Title: Heritability of Autism Spectrum Disorder in a UK population-based twin sample.

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Abstract (348 words)

Context: Most evidence to date highlights the importance of genetic influences on liability to autism and related traits. However, most of these findings are derived from clinically ascertained samples, possibly missing individuals with subtler manifestations, and obtained estimates may not be representative of the population.

Objective: (i) To establish the relative contributions of genetic and environmental factors in liability to autism spectrum disorder (ASD) and a broader autism phenotype in a large population-based twin sample; (ii) To ascertain the genetic/environmental relationship between dimensional trait measures and categorical diagnostic constructs of ASD.

Design: Joint continuous-ordinal liability threshold model fitting, using full information maximum likelihood to estimate genetic and environmental parameters of the (co)variance of the Childhood Autism Spectrum Test (CAST) and ASD diagnostic measures (the Development and Well-being Assessment (DAWBA), Autism Diagnostic Observation Schedule (ADOS), Autism Diagnostic Interview-Revised (ADI-R), and Best-estimate Diagnosis.

Setting: A population-based UK cohort of twins (Twins Early Development Study; TEDS).

Participants: Twin pairs included in the analysis: CAST n=6423 (mean age 7.9 years); DAWBA n=359 (mean age 10.26); ADOS and ADI-R n=203/205 (mean age 13.21) and Best-estimate Diagnosis n=207.

Main Outcome Measures: Population-based measure of autistic traits, CAST; structured diagnostic assessments (DAWBA, ADI-R, ADOS) and Best-estimate Diagnosis.

Results: On all ASD measures, MZ correlations (ranging from .77-.99) were significantly higher than DZ correlations (ranging from .22-.65), giving heritability estimates of 56-95%. The covariance of CAST and ASD diagnostic status (DAWBA, ADOS and Best-estimate Diagnosis) was largely explained by genetic factors (76-95%). For ADI-R only, shared environmental influences were significant (30%, 95%CI 8-47%), but smaller than genetic influences (56%, 95%CI 37-82%).

Conclusions and relevance: The liability to ASD and a more broadly defined high-autism trait phenotype in this large population-based twin sample derives primarily from genetic and, to a lesser extent, non-shared environmental effects. The largely consistent results across different diagnostic tools suggest the results are generalizable across multiple measures and assessment methods. Genetic factors underpinning individual differences in autistic-like traits show considerable overlap with genetic influences on diagnosed ASD. The robust methodology employed means that these estimates can be taken as a 'benchmark' for future research.

Introduction

Twin studies of autism, conducted from 1977 onwards, provided the first clear evidence that genetic factors were etiologically important [1-6]. Recent reviews of this literature [5, 7-9] show general agreement across studies: concordance for autism in monozygotic (MZ) twin pairs is typically at least double that in dizygotic (DZ) twin pairs, resulting in high heritability estimates (60-90%) [10-14] and suggesting little influence of shared environmental factors. Two twin studies stand in contrast and reported only moderate heritability (21-38%) with a substantial shared environmental component explaining 58-78% of the variance in liability to autism spectrum disorder (ASD) [15, 16]. In comparison, one recent twin study did not confirm significant shared environmental effects and reported heritability of 95% [17]. In addition, a large population study of extended families (~2 million individuals) reported estimates of 50% for both heritability and non-shared environmental factors [18]. Most recently, in the same population, molecular genetic analysis has indicated that 95% of variance in ASD is accounted for by common allelic variants, supporting a polygenic model [19]. This contrasts markedly with heritability estimates around 0 derived from SNP data (GCTA) in an arguably underpowered sample [20]. Given the interest in possible environmental factors in the etiology of autism, these contradictory findings have re-opened the discussion of high heritability and the possibility that findings may be biased by sample selection and screening. The first aim of the current study was to examine the relative importance of genetic and environmental factors in liability to ASD in a large systematically screened population-based twin sample.

