Online-Only Supplemental Materials

eTable 1. Demographics for ASD-Enriched Samples

Recruitment Population	Single sample recruited from an outpatient genetics clinic and via advertisements for participation in a clinical trial for children with NF1 and ASD.	Simons Simplex Collection[b]
ASD Instrument	SRS, SRS-2	SRS
Sample Size	79	2418
Median Age, years (range)	7.0 (4.0 – 13.0)	8.4 (4.0 – 18.0)
Males (%)	49 (62.0)	2114 (87.4)
Females (%)	30 (38.0)	304 (12.6)
Age < 18 years (%)	79 (100.0)	2417 (>99.9)
Age ≥18 years (%)	0 (0.0)	1 (<0.01)
Sporadic Transmission (%)	17 (21.5)[a]	2418 (100.0)
Familial Transmission (%)	33 (31.8)[a]	0 (0.0)
Mean SRS T-Score (SD)	78.59 (14.51)	79.59 (10.39)
SRS-2 score ≥60 (%)	70 (88.6)	2307 (95.4)
SRS-2 score ≥70 (%)	57 (72.2)	1991 (82.3)
SRS-2 score ≥75 (%)	55 (69.6)	1731 (71.6)

[a]Total does not equal 100% due to presence of unknown values

[b]References: Frazier TW, Hardan AY. Equivalence of symptom dimensions in females and males with autism. *Autism*. 2016; Aug 7 [Epub ahead of print]; Frazier TW, Georgiades S, Bishop SL, Hardan AY. Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. *J Am Acad Child Adolesc Psychiatry*. 2014; 53(3):329-340.

eTable 2. SRS-2 Subscale Scores in Children with NF1; Comparison to Norms and Idiopathic ASD

Parent-Report SRS-2 for Boys (<18 years)	Standardization Sample[a]		NF1 Sample (n=208)			Simons Simplex Collection (n=2114)[b]		
	Raw Mean	SD	T-Score of Mean	Raw Mean	Raw SD	T-Score of Mean	Raw Mean	Raw SD
Social Awareness	5.69	3.18	59.3	8.69	4.22	71.5	12.53	3.63
Social Cognition	6.15	5.38	59.6	11.38	7.84	73.0	18.52	5.62
Social Communication	10.79	9.11	60.0	19.92	13.17	74.3	33.32	9.14
Social Motivation	5.95	4.87	56.9	9.30	6.20	68.7	14.73	5.67
DSM-5 Social Communication and Interaction	28.6	20.2	60.3	49.28	29.19	75.0	79.10	21.41
DSM-5 Repetitive Behaviors and Restricted Interests	5.02	5.67	61.0	11.18	8.12	74.1	18.67	6.76
SRS Total Score	33.6	25.2	60.5	60.46	36.36	78.9	97.77	26.79
Parent-Report SRS-2 for Girls (<18 years)	**Standardization Sample[a]**		**NF1 Sample (n=212)**			**Simons Simplex Collection (n=304)[b]**		
	Raw Mean	**SD**	**T-Score of Mean**	**Raw Mean**	**Raw SD**	**T-Score of Mean**	**Raw Mean**	**Raw SD**
Social Awareness	5.10	3.21	58.1	7.73	3.85	74.2	12.80	3.65
Social Cognition	5.39	5.13	57.3	9.24	6.53	77.3	19.27	5.24
Social Communication	8.98	8.71	57.4	15.49	10.53	79.6	34.59	9.61
Social Motivation	5.39	4.57	55.3	7.82	5.34	71.2	15.21	5.82
DSM-5 Social Communication and Interaction	24.9	19.4	57.8	40.28	23.65	79.0	81.87	20.64
DSM-5 Repetitive Behaviors and Restricted Interests	4.13	4.94	59.0	8.52	6.76	78.8	18.83	7.08
SRS Total Score	29.0	23.7	58.2	48.80	29.31	84.2	100.69	26.49

SD: Standard Deviation

[a]Constantino JN, Gruber CP. Social Responsiveness Scale, Second Edition. Los Angeles, CA: Western Psychological Services; 2012. Mean T-scores for all domains and total scores of the standardization sample are 50.0 with a standard deviation of 10.0.

