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

Author(s):

Andreas Brunklaus, MD[1, 2]; Eduardo Pérez-Palma, PhD[3, 4]; Ismael Ghanty, MD[1, 2]; Ji Xinge, PhD[5]; Eva Brilstra, PhD[6]; Berten Ceulemans, PhD[7]; Nicole Chemaly, PhD[8]; Iris de Lange, MD[6]; Christel Depienne, PhD[9]; Renzo Guerrini, MD[10]; Davide Mei, PhD[10]; Rikke S Møller, PhD[11, 12]; Rima Nabbout, PhD[8]; Brigid M Regan, BSc[13]; Amy L Schneider, MGenCouns[13]; Ingrid E Scheffer, PhD[13, 14]; An-Sofie Schoonjans, MD[7]; Joseph D Symonds, PhD[1, 2]; Sarah Weckhuysen, PhD[15, 16, 17]; Michael W Kattan, PhD[5]; Sameer M Zuberi, MD[1, 2]; Dennis Lal, PhD[4, 18, 19, 2]

Equal Author Contributions:

A.B., E.P.P. and I.G. contributed equally to the manuscript and are co-first authors; A.B. and D.L. are co-senior authors

Corresponding Author:

Andreas Brunklaus

andreas.brunklaus@glasgow.ac.uk

Affiliation Information for All Authors: 1. The Pediatric Neurosciences Research Group, Royal Hospital for Children, Glasgow, UK; 2. Institute of Health and Wellbeing, University of Glasgow, UK; 3. Universidad del Desarrollo, Centro de Genética y Genómica, Facultad de Medicina Clínica Alemana, Santiago, Chile; 4. Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, USA; 5. Department of Quantitative Health Sciences, Cleveland Clinic, USA; 6. Department of Genetics, University Medical Centre, Utrecht, Netherlands; 7. Department of child neurology, University Hospital Antwerp, Antwerp, Belgium; 8. Reference centre for rare epilepsies, Department of Pediatric Neurology, Hôpital Necker-Enfants Malades, Université de Paris, Paris, France; 9. Institute of Human Genetics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany; 10. Neuroscience Department, Children's Hospital A. Meyer-University of Florence, Italy; 11. The Danish Epilepsy Centre, Dianalund, Denmark; 12. Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark; 13. Department of Medicine, Epilepsy Research Centre, The University of Melbourne, Austin Health, Melbourne, Australia; 14. University of Melbourne, Royal Childrens Hospital, Florey and Murdoch Childrens Research Institutes, Melbourne, Australia; 15. Applied & Translational Neurogenomics Group, VIB-Center for Molecular Neurology, VIB, Antwerp, Belgium; 16. Neurology Department, University Hospital Antwerp, Antwerp, Belgium; 17. Institute Born-Bunge, University of Antwerp, Antwerp, Belgium; 18. Cologne Center for Genomics, University of Cologne, Cologne, Germany; 19. Epilepsy Center, Neurological Institute, Cleveland Clinic, Cleveland, USA; 20. Stanley Center for Psychiatric Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA;

Contributions:

Andreas Brunklaus: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Eduardo Pérez-Palma: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Ismael Ghanty: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Ji Xinge: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Eva Brilstra: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Berten Ceulemans: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Nicole Chemaly: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Iris de Lange: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Christel Depienne: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Renzo Guerrini: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Davide Mei: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Rikke S Møller: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Rima Nabbout: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Brigid M Regan: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Amy L Schneider: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Ingrid E Scheffer: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

An-Sofie Schoonjans: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Joseph D Symonds: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Sarah Weckhuysen: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Michael W Kattan: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data

Sameer M Zuberi: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data

Dennis Lal: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Number of characters in title: 96

Abstract Word count: 350

Word count of main text: 3622

References: 38

Figures: 6

Tables: 1

Supplemental: STARD Checklist Supplemental Files

Statistical Analysis performed by: Eduardo Pérez-Palma, PhD Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, USA; Ji Xinge, PhD Department of Quantitative Health Sciences, Cleveland Clinic, USA

Search Terms:

Acknowledgements: We would like to thank the Dravet Syndrome Foundation and Dravet Syndrome UK for their generous grants to support this work.

Study Funding: D.L. work was supported by funds from the Dravet Syndrome Foundation (grant number, 272016), the BMBF (Treat-ION grant, 01GM1907), NIH NINDS (Channelopathy-Associated Epilepsy Research Center, 5-U54-NS108874). A.B. and S.Z. received a grant from Dravet Syndrome UK for the Glasgow SCN1A database (grant number, 16GLW00).

Disclosures: 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. Grant recipient 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, grants from Merck, Inc., grants from Novartis, and grants from Boehringer Ingelheim Pharmaceuticals, Inc, outside the submitted work. E. Brilstra reports no disclosures relevant to the manuscript. B. Ceulemans has received research funding, Brabant, Zogenix; Consultant, Brabant, Zogenix; Patent, ZX008. BC and the KU Leuven University/Antwerp University Hospital may benefit financially from a royalty arrangement that is related to this research if Zogenix is successful in marketing its product, fenfluramine. N. Chemaly has received honoraria from Nutricia and Eisai for presentations at symposia. I. de Lange reports no disclosures relevant to the manuscript. C. Depienne reports no disclosures relevant to the manuscript. R. Guerrini has acted as an investigator for studies with Zogenix, Biocodex, Biomarin, UCB, Angelini, and Eisai Inc. He has been a speaker and on advisory boards for Zogenix, Biocodex, Novartis, Biomarin, GW Pharma, and Biocodex; serves/has served on the editorial boards of Epilepsia, Progress in Epileptic Disorders, Neuropediatrics, Journal of Child Neurology, Seizure, BMC Medical Genetics, Topics in Epilepsy, and Neurology. He receives/has received research support from the Italian Ministry of Health, The European Community, The Tuscany Region, the Mariani Foundation, The Pisa Foundation, The Fund of Epilepsy, The GKT Special Trustees, The Italian Federation for Epilepsy, and The Italian Association for Epilepsy. D. Mei reports no disclosures relevant to the manuscript. R.S. Møller reports no disclosures relevant to the manuscript. R. Nabbout received research funding from the European Union (Seventh Framework Programme), EJP-RD (European Joint program for rare diseases, Shire, Zogenix, GW Pharma, and Eisai and consultation and lecturer fees from Eisai, Zogenix, Takeda, GW Pharma, Advicenne, Biocodex, Nutricia, Supernus, Biogen and Novartis. Brigid M Regan BSc: brigidscally@gmail.com no COI reports no disclosures relevant to the manuscript. A.L. Schneider reports no disclosures relevant to the manuscript. I.E. Scheffer has served on scientific advisory boards for UCB, Eisai, GlaxoSmithKline, BioMarin, Nutricia, Rogcon, Chiesi, Encoded Therapeutics and Xenon Pharmaceuticals; has received speaker honoraria from GlaxoSmithKline, UCB, BioMarin, Biocodex and Eisai; has received funding for travel from UCB, Biocodex, GlaxoSmithKline, Biomarin and Eisai; has served as an investigator for Zogenix, Zynerba, Ultragenyx, GW Pharma, UCB, Eisai, Anavex Life Sciences, Ovid Therapeutics, Epigenyx, Encoded Therapeutics and Marinus; and has consulted for Zynerba Pharmaceuticals, Atheneum Partners, Ovid Therapeutics, Care Beyond Diagnosis, Epilepsy Consortium and UCB. She may accrue future revenue on pending patent WO61/010176 (filed: 2008): Therapeutic Compound; has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies; has a patent molecular diagnostic/theranostic target for benign familial infantile epilepsy (BFIE) [PRRT2] 2011904493 & 2012900190 and PCT/AU2012/001321 (TECH ID:2012-009). A. Schoonjans reports no disclosures relevant to the manuscript. J.D. Symonds reports no disclosures

