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Autism Spectrum Disorders and Other Mental Health Problems: Exploring Etiological Overlaps and Phenotypic Causal Associations

RH: The Aetiology of Comorbidity of ASD and Other Mental Health Problems

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ABSTRACT

Objective: Recent studies highlight the impact of co-existing mental health problems in autism spectrum disorders (ASD). No twin studies to date reported on individuals meeting diagnostic criteria of ASD. This twin study reports on the aetiological overlap between diagnosis of ASD and emotional symptoms, hyperactivity, and conduct problems measured with the Strengths and Difficulties Questionnaire.

Method: Genetic and environmental influences on the covariance between ASD and co-existing problems were estimated, in line with the correlated risks model prediction. Phenotypic causality models were also fitted to explore alternative explanations of comorbidity: that co-existing problems are the result of or result in ASD symptoms, that they increase recognition of ASD, or that they arise due to an over-observation bias/confusion when differentiating between phenotypes.

Results: Over fifty percent of twins with broad spectrum/ASD met the borderline/abnormal levels cut-off criteria for emotional symptoms or hyperactivity, and a quarter met these criteria for the three reported problems. In comparison, between 13%-16% of unaffected twins scored above the cut-offs. The phenotypic correlation between ASD and emotional symptoms was entirely explained by genetic influences and accompanied by a moderate genetic correlation (.42). The opposite was true for the overlap with conduct problems, as non-shared environmental factors had the strongest impact. For hyperactivity, the best-fitting model suggested a unidirectional phenotypic influence of hyperactivity on ASD.

Conclusion: Our findings suggest a possible effect of hyperactivity on identification of ASD. The lack of genetic influences on conduct problems–ASD overlap further supports the genetic independence of these two phenotypes. Finally, the co-occurrence of emotional symptoms in ASD, compared to other co-occurring problems, is completely explained by common genetic effects.

Key words: autism spectrum disorders, comorbidity, mental health, behavioral genetics
INTRODUCTION

Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorder characterised by impaired social communication and restricted and repetitive behaviours and interests. In 2010, 52 million individuals worldwide were estimated to have ASD. Research over the last decade highlighted the high rates and severe impact of additional (comorbid) mental health problems in ASD. This is acknowledged in the most recent version of the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders (DSM-5; APA, 2013), where additional diagnoses such as attention-deficit/hyperactivity disorder (ADHD) or anxiety disorders are allowed in people with ASD for the first time.

In genetic epidemiology, there are two common reasons for comorbidity: 1) one disorder has a causal role for another disorder (e.g., chronic kidney disease as an increasing risk for coronary heart disease); 2) the two disorders co-occur because of overlapping genetic, or environmental, reasons (e.g., major depression and alcoholism may co-occur in adulthood due to childhood maltreatment). Twin studies are particularly informative in disentangling the genetic and (shared/non-shared) environmental influences on comorbidity with potentially high scientific and practical importance for diagnosis and treatment.

Commonly reported mental health comorbidity in samples with clinical ASD include internalizing problems, such as excessive fears and worry (anxiety) and depression. Internalising problems have been postulated as a cause of ASD symptoms (e.g., “rigid preferences for sameness” resulting from extreme anxiety), or an effect (e.g., communication difficulties lead to isolation, sadness and anxiety). General population twin studies have shown a phenotypic correlation between autistic-like traits and internalising problems of .30-.35, which was stable across a 5-year period. This covariance was explained modestly by shared genetic factors and mostly by shared and non-shared environmental factors. Longitudinal direction of causation investigations from 7-8 to 12 years showed that autistic-like traits had a modest phenotypic influence on internalising problems over time, while the reverse effect was of smaller magnitude. Study of three autistic-like (social difficulties, communication problems, and repetitive/restrictive behaviours [RRB]) and four internalising traits (social and generalised anxiety, fears, and negative affect) showed that communication problems and RRBs correlated strongly with generalised anxiety and negative affect, whereas little overlap was found between social difficulties and internalising behaviours. Conversely, stronger genetic
correlation (.53) between autism and anxiety traits was reported in a large Swedish cohort.\textsuperscript{19} The moderate phenotypic overlap between the traits is somewhat surprising considering the high rates of internalising problems in samples with clinical ASD.\textsuperscript{4,5} Replication of these results in twins with a diagnosis of ASD is much needed.\textsuperscript{16}

ADHD-like traits are also commonly reported in ASD. Twin findings show a consistent pattern of moderate to strong genetic overlap between ADHD and autism traits.\textsuperscript{20–22} Longitudinal direction of causation cross-lagged models showed that ADHD traits at age 8 were strongly predictive of ASD traits at age 12 (particularly communication difficulties), with a significantly less strong converse effect.\textsuperscript{23} An examination of subdomains of ADHD revealed that hyperactivity correlated less strongly than inattentiveness and impulsivity with autism subdomains.\textsuperscript{24}

Comorbidity of ASD with conduct-like problems is not easily interpretable and might be explained by confusion over the phenotype and its appropriate definition. ASD-associated disruptive behaviours may resemble conduct problems. However, “meltdowns” reflect the intrinsic developmental difficulties of ASD, whereas conduct problems in individuals without ASD reflect different processes.\textsuperscript{25} Very few studies have explored conduct-like problems in ASD to date. Those that exist report an aetiological independence and show small to moderate genetic overlap (larger in boys than girls), with most covariance explained by shared and non-shared environmental factors.\textsuperscript{19,26,27, 50}