[b]For comparison purposes, raw score mean and standard deviation with corresponding T-scores of SRS-2 subscales for affected probands from the Simons Simplex Collection (SSC) are included; n=2418. Eigenvalues and highest-loading factors on the first principal component generated from unweighted least squares exploratory factor analysis for the SSC are reported in eTable 5C & 5D.

eTable 3. Score Profile of SRS-2 and Conners Subscales for Individuals for Above-Threshold Quantitative ASD Scores

ASD T-Scores (n=70)	Mean (SD)	Minimum	Maximum
Social Awareness	76.34 (10.71)	55	104
Social Cognition	81.97 (7.87)	65	100
Social Communication	82.04 (9.26)	65	105
Social Motivation	74.93 (10.66)	56	108
Social Communication and Interaction	82.96 (8.15)	73	107
Restricted Interest and Repetitive Behaviors	81.84 (9.14)	62	102
SRS-2 Total	83.69 (7.99)	75	108
ADHD T-Scores (n=36)	**Mean (SD)**	**Minimum**	**Maximum**
Inattentive	77.25 (10.37)	55	90
Hyperactivity	79.03 (11.34)	48	90
ADHD Index	79.41 (9.76)	54	90

SD: Standard Deviation

eTable 4. Exploratory Factor Analysis (Excluding ASD-Enriched NF1 Sample) - Eigenvalues for Extracted Components

Factor		Initial Eigenvalues		Rotation Sum of Squared Loadings
	Total	% of Variance	Cumulative %	
1	20.084	30.898	30.898	15.406
2	3.190	4.908	35.806	10.218
3	2.587	3.980	39.785	12.348
4	1.825	2.808	42.593	10.860
5	1.399	2.153	44.746	10.980
6	1.367	2.103	46.849	5.914
7	1.284	1.976	48.825	10.097
8	1.215	1.869	50.693	8.345
9	1.140	1.755	52.448	5.834
10	1.131	1.740	54.188	6.748
11	1.074	1.652	55.840	12.092
12	1.028	1.581	57.421	3.833
13	1.012	1.556	58.977	1.233

eTable 5. Exploratory Factor Analysis Including ASD-Enriched NF1 Sample and Simons Simplex Collection (SSC)

(A) Eigenvalues for Extracted Components – NF1 Sample

Factor		Initial Eigenvalues		Rotation Sum of Squared Loadings
	Total	% of Variance	Cumulative %	
1	22.274	34.267	34.267	19.067
2	3.021	4.648	38.915	12.682
3	2.457	3.780	42.694	11.501
4	1.809	2.783	45.478	9.169
5	1.361	2.094	47.572	11.847
6	1.292	1.988	49.560	8.327
7	1.193	1.835	51.395	11.203
8	1.162	1.788	53.182	11.759
9	1.089	1.675	54.857	9.068
10	1.031	1.586	56.443	6.889
11	1.021	1.571	58.014	8.696

(B) Item Loadings on First Principal Component – NF1 Sample

Item	Domain	Loading
Doesn't know when others take advantage	SCI	.806
Wanders aimlessly, one activity to another	SCI	.780
Literal, doesn't get the real meaning	SCI	.776
Trouble keeping up with conversations	SCI	.685
Clings to adults, seems too dependent	SCI	.683
Walks between two people who are talking	SCI	.666
Is not physically well coordinated	RRB	.619
Problems understanding cause and effect	SCI	.594
Does extremely well at a few tasks, but not at most	RRB	.591
Talks around the subject	SCI	.507
Is too silly or laughs inappropriately	SCI	.506

SCI: Social Communication and Interaction; RRB: Restricted Interests and Repetitive Behaviors