relevant to the manuscript. S. Weckhuysen received speaker and consultancy fees from UCB, Xenon, Zogenix, Lundbeck and Biocodex. M.W. Kattan is a consultant for Glaxo Smith Kline and Exosome Diagnostics. S.M. Zuberi has received honoraria for presenting at educational events, advisory boards and consultancy work for GW Pharma, Zogenix, Biocodex, Encoded Therapeutics, Stoke Therapeutics and Nutricia D. Lal has received honoraria for advisory board work for Encoded Therapeutics

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

Results: 1018 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) versus 52.0 (SD, 57.5; $p < 0.001$) and young age of onset 6.0 (SD, 3.0) months versus 14.8 (SD, 11.8; $p < 0.001$) months, were each associated with Dravet syndrome versus 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]).

Discussion: The prediction model allows objective estimation at disease onset whether a child will develop Dravet syndrome versus 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.

Search Terms: *SCN1A*; Dravet syndrome; GEFS+; Prediction; Risk Model

Introduction

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 one 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] Whilst 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 two conditions on clinical grounds alone is challenging in the first two years of life since 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 currently 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 (15.84/1000-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 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 commenced.[14] Therefore, 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 versus other GEFS+ phenotypes. To date, only two studies have attempted to predict Dravet syndrome versus GEFS+ based on clinical and genetic data.[15,16] Whilst 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, there are currently 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]

Assembling a large *SCN1A* patient cohort, we hypothesize 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 multi-center retrospective cohort study to develop and validate a statistical model combining age of seizure onset (febrile or afebrile, whichever occurred first) and the

SCN1A genetic score in predicting Dravet syndrome versus 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 1018 patients from seven 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 Dravet syndrome and GEFS+ patients carrying pathogenic *SCN1A* variants from the following sites: The Royal Hospital for Children, Glasgow,[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, and unpublished cases (eMethods and eTable 1 in the Supplement).

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; 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 age >24 months;

however, a number of Dravet syndrome patients 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 UK, France, Italy, Netherlands, and Denmark (n = 743). We then tested the prediction model in two blinded validation cohorts from Australia (Validation cohort 1, n = 203) and Belgium (Validation cohort 2, n = 72). As our model is based on age of onset and genetic data, we only included patients who had this data available.

Blinding of Validation Cohorts

Whilst clinical information (Dravet syndrome vs GEFS+) was available to the assessors for the training cohort, data for the two validation cohorts were supplied without disclosing the phenotype. Details on whether a subject 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 the *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

insertion/deletions and synonymous variants. Details on molecular analysis for each center are provided in eMethods in the Supplement. 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) of the observed substitution.[26] 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, ten 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 questions was as follows: What is the discriminative accuracy of a statistical model combining age of seizure onset and the *SCN1A* genetic score in predicting Dravet syndrome versus 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 of seizure onset in months (referred to as the Index '*SCN1A* score & Onset' model) as predictors of Dravet syndrome and GEFS+ (eMethods in the Supplement). The age of 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 yet, and has been shown to be a valuable prognostic factor in earlier studies.[15,16]

To compare our model, we constructed three additional models: 1) age of seizure onset 'Onset-only' model, 2) 'CADD & Onset' model, and 3) 'REVEL & Onset' model, following the same procedure. We compared our model against a 'six-months seizure onset threshold' model proposed previously[15] which served as reference standard since 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 Dravet syndrome patient. Patients with predictions below 50% were assigned a GEFS+ status. We calibrated and compared the models using the receiver operating characteristic (ROC) curve, calibration curves, and the index of prediction accuracy (IPA).[30] Area under the curve (AUC) and IPA confidence intervals (95% CI) were generated with 1000 bootstraps sets during cross-validation. Sensitivities, specificities, positive predictive values, negative predictive values and accuracies alongside their 95% CI were calculated following established guideline.[31] All patients with ages of seizure onset, genetic variants and their corresponding genetic score are detailed in eTable 1 in the Supplement.

Data Availability Statement

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 which 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%) GEFS+ patients and in validation cohort 2 was 60/72 (83%) with 12 (17%) GEFS+ patients. 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 of seizure onset or a higher *SCN1A* genetic score were each associated with a diagnosis of Dravet syndrome (Figures 2A and 2B). Despite the significantly earlier seizure onset in the Dravet syndrome (mean [SD] age, 6.04 [3.0] months) versus GEFS+ group (mean [SD] age, 14.82 [11.8] months; $p < 0.001$) and the higher *SCN1A* genetic score in the Dravet syndrome (mean [SD] score, 133.43 [78.53]) versus GEFS+ group (mean [SD] score, 52.90 [57.58]; $p < 0.001$), there was considerable overlap between both disorders (Figures 2A and 2B).

Using the training cohort, we generated four 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 & Onset' model outperformed the prediction model based solely on the age of 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 & Onset' model equally outperformed models based on two additional pathogenicity scores, namely CADD ('CADD & Onset'; AUC, 0.85 [95% CI, 0.82-0.89]; IPA = 31.2%), and REVEL ('REVEL & Onset'; AUC, 0.84 [95% CI, 0.80-0.88]; IPA = 31.6%). In addition, our '*SCN1A* score & Onset' model outperformed the 'six-

months seizure onset threshold‘ model proposed previously[15] (Figures 3 and 4, AUC, 0.79). Dominance analysis showed that age of seizure onset was 2.06 times more important than the *SCN1A* genetic score to the overall model (eFigure 1 and eTable 2 in the Supplement). Model performance was similar when focusing only on index individuals (eTable 3 in the Supplement).

