN1A*-associated phenotype, with a constantly unfavorable outlook and requiring the most complex management choices, we analyzed data with patients divided into 2 groups: Dravet (also including borderline forms) and non-Dravet (including FS, GEFS+, focal epilepsy). Patients in the non-Dravet group were all free of seizures at last follow-up and exhibited normal or slightly delayed cognitive development.

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained for each individual. The study was approved by the Pediatric Ethics Committee of the Tuscany Region, in the context of both the EU Project DESIRE–602531 and the RESIDRAS initiative.

Genetic analysis. Methods for genetic analysis are provided in appendix e-1.

Statistical analysis. We used the STATA 13 (T Stat s.r.l.) for statistical analysis and descriptive statistics to describe the participants' main variables. For each Dravet/non-Dravet outcome, we performed the Pearson χ^2 test of independence on tables of frequency for each categorical variable of interest (mutation type, sex, type of first seizure, age at seizure onset, presence/absence of fever at onset, first seizure duration, familial epilepsy, EEG discharges) and Student t test for unequal variance for each continuous variable. Age at seizure onset was analyzed both as a continuous and as a categorical variable with individuals grouped into 3 classes: 0 to 6, 6 to 12, or >12 months at onset (table 1).

The studied population also included probands' relatives, as ascertained by familial segregation of clinical manifestations and mutations. Because of the hierarchical structure of data, we fit a standard logistic regression model that was amended to have random effects for each family. More formally put, we used a sandwich estimator for the variance-covariance matrix.[10] We performed a sensitivity analysis for a multilevel logistic model using a stepwise method.

To assess and compare the diagnostic accuracy of all the parameters we chose for discriminating at first seizure those patients who would develop Dravet syndrome from those who would face a less severe outcome, we calculated sensitivity, specificity, receiver operating characteristic (ROC) curves, diagnostic odds ratios (DORs), and positive and negative predictive values. Finally, we combined information from the different parameters. When appropriate, confidence intervals (CIs) were calculated with the use of exact likelihood.[11] Level of significance was set at 5% 2 sided.

RESULTS **Patients.** We analyzed 200 consecutive individuals carrying *SCN1A* mutations (109 male, 91 female carriers) with an average age of 18.58 years at last follow-up (SD 18.08, range 2.06–81.06 years). Seizures were the presenting symptom in 182 patients belonging to 139 unrelated families. In 33 instances, an *SCN1A* mutation was present in more than one family member. Of the 200 mutation carriers, 97 (48.5%) had Dravet syndrome, including borderline forms. In the non-Dravet group, distribution of phenotypes included 49 patients (23.8%) with GEFS+, 30 (14.8%) with FS, and 6 (3.5%) with focal seizures and 18 (8.9%) healthy individuals >18 years old who had undergone genetic testing during family studies for mutation confirmation and inheritance determination. The overall penetrance was 77%. Pedigrees with incomplete penetrance are shown in figure 1.

Association of clinical and mutational data with Dravet syndrome. We used the Pearson χ^2 independence test to analyze the distribution of categorical clinical

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Table 1 Characteristics of the study population

	Total, n	%	Non-Dravet (n = 103), n	Dravet (n = 97), n	Missing data, n	p Value (χ^2)
Sex					0	0.596
Male	109	54.5	58	51		
Female	91	45.5	45	46		
Mutation type					0	<0.001
Missense	129	64.5	78	51		
Splicing	19	9.5	13	6		
Nonsense	19	9.5	4	15		
Fs/rearrangements	33	16.5	9	24		
Family history of seizures					1	<0.001
Yes	111	55.8	78	33		
No	88	44.2	25	63		
Age at seizure onset, mo					47	<0.001
0–6	81	52.9	12	69		
6–12	37	24.2	18	19		
>12	35	22.9	35	0		
Mean (SD)	10.80 (14.48)		18.4 (19.51)	5.19 (2.233)		<0.001
Fever at first seizure					33	0.019
Yes	134	80.2	67	67		
No	33	19.8	9	24		
Seizure types					32	0.003
Clonic-tonic/clonic	142	84.5	73	69		
Focal	20	11.9	2	18		
Myoclonic	5	3.0	1	4		
Absence	1	0.6	0	1		
Seizure duration, min					74	0.001
0–5	65	51.6	37	28		
5–30	37	29.4	9	28		
>30	24	19.0	5	19		
EEG abnormalities					112	0.207
Yes	38	43.2	11	27		
No	50	56.8	21	29		

