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ORIGINAL RESEARCH

A Multi-Center Study: Developing a Nomogram for Predicting Genetic Results of Trio-Based Whole-Exome Sequencing (Trio-WES) in Diagnosing Children with Syndromic Neurodevelopmental Disorders (s-NDDs)

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Objective: The diagnostic efficacy of empirical trio-based whole-exome sequencing (trio-WES) for unexplained syndromic neurodevelopmental disorders (NDDs) remains unsatisfactory. This study aimed to explore the diagnostic value of phenotypic indicators and establish a nomogram for predicting the genetic results of trio-WES for diagnosing patients with unexplained s-NDDs.

Methods: We retrospectively collected phenotypic and genotypic data from 265 children with s-NDDs who received trio-WES at Sun Yat-sen Memorial Hospital (training cohort), 38 and 97s-NDDs patients with trio-WES test at Shenshan Medical Center (validation cohort-1) and Weierkang Children's Rehabilitation Center (validation cohort-2), respectively. Logistic analysis was employed to identify the independent predictors of a positive trio-WES diagnosis in the training cohort for model establishment. The predictive performance and robustness of the model were assessed using receiver operating characteristic (ROC) and confusion matrix analyses, respectively.

Results: The analysis revealed that the severity of neurodevelopmental delays, head circumference abnormality, and complexity of neurodevelopmental comorbidities were independent predictive indicators for distinguishing s-NDDs patients with positive trio-WES results. The nomogram combining the three predictors showed good predictive performance with an area under the ROC (AUC) in the training cohort of 0.827 (95% CI: 0.775–0.879), yielding a confusion matrix with sensitivity, specificity, accuracy, precision, and F1 score of 78.23%, 78.01%, 78.11%, 75.78%, and 0.77, respectively. The model also had an excellent prediction in the external validation cohorts (AUC: 0.953; 95% CI: 0.881–0.998, sensitivity: 88.89%; specificity: 80.00%; accuracy: 84.21%; precision: 80.00%; and F1 score: 0.84 in validation cohort-1 and AUC: 0.910; 95% CI: 0.843–0.978, sensitivity: 83.33%; specificity: 85.45%; accuracy: 84.54%; precision: 81.40%; and F1 score: 0.82 in validation cohort-2).

Conclusion: The model can serve as a useful tool for assisting decision-making in applying trio-WES in the diagnostic strategy for s-NDDs, helping to implement personalized pre-diagnosis assessments for affected families.

Keywords: syndromic neurodevelopmental disorders, trio-based whole-exome sequencing, diagnostic yield, phenotype-driven nomogram

Introduction

Syndromic neurodevelopmental disorders (s-NDDs) are genetically and clinically heterogeneous syndromes characterized by a complex group of neurological diseases with observable neurodevelopmental deficits beginning at a very early

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age, commonly <6 months-old.[1] As our understanding of s-NDDs has increased, the prevalence has dramatically increased, accounting for almost 1–3% of children worldwide.[2,3] The rapidly increasing prevalence of s-NDDs has caused a substantial burden to society and affected families. The main clinical manifestations of s-NDDs include global developmental delay/intellectual disability (GDD/ID), autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and epilepsy (EP), of which GDD/ID is the core of s-NDDs.[4,5] Other common neurological manifestations, such as ADHD, ASD, and EP, often act as comorbidities, solely or jointly present in affected individuals, which dramatically adds layers of complexity to the diagnosis of s-NDDs.[6]

Currently, the etiology of s-NDDs remains unclear; however, genetic disturbances such as single-nucleotide variants (SNVs) and copy-number variants (CNVs) are considered to play a key role in the pathogenesis of s-NDDs.[7] Owing to significant advancements in identifying genetic alterations in human diseases, particularly the popularization of trio-based (parental-offspring pattern) whole-exome sequencing (trio-WES), the most common sequencing technology enabling the identification of exon-level variants, including SNVs and CNVs, and genetic disturbances are being found more frequently than before in many individuals with idiopathic s-NDDs.[8] Nonetheless, nearly half of s-NDDs patients cannot obtain a genetic diagnosis after undergoing the trio-WES test because of the complex and heterogeneous genetic components underlying s-NDDs,[9] and the diagnostic yield of trio-WES in s-NDDs remains unsatisfactory. Therefore, developing a simple-to-use approach for the early identification of patients with s-NDDs who can be diagnosed by trio-WES is important for clinicians, which may provide affected individuals with further assessment of related medical conditions earlier in the disease course. Meanwhile, a user-friendly tool is crucial to assist decision-making for trio-WES diagnostic strategy at admission, promoting individualized medical planning and reducing unnecessary financial and time burdens for affected families.

The nomogram is a common graphical calculator used to visualize the scoring system of the linear regression model[10] and is widely used to predict various clinical outcomes across many pediatric neurodevelopmental diseases such as ASD and ADHD.[11,12] However, to the best of our knowledge, few studies have developed nomograms to forecast clinical outcomes in individuals with s-NDDs. Here, we conducted a multicenter study with independent external validation based on the phenotype-driven concept, developing and validating a nomogram model for predicting the genetic results of trio-WES in diagnosing patients with s-NDDs.

Materials and Methods

Participants Enrollment and Screening

The phenotypic definition of s-NDDs in this study is as follows: (1) exhibiting various severities of GDD/ID. Clinical diagnosis and severity assessment of GDD/ID were performed by neuropsychiatric pediatricians according to the guidelines of the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V)[1] and the criteria of the International Classification of Disease Version 11 (ICD-11),[13] respectively. (2) Comorbid with at least one type of common neurodevelopmental manifestation including EP, ADHD, and ASD. Diagnoses of these comorbidities were made by neuropsychiatric pediatricians according to the DSM-V guidelines for ADHD and ASD and the International League Against Epilepsy guidelines for EP.[14] (3) Children with a clear non-genetic etiology, such as bilirubin encephalopathy or hypoxic-ischemic encephalopathy, were excluded from this study.

As depicted in Figure 1, information on 871 individuals with s-NDDs from Children's Medical Center of Sun Yat-sen Memorial Hospital (SYSMH) from September 1, 2016, to December 31, 2022, were retrospectively analyzed and used as the training cohort enrollment. After carrying out a series of phenotypic data, informed consents of genetic tests, and routine genetic (G-band karyotyping and fragile-X analysis) results screenings to exclude ineligible cases, including s-NDDs patients from out-patient department with unclear/incomplete clinical information (excluding 346 subjects), patients' parents/guardians who refused to undergo genetic tests or declined to use their genetic reports for publication due to the financial or other personal issues (excluding 239 subjects), and patients who harbored apparent chromosomal disorders, such as fragile-X syndrome and Down syndrome, for which it was not appropriate to use trio-WES as their diagnostic strategy (excluding 21 cases). Finally, 265 patients with unexplained s-NDDs and trio-WES test results were included in this retrospective study and served as the training set. Meanwhile, 92 patients with s-NDDs from the Shenshan Medical

