

Supplement

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A. Methods

A1. Modified Chinese version of ASRS (MC-ASRS)

We selected the Autism Spectrum Rating Scale (ASRS) as a screening tool for the school-age population because of its good psychometric properties, its norm-based nature, its applicability to the population age, and the availability of parallel parent and teacher forms. Having secured approval from the developers (Multi-Health Systems, Inc., Toronto, ON, Canada), we developed a Chinese version (MC-ASRS) using iterative back-translation procedures in a pilot study in four study sites (Shanghai, Harbin, Guangzhou, and Changsha) from January to July 2014 ^[1]. The English version of the ASRS contains 71 items that yield separate scores for three scales: screening, DSM-5, and treatment. The screening scale comprises 60 items, including Social/Communication (19 items), Unusual behaviors (24 items), and Self-Regulation (17 items). These three scales are combined into a single composite score (Total score). A cut-off value of ≥ 60 has been recommended for screening in Western populations (<https://www.mhs.com>). The MC-ASRS includes a three-factor structure with 59 items, and a cut-off of 60 has a sensitivity of 94.2% and a specificity of 82.0%. The MC-ASRS has been validated as a reliable tool for screening autistic symptoms in Chinese children; receiver operating characteristic analysis has shown that it has excellent discriminant validity with an area under the curve value of 0.952 (95% CI: 0.936–0.967) ^[2, 3].

A2. Sampling methods and geographic characteristics of study sites

Study sites were selected based on consideration of their representativeness for geography, socio-demographic features, and economic development as well as evidence of previous research experience in ASD. Mainland China has 32 provincial-level divisions: 23 provinces, four municipal cities (Beijing, Shanghai, Tianjin, and Chongqing), and five minority autonomous regions. After careful deliberation, eight cities (Shanghai, Guangzhou, Changsha, Chongqing, Chengdu, Wenzhou, Beijing, and Harbin) from five provinces (Zhejiang, Hunan, Sichuan, Guangdong, and Heilongjiang) and three municipalities (Shanghai, Beijing, and Chongqing) were selected to participate in the study from July 2014 to December 2016. These eight cities occupy 2.1 percent of China's land area and comprise ~10% of China's total population. The economic status based on average per capita income in these cities is representative of the different levels of economic development nationally (i.e., Shanghai, Beijing, and Guangzhou are in the top tier, Wenzhou and Changsha in the middle, and Chengdu, Harbin, and Chongqing in the low tier). Each city is divided into a variable number of districts and streets, and we selected 82 streets from 17 districts in the eight cities from the local Public Security Bureau Household Registration System (PSBHRS) of each city. Combination of the following factors was considered when selecting the streets and districts: (1) population from 5,000 to 15,000; (2) lowest rate of migrant influx in the past year; and (3) a good level of cooperation expected from the local government.

The eligible children were identified in each selected district through the local PSBHRS. The inclusion criteria were: (1) aged 6 to 12 years (born between January 1, 2002 and December 31, 2008). These ages were chosen for two reasons: (a) a definitive diagnosis of ASD can be obtained at age 4 or later ^[4]; and (b) ~99% of these children were attending public school because of government mandate, and these schools are free by government policy; and (2) the children's families were registered in the selected areas, lived in the selected regions for at least 6 months during the last 12 months, and were enrolled during 2014–2016.

A3. Sample Size

Sample size calculations were performed with an assumption of a prevalence of 1%, an alpha of 0.05, power of ≥ 0.8 , and allowing for 5/1000 error. Based on this calculation, ~15,000 participants for each site, and a total of 120,000 participants for the entire study were determined. This study sample represented ~0.12% of the total population aged 6–12 years in China during the study period. The total number of Chinese children aged 6–12 years was estimated at ~100 million (N = 99,939,042) (<http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/indexch.htm>).