Previous studies demonstrate modest to strong genetic influences on autism traits and comorbid internalising and ADHD-related problems, and an aetiological independence for conduct-like problems. Here, for the first time, to our knowledge, we examine in this study the aetiology of these three comorbidities in population-based twin samples with ASD diagnosed with gold-standard instruments. First, we examined whether comorbidities are due to correlated genetic and environmental risks. Secondly, we tested phenotypic causal effects, with the direction of causation (ASD–hyperactivity/internalising/externalising) to be determined. Each of these hypotheses has implications for potential intervention strategies.\textsuperscript{28}

METHOD

Participants–The Social Relationship Study (SRS) Sample

Twins “at risk” of ASD were identified from a general population sample of twins (Twins Early Development Study, TEDS)\textsuperscript{29} on the basis of high scores on the Childhood Autism Spectrum Test.
and/or parental report of suspected/diagnosed ASD (for full details see Supplement 1, available online). Best-estimate diagnosis (BeD) was based on in-person assessments using standard diagnostic instruments, parent interview, and observational information. In addition to families with one or both twins meeting criteria for ASD, the SRS recruited control twins who were low in ASD traits (scoring <12 on CAST). Overall, BeD data were available for N=207 twin pairs (discordant/concordant for broad spectrum/ASD: 73/54; 80 pairs were controls) with a mean age of 13.16 years. Information on other mental health problems was collected from parents using the Strengths and Difficulties Questionnaire (SDQ). SDQ scores were available for n=166 (concordant/discordant: 40/55 and 71 control pairs) of the 207 twin pairs. Of 166 twins, 144 were 12 years or older, and 22 were below the age of 12.

**Measures**

**BeD for ASD**

The full procedure related to establishing best-estimate diagnosis (forthwith referred to as “ASD–BeD”) can be found in Supplement 2, available online. SRS participants were evaluated for ASD with a range of diagnostic measures: Development and Well-Being Assessment (DAWBA), Autism Diagnostic Observation Schedule (ADOS), and Autism Diagnostic Interview–Revised (ADI-R). ASD–BeD was made according to DSM-IV and International Classification of Diseases–10th Revision (ICD-10) criteria and based primarily on scores from the ADI-R and ADOS. The additional sub-category of Broad Spectrum was included in the classification to capture individuals with high-level autism traits that fell just short of an ASD diagnosis. ASD–BeD, therefore, consists of 3 ordinal classes: 0=no ASD/controls, 1=broad spectrum, 2=ASD.

**Strengths and Difficulties Questionnaire (SDQ)**

The SDQ is a well-regarded measure of mental health for 2- to 17-year-olds and widely used in clinical settings. It contains five subscales (5 items each) adapted to measure emotional symptoms, conduct problems, hyperactivity (inclusive of inattention symptoms), peer relationship problems, and pro-social behaviours, with each item given a three-point rating: 0=Not True, 1=Somewhat True, 2=Certainly True (maximum score=10).

Information on emotional symptoms, hyperactivity, and conduct problems were used as a continuous measure in the genetic model fitting analyses. We disregarded the peer relations
problems, as they are already captured by an ASD diagnosis and the prosocial scale, as it measures positive and not negative comorbidities (the main focus of this study).

For descriptive statistics and phenotypic analyses, the lower (borderline) threshold is used to indicate elevated rates of each SDQ problem; relatively few participants passed the borderline but not the abnormal thresholds, but inspection of the data suggested these participants were more properly grouped with abnormal than with unaffected participants. Cut-offs for borderline and abnormal levels (out of 10 points), respectively, are: emotional symptoms 4, 5; hyperactivity 6, 7; and conduct problems, 3, 4. SDQ revealed good internal consistency levels: emotional symptoms Cronbach’s \( \alpha = 0.72 \), hyperactivity \( \alpha = 0.81 \), and conduct problems \( \alpha = 0.67 \).

**Statistical Analyses**

**Twin Correlations**

The principles of the twin model fitting are provided elsewhere. Structural equation modeling (SEM) was performed in OpenMx using full information maximum likelihood estimation for the (genetic and environmental) variance and covariance decomposition. Continuous SDQ scores and ordinal ASD–BeD were analysed jointly. For ASD, a liability threshold model was assumed with a standard normal distribution underlying the ordered categories, with individuals receiving the diagnosis as they cross the disease threshold on this liability distribution. The joint multivariate normal distribution assumed between each measure of SDQ and ASD–BeD in a pair of twins allows estimation of the within- and across-twin (monozygotic [MZ]/dizygotic [DZ]) correlations. The ratio of MZ/DZ correlations indicate whether genetic or shared-environmental influences are responsible for variation within traits and on the covariance between them, which are formally estimated in the bivariate genetic twin model (explained below). Due to the selected nature of the sample, the thresholds on the ASD–BeD liability were fixed to population “known” values of ASD prevalence: first threshold of 5% separated the unaffected and twins with broad spectrum; second threshold of 1% separated the broad spectrum and twins with ASD.