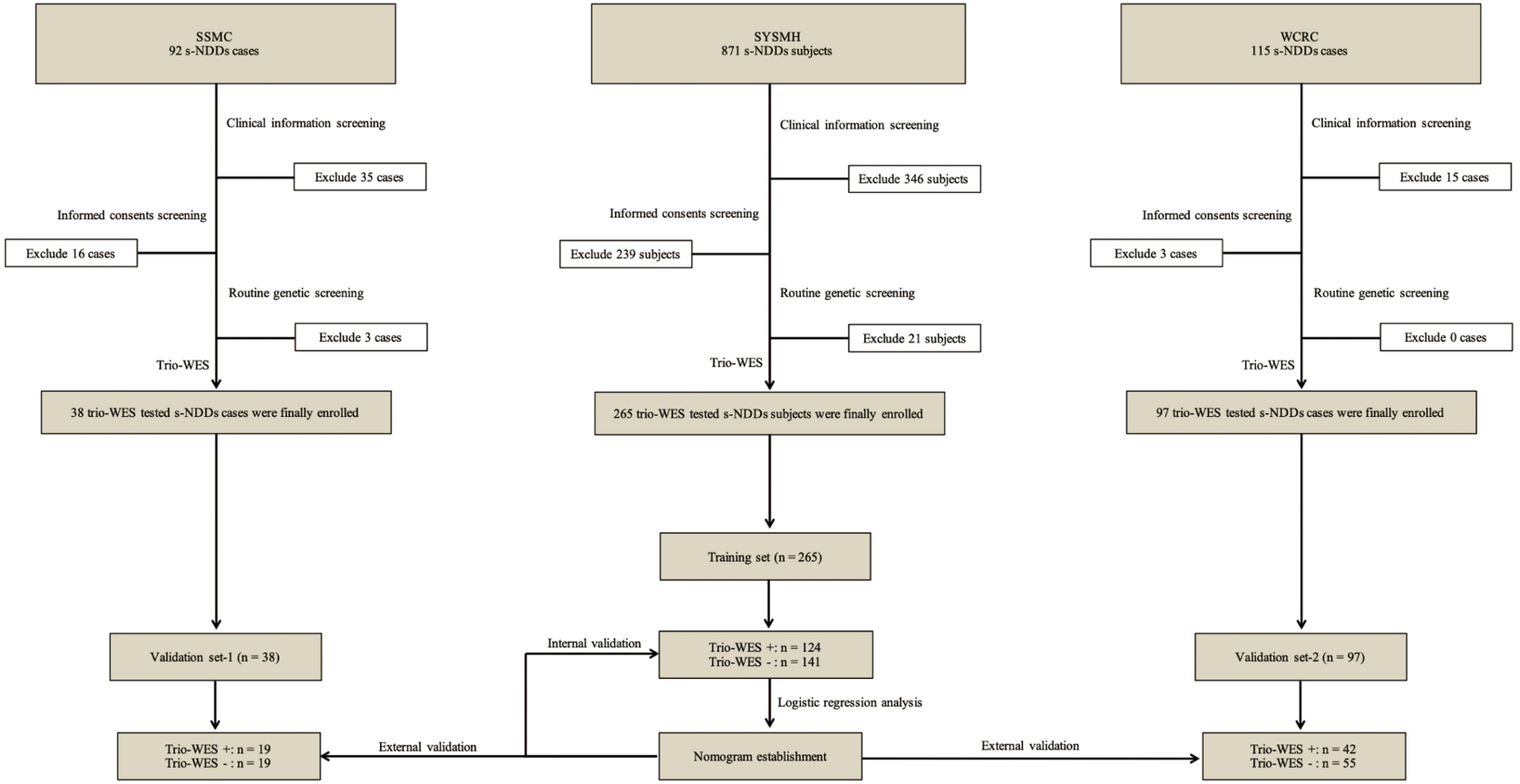

Figure 1 Flowchart of this retrospective multi-center study. "±" means enrolled subjects obtained a positive genetic results or a negative genetic results.
Abbreviations: s-NDDs, syndromic neurodevelopmental disorders; trio-WES, trio-based whole-exome sequencing; SYSMH, Sun Yat-Sen Memorial Hospital; SSMC, Shenshan Medical Center; GDD/ID, WCRC, Weierkang Children's Rehabilitation Center; global developmental delay/intellectual disability.

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Center (SSMC) on July 1, 2024, and July 31, 2025, were retrospectively analyzed and used as an external validation cohort enrollment. After performing a series of screenings (same as the screenings in the training set), 38 trio-WES-tested children with unexplained s-NDDs were included in this retrospective study and served as the validation set-1 in the current study. Moreover, 115 subjects with s-NDDs from the Weierkang Children's Rehabilitation Center (WCRC), a specialized children's neurorehabilitation clinic focusing on diagnosis and rehabilitation of pediatric neurodevelopmental disorders, from January 1, 2023, to December 31, 2023. After carrying out the same process of screenings, 97s-NDDs children with trio-WES test results were enrolled and served as the validation set-2 in the current study.

Ethical Compliance

This retrospective study was approved by the Ethical Committee of Sun Yat-sen Memorial Hospital (Approval Number: SYSKY-2025-244-01) and Shenshan Medical Center (Approval Number: 2025-SSKY-111-01). Written informed consent for genetic testing and the publication of related anonymous genetic reports were obtained from the parents/guardians of all 400 participants.

Definition of the Outcome Indicator

The binary outcome indicator in this study was the genetic results obtained via trio-WES in participants with s-NDDs, and the definition of trio-WES detected exon-level variants (including SNVs and CNVs) was based on the American College of Medical Genetics (ACMG) guidelines. Specifically, the pathogenicity of detected SNVs was assessed according to the 2015 ACMG guideline,[15] and these SNVs were accordingly divided into "pathogenic/likely pathogenic SNVs" and "benign/uncertain significance SNVs". The pathogenicity of the identified CNVs was evaluated according to the 2019 ACMG guideline for CNVs,[16] and these CNVs were also divided into "pathogenic/likely pathogenic CNVs" and "benign/uncertain significance CNVs" accordingly. Based on their pathogenicity evaluations, the participants in the current study were divided into s-NDDs patients with a positive trio-WES result (+, having pathogenic/likely pathogenic SNVs or CNVs from their genetic reports) and patients with negative trio-WES results (−, having benign/uncertain significance SNVs or CNVs from their genetic reports).

Interpretation of the Candidate Predictors

Candidate predictors in this study were retrospectively collected from patients' medical records in the hospital information system. This included (a) Baseline demographic characteristics such as *sex, admission date*, and *onset age*. (b) Phenotypic predictors: 1) GDD/ID severity evaluated via ICD-11: a participant under 5-years-old who had the developmental quotient (DQ) scores <35 points can be regarded to have *severe-profound GDD*, otherwise, this subject with DQ scores ranging from 35 to 75 points was regarded to have *mild-moderate GDD*. A child older than (≥) 5-years-old who presented the intelligence quotient (IQ) scores <40 points can be considered to have *severe-profound ID*, otherwise, this child with IQ scores ranging from 40 to 70 points was considered to have *mild-moderate ID*. 2) Comorbidity condition defined as follows: *single*, an s-NDDs participant coexisting only with one type of neurodevelopmental comorbidities, including ASD, ADHD, and EP; *multiple*, an s-NDDs participant coexisting with two or more types of neurodevelopmental comorbidities (ASD, ADHD, and EP). 3) ASD: *yes* or *no*. 4) ADHD: *yes* or *no*. 5) EP: *yes* or *no*. 6) HCA: *yes* (a participant exhibiting microcephaly or macrocephaly) or *no* (a participant has normal head phenotype). 7) BM detected by cranial magnetic resonance imaging (c-MRI): *yes* (a participant with pathological brain structure/parenchyma malformation, mainly including cerebral white matter changes, basal ganglia lesions, and dysplasia of the corpus callosum, revealed by c-MRI) or *no* (a participant with normal brain structure detected via c-MRI). 8) Hearing impairment and ear anomaly diagnosed by auditory brainstem responses (ABR) and Human Phenotype Ontology (HPO):[17] *yes* (a participant with hearing impairment diagnosed via the ABR and/or ear malformation according to the HPO) or *no* (a participant with normal hearing and normal ear phenotype). 9) Visual impairment and ocular malformation diagnosed by visual evoked potentials (VEP) and HPO: *yes* (a participant with visual impairment detected via the VEP and/or ocular malformation on the basis of the HPO) or no (a participant showing normal visual function with normal ocular phenotype).

