Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. The Social Relationships Study Recruitment Process

The SRS sample was drawn from the Twins Early Developmental Study (TEDS), which comprises twins born between 1994 and 1996 in England and Wales, recruited through birth records, and considered representative of the general population [1]. The SRS sample underwent a two-stage selection process. The main aim was to include all families in which one or both twins were suspected or confirmed to have an Autism Spectrum Disorder (ASD). The first stage involved identification of families who had at least one twin scoring at or above 15 points on the Child Autism Screening Test (CAST) completed when twins were 8 years old [2].

To ensure that there were no systematic biases in the employed sampling technique and to quantify any selective attrition, letters and CAST questionnaires were also sent to 1,900 families from the original TEDS sample that had ended their participation in the study at an early stage; this was done at the SRS time point (age 12-15). These included families who were excluded from the main study due to severe medical and genetic conditions, such as severe developmental delay. This process yielded 34 families where at least one twin scored at or above 15 or where an ASD was reported. The two CAST mail-outs yielded 289 families. In addition, 210 families had reported an ASD diagnosis to TEDS (via phone/mail). Some of those reporting a diagnosis were also in the group identified as above the CAST cut-off, and the pool of potential families with suspected ASD was 412. Of these families, 82 (20%) could not be contacted, either due to address changes or because they had subsequently dropped out of TEDS, or refused participation.

In total, 330 families were then asked to complete the ASD module of the Development and Well-Being Assessment (DAWBA) [3] via telephone interview, as the second stage of SRS sample selection. As a result, after exclusion of 10 pairs on the basis of missing zygosity information or other medical conditions (e.g. Down's syndrome and profound deafness) [4], the DAWBA identified 230 families with at least one child who met criteria for an ASD.

To increase the likelihood of capturing the most complete sample, all child psychiatrists in the UK were sent a letter asking for details of twins born between 1994 and 1996 and suspected of ASD. These were checked to ensure that they were not already part of TEDS and if not they were sent information packs and CAST questionnaires. In addition adverts were placed in the Twins and Multiple Births Association newsletter and on the National Autistic Society's website. These additional recruitment methods yielded five further families who were not part of the main TEDS population, bringing the total SRS sample to 235 families with at least one twin suspected of, or diagnosed with, an ASD.

From this group, 89 families could not be contacted or declined to participate in the study; 17 families opted to complete only the questionnaire section of the project. 129 families (62%) had home (or research centre) visits.

In order to categorise the sample, the gold standard diagnostic tools of the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R) were used. Two researchers worked with each family, one carrying out the ADI-R and the other the ADOS for one twin and then swapping for the second twin. This design meant that different assessors carried out the ADI-R and ADOS assessments within each pair in order to minimize any effects of rater bias. In total, ADOS assessments were conducted for 249 individual twins (spread over 124 pairs) and ADI-R interviews were carried out for 253 individual twins (spread over 126 pairs). The advantage of using different diagnostic tools was that it allowed comparison of parent and observer rated measures of autistic symptoms. For 89 cases (37%), they did not lead to the same diagnosis. All cases with diagnostic disagreement were referred to a team of psychiatrists who reviewed all available sources of information and reached a consensus decision (see Supplementary Materials 2: Best-estimate Diagnosis procedure). The weighted kappa statistic for ADI-R and ADOS was .67, indicating a substantial agreement and in keeping with the weighted kappa of .79 reported by Bolte and Poustka (2004) [5].