(C) Eigenvalues for Extracted Components – SSC

Factor		Initial Eigenvalues		Rotation Sum of Squared Loadings
	Total	% of Variance	Cumulative %	
1	13.060	20.093	20.093	5.410
2	3.950	6.077	26.170	6.520
3	2.645	4.070	30.240	6.687
4	2.190	3.369	33.609	6.337
5	1.801	2.770	36.379	7.743
6	1.642	2.526	38.905	7.495
7	1.560	2.400	41.305	7.405
8	1.406	2.163	43.468	3.868
9	1.267	1.949	45.416	2.405
10	1.152	1.772	47.189	5.483
11	1.080	1.661	48.850	2.142

(D) Item Loadings on First Principal Component – SSC

Item	Domain	Loading
Responds appropriately to mood changes in others	SCI	.607
Offers comfort to others when they are sad	SCI	.576
Is aware of what others are thinking and feeling	SCI	.565
Is able to understand the meaning of other people's tone of voice and facial expressions	SCI	.444
Has a sense of humor, understands jokes	SCI	.410
Is able to imitate others' actions	SCI	.404
Recognizes when something is unfair	SCI	.389
Is able to communicate his or her feelings to others	SCI	.381
Is imaginative, good a pretending (without losing touch with reality)	SCI	.349
Focuses his or her attention to where others are looking or listening	SCI	.311
Knows when he or she is talking too loud or making too much noise	SCI	.247

SCI: Social Communication and Interaction; RRB: Restricted Interests and Repetitive Behaviors

eTable 6. Fit Indices for 1-6 Class Mixture Models using Empirically-Derived SRS Factors

Class #	Par	LL	AIC	BIC	saBIC	ΔBIC	Entropy
1-class	20	-1829.61	3699	3788	3724		
2-class	26	-1730.22	3512	3628	3545	-160	0.910
3-class	32	-1673.45	3411	3553	3451	-75	0.896
4-class	38	-1635.51	3347	3515	3395	-37	0.865
5-class	44	-1608.41	3305	3500	3360	-16	0.887
6-class	50	-1602.98	3306	3527	3369	28	0.840

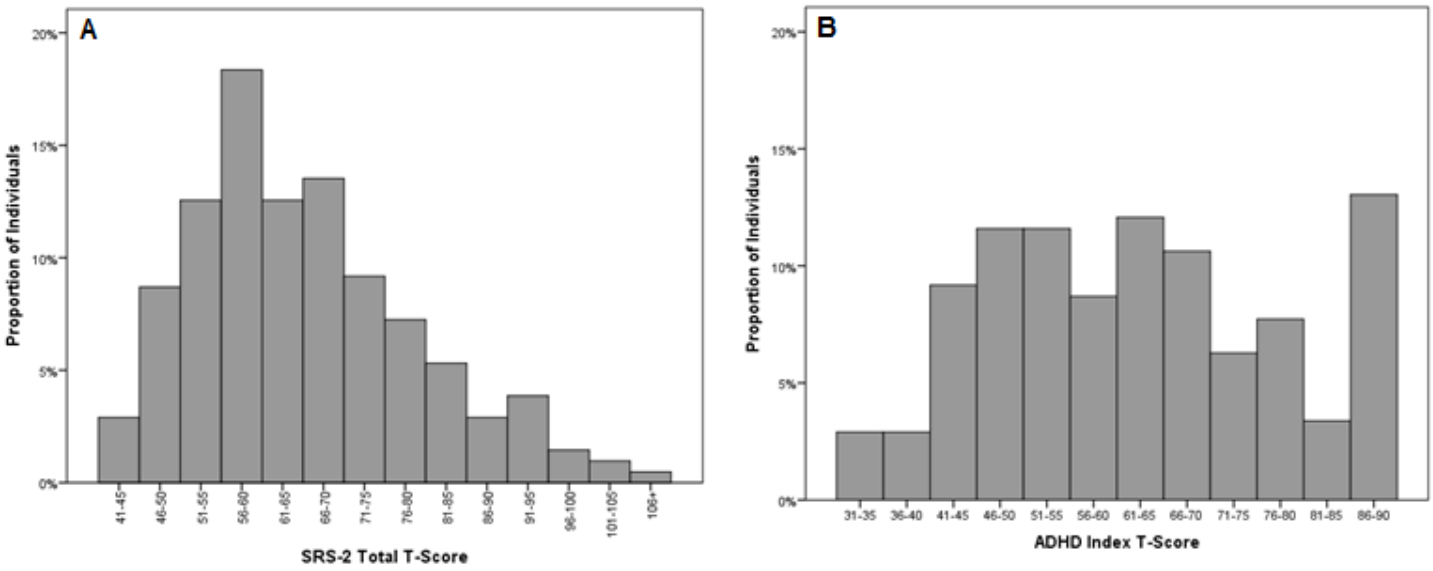
Par=number of estimated parameters, LL=Log-likelihood, AIC=Akaike Information Criterion, BIC=Bayesian Information Criterion, saBIC=sample-size adjusted Bayesian Information Criterion.

