RESEARCH ARTICLE

Development and Validation of a Prediction Model for Early Diagnosis of *SCN1A*-Related Epilepsies

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Abstract

Background and Objectives

Pathogenic variants in the neuronal sodium channel α1 subunit gene (*SCN1A*) are the most frequent monogenic cause of epilepsy. Phenotypes comprise a wide clinical spectrum, including severe childhood epilepsy; Dravet syndrome, characterized by drug-resistant seizures, intellectual disability, and high mortality; and the milder genetic epilepsy with febrile seizures plus (GEFS+), characterized by normal cognition. Early recognition of a child's risk for developing Dravet syndrome vs GEFS+ is key for implementing disease-modifying therapies when available before cognitive impairment emerges. Our objective was to develop and validate a prediction model using clinical and genetic biomarkers for early diagnosis of *SCN1A*-related epilepsies.

Methods

We performed a retrospective multicenter cohort study comprising data from patients with *SCN1A*-positive Dravet syndrome and patients with GEFS+ consecutively referred for genetic testing (March 2001–June 2020) including age at seizure onset and a newly developed *SCN1A* genetic score. A training cohort was used to develop multiple prediction models that were validated using 2 independent blinded cohorts. Primary outcome was the discriminative accuracy of the model predicting Dravet syndrome vs other GEFS+ phenotypes.

Results

A total of 1,018 participants were included. The frequency of Dravet syndrome was 616/743 (83%) in the training cohort, 147/203 (72%) in validation cohort 1, and 60/72 (83%) in validation cohort 2. A high *SCN1A* genetic score (133.4 [SD 78.5] vs 52.0 [SD 57.5]; $p < 0.001$) and young age at onset (6.0 [SD 3.0] vs 14.8 [SD 11.8] months; $p < 0.001$) were each associated with Dravet syndrome vs GEFS+. A combined *SCN1A* genetic score and seizure onset model separated Dravet syndrome from GEFS+ more effectively (area under the curve [AUC] 0.89 [95% CI 0.86–0.92]) and outperformed all other models (AUC 0.79–0.85; $p < 0.001$). Model performance was replicated in both validation cohorts 1 (AUC 0.94 [95% CI 0.91–0.97]) and 2 (AUC 0.92 [95% CI 0.82–1.00]).

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Glossary

AUC = area under the curve; **CADD** = Combined Annotation Dependent Depletion; **GEFS+** = genetic epilepsy with febrile seizures plus; **IPA** = index of prediction accuracy; **NPV** = negative predictive value; **PPV** = positive predictive value; **PTV** = protein truncating variant; **REVEL** = Rare Exome Variant Ensemble Learner; **SUDEP** = sudden unexpected death in epilepsy.

Discussion

The prediction model allows objective estimation at disease onset whether a child will develop Dravet syndrome vs GEFS+, assisting clinicians with prognostic counseling and decisions on early institution of precision therapies (http://scn1a-prediction-model.broadinstitute.org/).

Classification of Evidence

This study provides Class II evidence that a combined *SCN1A* genetic score and seizure onset model distinguishes Dravet syndrome from other GEFS+ phenotypes.

Epilepsy affects an estimated 50–65 million individuals worldwide.[1] The majority of epilepsies are thought to be genetic in origin due to single gene disorders or complex inheritance.[2] Pathogenic variants in the sodium voltage-gated channel alpha subunit 1, *SCN1A* (OMIM 182389), are the most common monogenic cause of epilepsy, affecting 1 in 12,200 live births.[3] Clinical presentation is highly variable and includes the severe infantile-onset Dravet syndrome as well as phenotypes within the mild genetic epilepsy with febrile seizures plus (GEFS+) spectrum.[4] Whereas Dravet syndrome leads to a significant developmental and epileptic encephalopathy with difficult-to-treat seizures and severe intellectual disability,[5,6] individuals with other GEFS+ phenotypes live independent lives with normal cognition and very mild epilepsy.[7] Distinction of these 2 conditions on clinical grounds alone is challenging in the first 2 years of life because the encephalopathy associated with Dravet syndrome is insidious and early development is within normal limits. Genotype–phenotype correlations are not well-established and when a pathogenic *SCN1A* variant is found, it is not possible for clinicians to accurately predict whether a child will develop Dravet syndrome or other GEFS+ phenotypes.[8] Both disorders may present with recurrent, often prolonged febrile seizures in an otherwise apparently normal infant. The full Dravet syndrome phenotype only emerges in the second and third year of life and is associated with high epilepsy mortality in early childhood (15.84/1,000 person-years), due to status epilepticus and sudden unexpected death in epilepsy (SUDEP).[5,6,9]

Accurate prediction of whether a young child with a pathogenic *SCN1A* variant will develop the severe epilepsy Dravet syndrome or milder GEFS+ phenotypes is important for counseling, patient management, and treatment planning. Clinicians often miss the opportunity for early intervention as they wait for symptoms such as developmental delay to emerge before making a diagnosis of Dravet syndrome. Treatment strategies have focused on achieving better seizure control with stiripentol, clobazam, and sodium valproate, as well as the use of cannabidiol and fenfluramine.[10-13] New gene-specific, disease-modifying therapies have been shown to significantly reduce seizure burden and mortality in Dravet rodent models when given early and the first-in-human trial of gene-based therapy in Dravet syndrome recently began.[14] Prompt diagnosis is important to enable timely administration of new treatments in Dravet syndrome and to avoid unnecessary and possibly harmful treatment in other GEFS+ phenotypes.