Abbreviation: Fs = frameshift mutations.
Seizure duration was grouped into 3 classes: 0 to 5, 5 to 30, and >30 minutes. Age at seizure onset was grouped into 3 classes: 0 to 6, 6 to 12, and >12 months. Seizure type, fever, seizure duration, age at seizure onset, and EEG discharges were calculated on 182 patients, excluding 18 individuals who did not experience seizures.

variables in the Dravet and non-Dravet groups and the Student *t* test for unequal variance for each continuous variable of interest. We analyzed mutation type and 7 parameters that are usually available at clinical presentation, including sex, family history of epilepsy, age at seizure onset, type and duration of first seizure, fever at first seizure, and epileptiform discharges (sharp waves, spikes, spikes and waves) at first EEG. Six of the analyzed parameters, with the exception of sex and EEG abnormalities, had a significantly different distribution in the 2 groups (table 1). The Student *t* test showed a significantly different age at seizure onset in patients with Dravet syndrome vs individuals without Dravet (5.19 ± 2.23 vs 18.4 ± 19.51 months; $p < 0.001$).

We fit a multilevel logistic model with the significant parameters and adjusted for age at last follow-up (table 2). The multivariate model showed that age at seizure onset (OR = 0.647, 95% CI 0.541–0.774, $p < 0.001$) and frameshift vs missense mutations (OR = 8.567, 95% CI 1.828–40.140, $p = 0.006$)

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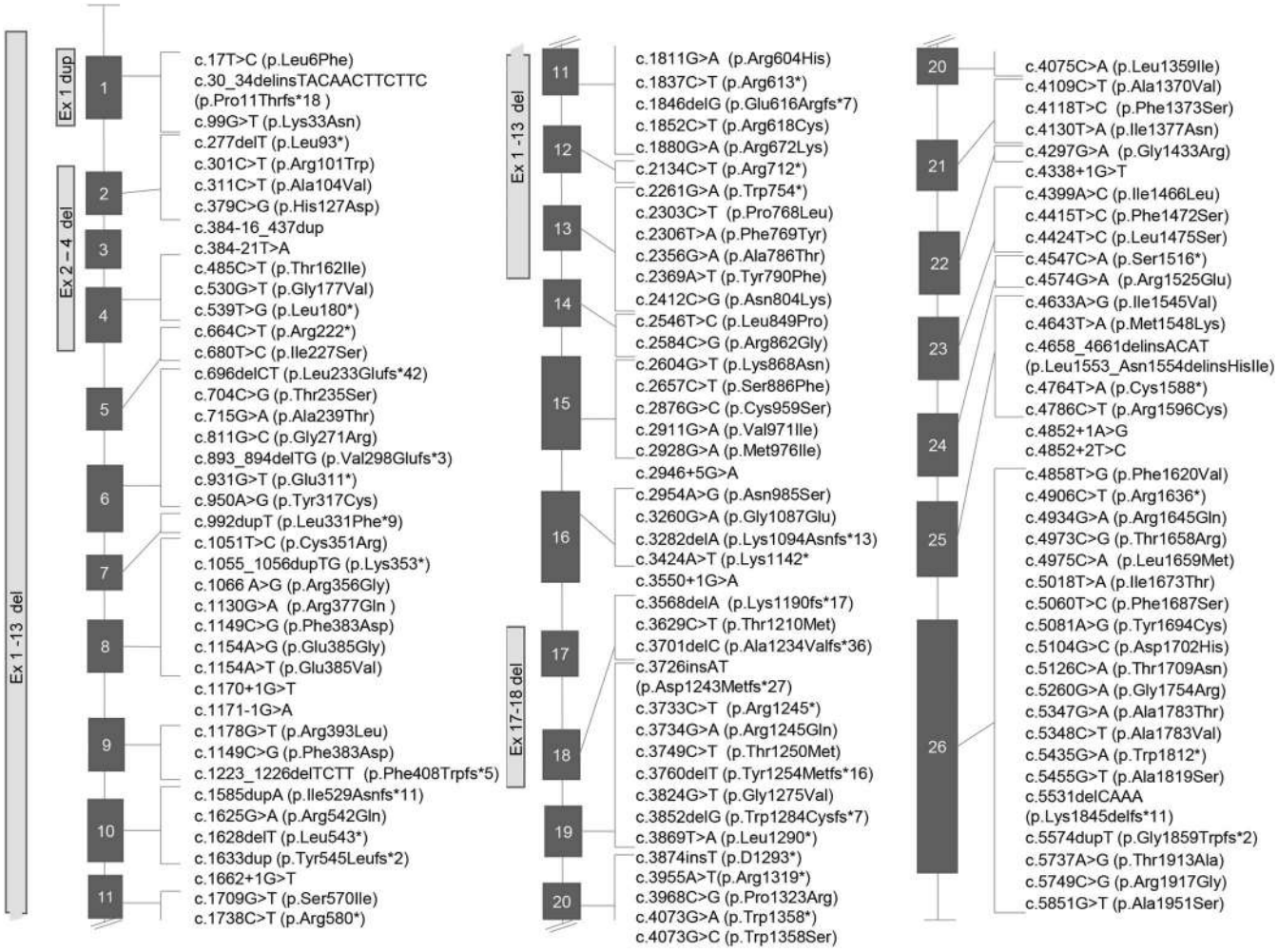
Figure 1 Schematic representation of *SCN1A* mutations identified in this study