**The Bivariate Genetic Model**

The (co)variance of each SDQ subscale and ASD–BeD was partitioned into additive genetic (A), shared-environmental (C), and non-shared environmental (inclusive of measurement error) E effects. In the absence of a specific order of traits in the model, the standardized correlated factor solution is interpreted such that the path from the \( A_1 \) factor to the SDQ subscale and the \( A_2 \) factor to
ASD–BeD are the square roots of their respective standardized path estimates (heritabilities) and the correlation path between $A_1$ (SDQ) and $A_2$ (ASD–BeD) is the genetic correlation between the variables ($r_A$). The same principle is applied to non-shared environmental effects (E). We then calculated the proportion of the phenotypic correlation ($r_{PH}$) due to correlated additive genetic effects A ($r_{PH_A} = \sqrt{h_1^2} \times r_A \times \sqrt{h_2^2}$) and due to correlated non-shared environmental effects E ($r_{PH_E} = \sqrt{e_1^2} \times r_E \times \sqrt{e_2^2}$) expressed as proportions of $r_{PH}$. Note that as shared environmental factors did not influence ASD–BeD, they cannot explain the covariance with comorbid traits and are hence excluded from above calculations.

**The Direction of Causation (DoC) Models**

The specific genetic and environmental effects on each of the SDQ measures and ASD–BeD are modelled as in the full bivariate genetic model, but with the genetic and environmental correlation between the traits substituted by causal effects of one trait on the other. In Figure 3, a reciprocal model is presented with $r$ and $r'$, reflecting both alternative causal pathways. Within twin data, the differentially predicted cross-twin cross-trait covariance is the basis of the direction of causation (DoC) model, and will only work if the relative proportions of variance (i.e. the h2) are sufficiently different across traits. For example, if SDQ problems are caused by ASD–BeD (SDQ ← ASD–BeD), then the expected cross-trait cross-twin covariance will be dominated by additive genetic effects. Thus because of the high heritability for ASD–BeD, a higher MZ compared to DZ covariance will be observed compared to if the relationship was the reverse (SDQ → ASD–BeD). To establish the direction of causation, the reciprocal (bidirectional) model is fitted first, and the unidirectional models ($r$ and $r'$) are the nested sub-models compared by using likelihood ratio and fit statistics (Akaike Information Criterion [AIC] and Bayesian Information Criterion [BIC]). Theoretically, if the variables only have two sources of variance (A and E), we can only proceed with causal hypothesis testing if we assume that both traits are measured without error or that the measurement errors are equal in magnitude.

**RESULTS**

**Descriptive Statistics**

Figure 1 shows the mean (and standard errors) for each SDQ subscale, divided into males and females meeting criteria for ASD, broad spectrum, and unaffected (comprising control twins and the unaffected co-twins of probands).

Figure 1 appears about here
Comorbid SDQ Problems Among Proband Twins

In Figure 2, we define “proband” as any individual assigned an ASD or broad spectrum best-estimate diagnosis. Of 135 individual probands, 71 (52%) passed the cut-off for emotional symptoms, 72 (53%) for hyperactivity, and 45 (33%) for conduct problems. Twenty-four (18%) probands met the cut off for all three mental health problems. Thirty three probands (24%) had no comorbid mental health problems. Of 40 ASD–BeD concordant proband pairs (MZ n=18, DZ n=22), 45% were also concordant for emotional symptoms (n=18); 40% for hyperactivity (n=16); 18% for conduct problems (n=7), and 22% were concordant for any two or more problems (n=9). Table S1, available online, shows that MZ twins concordant on ASD–BeD display a higher concordance rate on SDQ problems in comparison to DZ twins.

Comorbid SDQ Problems Among Discordant Twins

Six of the 55 ASD–BeD discordant pairs (11%) were concordant on emotional symptoms, four (7%) on hyperactivity, and 4 (7%) on conduct problems (Figure 2). Three pairs (5%) were concordant for any two or more problems. Among ASD–BeD discordant pairs, 3 were MZ and 52 were DZ. One MZ proband and 2 co-twins displayed no SDQ problems by parent report. In the DZ group, both individuals in 12 pairs and co-twin only in 23 pairs were free of SDQ problems; 23% (n=12) of pairs were concordant on at least one SDQ problem.

Twin Correlations

Table 1 shows polychoric and polyserial correlation estimates from the three bivariate models. The cross-twin (MZ/DZ) within-trait correlations (columns 1 and 2) for emotional symptoms (.70/.19) and hyperactivity (.66/.08) indicate dominance rather than additive genetic effects. However, our small sample is underpowered to detect dominance effects, thus we report broad-sense heritability by fitting additive genetic effects only. Estimates for conduct problems were .63/.25, and for ASD–BeD .91/.46, consistent with an AE model. The highest phenotypic overlap (r_PH) was between emotional symptoms and ASD–BeD (.33), followed by hyperactivity (.28) and conduct problems (.13) (all significant at 95% CI).

Bivariate Genetic Model Results
Table 2 shows the model-fit results and fit statistics ($\chi^2$, AIC, BIC) for the three bivariate models in separate sections. The goodness-of-fit test of two competing models was compared using the chi-square statistic ($\chi^2$) and the difference in degrees of freedom (DF). A non-significant $\chi^2$ value (p>.05) means the tested model is consistent with the data. A significant $\chi^2$ value (p<.05) means the model poorly fits the data and is rejected. For $\chi^2$, an increase of 3.84 or more for 1DF indicates a significantly worse fit. In each section, the first $\chi^2$(DF) concerns the difference in minus two log likelihood (-2LL) values of models 3 and 2 (testing the significance of parameter C), and the second of models 3a and 3 (testing the significance of either parameter $a_{21}$ or $e_{21}$). Models 2 to 3a were consistent with the twin correlations and showed that shared environmental factors did not influence the (co)variance of SDQ and ASD–BeD (model 3). The AE models could be further reduced for the emotional symptoms– ASD–BeD analysis (AE model 3a with path $e_{21}$ removed) and conduct problems (AE model 3a with $a_{21}$ removed). The estimates of genetic influences (Figure 3) ranged from 50% for hyperactivity, 60% for conduct problems, and 62% for emotional symptoms to 91% for ASD–BeD across the three models. The strongest phenotypic overlap ($r_{PH}=.32$, column 2, Table 2) between emotional symptoms and ASD–BeD was entirely accounted for by genetic factors (column 3, Table 2), as well as revealing a strong genetic correlation ($r_A=.42$, Figure 3).