Nomogram Establishment and Validation of Model Performance

Univariate and multivariate logistic regression with collinearity diagnostic analysis (variance inflation factor [VIF] < 5 and tolerance > 0.2) was used to screen independent predictive factors ($P < 0.05$) in the training cohort. A nomogram prediction model was constructed based on the regression coefficients of the included independent predictive factors and was visualized using the R package.

For the internal assessment of model performance in the training cohort, the stability of the nomogram was evaluated using a calibration curve with the Hosmer–Lemeshow (H-L) test. The H-L test ($P > 0.05$) indicated a good fit between the model-predicted calculation and the observed data. The receiver operating characteristic (ROC) curve with the area under the curve (AUC) was used to evaluate the discriminative ability of the model, with a value exceeding 0.7 indicating good discriminative ability. The clinical application value of the model was evaluated using a clinical impact curve (CIC) and a decision curve analysis (DCA). Moreover, we also used the 10-fold cross and the 1000-times bootstrap resampling validation methods to evaluate the reliability of the model, with a concordance index (C-index) exceeding 0.7 from both methods, revealing good reliability of the nomogram.

For external evaluation of model performance, the optimal cutoff value of the nomogram-total point was first calculated based on the maximal Youden index value of the training cohort. Then, according to this optimal cut-off value, all subjects in the external validation cohort were transformed into cases with "nomogram-predicted positive genetic results" or "nomogram-predicted negative genetic results". Finally, ROC, calibration curve, and DCA/CIC were used to evaluate the performance of the nomogram model in the transformed external cohort. We also calculated the model's sensitivity, specificity, accuracy, precision, and F1 score in the training and external validation sets to assess the predictive power of this nomogram comprehensively.

Statistical Analysis

In the current study, count data were expressed as the number of cases (%), and the χ^2 test was used for comparisons between groups. For quantitative data, a normal distribution test was performed first. The data conformed to a normal distribution and were expressed as mean ± standard deviation (SD), and the *t*-test was used for comparison between two groups. All data analyses and result visualizations were performed in the R environment (version 4.3.1). Following previous works used R packages for statistical analyses and visualization,[18–20] the R packages used in this study are mainly from Bioconductor (https://bioconductor.org). $P < 0.05$ was considered statistically significant.

Results

Comparisons of Clinical Features Between Training and Validation Sets

In this multicenter retrospective study, 265s-NDDs cases from SYSMH were enrolled in the training cohort to establish the nomogram model. 38 and 97s-NDDs subjects from SSMC and WCRC were enrolled as the two independent validation cohorts to verify the generalization performance of the established model. In the training set, the global diagnostic yield of trio-WES was 46.79%, while the global diagnostic rate of trio-WES in the validation set was 50.00%. Comparisons of the baseline demographics and phenotypic information between the three cohorts are presented in Table 1. The raw data of training set, external validation set-1 and external validation set-2 can be found in the Supplementary Table 1–3, respectively.

Independent Predictive Indicators Selection

A univariate Logistic regression analysis was performed to screen for potential diagnosis-related indicators. Of the 265 subjects in the training cohort, 124 were genetically diagnosed using the trio-WES test. As shown in Table 2, compared with patients with negative trio-WES outcomes, patients with positive trio-WES outcomes appeared to have severe-profound GDD/ID, multiple neurodevelopmental comorbidities, ASD, HCA, and BM more often (all $P < 0.05$), which indicated that the five variables may have potential positive associations with a high possibility of receiving a genetic diagnosis via trio-WES.

Table 1 Comparison of Baseline Clinical Features Between Training and the Two External Validation Cohorts

Indicators or Variables		Training Cohort	External Validation Cohort-1	*t*/χ2 value	*P*-value*	External Validation Cohort-2	*t*/χ2 Value	*P*-Value**
Demographic parameters								
Case number (n)		265	38			97		
Sex [n (%)]	Female	83 (31.3%)	17 (44.7%)	2.705	0.100	28 (28.9%)	0.201	0.654
	Male	182 (68.7%)	21 (55.3%)			69 (71.1%)		
Admission Periods (MM/YY ~ MM/YY)		09/2016 ~ 12/2022	07/2022 ~ 07/2025			01/2023 ~ 12/2023		
Onset age [Mean ± SD/(y)]		4.48 ± 3.74	5.30 ± 4.53	1.070	0.290	4.92 ± 2.97	1.234	0.218
Patient Sources		SYSMH	SSMC			WCRC		
sNDDs-related manifestation variables								
GDD/ID severity [n (%)]	Mild-moderate	156 (58.9%)	19 (50.0%)	1.071	0.301	63 (64.9%)	1.099	0.295
	Severe-profound	109 (41.1%)	19 (50.0%)			34 (35.1%)		
Comorbidity-ASD [n (%)]	Yes	168 (63.4%)	16 (42.1%)	6.317	0.012	58 (59.8%)	0.393	0.531
	No	97 (36.6%)	22 (57.9%)			39 (40.2%)		
Comorbidity-ADHD [n (%)]	Yes	65 (24.5%)	9 (23.7%)	0.013	0.910	33 (34.0%)	3.241	0.072
	No	200 (75.5%)	29 (76.3%)			64 (66.0%)		
Comorbidity-EP [n (%)]	Yes	104 (39.3%)	24 (63.2%)	7.789	0.005	35 (36.1%)	0.300	0.584
	No	161 (60.7%)	14 (36.8%)			62 (63.9%)		
Comorbidity condition [n (%)]	Single	210 (79.3%)	27 (71.0%)	1.309	0.253	69 (71.1%)	2.644	0.104
	Multiple	55 (20.7%)	11 (29.0%)			28 (28.9%)		
sNDDs-related malformation/malfunction variables								
HCA [n (%)]	Yes	77 (29.1%)	14 (36.8%)	0.959	0.328	27 (27.8%)	0.052	0.820
	No	188 (70.9%)	24 (63.2%)			70 (72.2%)		
BM [n (%)]	Yes	47 (17.7%)	14 (36.8%)	7.545	0.006	31 (32.0%)	8.497	0.004
	No	218 (82.3%)	24 (63.2%)			66 (68.0%)		
Hearing impairment/ear anomaly [n (%)]	Yes	28 (10.6%)	4 (10.5%)	0.000	1.000	9 (9.3%)	0.128	0.720
	No	237 (89.4%)	34 (89.5%)			88 (90.7%)		
Visual impairment/ocular malformation [n (%)]	Yes	10 (3.8%)	0 (0%)	0.536	0.464	10 (10.3%)	5.811	0.016
	No	255 (96.2%)	38 (100%)			87 (89.7%)		
Trio-WES outcomes								
Genetic results [n (%)]	Positive	124 (46.8%)	19 (50.0%)	0.137	0.711	42 (43.3%)	0.349	0.555
	Negative	141 (53.2%)	19 (50.0%)			55 (56.7%)		

Notes: *, training cohort vs. external validation cohort-1; **, training cohort vs. external validation cohort-2.

Abbreviations: s-NDDs, syndromic neurodevelopmental disorders; trio-WES, trio-based whole exome sequencing; SYSMH, Sun Yat-Sen Memorial Hospital; SSMC, Shen-shan Medical Center; WCRC, Weierkang Children's Rehabilitation Center; GDD/ID, global developmental delay/intellectual disability; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; EP, epilepsy; HCA, head circumference abnormality; BM, brain malformation.