A4. ASD screening procedures for children in Sources 1 and 2

Different procedures were applied to the children from the different sources (**Figure 1**). For the children in Sources 1 and 2, we implemented a two-step screening procedure (steps A and B):

Step A: Use of parent and teacher MC-ASRS scales for screening: We used parent and teacher MC-ASRS scales that were validated in our pilot study for screening ^[1-3]. For students enrolled in regular schools within the sampled districts (Source 1), parents of all eligible children received an information and consent letter from the school. Parents and teachers were given a screening booklet containing an information sheet about ASD and the study, the MC-ASRS questionnaire, and the instructions for completing it, along with an envelope to return the completed questionnaire. Contact information for a trained investigator from the study team was provided to parents and teachers should they have questions about the study. Written parental consent was obtained. Principal classroom teachers were requested

to score each student using the MC-ASRS teacher version. Completed scales were checked on-site by field investigators before data entry. A total score of ≥ 60 on either the parent or teacher scale was considered positive. For students enrolled in schools outside their residential districts (Source 2), the City Student Information System was consulted to identify the schools attended. With the assistance of the Education Bureau, the study documentation and questionnaire packages were mailed to parents and teachers through the school administrative system.

Step B: Screen by group interview and observation: For children who were positive for screening in step A, a face-to-face group interview and classroom observation were conducted by medical professionals at schools to further identify participants with a high likelihood of ASD who needed to go to a hospital for final diagnosis. The medical professionals responsible for the group screening and interviews had been trained and were qualified to perform DSM-5 for ASD diagnoses. Children in groups of 5–6 were observed and interviewed, and those with one or more positive scores on the scale were recorded by the interviewer and defined as positive (**see group observation and interview sheet**); this took at least 15–20 min for each child. The screening procedure was as follows: (1) direct observation of children's activities in the classroom related to social interactions with peers, repetitive physical movements, and atypical sensory processing behaviors; (2) a brief conversation with several semi-structured questions, which were selected from the Autism Diagnostic Observation Scale Module 3 (ADOS-M3): (i) friends and relationships: Do you have some friends? Can you tell me about them?

What do you like doing together?; (ii) emotions: What do you like doing that makes you feel happy or angry? What kinds of things make you feel this way? Can you describe it?; and (iii) reporting of events: Can you describe what happened recently? (e.g., a holiday, vacation, or shopping trip). During this interaction, the interviewer used these questions to understand the individual's eye-contact modulation, non-verbal communication, facial and other emotional expressions, language and communication competence, and atypical behaviors. In addition, in order to minimize false negatives in step A, children who were screened negative, but recommended by their teachers as having behavioral problems, were also included in Step B screening. The behavioral problems included inattention, hyperactivity, anxiety, aggression, indiscipline, and social withdrawal. Children who were positive for step B, along with their parents, were invited to participate in an in-person autism diagnostic evaluation in dedicated clinics at each study site.

Group observation and interview sheet

Basic information
Subject name and study ID: Gender: <input type="radio"/> Male <input type="radio"/> Female
Date of birth: ___ Year ___ Month ___ Day
School name:
Class:
ASRS score:
Observation and interview
A. Direct observation of children's activity in the classroom
1. Abnormal social interactions with peers (e.g., whether initiated conversation with peers) <input type="radio"/> Yes <input type="radio"/> No
2. Repetitive physical movements (e.g., jumping or whirling) <input type="radio"/> Yes <input type="radio"/> No
3. Atypical sensory processing behaviors (e.g., ring sensitive) <input type="radio"/> Yes <input type="radio"/> No
B. Conduct semi-structured questions
1. Friends and relationships Interview questions: Do you have some friends? Can you tell me about them? What do you like doing together?
2. Emotions

Interview questions: What do you like doing that makes you feel happy or angry? What kinds of things make you feel this way? Can you describe it?

3. Reporting of events

Interview questions: Can you describe what happened recently? (e.g., a holiday, vacation, or shopping trip).

C. Observe and evaluate the following behaviors and performance while conducting semi-structured questions.

Abnormal eye contact modulation

Yes No

Abnormal non-verbal communication

Yes No

Abnormal facial and other emotional expressions

Yes No

Abnormal reciprocal conversation

Yes No

Rigidity or restricted interest

Yes No

Atypical behaviors

Yes No

Note: Children with one or more “Yes” in sections A and C are defined as positive.