The hyperactivity ASD–BeD overlap was of similar size ($r_{PH}=.29$) and strongly influenced by genetics (62%) and by non-shared environmental influences (38%; column 4, Table 2). However, the non-shared environmental factors showed a much stronger correlation ($r_E=.51$) than the genetic factors ($r_A=.27$). A small but significant phenotypic association ($r_{PH}=.10$) between conduct problems and ASD–BeD was entirely explained by strongly associated non-shared environmental influences ($r_E=.50$). Overall, looking at the most parsimonious models, the results provide considerable support for a genetic basis for the comorbidity of emotional symptoms/hyperactivity and ASD–BeD.

Figure 3 + Table 2 appear about here

Direction of Causation Model Results

We fitted DoC models to determine the most likely direction of causation by comparing models 4, 5, and 6 in Table S2, available online. The reciprocal AE model (model 4) will have the same fit as the correlated factors AE model (model 3). For emotional symptoms – ASD–BeD, no evidence for either unidirectional model was seen based on $\chi^2$. However, the AIC and BIC fit statistics are in favour of unidirectional model 5, in which the phenotypic path ($r$) from emotional symptoms to
ASD–BeD (Emo→ ASD–BeD) is dropped and only path Emo← ASD–BeD (path r') is modelled and estimated at .32 (95%CI .23-.40). For hyperactivity – ASD–BeD relationship, there is strong evidence for unidirectional model 6 as the best-fitting model (i.e. a model with the Hyp→ ASD–BeD path only), based on the significant decline in fit when this path (r=.28, 95%CI .20-.35) is dropped in model 5 (χ²=4.29, p=.04), as well as the lowest AIC and BIC values. Similarly, for conduct problems -- ASD–BeD relationship model 6 was the best fitting model, if the decision is made on the lowest AIC and BIC fit statistics (r=.14, 95%CI .06-.22).

DISCUSSION

The aetiology of three co-occurring mental health problems was examined in a sample of twins assessed for ASD. Overall, half of probands met the criteria for borderline/abnormal levels for emotional symptoms or hyperactivity, and a quarter met the cut-off for all three problems. Only one other twin study to date has reported on multiple comorbid disorders in ASD using health record data (Lundstrom et al. 2014). 6 In a sample of 272 twins, 50% had four or more coexisting disorders (although these were not identical to those considered here); on average, 65% of co-twins without ASD of probands had at least one other disorder, suggesting genetic origins. We found lower hyperactivity but similar conduct problems levels when compared to the oppositional defiant disorder levels reported in Lundstrom et al.’s 6 sample, clustering all three under externalising problems. Internalising problems were not measured in the Swedish study. It is worth noting that the current study sample was identified via several screening stages, followed by face-to-face assessment, whereas Lundstrom et al. 6 used parent telephone interview data/national registry information as a proxy clinical diagnosis, a limitation noted by the authors. This could result in under or overestimation of probands with coexisting difficulties.

In comparison to non-twin samples, our prevalence rates for co-existing disorders in ASD exceeded those of Leyfer et al. 4 but were comparable to those of Simonoff et al (2008). 12 This could be due to use of different measures. However, recent reports suggest the same psychometric properties as the SDQ’s performance in adolescents with ASD and adult samples were comparable to samples without ASD. The SDQ shows good external validity when compared to several clinically utilized measures of anxiety, depression, obsessive-compulsive disorder (OCD), and ADHD (Findon et al, 2015, unpublished manuscript).

Aetiology of ASD and SDQ problems –
Emotional Symptoms

The heritability of emotional symptoms (62%) was comparable to estimates in typically developing samples (50-60%). The phenotypic emotional symptoms – ASD–BeD overlap (.32) was comparable to previous general-population twin studies and was entirely explained by genetic influences. We found no influence of shared environment on this overlap in contrast to previous studies, but this might reflect limited power. The genetic correlation was comparable to typically developing samples of similar ages. Our unique data provide evidence that internalising problems in populations with ASD diagnosis are partly due to genetic influences.

Hyperactivity

The heritability of hyperactivity (50%) was closer to the lower range of those reported on typically developing samples (50-80%). Genetic influences explained 62% of the phenotypic correlation \( r_{PH} = .29 \) between hyperactivity and ASD–BeD, with the remainder explained by non-shared environmental influences. The moderate genetic correlation (.27) is comparable to that reported in typically developing adolescent samples (.12-.33) for social difficulties and repetitive behaviours and ADHD traits but stands in contrast to data from middle childhood studies (~.50).

It has been demonstrated that the communication difficulties aspects of the ASD triad, but not others, correlate more strongly with ADHD-like traits. Future analysis should take this into account.