eTable 7. Family Relationship of First Degree Relative Pairings Concordantly Affected by NF1

	1st Affected Family Member			2nd Affected Family Member			Site
	Sex (Male/Female)	Age (years)	Relationship	Sex (Male/Female)	Age (years)	Relationship	
1	M	8	Son	F	25	Mother	WUSM
2	M	11	Brother	F	8	Sister	WUSM
3	F	19	Twin Sister	F	19	Twin Sister	WUSM
4	F	15	Sister	F	12	Sister	WUSM
5	M	10	Son	F	33	Mother	WUSM
6	F	12	Sister	F	8	Sister	WUSM
7	M	13	Son	F	36	Mother	WUSM
8	F	23	Daughter	M	58	Father	WUSM
9	F	11	Sister	M	3	Brother	WUSM
10	F	6	Daughter	F	28	Mother	WUSM
11	M	19	Son	F	49	Mother	WUSM
12	F	9	Daughter	M	40	Father	WUSM
13	F	5	Daughter	F	35	Mother	WUSM
14	F	16	Daughter	F	51	Mother	WUSM
15	M	24	Brother	M	13	Brother	WUSM
16	M	5	Son	M	31	Father	WUSM
17	F	11	Daughter	M	49	Father	WUSM
18	M	10	Son	M	57	Father	WUSM
19	F	44.5	Daughter	F	83.9	Mother	UCSF
20	M	9	Son	M	41	Father	UCSF
21	M	2.7	Son	F	29.5	Mother	UCSF
22	F	29.8	Daughter	F	52.5	Mother	UCSF
23	F	3.4	Daughter	F	34.6	Mother	UCSF
24	F	9.7	Daughter	F	31	Mother	UCSF
25	M	12	Brother	F	15	Sister	UCSF
26	F	12	Twin Sister	F	12	Twin Sister	UCSF
27	F	10	Sister	F	9	Sister	UM
28	F	8	Sister	F	11	Sister	UM
29	M	11	Brother	F	14	Sister	UM
30	F	10	Sister	F	8	Sister	UM
31	M	11	Brother	M	13	Brother	UM
32	F	6	Sister	F	10	Sister	UM
33	M	11	Brother	F	12	Sister	UM
34	F	11	Sister	F	8	Sister	UM
35	F	9	Sister	F	6	Sister	UM
36	F	9	Sister	M	6	Brother	UM
37	M	5.9	Brother	M	10	Brother	UM
38	F	7	Sister	M	12	Brother	CNHS
39	F	16	Twin Sister	F	16	Twin Sister	CNHS