The crucial aspect in deciding the best treatment approach and timing is the infant's odds of developing Dravet syndrome vs other GEFS+ phenotypes. To date, only 2 studies have attempted to predict Dravet syndrome vs GEFS+ based on clinical and genetic data.[15,16] These studies showed a moderate association between single outcome predictors such as early seizure onset or truncating variants being linked to a more severe phenotype, but there are no validated actionable prediction models available to guide clinical decision-making.[15,16]

The challenge of outcome prediction is not unique to genetic epilepsies, and risk prediction models are routinely used to aid decision-making in cardiovascular disease and cancer.[17,18]

Using a large *SCN1A* patient cohort, we hypothesized that combining clinical and genetic data will allow us to develop a statistical model for the early prediction of *SCN1A*-related epilepsy phenotypes.

Methods

Study Design, Participants, and Clinical Assessments

We conducted a multicenter retrospective cohort study to develop and validate a statistical model combining age at seizure onset (febrile or afebrile, whichever occurred first) and the *SCN1A* genetic score in predicting Dravet syndrome vs other GEFS+ phenotypes. Results are reported using the Enhancing the Quality and Transparency of Health Research (EQUATOR) network Standards for Reporting of Diagnostic Accuracy (STARD) guidelines for diagnostic accuracy studies.[19] We developed the clinical–genetic prediction model from a retrospective cohort of 1,018 patients from 7 countries:

United Kingdom (n = 276), France (n = 201), Italy (n = 126), Netherlands (n = 109), Denmark (n = 31), Australia (n = 203), and Belgium (n = 72). All cases were identified from consecutive referrals for genetic testing in different centers in the respective countries or for research referral from March 2001 to June 2020. We included patients with Dravet syndrome and patients with GEFS+ carrying pathogenic *SCN1A* variants from the following sites: The Royal Hospital for Children (Glasgow, UK),[4,20] The Hôpital Necker-Enfants Malades (Paris, France),[21] The A Meyer Children's Hospital (Florence, Italy),[15] The University Medical Center Utrecht and Radboud University Nijmegen Medical Center (the Netherlands),[16] The Danish Epilepsy Centre Filadelfia (Dianalund, Denmark),[22,23] The University Hospital Antwerp (Belgium),[24] The Austin Health and Royal Children's Hospital (Melbourne, Australia), and unpublished cases (eMethods and eTable 1, links.lww.com/WNL/B785).

Phenotypes were classified by experts in the management of Dravet syndrome and GEFS+ according to the following criteria: Dravet syndrome was defined as generalized or hemiclonic seizures frequently triggered by fever and often prolonged, typically followed by other seizure types including myoclonic, focal impaired awareness, and absence seizures, and normal cognitive and psychomotor development prior to seizure onset with subsequent slowing including plateauing or regression of skills in the second year of life. Patients were given a diagnosis of other GEFS+ phenotypes if they had presentations consistent with the febrile seizures plus spectrum, with or without a relevant family history and normal intellect,[7] which in the context of this study excludes Dravet syndrome. In most cases diagnoses were made at age >24 months; however, a number of patients with Dravet syndrome were diagnosed at an earlier age if the phenotype was highly suggestive, including the plateauing or regression of skills.

We developed the prediction model using a training cohort, including patients from the United Kingdom, France, Italy, Netherlands, and Denmark (n = 743). We then tested the prediction model in 2 blinded validation cohorts from Australia (validation cohort 1, n = 203) and Belgium (validation cohort 2, n = 72). Because our model is based on age at onset and genetic data, we only included patients who had these data available.

Blinding of Validation Cohorts

Whereas clinical information (Dravet syndrome vs GEFS+) was available to the assessors for the training cohort, data for the 2 validation cohorts were supplied without disclosing the phenotype. Details on whether a patient had Dravet syndrome or other GEFS+ phenotypes was only made available after the prediction analysis had been completed.

Standard Protocol Approvals, Registrations, and Patient Consents

Retrospective review of anonymized clinical referral data and variant findings was approved by the relevant institutional review boards (West of Scotland Research Ethics Committee, reference number 16/WS/0203).