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Table 2 Independent Variables Selection for Constructing a Nomogram of Predicting Genetic Results of Trio-WES in Diagnosing Cases with s-NDDs in Training Cohort Using Logistic Regression Analysis

Univariate Logistic Analysis			Multivariate Logistic Analysis			Final Incorporated Variables		
Candidate Indicators	**OR (95% CI)**	***P value***	**Candidate Indicators**	**OR (95% CI)**	***P value***	**Candidate Indicators**	**OR (95% CI)**	***P value***
Onset age	1.028 (0.964–1.097)	0.396						
GDD/ID severity	11.611 (6.451–20.897)	<0.001	GDD/ID severity	7.119 (3.747–13.526)	<0.001	GDD/ID severity	7.683 (4.087–14.439)	<0.001
Comorbidity-ASD	2.138 (1.277–3.581)	0.004	Comorbidity-ASD	1.799 (0.936–3.460)	0.078			
Comorbidity-ADHD	1.341 (0.765–2.349)	0.306						
Comorbidity-EP	0.844 (0.514–1.385)	0.502						
Comorbidity condition	2.906 (1.553–5.438)	0.001	Comorbidity condition	2.559 (1.198–5.468)	0.015	Comorbidity condition	2.897 (1.383–6.071)	0.005
HCA	7.564 (4.036–14.179)	<0.001	HCA	3.678 (1.780–7.602)	<0.001	HCA	3.893 (1.905–7.958)	<0.001
BM	3.720 (1.859–7.443)	<0.001	BM	2.202 (0.955–5.076)	0.064			
Hearing impairment/ear anomaly	1.593 (0.722–3.512)	0.249						
Visual impairment/ocular malformation	4.793 (0.998–23.015)	0.05						

Abbreviations: trio-WES, trio-based whole exome sequencing; s-NDDs, syndromic neurodevelopmental disorders; GDD/ID, global developmental delay/intellectual disability; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; EP, epilepsy; HCA, head circumference abnormality; BM, brain malformation; OR (95% CI), odds ratio (95% confidence interval).

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Following the univariate logistic-based selection, those five phenotypic indicators were incorporated into the multivariable logistic regression model. As shown in Table 2, GDD/ID severity (mild to moderate vs. severe to profound), comorbidity condition (single vs. multiple), and HCA (yes vs. no) were strong independent predictors of a positive trio-WES diagnosis (all $P < 0.05$). In contrast, ASD and BM were not independently associated with positive trio-WES diagnosis (both $P > 0.05$). Finally, we excluded ASD and BM from our multivariable logistic regression model for model adjustment, and the results showed that the three final-included variables (*GDD/ID severity*, OR 7.683, 95% CI 4.087–14.439, $P < 0.001$; *comorbidity condition*, OR 2.897, 95% CI 1.383–6.071, $P = 0.005$; *HCA*, OR 3.893, 95% CI 1.905–7.958, $P < 0.001$) were all independently associated with positive trio-WES outcomes in children with s-NDDs (Table 2).

Moreover, the three final-incorporated predictors were further analyzed using a collinearity diagnosis to exclude potential interdependencies among them. As shown in Table 3, the tolerances of the three predictors (ranging from 0.792 to 0.802) were all >0.2, while their VIF values (ranging from 1.015 to 1.263) were all <5, indicating that there were no significant interdependencies among the three phenotypic predictors.

Finally, a regression model was established to predict the genetic outcomes of trio-WES for diagnosing children with s-NDDs. The constructed model formula was as follows: logit (P) = 2.039 × *GDD/ID severity* + 1.064 × *comorbidity condition* + 1.359 × *HCA* − 1.556. The full regression coefficients (such as β, SE, and Wald) of the model are listed in Table 4.

Nomogram Construction and Evaluation of Model Discriminatory Performance

A prediction nomogram model (Figure 2A) and its related points system ("Nomogram points" column in Table 4) was visualized using R packages ("rms" and "rmda") based on three independent phenotypic indicators (GDD/ID severity, comorbidity condition, and HCA) identified by multivariate logistic regression analysis. For each predictor, a point is calculated by drawing a vertical line from the corresponding answer to the point bar at the top of the diagram. The points for all predictors were aggregated into total points. The total point was subsequently converted into the probability (*p)* of obtaining a trio-WES diagnosis by drawing a vertical line upward from the total point bar to the probability bar. The nomogram plot provided an estimate of the

Table 3 The Collinearity Diagnostic Analysis of Incorporated Variables for Predicting Genetic Results of Trio-WES in Diagnosing Cases with s-NDDs in Training Cohort

Incorporated Variables	Tolerance	VIF
GDD/ID severity	0.792	1.263
Comorbidity condition	0.985	1.015
HCA	0.802	1.247

Abbreviations: trio-WES, trio-based whole exome sequencing; s-NDDs, syndromic neurodevelopmental disorders; GDD/ID: global developmental delay/intellectual disability; HCA, head circumference abnormality; VIF, variance inflation factor.

Table 4 Coefficients of Established Model for Prediction of Obtaining Genetic Diagnosis via Trio-WES in Individuals with s-NDDs in Training Cohort

Incorporated Variables	B	S.E.	Wald	P value	Nomogram Points
GDD/ID severity	2.039 (β1)	0.322	40.108	< 0.001	100*
Comorbidity condition	1.064 (β2)	0.377	7.942	0.005	52 (100 × β2÷β1)
HCA	1.359 (β3)	0.365	13.885	< 0.001	67 (100 × β3÷β1)

Note: *, The variable showing the max β value and being set 100-point as the reference-point for other included variables.

Abbreviations: trio-WES, trio-based whole exome sequencing; s-NDDs, syndromic neurodevelopmental disorders; GDD/ID, global developmental delay/intellectual disability; HCA, head circumference abnormality; B, β value; S.E., standard error.