Conduct Problems

The heritability of conduct problems was comparable to those reported in the general literature (overlapping CIs). The small phenotypic overlap \( r_{PH} = .10 \) between conduct problems and ASD–BeD was explained entirely by non-shared environmental effects, which is in agreement with O’Nions et al. (2015), which showed almost complete genetic independence of autistic-like traits from callous-unemotional traits in the general twin population (TEDS). This, however, stands in contrast to studies of larger samples reporting genetic overlap of .14 in boys for conduct problems and ASD, and .35 for oppositional defiant disorder and ASD, measures derived from parent telephone interviews. These differences could be explained as informant-dependent effects. A recent report suggests that the severity of conduct problems varies as a function of language ability as children with ASD without phrase speech exhibited the highest levels of such behaviours. The low levels of conduct problems and little overlap with ASD in the current sample could be explained by the high proportion of diagnosed twins with verbal fluency (90%).
**Direction of Causation**

In twin studies, covariation predominantly due to E (indicated by a significant within-person cross-trait correlation but a non-significant cross-trait cross-twin correlation) could indicate a phenotypic causal relationship.\(^{39}\) From our data, following that logic, the relationship most likely to be phenotypically causal would be that between conduct problems and ASD–BeD (all E), and the one least likely to be causal would be that between emotional problems and ASD–BeD (all A). However, the most convincing statistical evidence for direction of causation is seen for the hyperactivity – ASD–BeD relationship, where the unidirectional model with causal path Hyp → ASD–BeD showed the best overall fit across all models (including the correlated risk models) based on lowest BIC fit index and, importantly, significance of this causal path indicated by chi-square test. Similar direction of causation effects was found in the general population twin study of ADHD traits at age 8 predicting autistic traits at age 12.\(^{23}\) Furthermore, a study in the independent epidemiological Avon Longitudinal Study of Parents and Children (ALSPAC) sample showed that children exhibiting high probability for abnormal SDQ hyperactive-inattentive symptoms were at greater probability of persistent social communication deficits and that this interrelationship was not reciprocal.\(^{47}\) These findings can be explained in two ways. First, it is possible that greater hyperactivity raises the likelihood of ASD symptoms being noticed and reaching diagnostic threshold. In previous work with the TEDS sample, we have shown that low IQ and/or teacher-noted externalising problems increase the likelihood that females with high ASD traits meet diagnostic criteria for ASD.\(^{48}\) Secondly, it is possible that the findings reflect the existence of a combined clinical entity of autistic/hyperactive-inattentive syndrome.\(^{47}\) Alternatively, this “phenotypic causality” is not a direct effect, but an association due to correlated independent factors that we have not accounted for in our analysis. For example, a family history of alcohol abuse was suggested to be a potential risk factor for both autism and ADHD diagnoses in a recent study.\(^{49}\) Another example is maternal tobacco smoking during the first trimester and teenage pregnancy, which were identified as shared prenatal/perinatal predictors of ASD- and ADHD-like trait trajectories across the child’s lifespan.\(^{47}\) Future (longitudinal) studies are necessary to test these alternative accounts.

Sampling bias is important to consider in studies of comorbid conditions, since individuals with more than one mental disorder are more likely to become part of a clinical sample,\(^{11}\) leading to artificially increased comorbidity rates when using clinically ascertained groups. Our sample is
population-based rather than clinic-ascertained. However, a child's ASD may still affect the likelihood that other problems are picked up by parents and clinicians. “Over-observation” is a possibility for inflating reports of additional problems. Diagnostic overshadowing, conversely, may mean that all difficulties are attributed to the ASD or hyperactivity and co-existing problems are missed. However, if either of these general biases were driving our findings, uniformly high or low correlations might be expected between ASD and all three SDQ problems, which was not the case in our sample.

The small sample size meant that we had limited power to detect possible shared environmental influences; in DoC models, it meant strongly inferring that traits were measured without error or that measurement errors were equal in magnitude. Another limitation was reliance on parent-report SDQ ratings rather than direct assessment of the children. Ideally, future studies ought to validate the SDQ against the functional impairment characteristic in ASD while using in-person clinical ratings. Additionally, our finding could be explained as correlated error variance, reflecting the same raters’ difficulties evaluating ambiguous communication styles resulting in erroneous evaluations of the child with ASD. To exclude potential rater biases, SDQ measures should ideally be collected from multiple informants in future studies.

Comorbidity of other mental health problems in ASD is important to identify because it is often treatable. Our findings suggest a possible effect of hyperactivity on identification of ASD, which could be the focus of future studies to clarify behavioural patterns as predictive of ASD or other diagnoses. Conversely, relatively little overlap between ASD and conduct problems found here suggests the importance of discriminating apparently similar (e.g. socially disruptive) behaviours due to different causes. Finally, the overlap of ASD and emotional symptoms explained as completely due to genes further strengthened the genetic aetiology demonstrated by previous twin studies. Clinicians may wish to be alert to possible internalising/externalising difficulties in siblings of those with ASD.

**CLINICAL GUIDANCE**

- Hyperactivity/inattention symptoms can be predictive of ASD or other diagnoses.
- Little overlap between conduct problems and ASD highlights the importance of reliably discriminating between apparently similar (e.g. socially disruptive) behaviours.
- Clinicians should be alert to possible internalising/externalising difficulties in siblings of those with ASD.
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**Figure Titles and Captions:**

**Figure 1** Means for each Strengths and Difficulties Questionnaire (SDQ) subscale in each diagnostic autism spectrum diagnosis (ASD)–best-estimate diagnosis (BeD) group (n=332 of twin individuals).