Genetic Analysis and *SCN1A* Genetic Score

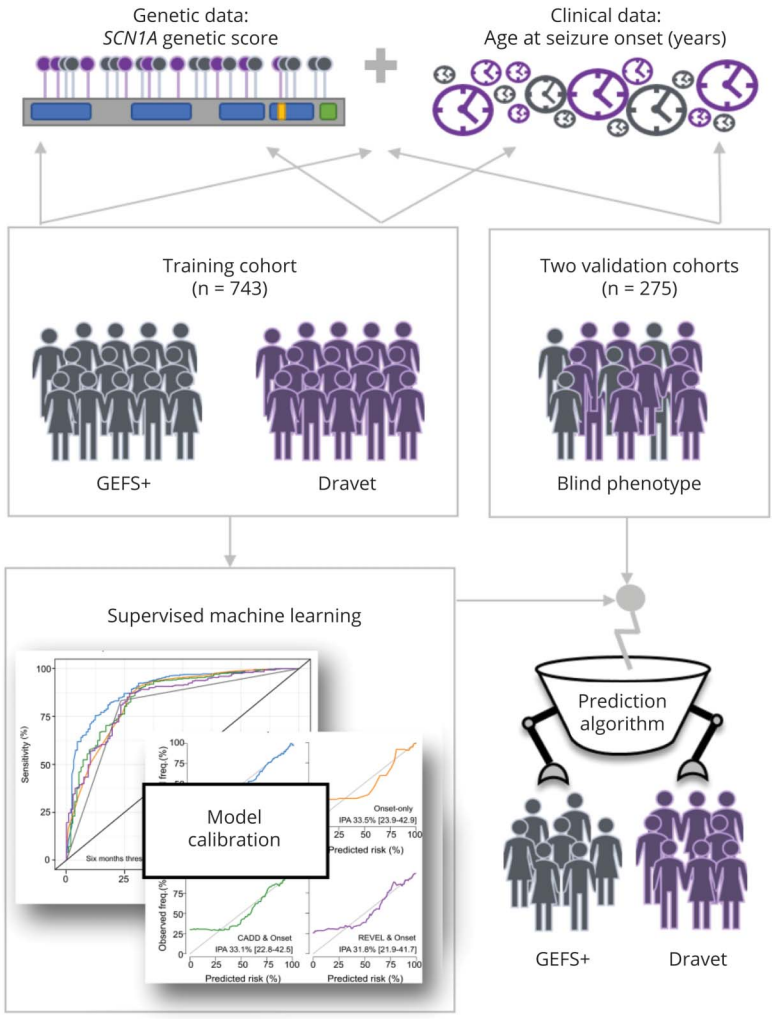
We included missense and protein truncating variants (PTVs). PTVs were composed of premature stop codons, frameshifts leading to stop codons, large deletions, and whole gene deletions. Variants whose effect cannot be predicted based on position, amino acid exchange, or truncation were excluded from the study. This applied to splice variants, in-frame small insertions/deletions, and synonymous variants. Details on molecular analysis for each center are provided in the eMethods (links.lww.com/WNL/B785). For each pathogenic variant, we generated a *SCN1A*-specific genetic score by combining paralog conservation of the mutated amino acid position[25] with the physicochemical properties (Grantham score[26]) of the observed substitution. Paralog conservation accounts for the degree of amino acid conservation across a single gene family alignment. In the case of the voltage-gated sodium channels gene family, 10 genes were aligned to calculate the paralog score: *SCN1A*–*SCN11A*. The score ranges from amino acid positions with −2.06 (least conserved) to 1.23 (most conserved) and is independent of the exchange observed. Paralog conserved sites are particularly enriched for pathogenic variants in voltage-gated sodium channels and high Grantham scores reflect radical amino acid substitutions that are more likely to be deleterious.[25,26] The *SCN1A* genetic score ranged from 0 (similar) to 207 (dissimilar) and is the result of the paralog score observed in the position multiplied by the Grantham score associated with the amino acid exchange. PTVs are assumed deleterious for protein function and were assigned the maximum *SCN1A* genetic score observed (207). We compared performance of the *SCN1A* genetic score with established variant interpretation tools such as CADD (Combined Annotation Dependent Depletion)[27] and REVEL (Rare Exome Variant Ensemble Learner).[28]

Statistical Analysis and Prediction Model Development

Our primary research question was as follows: What is the discriminative accuracy of a statistical model combining age at seizure onset and the *SCN1A* genetic score in predicting Dravet syndrome vs other GEFS+ phenotypes? This study provides Class II evidence relating to this research question.

Model development and validation was performed according to Transparent Reporting for Individual Prognosis or Diagnosis (TRIPOD) guidance of multivariable prediction models.[29] We applied a supervised machine learning approach and trained a generalized linear model using the *SCN1A* genetic score and the age at seizure onset in months (referred to as the Index *SCN1A* score and Onset model) as predictors of Dravet syndrome and GEFS+ (eMethods, links.lww.com/WNL/B785). The age at seizure onset was identified as the earliest clinical feature that could easily and reliably be assessed in the first year of life when most other clinical signs have not emerged and has been shown to be a valuable prognostic factor in earlier studies.[15,16]

Figure 1 Study Overview



Study workflow. Genetic data (*SCN1A* genetic score) and clinical data (age at seizure onset in months) from 743 patients (training cohort) were introduced to a supervised machine learning approach to produce a prediction model. We tested the prediction model with 2 independent blinded validation cohorts (n = 275). GEFS+ = genetic epilepsy with febrile seizures plus.

To compare our model, we constructed 3 additional models: (1) age at seizure onset Onset-only model, (2) CADD and Onset model, and (3) REVEL and Onset model, following the same procedure. We compared our model against a 6-months seizure onset threshold model proposed previously,[15] which served as reference standard as it was the only predictive model available prior to our study. For all models tested (including index and reference standard models), we used a 50% cutoff threshold to positively predict a case of Dravet syndrome. Patients with predictions below 50% were assigned a GEFS+ status. We calibrated and compared the models using the receiver operating characteristic curve, calibration curves, and the index of prediction accuracy (IPA).[30] Area under the curve (AUC) and IPA 95% CIs were generated with 1,000 bootstrap sets during cross-validation. Sensitivities, specificities, positive predictive values (PPVs), negative predictive values (NPVs), and accuracies alongside their 95% CIs were calculated following established guidelines.[31] All patients with ages at seizure onset, genetic variants, and their corresponding genetic score are detailed in eTable 1 (links.lww.com/WNL/B785)