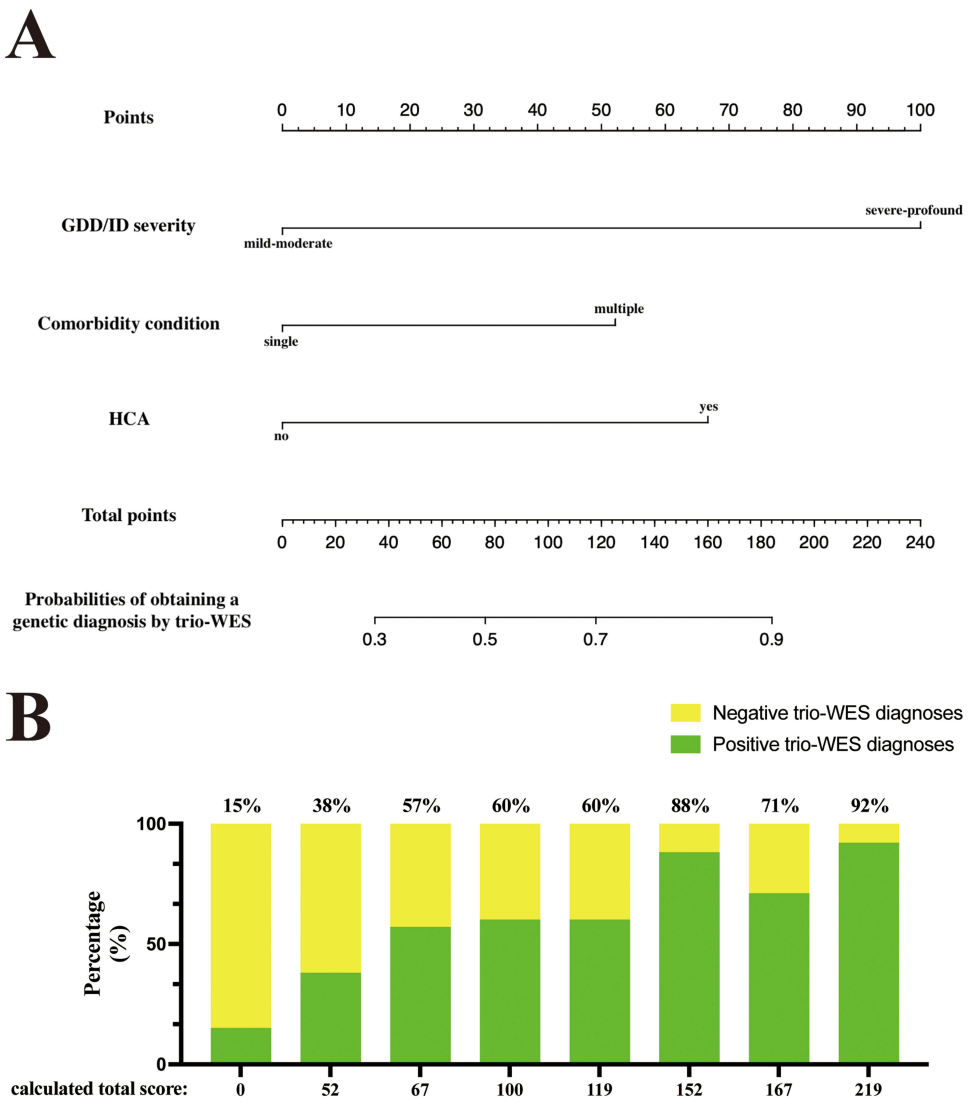

Figure 2 Logistic regression model visualized by the nomogram and the evaluation of the nomogram scoring system across 265 individuals in the training set. (**A**) There are two parts in the nomogram diagram: the upper part (from "Points" to the last variable, "HCA") showing the calculation of points for each incorporated variables, and the lower part (From "Total points" to "Probabilities of obtaining a genetic diagnosis by trio-WES") showing the probability of getting a genetic result via trio-WES testing. (**B**) Bar charts showing the percentage distributions of individuals having positive genetic diagnosis at each score in training cohort.
Abbreviations: GDD/ID, global developmental delay/intellectual disability; HCA, head circumference abnormality; trio-WES, trio-based whole-exome sequencing.

probability of obtaining positive trio-WES outcomes via the point contributions of the three independent predictors and can assist decision-making in using trio-WES in the initial diagnostic strategy of an s-NDDs individual.

According to the aggregate points across all subjects in the training set, these cases could be classified into different subgroups. As shown in Figure 2B, the bar chart revealing the percentage of subjects who had positive trio-WES outcomes in subgroups with different aggregate points across the 265 cases in the training cohort showed that a higher aggregate point was consistent with a higher percentage of subjects with a positive trio-WES result, indicating the robust discriminatory power of the nomogram scoring system we constructed.

Internal Evaluation and Validation of the Nomogram Performance

As indicated in Figure 3A, the calibration curve with the H-L test demonstrated that both the nomogram predictions from one-time calculation (apparent line) and 1000-times bootstrap-resampling (bias-corrected line) were closely aligned with the diagonal line in the training set with $\chi^2 = 1.175$, df = 4, and P value = 0.882 from the H-L test, indicating excellent consistency between the predicted probabilities and the actual observed values. As shown in Figure 3B, ROC plots

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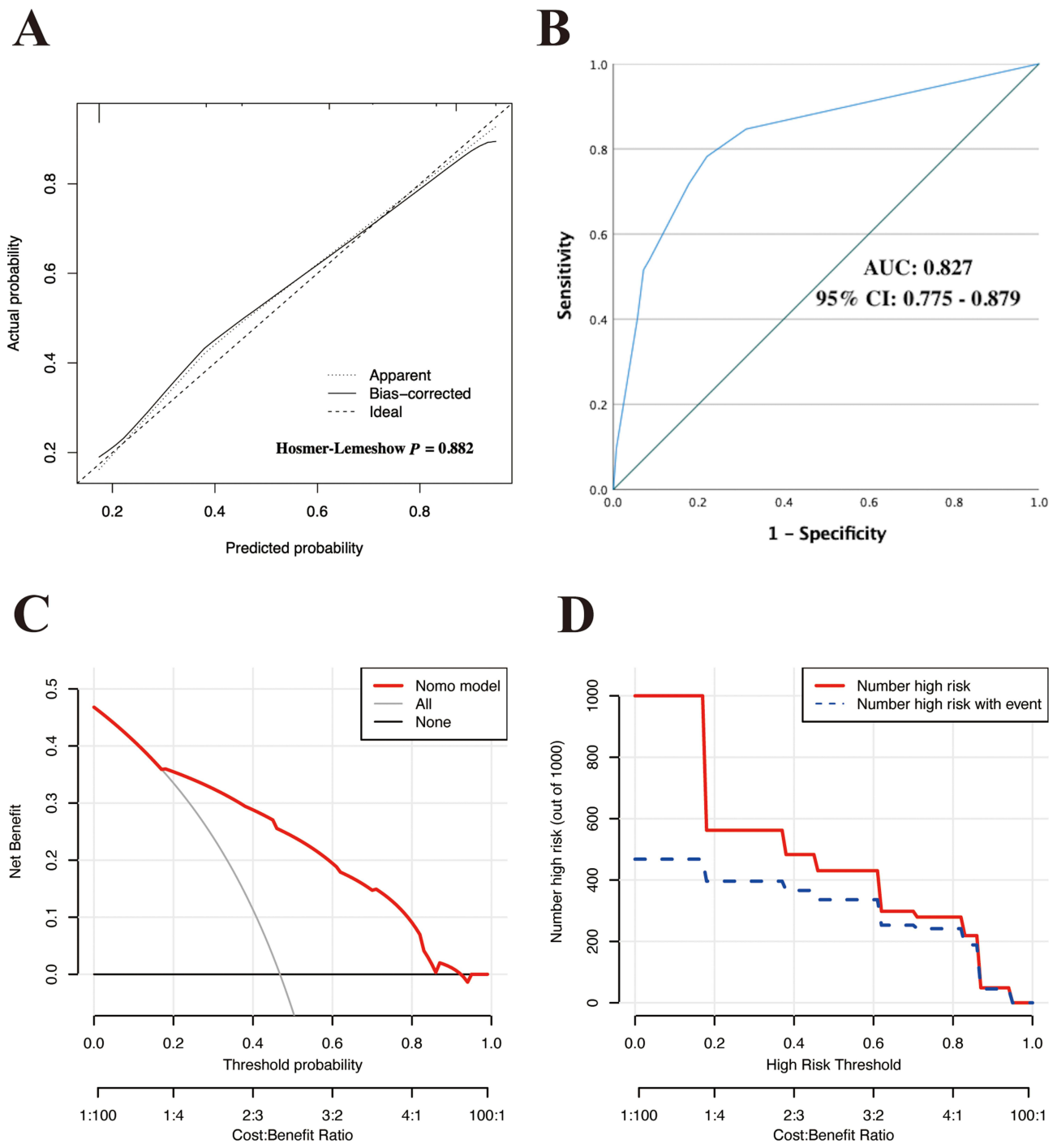

Figure 3 Discriminatory performance of the nomogram in the training cohort. (**A**) Calibration plot. (**B**) ROC curve. (**C**) DCA and (**D**) CIC for assessment of the predicted clinical utility and impact of the nomogram in s-NDDs individuals of the training set.