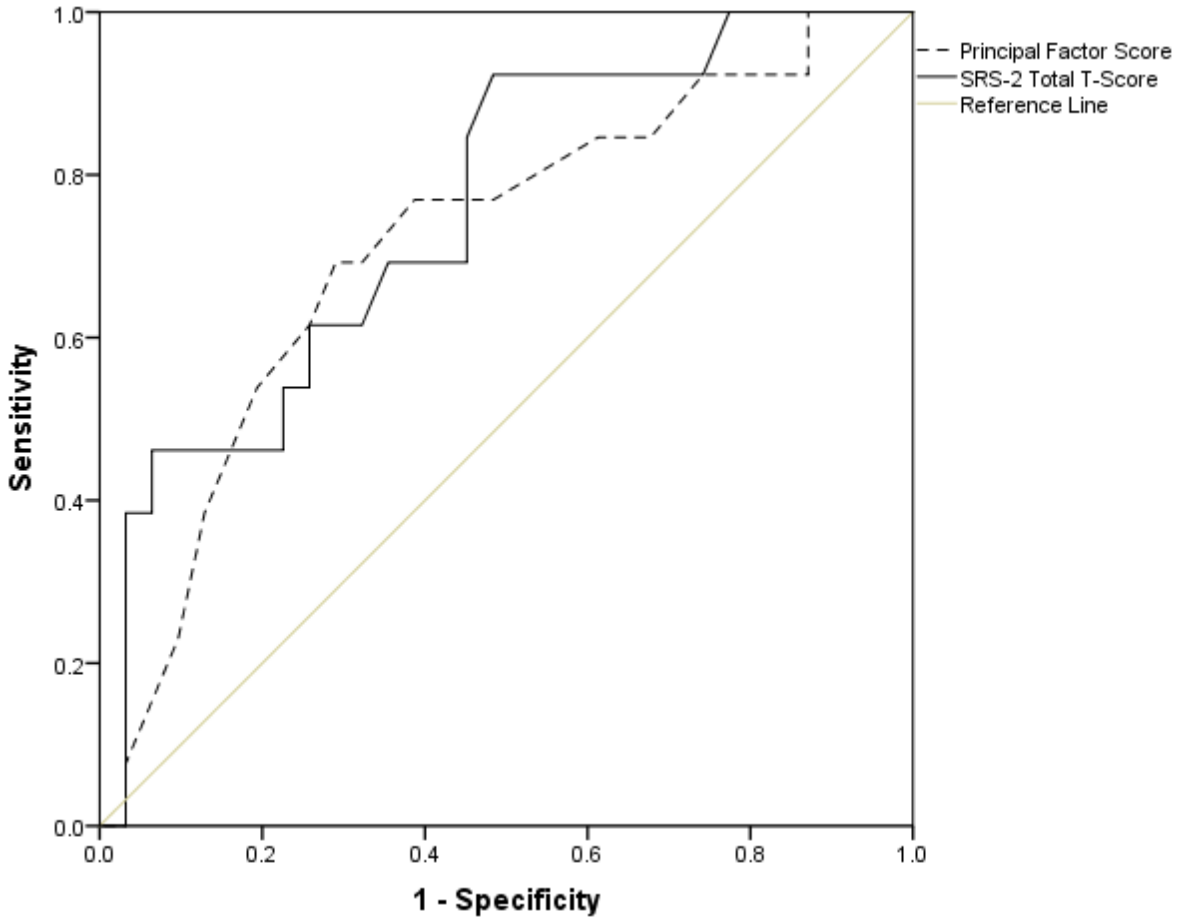
WUSM: Washington University School of Medicine; UCSF: University of California – San Francisco; UM: The University of Manchester; CNHS: Children's National Health System

eFigure 1. Standardized Quantitative Trait Distribution for ASD – Restricted Sample