A5. Screening and diagnostic procedures for children in Source 3

Children with significant intellectual disability (ID) and ASD do not usually attend regular or mainstream schools in China. Instead, these children attend various special education schools and rehabilitation centers or stay at home. To receive welfare from the government, children with disabilities must have a confirmed diagnosis from hospitals approved by the local Disabled Persons' Federation (DPF) and register with the DPF. Therefore, all children in Source 3 were considered to be at risk for ASD (without preliminary screening), and were directly invited to participate in the diagnostic assessment for ASD at study clinical sites.

A6. ASD clinical assessment teams

The clinical assessment team at each study site was led by a senior clinician with extensive experience in the diagnostic assessment of children with ASD. The diagnostic evaluation of autism was performed in the clinics of Children's Hospitals affiliated with Medical Schools at each study site. The clinicians responsible for the clinical and diagnostic assessment had received project-specific training on DSM-5 and were trained and certified to administer the Chinese versions of the Autism Diagnosis Observational Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R) under the supervision of Western Psychological Service-certified Chinese trainers.

A7. Method to estimate the prevalence of ASD

The population denominators observed and the estimated numbers of ASD cases used to estimate ASD prevalence are shown by site, sampling source, and overall in **Table S5**. The overall observed ASD prevalence was 0.29% (95% CI: 0.26–0.32%). In regular school samples (Source 1: sampled districts), the response (Rate 1) between the screening and assessment phases (**Figure 1**) varied by study site and ranged from 20% to 100%. We had very poor response rates across the eight sites for screening at non-sampled districts (Source 2). According to the definition of “study population”, we included these children to minimize possible selection bias. The sex ratios and age distributions of children who attended the schools within *versus* outside the sampled districts were comparable ($\chi^2 = 0.04$, $df = 1$, $P = 0.09$). Therefore, we estimated the numbers of ASD cases in those schools based on the observed rate from Source 1. For Source 3 (special education contexts), the observed response rates (Rate 3 in **Table S4**) from four pilot study sites were averaged and used to estimate the ASD numbers among non-responders across all sites. Using this approach, the estimated number of ASD cases was 867 for the target population sample, and the contributions of Sources 1, 2, and 3 were 38.5% (334), 3.4% (29), and 58.1% (504), respectively, thereby achieving a prevalence of 0.70% (95% CI: 0.64–0.74%).

A8. Quality control

We implemented several strategies to ensure the quality of data collection, including the following:

(1) Project-specific training: All site investigators and team members responsible for data collection received project-specific training. Clinicians responsible for ASD screening and diagnostic assessment received extensive training in the use of MC-ASRS and DSM-5 and were certified to reliably administer ADOS and ADI-R.

2) Maximization of the response and participant rates: The investigators and the team exerted their best efforts to disseminate the study information and educate the public about ASD in the community and schools.

3) Quality check for ASD case determination: Approximately 10% of ASD cases reported from each site were randomly selected by the principal investigator's team at the Children's Hospital of Fudan University for independent review and re-assessment. The review team included a panel of three senior and experienced clinicians with expertise in autism diagnosis who did not participate in the initial case evaluation.

B. Tables

Table S1. Gender distribution by study site and participation status

Site	Participants					Non participants	
	Male	%	Female	%	Total	Male	%
Shanghai	9,076	52.1	8,345	47.9	17,421	1,510	55.0
Guangzhou	7,120	54.8	5,867	45.2	12,987	907	53.1
Changsha	5,861	52.4	5,323	47.6	11,184	157	52.7
Harbin	12,260	52.8	10,978	47.2	23,238	660	53.0
Beijing	4,216	53.2	3,704	46.8	7,920	1,686	50.3
Chongqing	11,698	52.5	10,577	47.5	22,275	1,620	53.8
Chengdu	8,504	52.9	7,587	47.2	16,091	340	54.1
Wenzhou	7,952	54.1	6,738	45.9	14,690	1,862	56.6
Total	66,687	53.0	59,119	47.0	125,806	8,742	53.7

Gender did not differ between participants and non-participants ($\chi^2 = 2.8$, $df = 1$; $P = 0.097$) and differed significantly among the eight study sites ($\chi^2 = 35.1$, $df = 7$, $P < 0.001$).