Data Availability

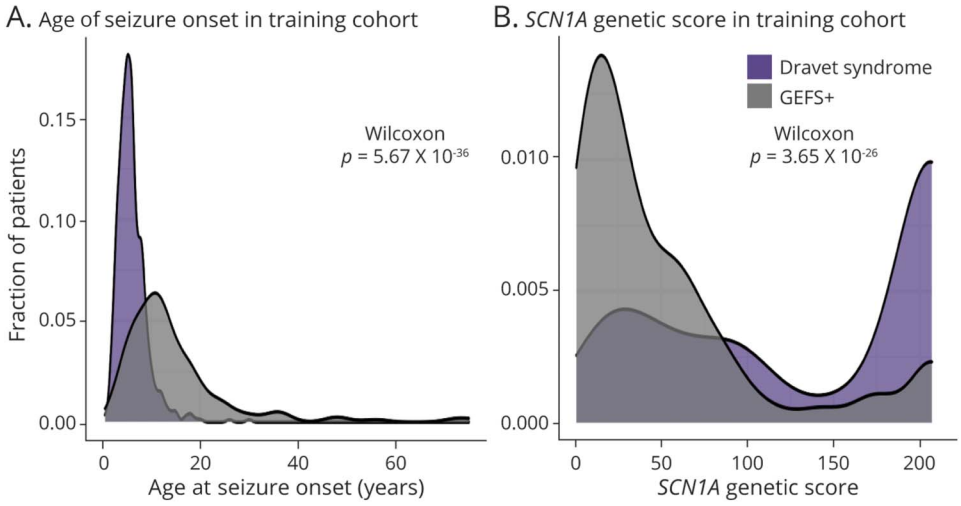
Anonymized data not published within this article will be available from the lead author by email on reasonable request.

Results

Of an original 862 patients, 119 (14%) carried variants whose effect cannot be predicted and were excluded. The training cohort included 743 patients, of whom 616 (83%) had Dravet syndrome and 127 other GEFS+ phenotypes (17%). The frequency of Dravet syndrome in validation cohort 1 was 147/203 (72%) with 56 (28%) patients with GEFS+ and in validation cohort 2 60/72 (83%) with 12 (17%) patients with GEFS+. The training cohort had 447 missense variant (60%) and 296 PTV (40%) carriers, validation cohort 1 had 134 missense variant (66%) and 69 PTV (34%) carriers, and validation cohort 2 had 44 missense variant (61%) and 28 PTV (39%) carriers.

A summary of the study outline is shown in Figure 1. Among the training cohort, a younger age at seizure onset or a higher

Figure 2 Training Cohort Data



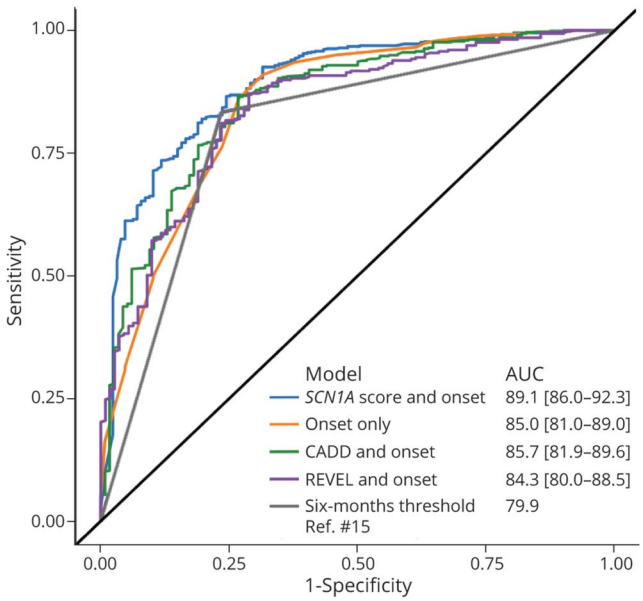
(A) Density plot showing the distribution of the age at seizure onset in training cohort patients with Dravet syndrome (purple area) and genetic epilepsy with febrile seizures plus (GEFS+) (gray area). (B) Density plot showing the distribution of the *SCN1A* genetic score in training cohort patients with Dravet syndrome (purple area) and GEFS+ (gray area). Statistical difference between the observed means was evaluated with the Wilcoxon test.

SCN1A genetic score were each associated with a diagnosis of Dravet syndrome (Figure 2, A and B). Despite the significantly earlier seizure onset in the Dravet syndrome (mean [SD] age, 6.04 [3.0] months) vs GEFS+ group (14.82 [11.8] months; $p < 0.001$) and the higher *SCN1A* genetic score in the Dravet syndrome (133.43 [78.53]) vs GEFS+ group (52.90 [57.58]; $p < 0.001$), there was considerable overlap between the disorders (Figure 2, A and B).

Using the training cohort, we generated 4 different models to discriminate between Dravet syndrome and other GEFS+ phenotypes. With an AUC of 0.89 (95% CI 0.86–0.92) and an IPA of 38.7%, the clinical–genetic *SCN1A* score and Onset model outperformed the prediction model based solely on the age at seizure onset (Onset-only: AUC 0.84 [95% CI 0.80–0.88]; IPA = 33.6%; $p < 0.001$; Figures 3 and 4). The *SCN1A* score and Onset model equally outperformed models based on 2 additional pathogenicity scores, namely CADD (CADD and Onset: AUC 0.85 [95% CI 0.82–0.89]; IPA = 31.2%) and REVEL (REVEL and Onset: AUC 0.84 [95% CI 0.80–0.88]; IPA = 31.6%). In addition, our *SCN1A* score and Onset model outperformed the 6-months seizure onset threshold model proposed previously[15] (Figures 3 and 4; AUC 0.79). Dominance analysis showed that age at seizure onset was 2.06 times more important than the *SCN1A* genetic score to the overall model (eFigure 1 and eTable 2, links.lww.com/WNL/B785). Model performance was similar when focusing only on index cases (eTable 3, links.lww.com/WNL/B785). Next, we tested the performance of the *SCN1A* score and Onset model in 2 independent blinded validation cohorts of *SCN1A* epilepsy. Model performance achieved an AUC of 0.94 (95% CI 0.91–0.97) in validation cohort 1 and an AUC of 0.92 (95% CI 0.82–1.00) in validation cohort 2.