Nomenclature of mutations followed recommendations of the Human Genome Variation Society.

were significantly associated with Dravet syndrome. The sensitivity analysis confirmed the results obtained in the multilevel logistic models (table 2).

Association of age at seizure onset with Dravet syndrome. Age at seizure onset, analyzed as both a continuous and a categorical variable, was distributed differently in patients with Dravet and those without Dravet syndrome. Mean age at first seizure was 5.19 months for patients with Dravet and 18.4 months for those without Dravet syndrome (table 1). Multilevel logistic regression analysis showed an OR of 0.647, meaning that an older age at seizure onset represents a protective factor against the risk of developing Dravet syndrome (table 2). None of the patients who experienced their first seizure after 12 months of age developed Dravet syndrome.

Association of mutations with Dravet syndrome. We found 123 different mutations, 65 of which were novel, in 200 individuals (table e-1). Fifty-four mutations (42.2% of 128 individuals for whom heritability was tested) were de novo and 74 (57.8%) were inherited, 6 of which were from a parent with somatic mosaicism. Mutations were distributed throughout the gene (figure 2), and except for familial cases, only 11 were observed in more than one individual.

We evaluated the effect of missense mutations using different bioinformatic tools (appendix e-1) based on functional prediction scores and conservation scores. We deemed as damaging all substitutions predicted to be deleterious by at least 2 conservation and 2 prediction algorithms and all the mutations resulting in loss of function (nonsense, frameshift, splicing, and genomic rearrangements). To estimate the allelic frequency of the mutations, we interrogated public frequency databases (appendix e-1). We identified 71 different missense mutations, of which 62 were not present in databases, 6 were reported in the Exome Aggregation Consortiumto have a frequency of $<0.0001\%$, and 3 (p.Thr1250Met, p.Arg542Gln, and p.Arg604His) with a frequency

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Table 2 Multilevel logistic regression with robust estimator for standard error

	OR	95% CI	p Value
Mutation type			
Missense mutations outside the pore region	1.000		
Missense mutations in the pore region	0.580	0.080–4.215	0.590
Splicing	0.300	0.051–1.759	0.182
All truncating	1.542	0.326–7.294	0.585
Nonsense	0.686	0.108–4.376	0.691
Fs/rearrangements	8.567[a]	1.828–40.140	0.006
Family history			
No	1.000		
Yes	0.492	0.144–1.689	0.260
Age at last follow-up	0.997	0.990–1.004	0.437
Age at seizure onset	0.647[a]	0.541–0.774	0.000
Fever at first seizure			
No	1.000		
Yes	3.016	0.703–12.933	0.137
Seizure types			
Clonic-tonic/clonic	1.000		
Focal	7.174	0.698–73.747	0.097
Myoclonic	4.465	0.199–99.936	0.345
Absence	—		
Seizure duration, min			
0–5	1.000		
5–30	2.052	0.490–8.589	0.325
>30	3.330	0.692–16.017	0.133

Abbreviations: CI = confidence interval; Fs = frameshift mutations; OR = odds ratio.
Age at seizure onset was analyzed as a continuous variable. Seizure duration was divided into 3 classes: 0 to 5, 5 to 30, and >30 minutes.
[a] Significant values according to CIs and p values.

of ≥0.0001% (table e-1). We grouped mutations into 5 classes: missense, missense falling into the pore-forming region, splicing, nonsense, and frameshift (including rearrangements of entire exons). Multilevel logistic regression revealed that frameshift mutations and rearrangements confer a significantly higher risk of developing Dravet syndrome (OR = 8.567, 95% CI 1.828–40.140, $p = 0.006$, table 2) with respect to missense mutations.