Next, we tested the performance of the '*SCN1A* score & Onset' model in two independent blinded validation cohorts of *SCN1A* epilepsy patients. 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 positive and negative predicted values (PPV/NPV) as well as sensitivities 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 = 1018) 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 & Onset’ model yielded an AUC, 90.4 (95% CI, 87.5-93.2) in the random training cohort and an AUC, 91.5, (95% CI, 87.1-95.9) in the random validation cohort (eFigure 2 in the Supplement).

Finally, we developed the prediction model into an online tool designed to evaluate any missense or protein truncating variant (PTV) found in a given patient with an *SCN1A* pathogenic variant combined with the age of seizure onset (http://scn1a-prediction-model.broadinstitute.org/). The *SCN1A*-epilepsy prediction model will calculate a patient's probability (%) of developing Dravet

syndrome versus other GEFS+ phenotypes in a user-friendly platform that is freely available online (eFigure 3 in the Supplement).

Discussion

In this large, multicenter cohort study, we found that a clinical-genetic prediction model, combining the age of seizure onset with a newly developed *SCN1A* genetic score, allows an objective early estimation as to whether a child will develop Dravet syndrome versus 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 versus other GEFS+ phenotypes in *SCN1A* positive patients, judgments about diagnosis and prognosis are challenging, particularly for non-expert clinicians. Consider the example of a nine-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 of 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 of 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 of seizure onset. Whilst model prediction is mainly determined by age of onset, these examples illustrate how both, the

genetic information as well as the age of onset, play an important part in the outcome prediction model.

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 patients,[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 option 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 three years of life, emphasizing all the more 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 two 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 up 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 anti-seizure 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. Modelling 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 modelling 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 further improve prediction accuracy. Our logistic regression model achieves an excellent to outstanding fitting (AUC=89.1) and the use of more complex modelling strategies might lead to

overfitting with little opportunity to increase performance. Future larger cohorts with additional phenotypical 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 (NMD) 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, that 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* versus familial) which might contribute to the predictive power of the model, as inherited cases are often associated with milder phenotypes. However, this data is often not available, particularly in healthcare settings where this 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 is available.

Conclusions

Our findings suggest that routinely accessible biomarkers such as age of seizure onset combined with an *SCN1A* genetic score can be used to predict Dravet syndrome. Whilst 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.

Acknowledgement

We would like to thank the Dravet Syndrome Foundation and Dravet Syndrome UK for their generous grants to support this work.

Study Funding

D.L. work was supported by funds from the Dravet Syndrome Foundation (grant number, 272016), the BMBF (Treat-ION grant, 01GM1907), NIH NINDS (Channelopathy-Associated Epilepsy Research Center, 5-U54-NS108874). A.B. and S.Z. received a grant from Dravet Syndrome UK for the Glasgow *SCN1A* database (grant number, 16GLW00). The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclosure

A.B. has received honoraria for presenting at educational events, advisory boards and consultancy work for Biocodex, Encoded Therapeutics, GW Pharma, Nutricia, Stoke Therapeutics and Zogenix.

Author Contributions

A.B. has access to all the data and takes responsibility for the data, accuracy of the data analysis, and the conduct of the research. A.B., E.P.P., I.G., J.X., and D.L. were responsible for study conception, design, data acquisition, analysis, and interpretation and drafting of the manuscript. A.B. and E.P.P. verified the underlying data. E.P.P. was responsible for imaging analysis. E.B., B.C., N.C., I.D.L., C.D., R.G., D.M., R.S.M., R.N., B.M.R., A.L.S., I.E.S., A.S.S., J.D.S., S.W.,

M.W.K. and S.M.Z. were responsible for acquisition of data and for substantial revision of the manuscript for intellectual content.

A.B., E.P.P. and I.G. contributed equally to the manuscript.

References

1. Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. Estimation of the burden of active and life-time epilepsy: A meta-analytic approach. *Epilepsia*. 2010;51(5):883–890.

2. Thomas RH, Berkovic SF. The hidden genetics of epilepsy-a clinically important new paradigm. *Nat Rev Neurol*. 2014;10(5):283–292.

3. Symonds JD, Zuberi SM, Stewart K, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. *Brain*. 2019;142(8):2303–2318.

4. Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype–phenotype associations in SCN1A-related epilepsies. *Neurology*. 2011;76(7):594–600.

5. Dravet C, Oguni H. Chapter 65 - Dravet syndrome (severe myoclonic epilepsy in infancy). In: Dulac O, Lassonde M, Sarnat HB, eds. Handbook of Clinical Neurology. Elsevier, 2013:627–633.

6. Brunklaus A, Ellis R, Reavey E, Forbes GH, Zuberi SM. Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome. *Brain*. 2012;135(8):2329–2336.

7. Zhang Y-H, Burgess R, Malone JP, et al. Genetic epilepsy with febrile seizures plus: Refining the spectrum. *Neurology*. 2017;89(12):1210–1219.

8. Brunklaus A, Schorge S, Smith AD, et al. SCN1A variants from bench to bedside—improved clinical prediction from functional characterization. *Hum Mutat*. 2020;41(2):363–374.

9. Cooper MS, Mcintosh A, Crompton DE, et al. Mortality in Dravet syndrome. *Epilepsy Res*. 2016;128:43–47.

10. Chiron C, Marchand MC, Tran A, et al. Stiripentol in severe myoclonic epilepsy in infancy: a randomised placebo-controlled syndrome-dedicated trial. *Lancet*. 2000;356(9242):1638–1642.

11. Devinsky O, Cross JH, Laux L, et al. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N Engl J Med*. 2017;376(21):2011-2020.

12. Lagae L, Sullivan J, Knupp K, et al. Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2019;394(10216):2243–2254.

13. Nabbout R, Mistry A, Zuberi S, et al. Fenfluramine for Treatment-Resistant Seizures in Patients With Dravet Syndrome Receiving Stiripentol-Inclusive Regimens: A Randomized Clinical Trial. *JAMA Neurol*. 2020;77(3):300-308.

14. Han Z, Chen C, Christiansen A, et al. Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. *Sci Transl Med*. 2020:12(558):eaaz6100. doi:10.1126/scitranslmed.aaz6100.

15. Cetica V, Chiari S, Mei D, et al. Clinical and genetic factors predicting Dravet syndrome in infants with SCN1A mutations. *Neurology*. 2017;88(11):1037–1044.