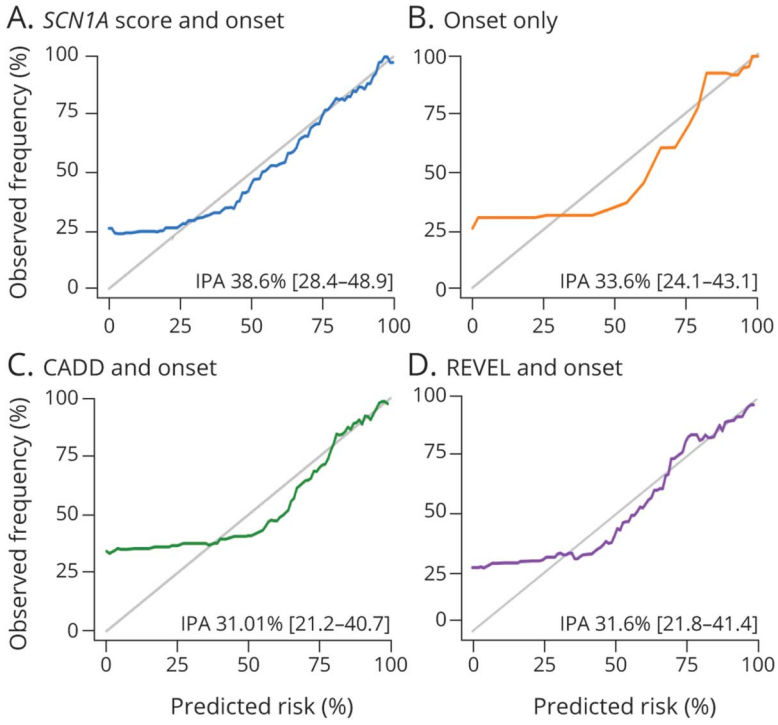
In the model evaluation, patients with higher probability values are predicted to have Dravet syndrome and patients with lower values are predicted to have other GEFS+ phenotypes (Figures 5 and 6). Table 1 illustrates the model performance detailing PPVs and NPVs as well as sensitivities

Figure 3 Training Cohort Model Performance: ROC Curve Analysis



Receiver operating characteristic (ROC) curves showing the relationship between the observed sensitivity and specificity for different models using genetic scores and seizure age at onset: *SCN1A* score and Onset (blue line, n = 743), onset only (orange line, n = 743), CADD (Combined Annotation Dependent Depletion) score and Onset (green line), and REVEL (Rare Exome Variant Ensemble Learner) score and Onset (purple line). Because CADD and REVEL scores are not available for all variants contained in the training cohort, the CADD and Onset and REVEL and Onset models were built with a subset of 651 and 438 training cohort patients, respectively (eTable 1, links.lww.com/WNL/B785). The 6-months seizure onset threshold model (gray line) proposed previously[15] is shown for comparison. Area under the curve (AUC) values and 95% CIs are shown at the bottom right corner of the plot.

Figure 4 Calibration Curves per Model



Training cohort model performance. Individual calibration curves showing the relationship between the predicted risk and the observed frequency for each of the tested models. Index of prediction accuracy (IPA) is shown below each model. Color code: *SCN1A* score and Onset (blue line), Onset-only (orange line), CADD (Combined Annotation Dependent Depletion) score and Onset (green line), and REVEL (Rare Exome Variant Ensemble Learner) score and Onset (purple line).

and specificities observed at different thresholds for both validation cohorts individually and combined (n = 275; Dravet syndrome = 207, GEFS = 68).

To explore potential performance confounders due to patient country ascertainment, we combined and randomly split the entire cohort (n = 1,018) into an additional training cohort with 70% of patients (n = 713) and a single validation cohort with 30% of patients (n = 305). In keeping with our previous results, the *SCN1A* score and Onset model yielded an AUC of 90.4 (95% CI 87.5–93.2) in the random training cohort and an AUC of 91.5 (95% CI 87.1–95.9) in the random validation cohort (eFigure 2, links.lww.com/WNL/B785).