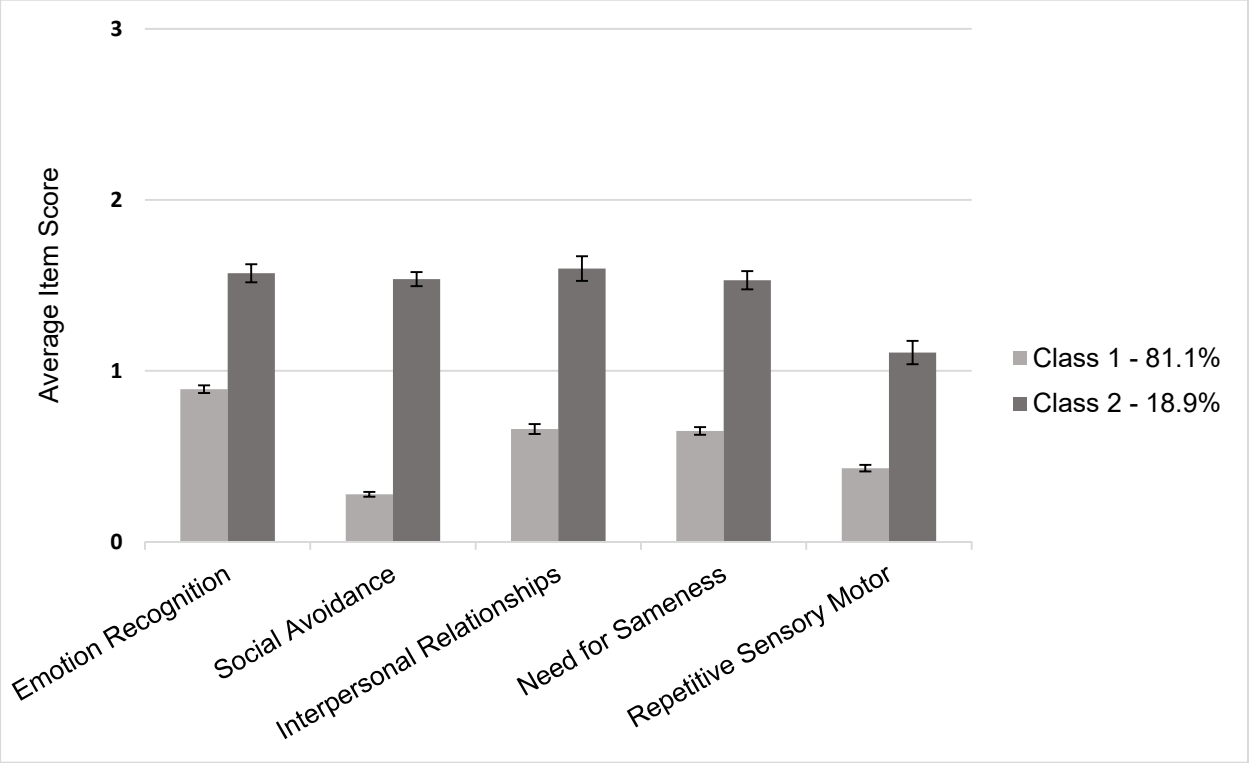
Distribution of SRS-2 Total T-scores (A) and ADHD Index T-scores (B) for individuals with available ADHD data (n=207)

eFigure 2. Receiver Operating Characteristics (ROC) Curves Comparing the Performance of the Principal Factor Score and SRS-2 Total T-Score in Identifying Categorical Diagnoses of ASD