Table S2. Age distribution by study site

Site	6 years		7 years		8 years		9 years		10 years		11 years		12 years		Total	
	n	%	N	%	N	%	n	%	N	%	n	%	N	%	n	%
Shanghai	3,482	20.0	3,281	18.8	2,008	11.5	2,455	14.1	2,837	16.6	3,261	18.9	97	0.6	17,421	100.0
		0		8		5		1		3		7				0
Guangzhou	1,707	13.1	1,892	14.6	1,612	12.4	1,582	12.2	1,726	13.3	1,632	12.6	2,836	21.8	12,987	100.0
		1		6		4		2		3		6		8		0
Changsha	1,591	14.2	1,781	15.9	1,761	15.8	1,818	16.6	1,899	17.3	1,542	13.9	792	7.0	11,184	100.0
		2		9		8		3		0		8				0
Harbin	12	0.1	4,081	17.3	3,366	14.5	3,304	14.3	3,567	15.5	2,351	10.3	6,557	28.2	23,238	100.0
				6		5		2		4		0		2		0
Beijing	1,062	13.4	1,857	23.4	1,346	17.1	1,238	15.6	1,293	16.4	669	8.5	455	5.7	7,920	100.0
		4		5		0		6		3						0
Chongqing	2,478	11.1	3,811	17.4	3,459	15.6	3,249	14.5	3,558	16.1	3,195	14.4	2,525	11.2	22,275	100.0

		1	1	5	6	1	3	3	0							
		16.	17.	13.	13.	14.	13.	10.	100.							
Chengdu	2,682	2,816	2,224	2,171	2,301	2,210	1,687	16,091								
		7	5	8	5	3	7	5	0							
		14.	14.	13.	13.	15.	13.	15.	100.							
Wenzhou	2,056	2,055	2,020	1,942	2,294	2,031	2,292	14,690								
		0	0	8	2	6	8	6	0							
Total	15,07	12.	21,57	17.	17,79	14.	17,75	14.	19,47	15.	16,89	13.	17,24	13.	125,80	100.
	0	0	4	2	6	2	9	1	5	4	1	4	1	7	6	0

$\chi^2 = 13,000, df = 42, P < 0.0001$

Table S3. Age distribution and participation by age

Study population	6yr	7yr	8yr	9yr	10yr	11yr	12yr	Total
Participants (n)	15,070	21,574	17,796	17,759	19,475	16,891	17,241	125,806
(%)	12.0	17.2	14.2	14.1	15.4	13.4	13.7	100.0
Non-participants (n)	2,736	2,680	2,139	1,952	2,138	1,650	2,985	16,280
(%)	16.8	16.5	13.1	12.00	13.1	10.1	18.4	100
Total	17,806	24,254	19,935	19,711	21,613	18,541	20,226	142,086
	12.5	17.1	14.0	13.9	15.2	13.1	14.2	100.0

$\chi^2 = 718.4, df = 6, P < 0.0001$

Table S4. Estimated prevalence of ASD by source and site

Site	N_total	Observed									Estimated						
		AS D	Source1			Source2		Source3			n1	n2	n3	n_tota 1	P, ‰*	95% CI#	
			N1	Rate	n1	N2	n	N3	n3	Rate3						L1, ‰	U1, ‰
				1			2										
Shanghai	17,421	41	14,490	0.6	25	2,731	-	20 0	16	0.22	38	7	57	102	5.9	4.7	7.0
Guangzhou	12,987	51	12,398	0.7	21	403	-	18 6	30	0.34	36	1	10 7	144	11.1	9.3	12.9
Changsha	11,184	31	9,744	1	23	1,425	-	15	8	0.20	36	5	29	70	6.2	4.8	7.7
Harbin	23,238	81	22,376	0.7	71	811	-	51	10	0.33	97	3	36	136	5.9	4.9	6.9
Beijing	7,920	46	7,112	0.6	34	783	-	25	12	-	61	7	43	111	14. 0	11.4	16.6
Chongqing	22,275	56	20,010	0.2	16	2,167	-	98	40	-	28	3	14	174	7.8	6.7	9.0