A comparison group was also included in the study, consisting of 79 families from the TEDS sample who scored below 12 on the CAST at age 8 and who lived in the South East of England. This group was matched to the suspected ASD group in terms of gender, zygosity, age and SES. They completed the same battery of assessments (e.g., measures of IQ) as the suspected ASD sample but, because they were selected to be at low risk for ASD, did not complete the diagnostic assessments (i.e. ADOS and ADI-R). A subsample (n = 29) completed the ASD module of the DAWBA either online or by telephone interview.

eAppendix 2. Diagnostic Assessments and Classifications

ADI-R (the following text is taken from www.agre.org)

1. Autism is identified using the well-validated ADI-R scoring algorithm [6].

2. NQA (Not Quite Autism) represents individuals who are no more than one point away from meeting autism criteria on any or all of the 3 "content" domains (i.e., social, communication, and/or behavior), and meet criteria on the “age of onset” domain; or, individuals who meet criteria on all 3 "content" domains, but do not meet criteria on the "age of onset" domain.

3. Broad Spectrum defines individuals who show patterns of impairment along the spectrum of pervasive developmental disorders. This is a broad diagnostic category that encompasses individuals ranging from mildly- to severely-impaired. This category potentially includes such pervasive developmental disorders as PDD-NOS and Asperger's syndrome, which are used in many genome scans; however, this classification is not based on any validated algorithms.

AGRE AFFECTED STATUS ALGORITHMS

Note: All of the following algorithms are based on ADI-R domain scores.

I. "AUTISM" classification:

Uses the well-validated ADI-R diagnostic algorithm [6]:

1. social >= 10, and

2. communication: verbal >= 8; nonverbal >= 7, and

3. behavior >= 3

PLUS age of onset >= 1

II. "NOT QUITE AUTISM (NQA)" classification:

EITHER:

(A) Meets cut-offs on all 3 "content" domains, but not age of onset domain:

1. social >= 10, and

2. communication: verbal >= 8; nonverbal >= 7, and

3. behavior >= 3

PLUS age of onset = 0

OR:

(B) Is no more than 1 point below cut-off on any, or all, of the 3 "content" domains, and meets “age of onset” domain:

1. social >= 9, and

2. communication: verbal >= 7; nonverbal >= 6, and

3. behavior >= 2

PLUS age of onset >=1

III. "BROAD SPECTRUM" classification:

Age of onset >= 0;

PLUS does not meet criteria for Autism or NQA;

PLUS meets one or more of the following (A, B, or C):

(A) Shows severe deficit on at least one domain; severe is defined by scores at one or more of the following levels (e.g., 1 or 2 or 3):

1. social >= 8, or

2. communication: verbal >= 7; nonverbal >= 6, or

3. behavior >= 3

(B) Shows more moderate deficits in at least two domains; moderate is defined by scores at two or more of the following levels (e.g., 1 + 2, or 2 + 3, or 1 +3):

1. social >= 4

2. communication >= 3 (nonverbal or verbal)

3. behavior >= 2

(C) Shows only minimal deficits, but in all three domains at the following levels:

1. social >= 3, and

2. communication >= 2 (nonverbal or verbal), and

3. behavior >= 1

ADOS

The table below summarises the cut-offs for ADOS algorithm used in the current study:

Module	**Extra info**	**Cut-off**
One	No words	Autism = 16
		ASD = 11
One	Some words	Autism = 12
		ASD = 8
Two	Over 5 years of age	Autism = 9
		ASD = 8
Three		Autism = 9
		ASD = 7

In addition to the standard cut-offs, we have chosen to implement a broader spectrum category, whereby we take 2 points below the ASD cut-off, this translates as:

Module one no words = 9
Module one some words = 6
Module two over 5 = 6
Module three = 5

Best-estimate Diagnosis (BeD)

Diagnosis was made according to DSM-IV and ICD-10 criteria and based primarily on scores from the ADI-R and ADOS. After regrouping cases on their ADOS scores (amalgamating the Autism and ASD categories to make one ASD category) and ADI-R scores (amalgamating the Autism and NQA categories to make one ASD category) there were 154 cases with agreement between ADOS and ADI, leaving 89 with disagreement in the ratings. Following review of all available information (ADOS, ADI-R, and DAWBA scores as well as notes from interviewers and case notes, Patrick Bolton (PB) and Emma Colvert (EC) were able to assign a Best-estimate Diagnosis (BeD) in 59 cases. In these cases, discrepancies in classification were small, with cases falling just short of threshold on one of the measures. In the remaining cases, data were missing on one or other diagnostic measure (n=10) or large differences in classification assignment were present (n=20).