Abbreviations: AUC, area under the ROC curves; 95% CI, 95% confidence interval; ROC, receiver operating characteristic; DCA, decision curve analysis; CIC, clinical impact curve; s-NDDs, syndromic neurodevelopmental disorders.

showed an AUC of 0.827 (95% CI: 0.775–0.879), indicating that the model had good predictive ability in the training cohort. Meanwhile, according to the optimal cutoff point that corresponded to the highest values of sensitivity and specificity in the ROC plot in the training cohort, the maximal Youden index was calculated to be 0.562, and its corresponding cutoff total point in the nomogram scoring system was 52, which generated a confusion matrix with the optimal values of sensitivity, specificity, accuracy, precision, and F1 score being 78.23%, 78.01%, 78.11%, 75.78%, and

Table 5 Predictive Parameters of the Established Phenotype-Driven Model in Training and Validation Cohorts

Predictive Parameters	Training Cohort	Validation Cohort-1	Validation Cohort-2
Sensitivity (%)	78.23%	88.89%	83.33%
Specificity (%)	78.01%	80.00%	85.45%
Accuracy (%)	78.11%	84.21%	84.54%
Precision (%)	75.78%	80.00%	81.40%
F1 score	0.77	0.84	0.82

0.77, respectively, in the training set (Table 5). These results further revealed that the nomogram model had a robust discriminatory ability to predict the genetic results of trio-WES in diagnosing patients with s-NDDs. Moreover, within a relevant threshold range of trio-WES diagnostic rate (approximately 0.18 ~ 0.50), DCA and CIC analyses demonstrated that using this nomogram for cases from the training cohort could obtain a greater net benefit compared with treat-none and treat-all diagnostic strategies. When the diagnostic rate threshold <0.18, using this model could also obtain a greater net benefit compared with treat-none diagnostic strategy and equal to the treat-all diagnostic strategy (Figure 3C and D), indicating that the predictive model had promising clinical application.

Finally, 10-fold-cross and 1000-times bootstrap-resampling analysis was used to internally verify the performance stability of the nomogram in the training set. As shown in Figure 4, the C-index values were 0.824 (95% CI: 0.757–0.879) and 0.821 (95% CI: 0.757–0.886), based on 10-fold-cross validation (Figure 4A) and 1000-times bootstrap validation (Figure 4B), suggesting that the predictive model had excellent stability and did not overfit.

External Evaluation of the Model's Generalization Performance

According to the optimal cutoff total point (52) calculated in the training set, the 39s-NDDs cases in the external validation cohort-1 from SSMC could be transformed into two subgroups (model-predicted positive trio-WES subgroup: 20 subjects and model-predicted negative trio-WE subgroup: 18 subjects). As illustrated in Figure 5A, the calibration curve with the H-L test showing both the model predictions from one-time calculation (apparent line) and 1000-times bootstrap-resampling (Bias-corrected line) were in good agreement with the diagonal line in the transformed external cohort with $\chi 2 = 1.047$, df = 4, and *P* value = 0.903. Additionally, the ROC plots (Figure 5B) revealed an AUC value of 0.953 (95% CI: 0.881–0.998). DCA and CIC analysis (Figure 5C and D) showed significant net benefits could be obtained in the transformed external cohort-1 using the nomogram within the threshold range of trio-WES diagnostic rate (approximately 0.10~0.50). Finally, based on the

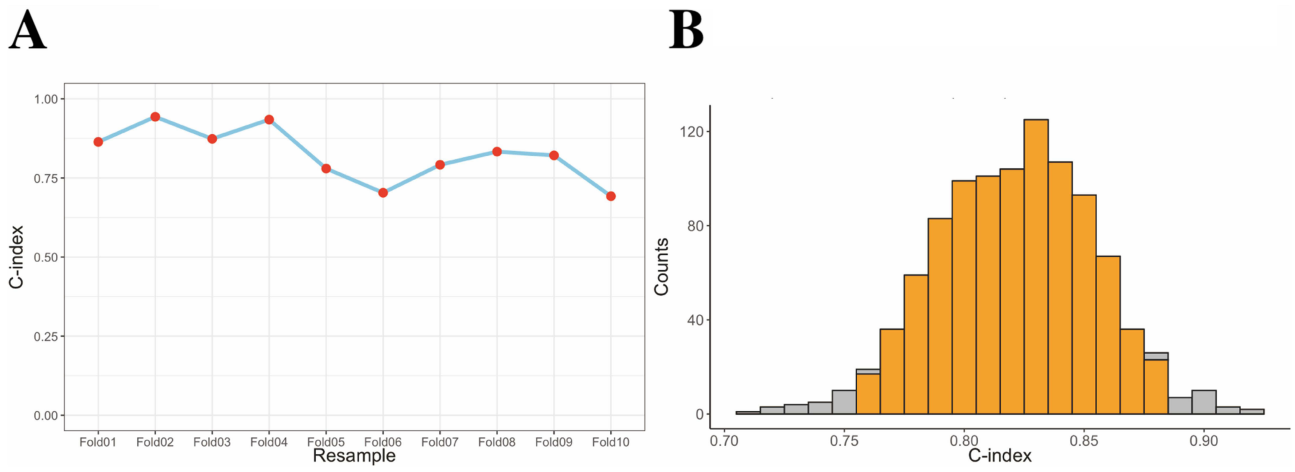

Figure 4 Internal verification of the robustness performance of the model across the training set. (**A**) 10-fold-cross validation revealing the model had excellent stable consistence with C-index (95% CI) being 0.824 (0.757–0.886). (**B**) 1000-time resampling bootstrap validation further verifying the model had good stability with C-index (95% CI) being 0.821 (0.757–0.886).
Abbreviations: C-index, concordance index; 95% CI, 95% confidence interval.