16. de Lange IM, Gunning B, Sonsma ACM, et al. Influence of contraindicated medication use on cognitive outcome in Dravet syndrome and age at first afebrile seizure as a clinical predictor in SCN1A-related seizure phenotypes. *Epilepsia*. 2018;59(6):1154–1165.

17. Zethelius B, Berglund L, Sundström J, et al. Use of Multiple Biomarkers to Improve the Prediction of Death from Cardiovascular Causes. *N Eng J Med*. 2008;358(20):2107–2116.

18. Mallett S, Royston P, Waters R, Dutton S, Altman DG. Reporting performance of prognostic models in cancer: a review. *BMC Med*. 2010;8:21. doi: 10.1186/1741-7015-8-21.

19. Cohen JF, Korevaar DA, AltmanDG, et al. STARD2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open. 2016;6(11):e012799.

20. Brunklaus A, Du J, Steckler F, et al. Biological concepts in human sodium channel epilepsies and their relevance in clinical practice. *Epilepsia*. 2020;61(3):387–399.

21. Depienne C, Trouillard O, Saint-Martin C, et al. Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. *J Med Genet*. 2009;46(3):183–191.

22. Møller RS, Larsen LHG, Johannesen KM, et al. Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies. *Mol Syndromol*. 2016;7(4):210–219.

23. Johannesen KM, Nikanorova N, Marjanovic D, et al. Utility of genetic testing for therapeutic decision-making in adults with epilepsy. *Epilepsia*. 2020;61(6):1234–1239.

24. Claes L, Ceulemans B, Audenaert D, et al. De novo SCN1A mutations are a major cause of severe myoclonic epilepsy of infancy. *Hum Mutat*. 2003;21(6):615–621.

25. Lal D, May P, Perez-Palma E, et al. Gene family information facilitates variant interpretation and identification of disease-associated genes in neurodevelopmental disorders. *Genome Med*. 2020;12(1):28. doi: 10.1186/s13073-020-00725-6.

26. Grantham R. Amino Acid Difference Formula to Help Explain Protein Evolution. *Science*. 1974;185(4154):862–864.

27. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 2019;47(D1):D886–894.

28. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am J Hum Genet*. 2016;99(4):877–885.

29. Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMJ*. 2015; 350(g7594). doi:10.1136/bmj.g7594.

30. Kattan MW, Gerds TA. The index of prediction accuracy: an intuitive measure useful for evaluating risk prediction models. *Diagn Progn Res.* 2018;2:7. doi: 10.1186/s41512-018-0029-2.

31. Mercaldo ND, Lau KF, Zhou XH. Confidence intervals for predictive values with an emphasis to case–control studies. *Stat Med*. 2007;26(10):2170–2183.

32. Cervellin G, Borghi L, Lippi G. Do clinicians decide relying primarily on Bayesians principles or on Gestalt perception? Some pearls and pitfalls of Gestalt perception in medicine. *Intern Emerg Med*. 2014;9(5):513–519.

33. Dutton SBB, Dutt K, Papale LA, Helmers S, Goldin AL, Escayg A. Early-life febrile seizures worsen adult phenotypes in Scn1a mutants. *Expl Neurol*. 2017;293:159–171. doi: 10.1016/j.expneurol.2017.03.026.

34. Steyerberg EW, Vickers AJ, Cook NR, et al. Assessing the Performance of Prediction Models: A Framework for Traditional and Novel Measures. *Epidemiology*. 2010;21(1):128–138.

35. Lange IM de, Koudijs MJ, Slot R van 't, et al. Mosaicism of de novo pathogenic SCN1A variants in epilepsy is a frequent phenomenon that correlates with variable phenotypes. *Epilepsia*. 2018;59(3):690–703.

36. Khajavi M, Inoue K, Lupski JR. Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur J Human Genet*. 2006;14(10):1074–1081.

37. Sadleir LG, Mountier EI, Gill D, et al. Not all *SCN1A* epileptic encephalopathies are Dravet syndrome: Early profound Thr226Met phenotype. *Neurology*.2017;89(10):1035-1042.

38. Berecki G, Bryson A, Terhag J, et al. *SCN1A* gain of function in early infantile encephalopathy. *Ann. Neurol*. 2019;85(4):514-525.

Figure 1. Study Overview.

Figure legend: Study workflow. Genetic data (*SCN1A* genetic score) and clinical data (age of seizure onset in months) from 743 patients (training cohort) was introduced to a supervised machine learning approach to produce a prediction model. We tested the prediction model with two independent blinded validation cohorts (n= 275).

Figure 2. Training Cohort Data.

Figure legend: A. Density plot showing the distribution of the age of seizure onset in training cohort patients with Dravet syndrome (purple area) and GEFS+ (grey area). **B**. Density plot showing the distribution of the *SCN1A* genetic score in training cohort patients with Dravet syndrome (purple area) and GEFS+ (grey area). Statistical difference between the observed means was evaluated with the Wilcoxon test.

Figure 3. Training Cohort Model Performance – ROC curve analysis.

Figure legend: Receiver operating characteristic (ROC) curves showing the relationship between the observed sensitivity and specificity for different models using genetic scores and seizure age of onset: '*SCN1A* score & Onset' (blue line, n=743), 'Onset-only' (orange line, n=743), 'CADD score & Onset' (green line) and 'REVEL score & Onset' (purple line). Since CADD and REVEL scores are not available for all variants contained in the training cohort, the 'CADD & onset' and 'REVEL & Onset' models were built with a subset of 651 and 438 training cohort patients, respectively (eTable 1 in the Supplement). The 'six months seizure onset

threshold' model (grey line) proposed previously[15] is shown for comparison. Area under the curve values (AUC) and 95% confidence interval (CI) are shown at the bottom right corner of the plot.

Figure 4. Calibration curves per model.

Figure legend: 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 & Onset' (blue line), 'Onset-only' (orange line), 'CADD score & Onset' (green line) and 'REVEL score & Onset' (purple line).

Figure 5. Validation Cohort 1 and 2 Prediction Results.

Figure legend: Patients with probability values above 50% were predicted to have Dravet syndrome and patients with values below 50% were predicted to have GEFS+. **A** and **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. True Dravet syndrome patients are shown in purple while true GEFS+ patients are shown in grey. Dotted horizontal line denotes a 50% threshold with values above 50% predicting Dravet syndrome and values below 50% predicting GEFS+ patients. Area under the curve (AUC) and index of prediction accuracy (IPA) confidence intervals (95% CI) are given.