																			3
Chengdu	16,091	32	15,021	0.7	23	1,035	-	35	9	-	26	2	32	60	3.7	2.8	4.7		
Wenzhou	14,690	25	13,108	0.8	9	1,558	-	24	16	-	12	1	57	70	4.8	3.7	5.9		
Total	125,80	363	114,25	-	22	10,91	-	63	14	0.28	33	29	50	867	6.9	6.4	7.4		
	6		9		2	3		4	1		4		4						

N, sample size or population denominator; n, number of diagnosed ASD cases; Rate 1 or 3, response rate in Source 1 or 3 calculated as the proportion of children screened positive in the DSM-5 assessment; *P, prevalence of ASD; N1, N2, N3, sample size of investigation of Sources 1, 2, and 3; n1, n2, n3, number of diagnosed ASD cases in Sources 1, 2, and 3

Table S5. IQ distribution of children with ASD (N = 185)

WIS-C Full-scale IQ	Shanghai	Guangzhou	Changsha	Harbin	Beijing	Chongqing	Chengdu	Wenzhou	Total
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Normal IQ (IQ ≥85)	5 (17.9)	10 (37.1)	2 (14.3)	30 (61.2)	4 (36.4)	1 (4.6)	11 (55.0)	3 (21.4)	66 (35.7)
Borderline (IQ 70–84)	3 (10.7)	6 (22.2)	2 (14.3)	10 (20.4)	2 (18.2)	2 (9.1)	6 (30.0)	4 (28.6)	35 (18.9)
Mild ID (IQ 50–69)	4 (14.3)	2 (7.4)	1 (7.1)	5 (10.2)	2 (18.2)	5 (22.7)	1 (5.0)	1 (7.1)	21 (11.4)
Moderate/severe ID (IQ <50)	16 (57.1)	9 (33.3)	9 (64.3)	4 (8.2)	3 (27.2)	14 (63.6)	2 (10.0)	6 (42.9)	63 (34.0)
Total	28 (100.0)	27 (100.0)	14 (100.0)	49 (100.0)	11(100.0)	22 (100.0)	20 (100.0)	14 (100.0)	185 (100.0)

ASD, autism spectrum disorder; IQ, intelligence quotient; ID, intellectual disability

Table S6. Gender differences in intellectual level, ASRS, ADOS, and ADI-R in children with ASD

Instrument	Boys with ASD		Girls with ASD		Cohen's <i>d</i>	<i>P</i> value
	<i>n</i>	mean ± SD	<i>n</i>	mean ± SD		
Total ASRS score	167	64.5 ± 13.6	44	64.5 ± 14.1	0.0	0.988
WIS-C (N = 185)						
Full IQ	148	73.9 ± 28.7	37	55.7 ± 20.9	0.7	<0.0001
Verbal IQ	148	72.8 ± 31.6	37	55.0 ± 23.2	0.6	0.002
Performance IQ	148	71.9 ± 29.8	37	57.9 ± 25.1	0.5	0.010
ADOS assessment (N = 164)						
Communication	130	4.8 ± 2.3	34	5.6 ± 2.9	0.3	0.090
Social interaction	130	9.1 ± 3.3	34	9.9 ± 4.1	0.1	0.250
Play	130	2.3 ± 3.1	34	2.1 ± 2.9	0.1	0.720
Stereotyped behavior	130	2.2 ± 1.9	34	2.3 ± 1.8	0.1	0.780
ADI-R assessment (N = 154)						

Social interaction	125	18.0 ± 7.6	29	22.7 ± 6.9	0.6	0.003
Communication–verbal	125	12.0 ± 5.3	29	13.2 ± 5.5	0.2	0.280
Communication–non-verbal	125	7.4 ± 4.7	29	10.0 ± 4.3	0.6	0.037
Stereotyped behavior	125	4.3 ± 2.8	29	4.7 ± 2.9	0.1	0.368

ASD, autism spectrum disorder; SD, standard deviation; ASRS, Autism Spectrum Rating Scale; WIS-C, Wechsler Intelligence Scale for Children; IQ, intelligence quotient; ADOS, Autism Diagnosis Observational Schedule; ADI-R, Autism Diagnosis Interview-Revised.