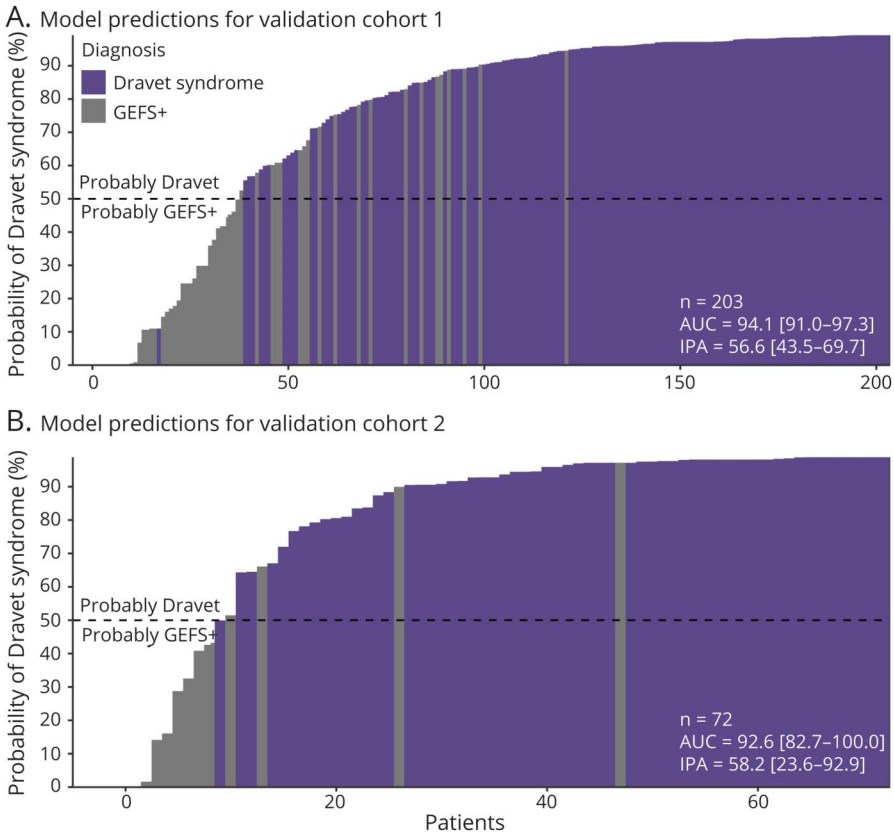
We developed the prediction model into an online tool designed to evaluate any missense or PTV found in a given patient with an *SCN1A* pathogenic variant combined with the age at seizure onset. The *SCN1A* epilepsy prediction model will calculate a patient's probability (%) of developing Dravet syndrome vs other GEFS+ phenotypes in a user-friendly platform that is available online at no cost (eFigure 3, links.lww.com/WNL/B785).

Discussion

In this large, multicenter cohort study, we found that a clinical–genetic prediction model, combining the age at seizure onset with a newly developed *SCN1A* genetic score, allows an objective early estimation as to whether a child will develop Dravet syndrome vs other GEFS+ phenotypes. We were able to show that our prediction model outperformed any previous or alternative models and represents a validated clinical tool to aid early differentiation between Dravet syndrome and GEFS+.[15,16]

In the absence of internationally validated expert-based guidelines for the prediction of Dravet syndrome vs other GEFS+ phenotypes in *SCN1A*-positive patients, judgments about diagnosis and prognosis are challenging, particularly for nonexpert clinicians. Consider the example of a 9-month-old infant presenting with recurrent febrile seizures and a pathogenic *SCN1A* variant. In this case, a previous recommendation[15] would predict that the risk of Dravet syndrome is moderate (51%), based on the age at onset alone. Yet additional information of a high *SCN1A* genetic score might increase that risk to >90%, whereas a low genetic score might reduce that risk to <10%. Consideration of the age at onset alone will not allow a confident distinction between Dravet syndrome and GEFS+ and the clinician is likely to wait until signs of developmental slowing start emerging in the second or third year of life before making a Dravet syndrome diagnosis.[6] In the same way, a PTV variant might suggest a diagnosis of Dravet syndrome; however, that probability will decrease the later the age at seizure onset. Whereas model prediction is mainly determined by age at onset, these examples illustrate how both the genetic information as well as the age at onset play an important part in the outcome prediction model.

Figure 5 Validation Cohort 1 and 2 Prediction Results



Patients with probability values above 50% were predicted to have Dravet syndrome and patients with values below 50% were predicted to have genetic epilepsy with febrile seizures plus (GEFS+). (A, B) Predicted values across validation cohorts 1 and 2 are shown, respectively. Each bar corresponds to a patient. The height of each bar represents the probability of that patient developing Dravet syndrome. Patients with true Dravet syndrome are shown in purple; patients with true GEFS+ are shown in gray. Dotted horizontal line denotes a 50% threshold with values above 50% predicting Dravet syndrome and values below 50% predicting GEFS+. Area under the curve (AUC) and index of prediction accuracy (IPA) 95% CIs are given.