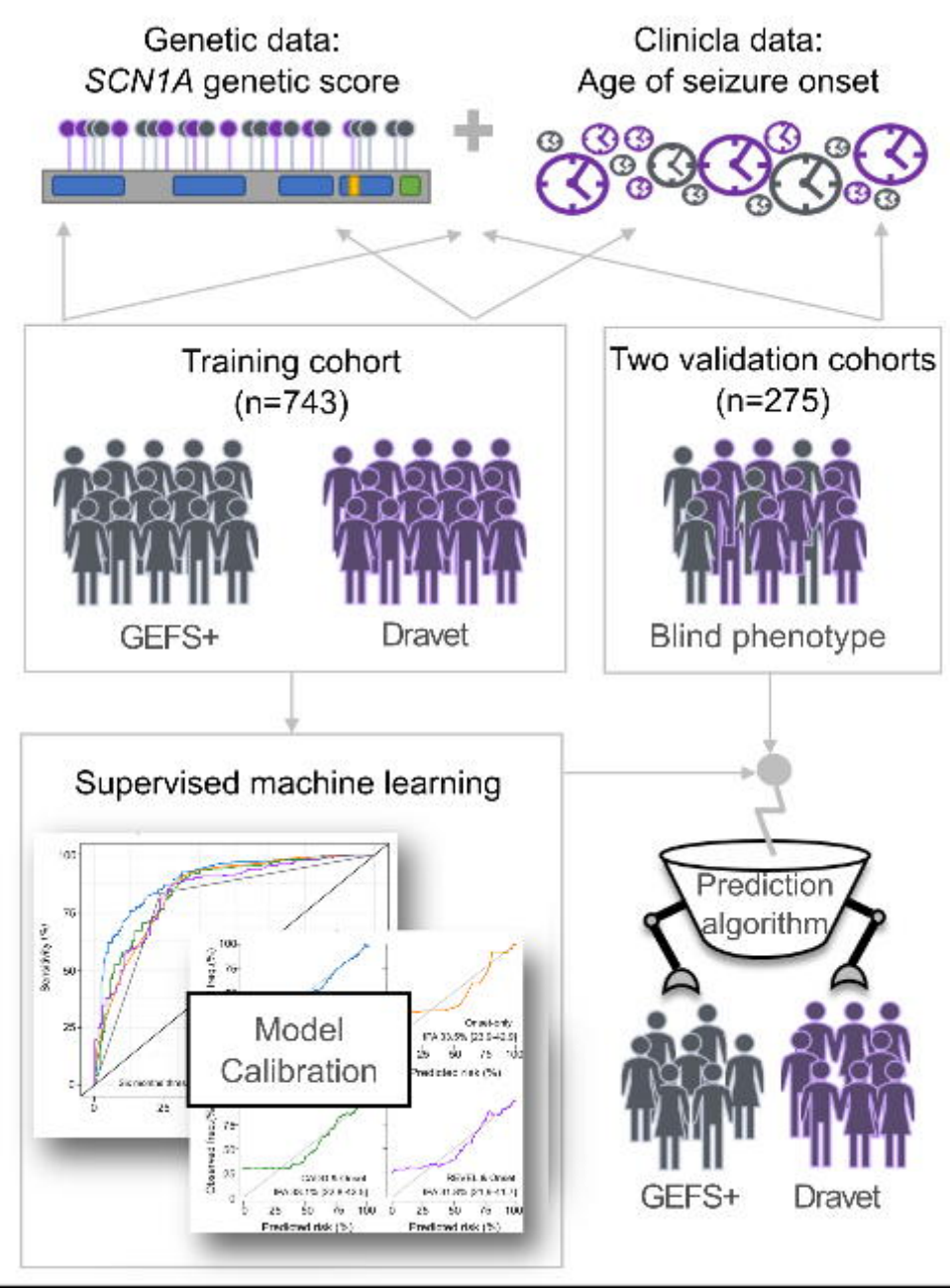
Figure 6. Validation Cohort 1 and 2 Phenotype Distribution.

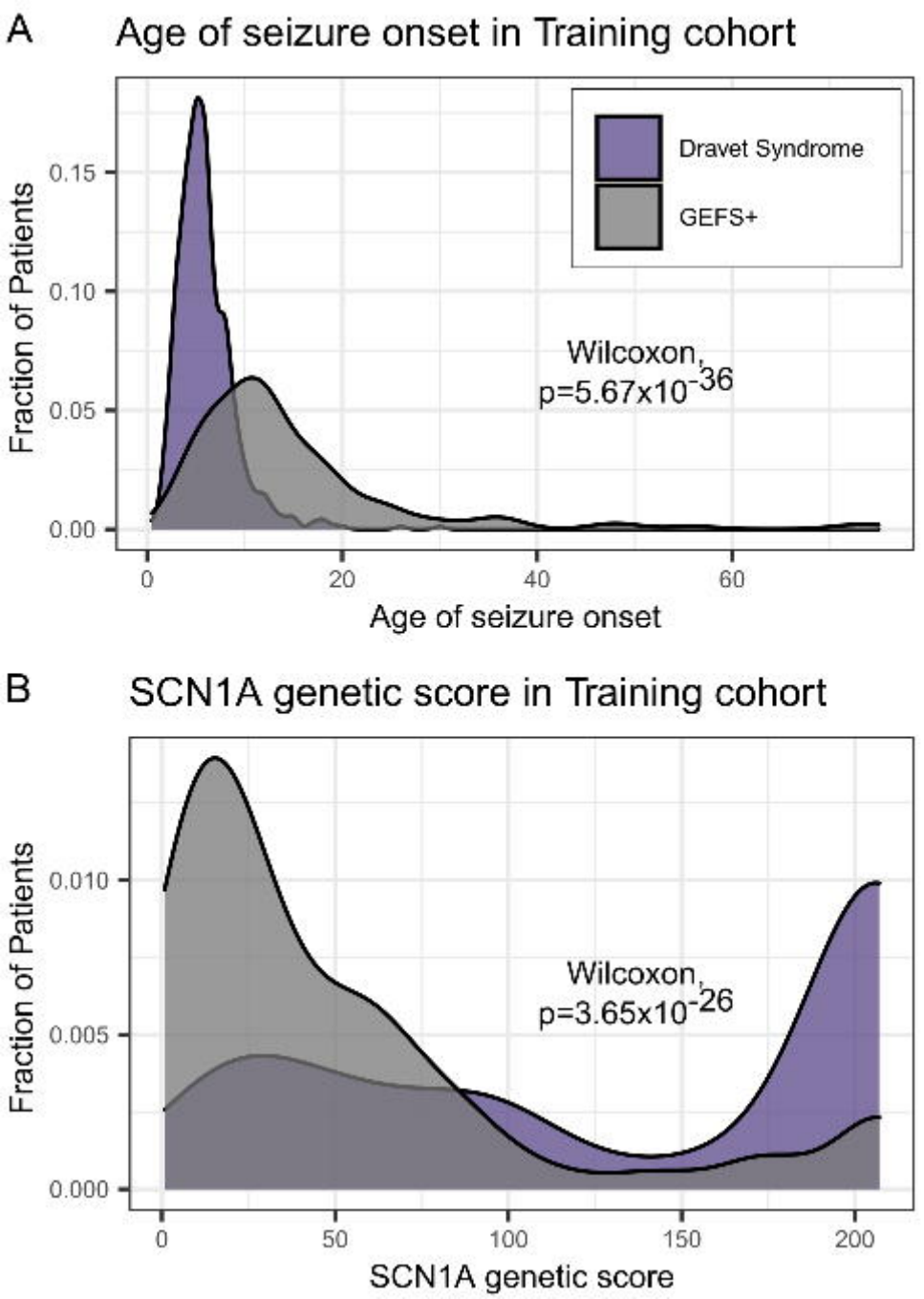
Figure legend: Phenotype distribution with density of prediction performed on validation cohorts 1 and 2 respectively. True Dravet syndrome and GEFS+ patients accumulate across their corresponding model predictions (horizontal axis). Dotted vertical line denotes a 50% threshold with values above 50% predicting Dravet syndrome and values below 50% predicting GEFS+ patients.