Diagnostic test. To compare the diagnostic accuracy of all parameters, we estimated sensitivity, specificity, ROC area, DOR, and positive and negative predictive values (table 3). We performed this analysis on the 119 patients for whom information on all 8 phenotypic and genotypic items was available (table 1). Patients carrying variants of uncertain significance (p.Thr1250Met, p.Arg542Gln, and p.Arg604His) were excluded from this analysis. ROC analysis showed age at seizure onset to accurately recognize patients with Dravet syndrome. On the basis of this parameter, we identified 3 subgroups within which the probability of developing Dravet syndrome was significantly different: 85% in the 0- to 6-month seizure-onset group, 51% in the 6- to 12-month group, and none after the 12th month of age. The optimal diagnostic cutoff was 6 months of age (sensitivity = 83.3%, 95% CI 72.7–91.1; specificity = 76.60%, 95% CI 62.0–87.7, table 3).

On the basis of the results shown in table 3, we explored a combination of parameters that, however, did not yield higher significativity than the test based on age at first seizure.

DISCUSSION Previous studies have examined the spectrum of *SCN1A* mutations associated with Dravet syndrome[8,12] and suggested clinical criteria for *SCN1A* screening based on early seizure characteristics.[13] These studies, performed on patient populations whose clinical characteristics were highly suggestive of Dravet syndrome, have contributed to delineate its genotypic and phenotypic spectra. The approaches used, however, could not address the opposite perspective of defining the risk of divergent outcomes in a population of mutation-positive patients whose clinical presentation is relatively similar at seizure onset. Considering that *SCN1A* screening is widely performed soon after early prolonged/repeated FS appear, at a stage when either benign or ominous outcomes are still possible, we attempted to identify reliable early clinical and mutational outcome predictors that can help clinicians to deal with a frequently encountered dilemma.

To gather a sample population that was representative of the spectrum of *SCN1A*-associated phenotypes, as a first step, we collected clinical details on 200 individuals with damaging *SCN1A* mutations, including healthy relatives of mutation carriers. Of the 182 patients with seizures, 53.6% had Dravet syndrome and 46.4% had milder conditions, including GEFS+ (27%), FS (16.2%), and focal seizures (3.2%). Eighteen remaining relatives of probands (9% of the whole sample) were mutation carriers who never experienced seizures. We then analyzed 7 clinical parameters usually available at seizure onset—sex, family history of epilepsy, age at/fever at/type of/duration of first seizure, and abnormalities at first EEG—and found 5 of them to exhibit a significantly different distribution in the Dravet and non-Dravet groups. While the Dravet group had a higher frequency of onset within the sixth month ($p < 0.001$), of focal seizures ($p = 0.003$), and of seizures lasting >5 minutes ($p = 0.001$), family history ($p < 0.001$) and evidence of fever at seizure onset ($p = 0.019$) were more frequent in the non-Dravet group.