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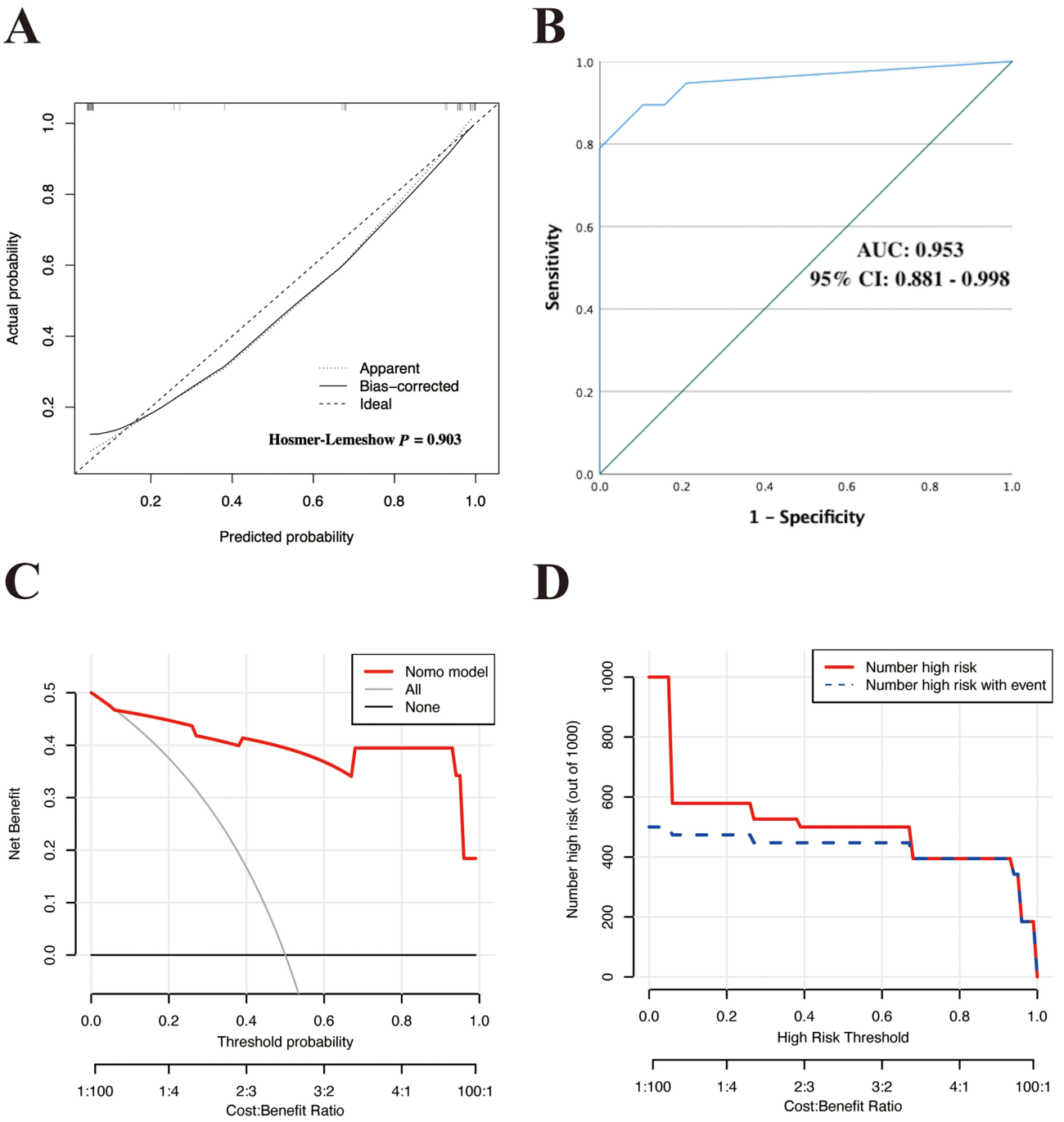

Figure 5 Discriminatory performance of the nomogram in the external validation cohort-1. (**A**) Calibration plot. (**B**) ROC curve. (**C**) DCA and (**D**) CIC for assessment of the predicted clinical utility and impact of the nomogram in s-NDDs individuals of an independent validation set.

Abbreviations: AUC, area under the ROC curves; 95% CI, 95% confidence interval; ROC, receiver operating characteristic; DCA, decision curve analysis; CIC, clinical impact curve; s-NDDs, syndromic neurodevelopmental disorders.

transformed data in the external cohort-1, the confusion matrix analysis showed that the sensitivity, specificity, accuracy, precision, and F1 score of the nomogram in the external validation cohort-1 were 88.89%, 80.00%, 84.21%, 80.00%, and 0.84, respectively (Table 5). Similarly, the calibration curve with the H-L test (χ^2: 1.399, df: 4, and *P* value: 0.844), ROC curve (AUC = 0.910 with 95% CI = 0.843–0.978) and DCA/CIC (obtaining a greater net benefit compared with treat-none and treat-all diagnostic strategies within the diagnostic rate threshold range of 0.10~0.50, approximately) were also performed in the external validation set-2 (Figure 6), and a confusion matrix analysis revealed the sensitivity, specificity, accuracy, precision,

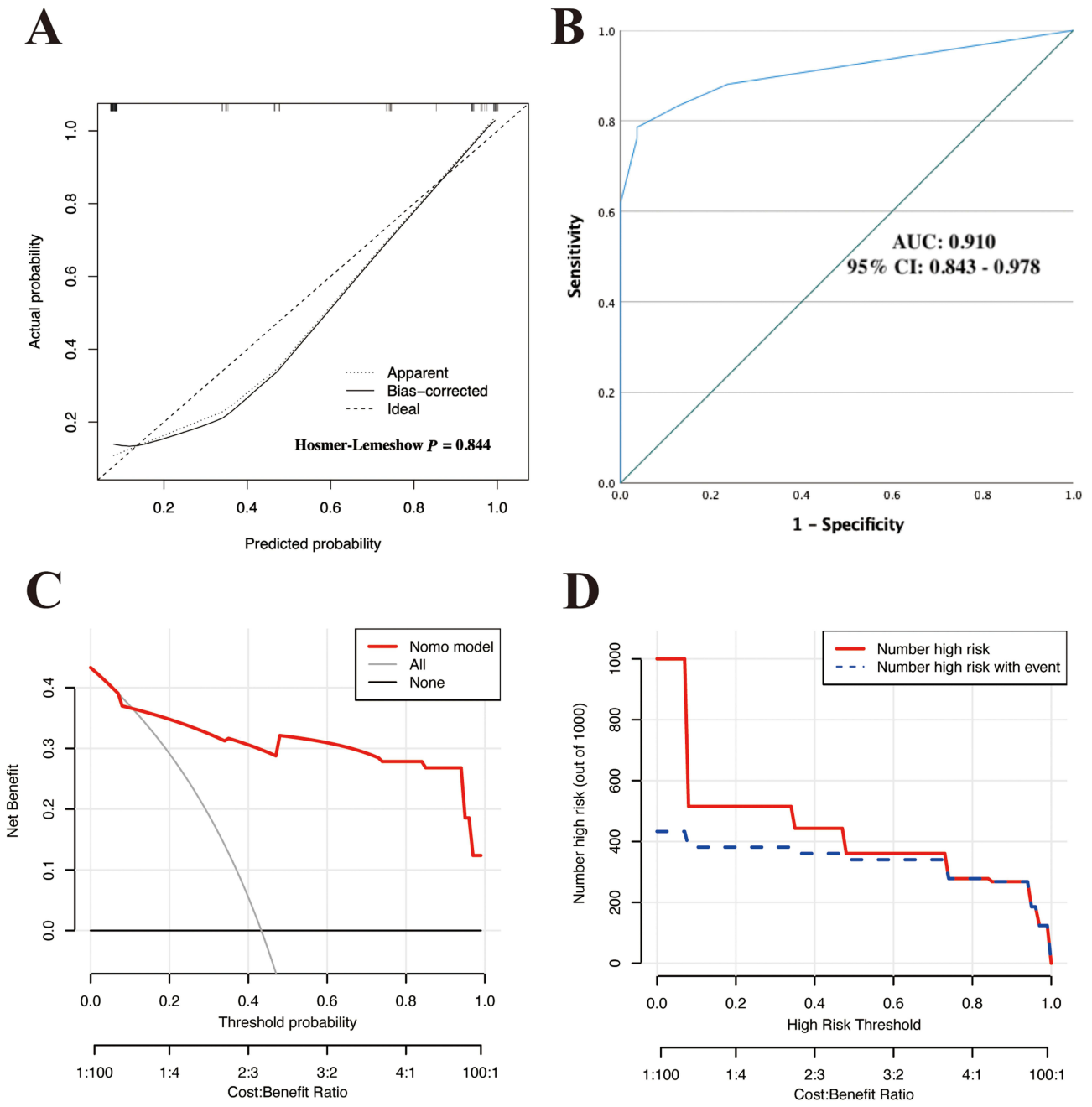

Figure 6 Discriminatory ability of the model in the external validation cohort-2. (**A**) Calibration plot. (**B**) ROC curve. (**C**) DCA and (**D**) CIC for evaluation of the predicted clinical utility and impact of the model in s-NDDs patients of another external validation cohort.
Abbreviations: AUC, area under the ROC curves; 95% CI, 95% confidence interval; ROC, receiver operating characteristic; DCA, decision curve analysis; CIC, clinical impact curve; s-NDDs, syndromic neurodevelopmental disorders.

and F1 score of the model in the external validation cohort-2 were 83.33%, 85.45%, 84.54%, 81.40% and 0.82, respectively. All these results from two independent external cohorts confirmed that the nomogram model exhibited good generalization ability and stable repeatability in other external sets.