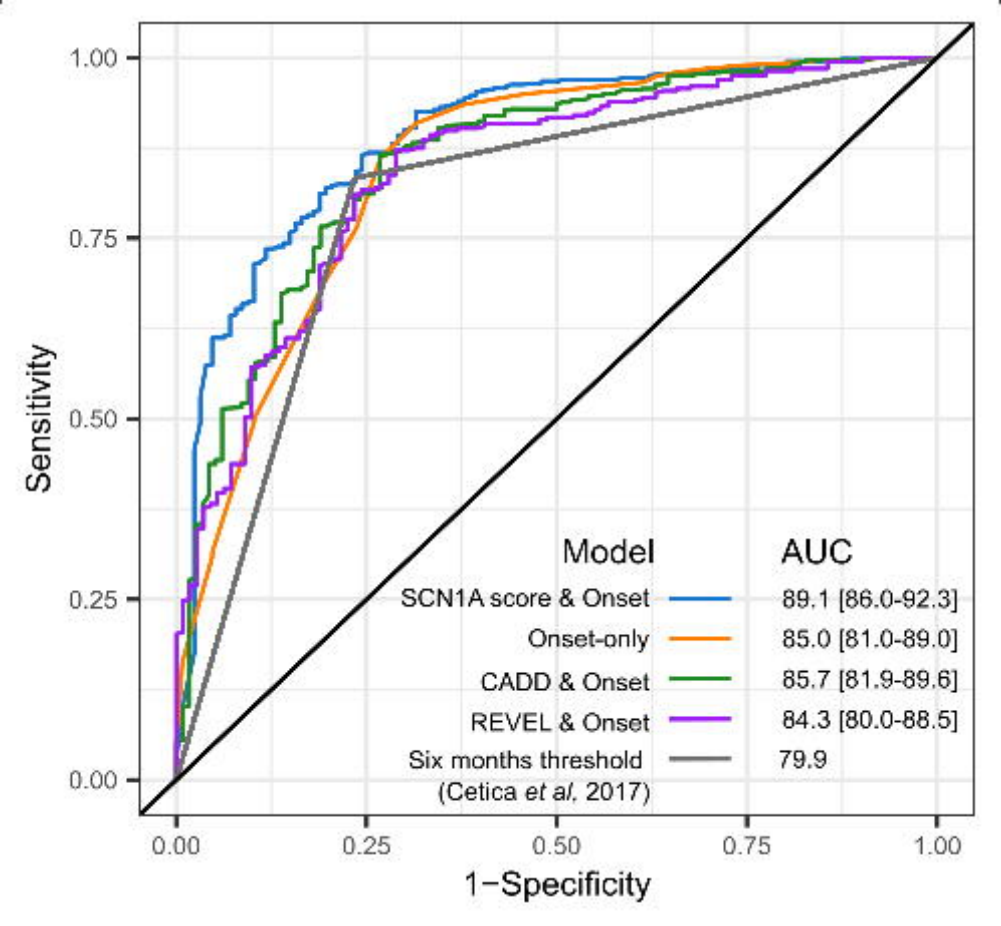
Table 1. Model Performance Parameters According to Different Thresholds