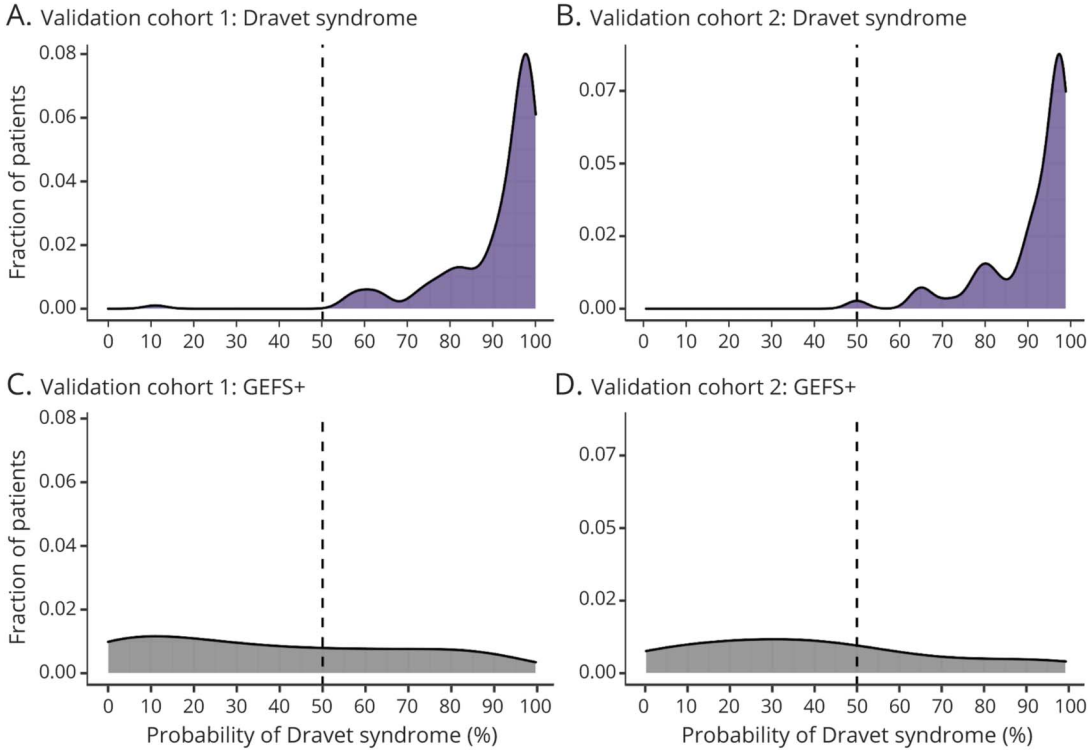
Most clinicians subjectively use patient and disease characteristics to predict outcome based on personal experience and knowledge.[32] Incorrect clinical stratification results in diagnostic delay, and valuable time in the child's early development, together with subtle slowing of development, may have occurred before precision treatment is started. A validated and quantifiable approach allows Dravet syndrome risk prediction much earlier, as soon as the genetic result is available, which could be within weeks of the child having presented with recurrent seizures.[20]

Early treatment in Dravet syndrome is important. Studies in *Scn1a* mutant mice illustrate that early-life febrile seizures are associated with impaired cognition and behavior in the long term.[33] Similarly, early use of contraindicated medication in the second year of life has been associated with adverse developmental outcomes in Dravet syndrome,[16] emphasizing that early diagnosis is essential to establish appropriate treatment as soon as possible.[10-13] Gene-specific therapy approaches are emerging as promising treatment options for Dravet syndrome when given early.[14] Notably, mortality rates in Dravet syndrome due to status epilepticus and SUDEP are high, particularly affecting very young children in their first 3 years of life, emphasizing the importance of early diagnosis and treatment.[9]

It is a strength that the prediction model was not only based on a large and well-phenotyped international training cohort using recognized disease criteria but has been independently retested and validated in 2 equally well-characterized blinded validation cohorts, as well as in additional random samples of the entire cohort, confirming the robustness of our findings. Our approach of using clinical and genetic data combined with machine learning techniques allowed us to better predict outcome than using clinical data or widely adapted variant pathogenicity scores such as CADD or REVEL in isolation. The prediction model uses data that are easily accessible to clinicians in any young infant presenting with a pathogenic *SCN1A* variant. Details can be entered electronically via a free web-based application generating a probability estimate that informs clinical decision-making. These features allow ease of access across health care settings globally, increasing the model's clinical usefulness.

Weighing possible disease outcomes in an individual patient is a complex task and decision curve analysis helps to determine thresholds of sensitivity and specificity. This allows the researcher to identify the most appropriate model performance measures. Depending on the clinical situation and the harm to benefit ratio, recommendations are likely to differ according to the type of treatment considered and the adverse events reported.[34] For instance, starting a young child on antiseizure

Figure 6 Validation Cohort 1 and 2 Phenotype Distribution



Phenotype distribution with density of prediction performed on validation cohorts 1 and 2, respectively. Patients with true Dravet syndrome and patients with genetic epilepsy with febrile seizures plus (GEFS+) accumulate across their corresponding model predictions (horizontal axis). Dotted vertical line denotes a 50% threshold with values above 50% predicting Dravet syndrome and values below 50% predicting GEFS+.

medication with potentially significant side effects has to be weighed against the benefit of possible seizure freedom. If the harm of unnecessary treatment is deemed limited, then a lower model threshold may be acceptable (Table 1). However, in the case of novel interventions, such as gene-specific therapy approaches, different thresholds might apply. Given these complexities, our prediction model is not intended to replace clinical judgment, but to inform and complement clinical decision-making based on objective and quantifiable data.

There are several limitations to this study. Modeling of disease outcomes based on *SCN1A* variant information will be affected by a number of modifying factors, including the unknown genetic and environmental background of the individual, epigenetics, as well as transcriptional and post-translational factors that are beyond our modeling capacity. Given that Dravet syndrome and other GEFS+ phenotypes are part of a disease spectrum, borderline presentations will be more difficult to predict, as shown in Figure 5 and Table 1. We acknowledge that our cohorts are biased towards Dravet syndrome cases and larger, more balanced datasets are needed to improve prediction accuracy. Our logistic regression model achieves an excellent to outstanding fitting (AUC 89.1) and the use of more complex modeling strategies might lead to overfitting with little opportunity to increase performance. Future larger cohorts with additional phenotypic data will allow the implementation of more complex models with increased granularity to better predict the complex heterogeneity of *SCN1A*-related epilepsies and will include types of variants where functional interpretation is more challenging.