Note: The range of scores for SDQ was 0 to 10 points; borderline/abnormal category cut-off for hyperactivity was 6 points, for emotional symptoms 4, and for conduct problems, 3 points. BS = broad spectrum; UN = unaffected.

**Figure 2** Coexisting Strengths and Difficulties Questionnaire (SDQ) problems in Social Relationship Study sample. Note: Headings “proband” and “co-twin” represent an individual. When squares to the right of proband and to the left of co-twin are shaded, it means this individual met the cut-off for borderline OR abnormal category for emotional symptoms (Emo; ≥4 points, medium fill), hyperactivity (Hyp; ≥6, light fill) and conduct problems (Con; ≥3, dark fill). Scores for concordant unaffected are not included in this figure but can be found in Table 1. Concordant = autism spectrum diagnosis (ASD)/broad spectrum in both twins; Discordant = twin 1 is ASD/broad spectrum, twin 2 is unaffected; DZ OS = dizygotic opposite-sex twin pairs; DZ SS = dizygotic same sex; MZ = monozygotic.

**Figure 3** Standardized estimates of the three AE (additive genetic and non-shared environmental influences) bivariate models of emotional symptoms (Emo) and autism spectrum diagnosis (ASD)–best-estimate diagnosis (BeD); hyperactivity (Hyp) and ASD–BeD and conduct problems (Con) and ASD–BeD. Note: The genetic correlation was fixed at zero for ASD–BeD and conduct problems, as this path was non-significant. Conversely, the environmental correlation was fixed at zero for ASD–BeD and emotional symptoms, as this path was non-significant (dashed lines). Prevalences for the ASD liability threshold were fixed at 5% (broad spectrum disorder) and 1% (ASD). $A_{1/2}$ and $E_{1/2}$ denote latent genetic and environmental factors on each trait individually; $r_A$ and $r_E$ denote the genetic and environmental correlations between the A and E factors influencing ASD–BeD and each Strengths and Difficulties Questionnaire (SDQ) measure. $r$ and $r’$ = phenotypic causal paths calculated in the direction of causation models instead of the $r_A$ and $r_E$ paths.
Table 1 Monozygotic (MZ) and Dizygotic (DZ) Within-Trait and Cross-Trait Twin Correlations Based on the Bivariate Analysis of Each Strengths and Difficulties Questionnaire (SDQ) Problem/Scale and Autism Spectrum Disorder–Best-Estimate Diagnosis (ASD–BeD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r_{MZ}^a$</th>
<th>$r_{DZ}^a$</th>
<th>Cross-twin cross-trait (MZ)$^b$</th>
<th>Cross-twin cross-trait (DZ)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD–BeD$^c$</td>
<td>.91 (.84-.95)</td>
<td>.46 (.36-.55)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Emotional symptoms</td>
<td>.70 (.53-.80)</td>
<td>.19 (.02-.27)</td>
<td>.26 (.15-.36)</td>
<td>.17 (.07-.27)</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>.66 (.46-.77)</td>
<td>.08 (.00-.24)</td>
<td>.20 (.09-.29)</td>
<td>.06 (.00-.15)</td>
</tr>
<tr>
<td>Conduct problems</td>
<td>.63 (.45-.75)</td>
<td>.25 (.07-.41)</td>
<td>.06 (.00-.18)</td>
<td>.01 (.00-.11)</td>
</tr>
</tbody>
</table>

Note: Significant estimates (i.e. 95% CI not spanning zero) are given in bold. n/a = not available.

$^a$ Maximum likelihood within-trait twin correlations ($r_{MZ}$ and $r_{DZ}$) estimated in a model with two thresholds on the liability to ASD fixed to population values of broad spectrum diagnosis (5%) and ASD (1%) prevalence.

$^b$ Maximum likelihood cross-twin cross-ASD–BeD correlations, obtained for each SDQ measure and ASD–BeD separately.

$^c$ For ASD–BeD, 3 sets of MZ and DZ correlations are available as 3 bivariate analyses were performed: here only one is provided (the other two were of values identical to one decimal place and with overlapping 95% CI).

Table 2 Phenotypic Overlap Due to Genetic and Environmental Effects

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r_{PH}^b$</th>
<th>$r_{PH,A}^c$</th>
<th>$r_{PH,E}^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional symptoms</td>
<td>.32 (.23-.40)</td>
<td>.32 (.23-.40) [100%]</td>
<td>-</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>.29 (.20-.37)</td>
<td>.18 (.06-.30) [62%]</td>
<td>.11 (.03-.20) [38%]</td>
</tr>
<tr>
<td>Conduct problems</td>
<td>.10 (.04-.16)</td>
<td>-</td>
<td>.10 (.04-.16) [100%]</td>
</tr>
</tbody>
</table>

Note: Significant estimates (i.e., 95% CI not spanning zero) are given in bold.

$^a$ Thresholds for the autism spectrum disorder (ASD) liability were set at 5% (broad spectrum disorder) and 1% (ASD).

$^b$ $r_{PH}$ - phenotypic correlation between ASD–best-estimate diagnosis (BeD) and each Strengths and Difficulties Questionnaire (SDQ) measure (95% CI).