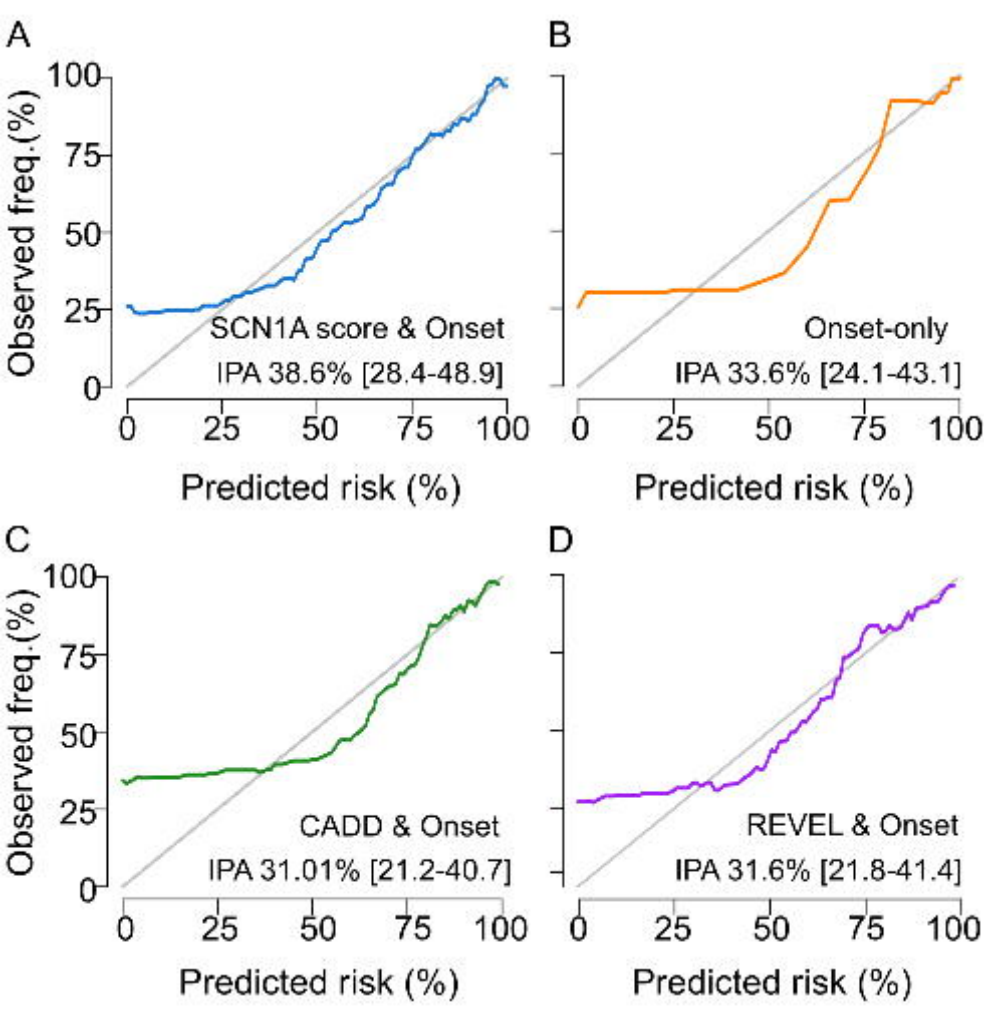
Validation Cohort 1 (n = 203)					
Threshold	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
50%	99.3% (96.3-100)	64.3% (50.4-76.6)	88.0% (82.0-92.5)	97.3% (85.8-99.9)	89.7% (84.6-93.5)
60%	95.9% (91.3-98.5)	67.9% (54.0-79.7)	88.7% (82.7-93.2)	86.4% (72.6-94.8)	88.2% (82.9-92.3)
70%	92.5% (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)					
Threshold	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
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 & 2 (n = 275)					
Threshold	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
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)

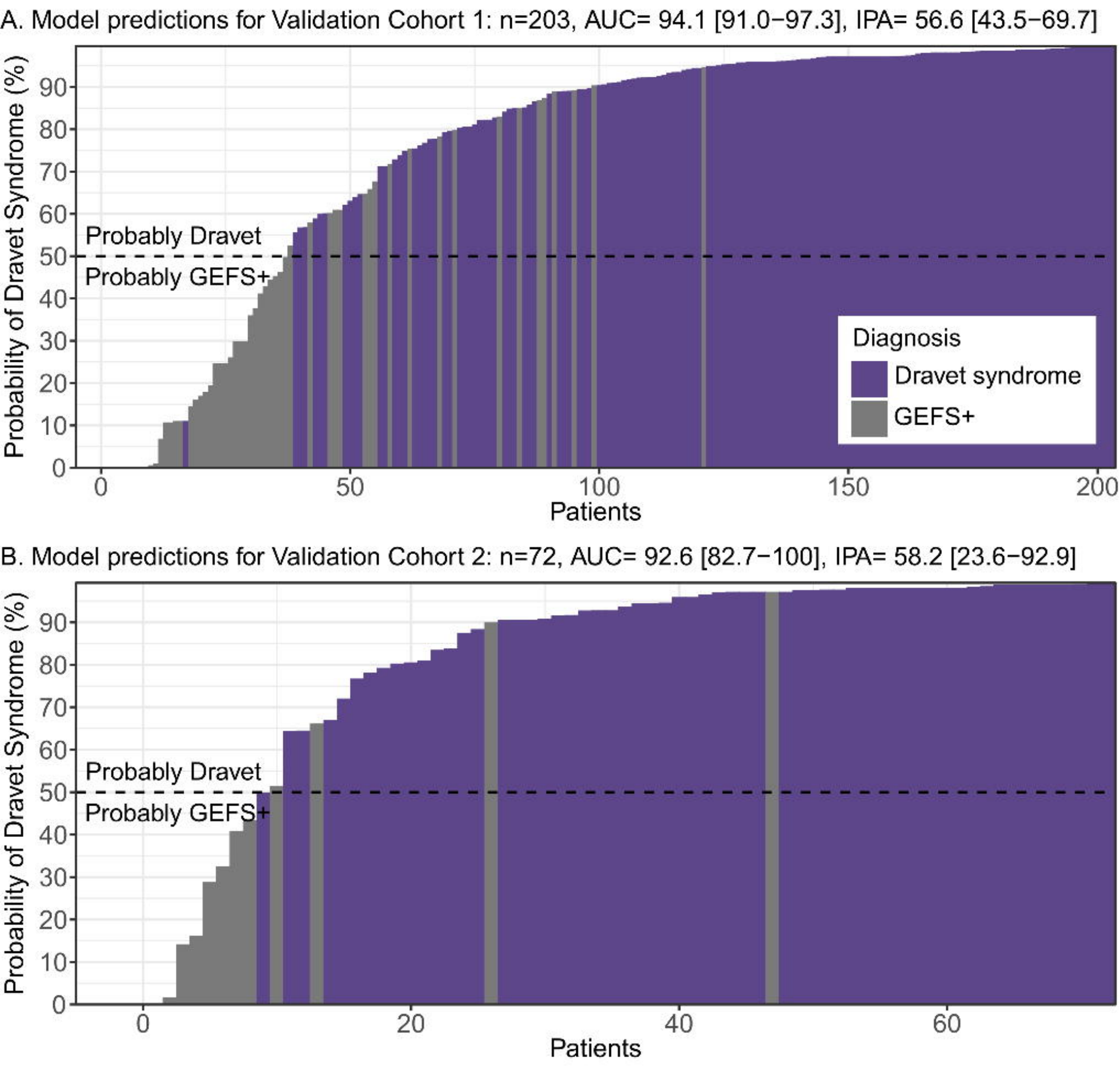
Table 1 legend. Model performance parameters of validation cohort 1, validation cohort 2 and the combined validation cohorts 1 and 2 (n=275; Dravet syndrome=207; GEFS+=68) according to different probability thresholds (50%, 60%, 70%, 80%, and 90%). This includes sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy observed using the '*SCN1A* score & Onset' model. Corresponding 95% confidence intervals (CI) are shown between parentheses.