Discussion

With the rapid popularization of next-generation sequencing technology in clinical practice, genetic components are being found more often than before in many individuals with idiopathic syndrome involving developmental abnormalities, including intellectual disability.[21] The diagnostic patterns of clinical genetics has been obviously changed since the

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popularization of trio-WES; trio-WES can bring us an efficient way to obtain a genetic diagnosis for many idiopathic disorders, preventing "diagnostic odysseys" experienced for many affected individuals and their parents.[22,23] However, we should be aware of the technical limitations of trio-WES (exon-level sequencing only), along with the complexity of the human genomic abnormalities (like deep intronic or non-coding variants) might inevitably hinder the diagnostic power of this technology. Whole genome sequencing (WGS) might definitely provide advantages over trio-WES via detecting both variants at exon-level and non-exon-level. However, due to its high-priced screening and more intense work requirement, the popularization of WGS in clinical practice is currently restricted in many developing countries, including China. Nowadays, trio-WES technology still be considered as the first-tier genetic diagnosis approach in the world.[24] Thus, it is insightful to develop effective tool to assist the decision-making of using trio-WES in diagnostic strategy of idiopathic syndrome, like s-NDDs.

In this study, by carefully and objectively collecting the phenotypic data that can potentially reflect neurodevelopmental abnormalities in children with s-NDDs, the global diagnostic yield of trio-WES in s-NDDs patients from the training and external validation cohort was 46.79% and 50.00%, respectively, which were both higher than those recorded in previous studies of individuals with GDD/ID alone with trio-WES detection (27% ~39%).[25,26] Our findings emphasize the importance of key phenotypic factors enrichment in enhancing diagnostic rate of using trio-WES, and reinforce the previous concept of phenotype-driven strategy[27] in genetic diagnosis, which is the core principle of the "DeepRare", an AI system powered by large language models accepted by *Nature* in 2025.[28] We therefore speculate on the possibility of developing a model for predicting the probability of positive trio-WES results by enriching core phenotypic signifiers that can well-reflect neurodevelopmental conditions in s-NDDs patients.

Among the three phenotypic indicators (GDD/ID severity, Comorbidity condition, and HCA) identified in current work, the strongest association with positive genetic results of trio-WES in s-NDDs patients was severe-profound GDD/ID (OR: 7.683, 95% CI: 4.087–14.439), followed by the existence of HCA (OR: 3.893, 95% CI: 1.905–7.958) and multiple comorbidities (OR: 2.897, 95% CI: 1.383–6.071). These findings indicated that severe neurodevelopmental delays, craniofacial anomalies and complicated neurodevelopmental comorbidities coexistence can share overlapping genetic backgrounds directly pointing to monogenic neurodevelopmental disorders, thus bringing higher diagnostic yield of trio-WES into patients with these three strong signifiers. As previous studies revealed, alterations in genes related to the functions of gene-expression regulation (mainly including chromatin remodeling, histone modification and DNA methylation) and neuronal communication can cause severe neurodevelopmental delays with a broad spectrum of neurodevelopmental comorbidities, like ASD and ADHD.[29] Meanwhile, in the craniofacial development, the process of crosstalk between neural crest cells and craniofacial ectoderm matrix plays an essential role in craniofacial patterning and morphogenesis, and the formation of neural crest requires complicated epigenetic modifications.[30] Disruptions in genes associated with chromatin remodeling, histone modification, DNA methylation and neuronal communication can severely impair the normal development of neural crest, resulting in a series of craniofacial anomalies, among which HCA is the prominent one.[31] Given these shared gene-functional abnormalities (dysfunctions of gene-expression regulation and neuronal communication) among phenotypes of severe neurodevelopmental delays, craniofacial anomalies and complicated neurodevelopmental comorbidity conditions, it is reasonable to speculate that an s-NDDs patient presenting with more of these phenotypic signifiers can have more probabilities of having genetic background, and thus can obtain a positive genetic result more easily by receiving trio-WES test.

Importantly, by combining the three easily assessed phenotypic signifiers that independently links to the genetic causes in s-NDDs subjects, we successfully constructed and validated a nomogram showing good precision (training set: 75.78%, validation set-1: 80.00%, and validation set-2: 81.40%) and accuracy (training set: 78.11%, validation set-1: 84.21% and validation set-2: 84.54%) in the prediction of genetic results of using trio-WES in s-NDDs patients, which indicates promising clinical applications. However, this work has some limitations. First, due to the lack of established and Chinese version scales for emotional disorders, such as childhood anxiety disorder, and sleep disturbances in young children, we cannot include those conditions or manifestations into candidate phenotypic indicators, which may inevitably bring potential confounding bias into the model, more candidate variables, such as childhood anxiety disorder and sleep disorder, are required in the future researches. Second, with the continue updates of ACMG interpretation frameworks and reference databases or the probands' phenotypic information expanding with age, a very small part of variants that assessed as uncertain significance previously can

be potentially re-assessed as pathogenic/likely pathogenic, which may bring potential measurement bias into the model. However, due to the nature limitations of the retrospective work, we cannot retrospectively change the original trio-WES genetic reports that had already been made at a previous fixed time point. Continuous updates on the variants interpretation frameworks and reference databases and phenotypic information of grow-up patients should be considered in the future studies. Third, due to the sample size for the external validation cohort-1 (n = 38) and external validation cohort-2 (n = 97) are all relatively small, which might affect statistical power of the model and limit its generalizability. Further validation with larger external cohorts is required. Finally, this pilot study was based on a retrospective analysis, leaving the model's performance in a prospective research undetermined.

Conclusion

In summary, GDD/ID severity, comorbidity condition, and HCA are independent phenotypic signifiers linked to positive trio-WES diagnoses in children with s-NDDs. The nomogram model based on them showed good calibration, discrimination, and clinical applicability, providing an accurate and effective tool for clinicians to identify children with s-NDDs who have a high probability of obtaining a genetic diagnosis via trio-WES in a timely manner, and for affected families to estimate their personalized diagnostic probability of receiving trio-WES test at the pre-diagnosis stage. However, it should be noted that due to the established model based on three medical centers with a small number of enrolled cases (training cases: 265; validation cases: 38+97), the statistical power and the robustness of the model is limited. The overall conclusions still require confirmation through studies with larger sample sizes and more complete data. Nonetheless, the current findings can provide a reference for the identification of cases with higher probability of obtaining genetic diagnosis via trio-WES in clinical practice.

Data Sharing Statement

The data supporting this study's findings are presented in this article. Further inquiries can be directed to the corresponding author (Dr. Wenting Tang) via Email with reasonable requests.

Ethics Approval and Consent to Participate

All procedures of the study were performed in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Sun Yat-sen Memorial Hospital (Approval Number: SYSKY-2025-100-01) and the Shenshan Medical Center (Approval Number: 2025-SSKY-111-01), respectively. Written informed consent was obtained from all enrolled subjects' parents or guardians.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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