The accuracy of a mutation-based prediction model is likely to be negatively influenced by specific genetic factors such as postzygotic mosaicism, which is seen in 7.5% of de novo pathogenic *SCN1A* variants.[35] In the same way, truncating *SCN1A* variants that are normally predicted to be deleterious for channel function might escape nonsense-mediated mRNA decay if occurring in the terminal portion of the gene.[36] As we did not observe any PTVs associated with Dravet syndrome beyond amino acid position 1930, the tool informs the user that our model does not provide a reliable prediction in such cases. These rare examples illustrate that in a minority of cases, truncating variants might not always be deleterious—an exception to the rule, which is difficult to model. Recently, very early onset cases of developmental and epileptic encephalopathy with movement disorder have been described that are not Dravet syndrome. Our tool alerts the user to consider such a phenotype for any patient presenting at less than 4 months of age.[37,38] Lastly, there may be additional predictors of disease outcome not included here, such as the mode of inheritance (de novo vs familial), which might contribute to the predictive power of the model, as inherited

Table 1 Model Performance Measures According to Different Thresholds

Threshold	Sensitivity	Specificity	PPV	NPV	Accuracy
Validation cohort 1 (n = 203)					
50%	99.3 (96.3–100)	64.3 (50.4–76.6)	88.0 (82.0–92.5)	97.3 (85.8–99.9)	89.7 (84.6–93.5)
60%	95.9 (91.3–98.5)	67.9 (54.0–79.7)	88.7 (82.7–93.2)	86.4 (72.6–94.8)	88.2 (82.9–92.3)
70%	92.5 (87.0–96.2)	78.6 (65.6–88.4)	91.9 (86.3–95.7)	80.0 (67.0–89.6)	88.7 (83.5–92.7)
80%	84.4 (77.5–89.8)	85.7 (73.8–93.6)	93.9 (88.4–97.3)	67.6 (55.5–78.2)	84.7 (79.0–89.4)
90%	70.1 (62.0–77.3)	96.4 (87.7–99.6)	98.1 (93.3–99.8)	55.1 (44.7–65.2)	77.3 (71.0–82.9)
Validation cohort 2 (n = 72)					
50%	98.3 (91.1–100)	66.7 (34.9–90.1)	93.7 (84.5–98.2)	88.9 (51.8–99.7)	93.1 (84.5–97.7)
60%	98.3 (91.1–100)	75.0 (42.8–94.5)	95.2 (86.5–99.0)	90.0 (55.5–99.7)	94.4 (86.4–98.5)
70%	93.3 (83.8–98.2)	83.3 (51.6–97.9)	96.6 (88.1–99.6)	71.4 (41.9–91.6)	91.7 (82.7–96.9)
80%	86.7 (75.4–94.1)	83.3 (51.6–97.9)	96.3 (87.3–99.5)	55.6 (30.8–78.5)	86.1 (75.9–93.1)
90%	75.0 (62.1–85.3)	83.3 (51.6–97.9)	95.7 (85.5–99.5)	40.0 (21.1–61.3)	76.4 (64.9–85.6)
Combined validation cohorts 1 and 2 (n = 275)					
50%	99.0 (96.6–99.9)	64.7 (52.2–75.9)	89.5 (84.8–93.2)	95.7 (85.2–99.5)	90.5 (86.5–93.7)
60%	96.6 (93.2–98.6)	69.1 (56.7–79.8)	90.5 (85.8–94.0)	87.0 (75.1–94.6)	89.8 (85.6–93.1)
70%	92.8 (88.3–95.9)	79.4 (67.9–88.3)	93.2 (88.9–96.2)	78.3 (66.7–87.3)	89.5 (85.2–92.8)
80%	85.0 (79.4–89.6)	85.3 (74.6–92.7)	94.6 (90.3–97.4)	65.2 (54.3–75.0)	85.1 (80.3–89.1)
90%	71.5 (64.8–77.5)	94.1 (85.6–98.4)	97.4 (93.4–99.3)	52.0 (42.8–61.1)	77.1 (71.7–81.9)

Values are % (95% CI). Model performance measures of validation cohort 1, validation cohort 2, and the combined validation cohorts 1 and 2 (n = 275; Dravet syndrome = 207; genetic epilepsy with febrile seizures plus = 68) according to different probability thresholds (50%, 60%, 70%, 80%, and 90%). This includes sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy observed using the *SCN1A* score and onset model.

cases are often associated with milder phenotypes. However, these data are often not available, particularly in health care settings where this screening incurs a direct cost to the patient.

The challenge of clinical decision-making is not limited to *SCN1A*-related epilepsies. Our approach of developing a clinical decision-support algorithm is generalizable and can be applied to many genetic disorders where genetic and clinical data are available.

Our findings suggest that routinely accessible biomarkers such as age at seizure onset combined with an *SCN1A* genetic score can be used to predict Dravet syndrome. Although the model can be employed at the time of diagnosis, expert clinical assessment will allow further delineation of the phenotype over time. The prediction model represents an important step towards evidence-based clinical outcome prediction, assisting clinicians with prognostic counseling and decisions on early institution of precision therapies.

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Disclosure

A. Brunklaus has received honoraria for presenting at educational events, advisory boards, and consultancy work for Biocodex, Encoded Therapeutics, GW Pharma, Nutricia, Stoke Therapeutics, and Zogenix. E. Pérez-Palma has received honoraria for consultancy work for the Friends of Faces foundation and a grant from Agencia Nacional de Investigación y Desarrollo of Chile (ANID, Grant PAI77200124) and the FamilieSCN2A foundation (2020 Action Potential Grant). I. Ghanty reports no disclosures relevant to the manuscript. J. Xinge holds grants from NovoNordisk, Inc.,

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Appendix *(continued)*

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