$^c$ $r_{PH,A}$, $r_{PH,E}$ - extent to which the phenotypic correlation ($r_{PH}$) is due to correlated genetic and non-shared environmental influences (95% CI). Values in square brackets are percentages of $r_{PH}$.
Table S1: Concordance/Discordance on Strengths and Difficulties Questionnaire (SDQ) Measures Among Monozygotic (MZ) and Dizygotic (DZ) Social Relationship Study Sample Twin Pairs, Split Into Groups by Concordance Status on Best-Estimate Diagnosis (BeD)

<table>
<thead>
<tr>
<th>BeD Concordance status (207 pairs)</th>
<th>Pairs with SDQ available (166 pairs)</th>
<th>Concordant affected on SDQ category</th>
<th>Discordant on SDQ category</th>
<th>Unaffected on SDQ category</th>
<th>Twin pairs missing SDQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrow ASD MZ=17, DZ=11</td>
<td>Emo 13, 9</td>
<td>8, 4 (62%) (45%)</td>
<td>3, 4 (23%) (45%)</td>
<td>2, 1 (15%) (10%)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Hyp 13, 9</td>
<td>6, 3 (46%) (33%)</td>
<td>2, 3 (15%) (33%)</td>
<td>5, 3 (39%) (33%)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Con 13, 9</td>
<td>1, 1 (8%) (11%)</td>
<td>3, 4 (23%) (44%)</td>
<td>9, 4 (69%) (44%)</td>
<td>6</td>
</tr>
<tr>
<td>Narrow ASD + broad spectrum MZ=7³, DZ=19¹</td>
<td>Emo 5, 13³</td>
<td>4, 2 (80%) (15%)</td>
<td>1, 6 (20%) (46%)</td>
<td>0, 5 (0%) (39%)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Hyp 5, 13³</td>
<td>2, 5 (40%) (39%)</td>
<td>2, 5 (40%) (39%)</td>
<td>1, 3 (20%) (22%)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Con 5, 13³</td>
<td>2, 3 (40%) (22%)</td>
<td>3, 5 (60%) (39%)</td>
<td>0, 5 (0%) (39%)</td>
<td>8</td>
</tr>
<tr>
<td>Narrow ASD + unaffected MZ=2, DZ=60</td>
<td>Emo 2, 43</td>
<td>1, 4 (50%) (9%)</td>
<td>0, 18 (0%) (42%)</td>
<td>1, 21 (50%) (49%)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Hyp 2, 43</td>
<td>0, 4 (0%) (9%)</td>
<td>1, 25 (50%) (58%)</td>
<td>1, 14 (50%) (33%)</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Con 2, 43</td>
<td>0, 4 (0%) (9%)</td>
<td>0, 12 (0%) (28%)</td>
<td>2, 27 (100%) (63%)</td>
<td>17</td>
</tr>
<tr>
<td>Broad spectrum + unaffected MZ=1, DZ=10</td>
<td>Emo 1, 9</td>
<td>0, 1 (0%) (11%)</td>
<td>0, 3 (0%) (33%)</td>
<td>1, 5 (100%) (56%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hyp 1, 9</td>
<td>0, 0 (0%) (0%)</td>
<td>0, 2 (0%) (22%)</td>
<td>1, 7 (100%) (78%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Con 1, 9</td>
<td>0, 0 (0%) (0%)</td>
<td>1, 7 (100%) (78%)</td>
<td>0, 2 (0%) (22%)</td>
<td>1</td>
</tr>
<tr>
<td>Unaffected MZ=29, DZ=51</td>
<td>Emo 24, 47</td>
<td>1, 1 (4%) (2%)</td>
<td>6, 10 (25%) (21%)</td>
<td>17, 36 (71%) (77%)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Hyp 24, 47</td>
<td>1, 1 (4%) (2%)</td>
<td>2, 11 (8%) (23%)</td>
<td>21, 35 (88%) (75%)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Con 24, 47</td>
<td>2, 2 (8%) (4%)</td>
<td>4, 13 (17%) (28%)</td>
<td>18, 32 (75%) (68%)</td>
<td>9</td>
</tr>
</tbody>
</table>