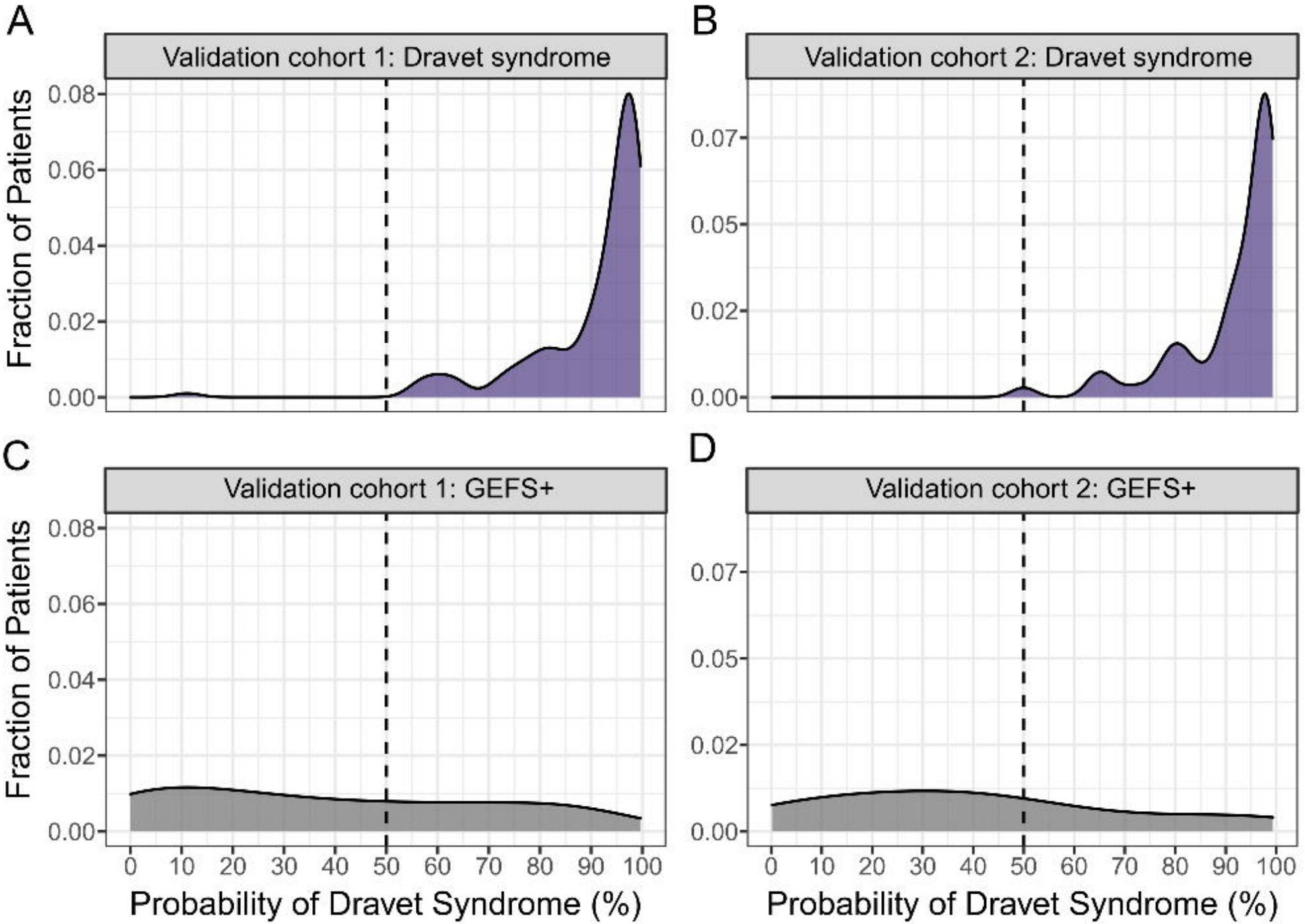

Genetic data:
SCN1A genetic score
Clinicla data:
Age of seizure onset
Training cohort
(n=743)
GEFS+
Dravet
Two validation cohorts
(n=275)
Blind phenotype
Supervised machine learning
Model
Calibration
Prediction
algorithm
GEFS+
Dravet


A
Age of seizure onset in Training cohort
Dravet Syndrome
GEFS+
Wilcoxon,
p=5.67x10-36
Fraction of Patients
0.15
0.10
0.05
0.00
0
20
40
60
Age of seizure onset
B
SCN1A genetic score in Training cohort
Wilcoxon,
p=3.65x10-26
Fraction of Patients
0.010
0.005
0.000
0
50
100
150
200
SCN1A genetic score


1.00
0.75
0.50
0.25
0.00
Sensitivity
0.00
0.25
0.50
0.75
1.00
1−Specificity
Model
AUC
SCN1A score & Onset
89.1 [86.0-92.3]
Onset-only
85.0 [81.0-89.0]
CADD & Onset
85.7 [81.9-89.6]
REVEL & Onset
84.3 [80.0-88.5]
Six months threshold
(Cetica et al, 2017)
79.9


A
Observed freq.(%)
100
75
50
25
0
SCN1A score & Onset
IPA 38.6% [28.4-48.9]
0
25
50
75
100
Predicted risk (%)
B
Onset-only
IPA 33.6% [24.1-43.1]
0
25
50
75
100
Predicted risk (%)
C
Observed freq.(%)
100
75
50
25
0
CADD & Onset
IPA 31.01% [21.2-40.7]
0
25
50
75
100
Predicted risk (%)
D
REVEL & Onset
IPA 31.6% [21.8-41.4]
0
25
50
75
100
Predicted risk (%)


A. Model predictions for Validation Cohort 1: n=203, AUC= 94.1 [91.0−97.3], IPA= 56.6 [43.5−69.7]
Probability of Dravet Syndrome (%)
0
10
20
30
40
50
60
70
80
90
Probably Dravet
Probably GEFS+
Diagnosis
Dravet syndrome
GEFS+
0
50
100
150
200
Patients
B. Model predictions for Validation Cohort 2: n=72, AUC= 92.6 [82.7−100], IPA= 58.2 [23.6−92.9]
Probability of Dravet Syndrome (%)
0
10
20
30
40
50
60
70
80
90
Probably Dravet
Probably GEFS+
0
20
40
60
Patients


A
Validation cohort 1: Dravet syndrome
Fraction of Patients
0.08
0.06
0.04
0.02
0.00
B
Validation cohort 2: Dravet syndrome
0.07
0.05
0.02
0.00
C
Validation cohort 1: GEFS+
Fraction of Patients
0.08
0.06
0.04
0.02
0.00
0 10 20 30 40 50 60 70 80 90 100
Probability of Dravet Syndrome (%)
D
Validation cohort 2: GEFS+
0.07
0.05
0.02
0.00
0 10 20 30 40 50 60 70 80 90 100
Probability of Dravet Syndrome (%)