Table S7. Clinical features of newly- and previously-diagnosed ASD cases

Clinical variable	Newly diagnosed		Previously Diagnosed		Cohen's <i>d</i>	<i>P</i> value
	<i>n</i>	Mean, SD	<i>n</i>	Mean, SD		
Total ASRS score	127	61.1±12.1	84	69.7±14.2	0.4	<0.0001
WISC-C (available participants)						
Total IQ	106	79.1±25.2	79	58.4±27.9	0.8	<0.0001
Verbal IQ	106	76.7±29.6	79	59.3±30.0	0.6	<0.0001
Performance IQ	106	75.9±28.4	79	59.9±28.4	0.6	<0.0001
ADOS assessment						
Communication	83	4.1±2.0	81	5.9±2.7	0.8	<0.0001
Social interaction	83	8.7±3.5	81	9.9±3.4	0.3	0.026
Play	83	2.6±4.1	81	2.0±1.5	-0.2	0.166
Stereotyped behavior	83	1.8±1.8	81	2.6±1.9	0.4	0.007
ADI-R assessment						

Social interaction	76	15.9±7.3	78	22.3±6.4	0.9	<0.0001
Communication–verbal	76	9.8±4.5	78	15.1±1.5	1.6	<0.0001
Communication–non-verbal	76	6.3±4.3	78	10.0±4.3	0.9	<0.0001
Stereotyped behavior	76	3.4±2.2	78	5.4±3.0	0.8	<0.0001

ASD, Autism Spectrum Disorder; SD, standard deviation; ASRS, Autism Spectrum Rating Scale; WIS-C, Wechsler Intelligence Scale for Children; IQ, intelligence quotient; ADOS, Autism Diagnosis Observational Schedule; ADI-R, Autism Diagnosis Interview-Revised.

Table S8. Medical and psychiatric comorbidity by new *versus* previous ASD case status N (%)

Characteristics	Number of participants with comorbidity	Newly-diagnosed ASD with comorbidity	Previously-Diagnosed ASD with comorbidity	<i>P</i>-value
Comorbidities assessed <i>via</i> medical records, n/N (%)*				
Epilepsy (N = 105)	6/105(5.7)	3/49(6.1)	3/56(5.4)	0.600
Febrile seizures (N = 105)	7/105(6.7)	5/49(10.2)	2/56(3.6)	0.167
Sleep disorder (N = 78)	15/78(19.2)	3/40(7.5)	12/38(31.6)	0.0007
Gastrointestinal problem (N = 87)	36/87(41.4)	18/46(39.1)	18/41(43.9)	0.412
Allergy (N = 76)	12/76(15.8)	6/40(15.0)	6/36(16.7)	0.587
Psychiatric comorbidities <i>via</i> Mini-kids interview*				
ADHD (available participants, N = 102)	44(43.1)	22/54(40.7)	22/48(45.8)	0.604
Specific phobias (available participants, N = 94)	10/94(10.6)	6/47(12.8)	4/47(8.5)	0.503

Agoraphobia (available participants, N = 94)	7/94(7.5)	4/47(8.5)	3/47(6.4)	0.694
Obsessive-compulsive disorder (available participants, N = 94)	6/94(6.4)	3/46(6.5)	3/48(6.3)	0.957
Social phobia (available participants, N = 95)	6/95(6.3)	5/48(10.4)	1/47(2.1)	0.097
Mania (available participants, N = 94)	5/94(5.3)	3/47(6.4)	2/47(4.3)	0.646
Tics (available participants, N = 95)	5/95(5.3)	4/48(8.3)	1/47(2.1)	0.176
Others (N = 1028)	23/1028(4.3)	10/511(2.0)	13/517(2.5)	0.307

ADHD, Attention Deficit Hyperactivity Disorder; Mini-kids, Mini International Neuropsychiatric Interview (MINI kid) for Children and Adolescents (parent version); others: oppositional defiant disorder, suicidal behavior, dysthymia, adjustment disorders, post-traumatic stress disorder, conduct disorder, panic disorder, psychotic disorders, anorexia nervosa, major depressive episode, separation anxiety disorder, alcohol dependence, substance dependence, bulimia nervosa, generalized anxiety disorder; *observed number of participants with comorbidity/number of all participants assessed with the comorbidity (percentage)

C. Team members of the LATENT NHC study

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