Note: All n refers to pairs available. Data are given as n of MZ, n of DZ, except where noted. Con = conduct problems; Emo = emotional symptoms; Hyp = hyperactivity. ¹ 1 MZ and 2 DZ pairs were concordant on broad spectrum; of these 3 pairs, 1 DZ pair had SDQ scores available.
Table S2: Comparison of Fit Indices of the Bivariate AE (Additive Genetic and Non-Shared Environmental Influences) Model, the Reciprocal and Unidirectional Causal Models for Strengths and Difficulties Questionnaire (SDQ) Mental Health Problems and Best-Estimate Diagnosis (BeD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model</th>
<th>-2LL</th>
<th>DF</th>
<th>np</th>
<th>AIC</th>
<th>BIC</th>
<th>$\chi^2$(DF)</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Emotional</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>symptoms</td>
<td>1 Correlations a</td>
<td>2770.477</td>
<td>737</td>
<td>9</td>
<td>1296.477</td>
<td>-1159.736</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2 ACE</td>
<td>2775.600</td>
<td>737</td>
<td>9</td>
<td>1301.600</td>
<td>-1154.614</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 AE</td>
<td>2775.642</td>
<td>740</td>
<td>6</td>
<td>1295.642</td>
<td>-1170.570</td>
<td>0.04(3)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>3a AE ($a_{21}=0$)</td>
<td>2777.029</td>
<td>741</td>
<td>5</td>
<td>1295.029</td>
<td>-1174.515</td>
<td>1.39(1)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>4 Reciprocal AE b</td>
<td>2775.642</td>
<td>740</td>
<td>6</td>
<td>1295.642</td>
<td>-1170.570</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 Drop r: Emo $\rightarrow$ BeD c</td>
<td>2775.849</td>
<td>741</td>
<td>5</td>
<td>1293.849</td>
<td>-1175.695</td>
<td>0.21(1)^d</td>
<td>0.65</td>
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<tr>
<td></td>
<td>6 Drop r': Emo $\leftarrow$ BeD</td>
<td>2778.214</td>
<td>741</td>
<td>5</td>
<td>1296.214</td>
<td>-1173.330</td>
<td>2.57(1)^d</td>
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<tr>
<td>Hyperactivity</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
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<td>1 Correlations a</td>
<td>2883.043</td>
<td>737</td>
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<td>1409.043</td>
<td>-1047.171</td>
<td>-</td>
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<tr>
<td></td>
<td>2 ACE</td>
<td>2890.503</td>
<td>737</td>
<td>9</td>
<td>1416.503</td>
<td>-1039.711</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 AE</td>
<td>2890.559</td>
<td>740</td>
<td>6</td>
<td>1410.559</td>
<td>-1055.653</td>
<td>0.06(3)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>4 Reciprocal AE b</td>
<td>2890.559</td>
<td>740</td>
<td>6</td>
<td>1410.559</td>
<td>-1055.653</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 Drop r: Hyp $\rightarrow$ BeD</td>
<td>2894.847</td>
<td>741</td>
<td>5</td>
<td>1412.847</td>
<td>-1056.698</td>
<td>4.29(1)^d</td>
<td>0.04</td>
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<tr>
<td></td>
<td>6 Drop r': Hyp $\leftarrow$ BeD</td>
<td>2891.150</td>
<td>741</td>
<td>5</td>
<td>1409.150</td>
<td>-1060.395</td>
<td>0.59(1)^d</td>
<td>0.44</td>
</tr>
<tr>
<td>Conduct</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>problems</td>
<td>1 Correlations a</td>
<td>2623.63</td>
<td>737</td>
<td>9</td>
<td>1149.63</td>
<td>-1306.58</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2 ACE</td>
<td>2624.25</td>
<td>737</td>
<td>9</td>
<td>1150.25</td>
<td>-1305.96</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3 AE</td>
<td>2624.32</td>
<td>740</td>
<td>6</td>
<td>1144.32</td>
<td>-1321.89</td>
<td>0.07(3)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>3a AE ($a_{21}=0$)</td>
<td>2625.53</td>
<td>741</td>
<td>5</td>
<td>1143.53</td>
<td>-1326.01</td>
<td>1.28(4)</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>4 Reciprocal AE b</td>
<td>2624.32</td>
<td>740</td>
<td>6</td>
<td>1144.32</td>
<td>-1321.89</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 Drop r: Con $\rightarrow$ BeD</td>
<td>2627.10</td>
<td>741</td>
<td>5</td>
<td>1145.10</td>
<td>-1324.43</td>
<td>2.79(1)^d</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>6 Drop r': Con $\leftarrow$ BeD</td>
<td>2624.53</td>
<td>741</td>
<td>5</td>
<td>1142.53</td>
<td>-1327.01</td>
<td>0.21(1)^d</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Note: Number of observed statistics (os) = 746 (same across all models); degrees of freedom (DF) = os-np (number of parameters). Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) are DF-penalised. In each section, the first $\chi^2$(DF) and p-value concern the difference in minus two log likelihood (-2LL) value of models 3 and 2 (testing the significance of parameter C); the second concern the difference in -2LL value of models 3a and 3 (testing the significance of either parameter $a_{21}$ or $e_{21}$); the third concern the difference in -2LL value of models 5 and 4 (testing the significance of parameter r); the fourth concern the difference in -2LL value of models 6 and 4 (testing the significance of parameter r'). The best-fitting models are highlighted in bold.

Con = conduct problems; Emo = emotional; Hyp = hyperactivity.

a Constrained correlation model: combined continuous SDQ problems and ordinal BeD analysis. To correct for ascertainment, threshold 1 and 2 on the liability to ASD are fixed to correspond to 5% and 1% prevalence. Correlations are further constrained to obtain: 1 overall within-person cross-trait correlation; 1 monozygotic (MZ) cross-trait cross-twin correlation, and 1 dizygotic (DZ) cross-trait cross-twin correlation.
b Because all C parameters were non-significant and estimated to be close to zero, the AE model was selected as the base reciprocal model.
c Best-fitting model (i) as compared to the reciprocal AE model, and based on (ii) the lowest AIC and BIC values.
d Compared to reciprocal causal model.
Supplement 1

The Social Relationship Study (SRS) Sample—Selection Procedure

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. 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.

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 (ages 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) 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), 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 having 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, advertisements 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 having, or diagnosed with, an ASD.

From this group, 89 families could not be contacted or declined to participate in the study, and 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. The weighted kappa statistic for ADI-R and ADOS was .67, indicating a substantial agreement and was 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 group with suspected ASD in terms of gender, zygosity, age, and socioeconomic status (SES). They completed the same battery of assessments (e.g., measures of IQ) as the sample with suspected ASD, but, because they were selected to be at low risk for ASD, they 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.

**Supplement 2**

**Best-Estimate Diagnosis Procedure**

Diagnosis was made according to *DSM-IV* and *International Classification of Diseases – 10th Revision (ICD-10)* criteria and based primarily on scores from the ADI-R and ADOS. After regrouping participants 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), Dr. Bolton and Dr. Colvert 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 Dr. Bolton and Sarah R. Curran 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.

**References:**