For these 30 cases the original DAWBA, ADOS and ADI-R schedules and interviewer notes were reviewed (where available). In addition, audio and video recordings of the ADI-R interviews and ADOS assessments were reviewed by PB and Sarah R Curran (SRC) independently. The review process considered the potential basis for discrepancies in diagnostic classification (reporting bias, developmental change, comorbid conditions, and administration problems) and a consensus BeD was assigned.

eAppendix 3. Concordance Rates

eTable 1 Number of unaffected/concordant/discordant pairs for each clinical measure of autism (DAWBA, ADI-R, ADOS and Best-estimate Diagnosis) as a three category variable (unaffected=0, Broad Spectrum=1, ASD=2).

DAWBA							
MZ	twin 2=0	twin 2=1	twin 2=2	**DZ**	twin 2=0	twin 2=1	twin 2=2
twin 1=0	47	2	3	twin 1=0	138	20	33
twin 1=1	6	5	3	twin 1=1	27	0	2
twin 1=2	5	1	15	twin 1=2	38	1	2
ADI-R							
MZ	twin 2=0	twin 2=1	twin 2=2	**DZ**	twin 2=0	twin 2=1	twin 2=2
twin 1=0	28	0	2	Twin 1=0	51	2	21
twin 1=1	0	1	7	Twin 1=1	4	3	16
twin 1=2	2	4	12	Twin 1=2	27	16	8
ADOS							
MZ	twin 2=0	twin 2=1	twin 2=2	**DZ**	twin 2=0	twin 2=1	twin 2=2
twin 1=0	32	0	3	twin 1=0	73	4	25
twin 1=1	2	1	2	twin 1=1	3	1	7
twin 1=2	2	1	12	twin 1=2	24	1	9
Best-estimate Diagnosis							
MZ	Twin 2=0	Twin 2=1	Twin 2=2	**DZ**	twin 2=0	twin 2=1	twin 2=2
Twin 1=0	29	0	0	twin 1=0	51	4	28
Twin 1=1	1	1	5	twin 1=1	6	2	10
Twin 1=2	2	1	17	twin 1=2	32	7	11

eTable 2 Number of DZ Same Sex and Opposite Sex concordant/discordant pairs for each clinical measure of autism (DAWBA, ADI-R, ADOS and Best-estimate Diagnosis) as a three category variable.

ASD [1]	MZ Discordant/ Concordant [3]	Proband-wise Concordance Rate	DZ Same Sex Discordant/ Concordant [3]	Proband-wise Concordance Rate	DZ Opposite Sex Discordant/ Concordant [3]	Proband-wise Concordance Rate

DAWBA	12/15	.71	35/2	.10	39/0	.00
ADI-R	15/12	.62	37/7	.28	43/1	.04
ADOS	8/12	.75	27/8	.37	30/1	.06
Best-estimate Diagnosis	8/17	.87	34/9	.35	43/2	.09
ASD+Broad Spectrum [2]	MZ Discordant/ Concordant [3]	Proband-wise Concordance Rate	DZ Same Sex Discordant/ Concordant[3]	Proband-wise Concordance Rate	DZ Opposite Sex Discordant/ Concordant [3]	Proband-wise Concordance Rate
DAWBA	16/24	.75	58/3	.09	60/2	.06
ADI-R	4/24	.92	25/26	.68	29/17	.54
ADOS	7/16	.82	27/13	.49	29/5	.26
Best-estimate Diagnosis	3/24	.94	32/20	.56	38/10	.35

[1] ASD rates reflect twins included in category 2 only.
[2] ASD+Broad Spectrum rates reflect pairs in which a child was either included in category 1 or 2.
[3] Number of Discordant and Concordant pairs included in the calculation.

References

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