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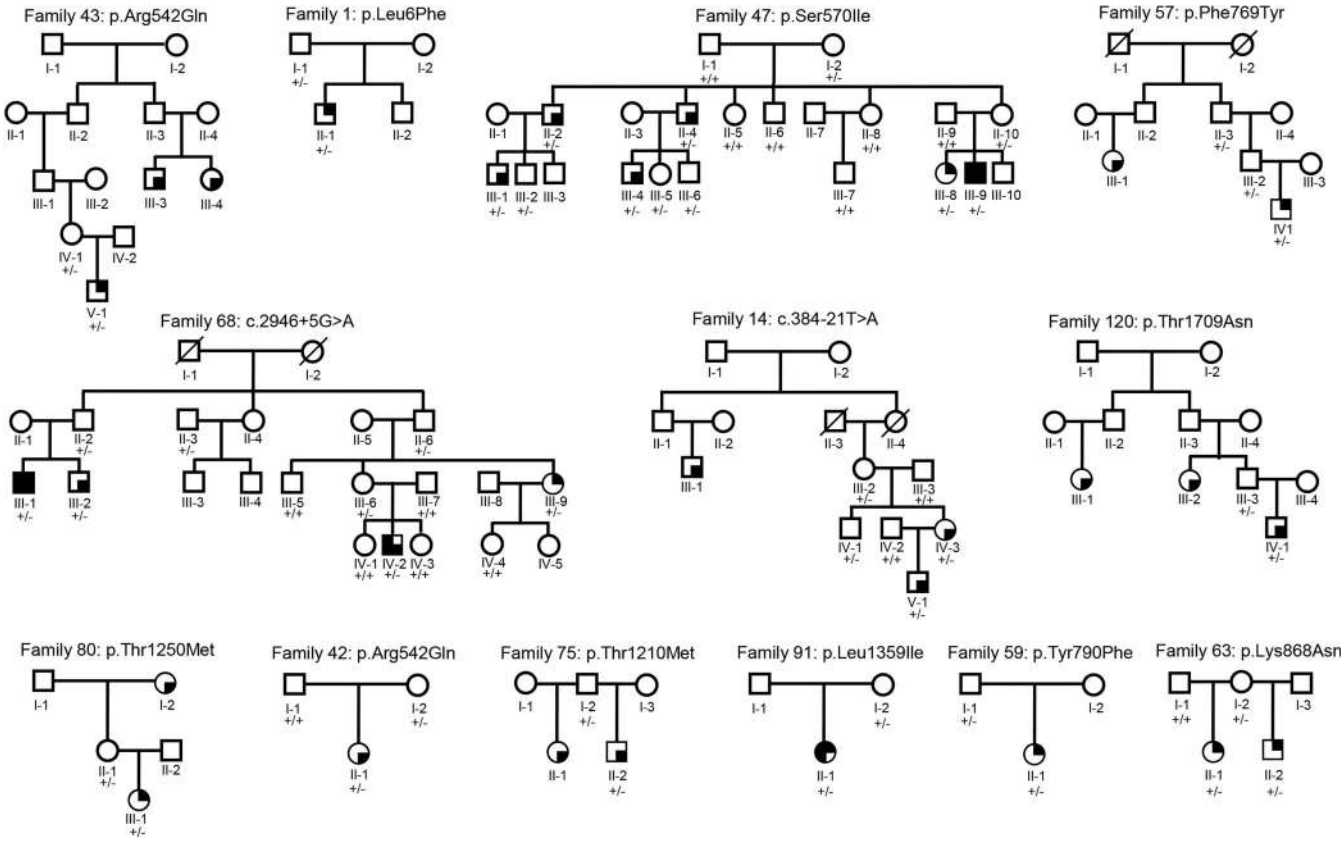
Figure 2 Pedigrees of families exhibiting incomplete penetrance of *SCN1A* mutations



■ ● = Dravet syndrome; ◪ ◔ = FS; ◪ ◔ = generalized/genetic epilepsy with febrile seizures plus; ■ ◕ = focal epilepsy; +/− = heterozygous *SCN1A* mutation; +/+ = absence of the *SCN1A* mutation.

Multilevel logistic regression showed that age at seizure onset was the more relevant parameter associated with Dravet syndrome (OR = 0.647, 95% CI 0.541–0.774, $p < 0.001$). The diagnostic ROC analysis identified age at seizure onset as the main and statistically relevant indicator, with the optimal diagnostic cutoff for age being 6 months (sensitivity = 83.33%; specificity = 76.60%). The risk for Dravet syndrome declined progressively according to the age group at seizure onset, being 85% in the 0- to 6-month group, 51% in the 6- to 12-month group, and 0% after the 12th month.

We observed 123 different *SCN1A* mutations. As previously reported,[14,15] truncating mutations were more represented in patients with Dravet than in those without Dravet syndrome (40% vs 13%). We also observed 13 truncating mutations associated with mild phenotypes and 37 missense mutations outside the pore-forming region in Dravet syndrome, thus confirming the variable phenotypic consequences of

Table 3 Diagnostic accuracy of analyzed parameters

	Sensitivity, %	Specificity, %	ROC area	DOR	PPV, %	NPV, %
Age at seizure onset, mo						
0–6	83.33	76.60	0.7996	16.4	84.50	75
0–12	97.22	51.06	0.7414	36.5	75.30	92.30
No family history	63.89	70.21	0.6705	4.17	76.70	55.90
First seizure duration (>5 min)	61.11	72.34	0.6673	4.11	77.20	54.80
Mutation (truncating)	48.61	80.85	0.6473	3.99	79.50	50.70
Focal seizures	19.44	95.74	0.5759	5.43	87.50	43.70
Afebrile seizures	27.78	82.98	0.5538	1.88	71.40	42.90

Abbreviations: DOR = diagnostic odds ratio; NPV = negative predictive value; PPV = positive predictive value; ROC = receiver operating characteristic. Truncating refers to nonsense, frameshift mutations, and rearrangements.