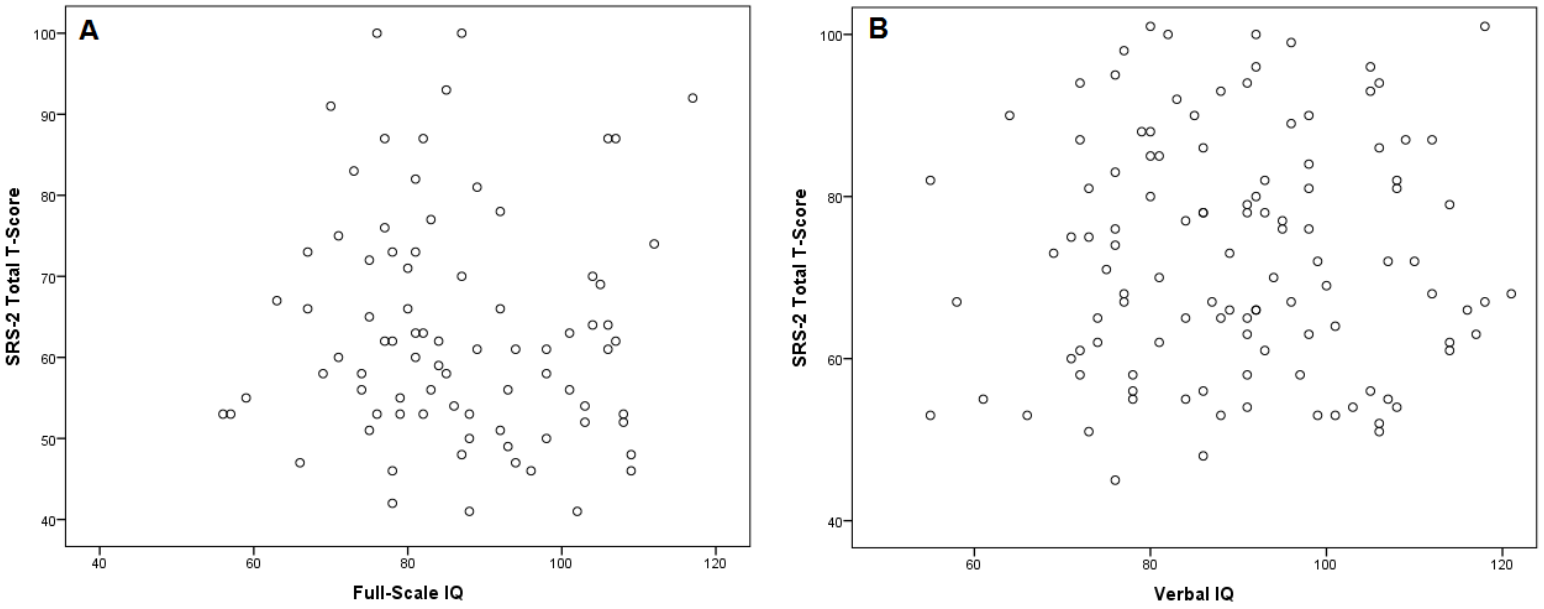
As observed in previous studies of the SRS-2, preservation of a higher proportion of the instrument's items (SRS-2 Total T-Score; solid line) outweighs advantages of the reduction in informant burden (constraining data acquisition to a single principal factor – Principal Factor Score; dotted line) when sensitivity and specificity are concerned. We note, also, that ASD case assignment within any ASD-associated monogenic syndrome could be expected to erode ROC characteristics from the ideal, especially when all or most patients bear some degree of elevated symptom burden for the discriminating characteristic or test (i.e. relative absence of true "normal"). Thus, in NF1, when ASD classification is made on the basis of diagnostic assessments validated exclusively for idiopathic ASD, we would expect less robust prediction from trait or symptom counts than would typically be observed when distinguishing idiopathic ASD from the normal distribution of such traits in the general population.

eFigure 3. Empirically-Derived SRS-2 Factor Scores by Latent Classes



Empirically-derived mean scores and standard error stratified by latent classes for all five SRS-2 factors. A uniform elevation in average item score for Class 2 (dark bars) is observed relative to Class 1 (light bars) representing the full range of autistic symptoms across both criterion domains of social communication and interaction, and restricted interests and repetitive behaviors.

eFigure 4. Within-Individual Correlation of Intelligence and Quantitative Autistic Trait Scores



A) Correlation between Full-Scale IQ (x-axis) and SRS-2 Total T-Score (y-axis); Pearson correlation coefficient, r= -0.06. (n=81 of 531; 15.2% of total sample). B) Correlation between Verbal IQ (x-axis) and SRS-2 Total T-Score (y-axis); Pearson correlation coefficient, r= 0.01. (n=112 of 531; 21.1% of total sample).

eMethods

Mixture Model

Using empirically-derived SRS factors, a series of mixture models were estimated with 1-6 latent classes specified.[25] Each model permitted nuisance covariance between SRS indicators. Model fit was estimated using the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), sample-size adjusted Bayesian Information Criterion (saBIC), and classification entropy.[26,27] Change in BIC from n to n-1 classes was evaluated to identify the number of classes that resulted in the greatest improvement in fit. Two separate hierarchical regression models were estimated to examine whether empirical classifications were predominantly due to ASD or ADHD symptomatology. In both models, age was entered in the first step to adjust for age-differences observed between classes. In steps 2 and 3, ASD or ADHD symptom scores were entered in alternating fashion to estimate specific and common variance associated with these predictors of empirical classifications.