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SCN1A mutations.[1] Although genotype-phenotype studies have pointed out that less severe phenotypes are more common with missense than truncating mutations[16–18] and that missense mutations in the pore-forming region tend to be associated with more severe phenotypes,[19] correlations between specific mutations and specific phenotypes are weak.[20] A meta-analysis of 155 missense *SCN1A* mutations indicated that the physicochemical properties of amino acid changes influence the epilepsy phenotype and could be used to predict the phenotype associated with each mutation.[21] Similarly, an attempt to correlate the Grantham score of missense mutations, a measure of physicochemical differences between amino acids, with phenotypic outcome demonstrated that the type of amino acidic substitution does not independently predict different phenotypes within the spectrum of *SCN1A*-related epilepsies.[15] Our estimation through ROC analysis of the value of different mutation types to predict the risk of developing Dravet syndrome sets the best cutoff grouping truncating mutations vs splicing and missense mutations but could not predict with high confidence Dravet syndrome vs milder phenotypes (sensitivity = 48.61%, specificity = 80.85%). Therefore, localization and type of *SCN1A* mutations are, on their own, less accurate predictors of outcome than assessment based on age at seizure onset. Combining mutational data with clinical parameters did not significantly improve the discrimination performance of the test.

Of the 33 families included in our cohort, all 20 families exhibiting complete penetrance carried truncating mutations, while the remaining 13 exhibiting incomplete penetrance carried either splicing or missense mutations (figure 2). Overall, penetrance was highly mutation-dependent, reaching 100% in families with truncating mutations and 77% in those with segregating missense mutations. Nonpenetrance for *SCN1A* mutations, observed in 9% of our sample, has already been reported in GEFS+[22] and less frequently in Dravet syndrome,[23] but its frequency had not been assessed on large series before. We also observed wide intrafamilial phenotypic variability, with only 5 of 33 families exhibiting the same phenotype in all affected members. High phenotypic variability within the same family[15,23–27] is interpreted as a consequence of epistasis.

From a pathophysiologic perspective, there seems to be an age-at-seizure-onset–dependent response of the brain carrying an *SCN1A* mutation to early epileptogenesis whereby onset within the sixth month almost regularly progresses as an epileptic encephalopathy, while onset after the 12th month never does. It is unlikely that this course was influenced by treatment choices in the population studied because no uniform attitude or protocol exists, with either immediate treatment of seizures or long-term medication currently being started on the basis of individual preferences concerning drug(s) and timing.

It remains to be clarified whether the worst prognosis related to a younger age at seizure onset can be entirely explained by genetic factors, with earlier onset just being an expression of a lower seizure threshold prompted by the most damaging mutations. Earlier onset of seizure activity can actually by itself induce changes that permanently lower seizure threshold and cause cognitive impairment[28] above and beyond what is caused by the underlying mutation. Studies on animal models have demonstrated that the deleterious consequences of seizures strongly depend on the developmental stage at which they occur: immature neurons having few synapses and more developed neurons that express a multitude of functional synapses endure different consequences.[28,29] Long-lasting effects of seizures may derive from seizure-induced transformation of a naive network to one that has increased seizure susceptibility. In particular, converging evidence has been gathered that in the rat brain, early/prolonged hyperthermic seizures cause permanent changes resulting in long-standing increased excitability.[28] Although it is still unclear whether similar changes occur in the human brain, analogies with the observations that Dravet syndrome develops only when early/prolonged hyperthermic seizures appear in the first year of life, particularly in the first 6 months, are strong.

In pediatric epilepsy practice, young infants with prolonged/repeated fever-related seizures and *SCN1A* mutations pose considerable concerns in terms of their risk of developing Dravet syndrome, a risk that our study sets at ≈50% overall but at 0% in those with seizure onset after the 12th month. It is of primary importance to discern those at higher risk of severe outcomes and to promptly organize management accordingly. Because this study indicates that age at seizure onset is a reliable indicator of outcome, we suggest that seizure onset within the first year of life should prompt appropriate treatment choices, for example, introduction of the stiripentol-clobazam combination[30] before the epileptic encephalopathy is established. Of course this suggestion is valid provided no precious time is lost delaying mutation analysis.[4] Although no controlled evidence exists that more appropriate earlier treatment can limit the progression toward the severe end of the *SCN1A* spectrum, this possibility should be explored through a dedicated trial, which might use the results of this study as a comparator.

AUTHOR CONTRIBUTIONS

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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