Twin and family studies [21, 22] have also shown that the genetic liability to autism confers a risk for a broader range of impairments in social communication, interest patterns and behaviors that extends beyond the traditional diagnostic boundaries for autism [9, 23, 24]. These pioneering studies contributed to the revision and broadening of diagnostic criteria and to the conceptualization of autism as a spectrum encompassing subtypes of pervasive developmental disorders like Asperger's syndrome, atypical autism and subtler presentations [25, 26]. Recent research has explored autistic-like traits in community samples and have provided evidence that there is a genetic correlation between autistic-like traits at the extremes and in the rest of the population [27-31]. Our second aim was therefore to

quantify the genetic and environmental relationship between dimensional trait measures and the categorical diagnostic constructs of ASD (from gold-standard instruments), which has not been done before.

In order to provide a more definitive picture that addresses these two aims and incorporates current diagnostic concepts, we employed rigorous approaches and screened an age-specific epidemiological sample of twins, to ascertain all twins with possible ASD. We then undertook independent, in-depth evaluations of the twins, utilizing a further screening instrument and well-established diagnostic assessment tools. The purpose was to minimize methodological artifacts and provide results that can be used as a 'benchmark' for comparison in future research.

Our approach is novel and contrasts with other recent twin and family studies in both sample ascertainment and analytic methods. Previous studies [15, 16] have identified their twin samples through clinical services. Such a strategy could result in sampling bias; if registration or participation is influenced by concordance, proband-wise concordance rates in DZ pairs might be raised, resulting in inflated estimates of common environmental influence. Additionally, relying solely on clinical ascertainment could result in under-identification of high-functioning cases [32]. It is important to include these cases and define the genetic liability as a continuous distribution that extends beyond stringent diagnostic categories. Our study is novel in using gold-standard, in-person, clinical diagnostic tools with a population-based (versus clinic) sample in which ascertainment was good (62% response rate from the eligible sample, compared with e.g. 17% in Hallmayer et al's study [15]).

In summary, our first aim was estimating heritability of the liability to ASD using a population-based sample, selected via several screening instruments sent to all twins in a three-year birth cohort. The second aim was to study the genetic/environmental relationship between dimensional trait measures and categorical diagnostic constructs of ASD. In contrast to previous approaches [15, 18], we assumed a continuous liability distribution underlying ASD and a more broadly defined phenotype with high level autism traits that fell short of thresholds for an ASD diagnosis. We predicted a strong

genetic overlap between dimensional and diagnostic measures in keeping with previous extremes-based twin analyses [21-23].

Methods

Participants

TEDS and Social Relationship Study (SRS) samples

The participants were recruited from the Twins Early Development Study (TEDS), a longitudinal study of twin pairs ascertained from population records of twin births in England and Wales between 1994 and 1996. The TEDS sample is considered representative of the UK in terms of maternal ethnicity (92.8% white) and education (40.1% with A-levels or higher, the equivalent of some college education in the United States) [33].

The ASD and co-twin sample were selected following a two-stage screening process outlined in Figure 1 and Supplementary Materials (section 1). Of the 412 eligible families at stage one, 80% completed DAWBA interview. At stage two, of the 235 eligible families 62% had diagnostic evaluations. 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.

**********INSERT FIGURE 1 ABOUT HERE**********

Within the final sample those with ASD were broadly comparable to those eligible for participation (CAST ≥ 15 or suspected ASD) but who did not take part, with the exception of gender (Zygosity $\chi^2(1) = 1.5$, p=.23; Socioeconomic Status t(397) = -1.2, p=.25; CAST t(420) = -1.5, p=.14; Gender $\chi^2(1) = 20.1$, p<.001, 36% of the high CAST/suspected ASD group were female, versus 17% of the final sample).

Measures

The Childhood Autism Spectrum Test (CAST)

The CAST is an informant-completed questionnaire based on behavioral descriptions of ASD as delineated in ICD-10 and DSM-IV. The 31 items are scored yes/no and summed; a cut-off score of 15 or above is reported to have 100% sensitivity, 97% specificity and a positive predictive value of 50% for a diagnosis of ASD [28]. CAST data from at least one twin were available from 6423 pairs (MZ=2261, DZ Same Sex=2097, DZ Opposite Sex=2065), with a mean age of 7.9 years (SD=0.53). 289 pairs (4.1% of all pairs; 317 individuals) scored above cut-off.

The Development and Well-being Assessment (DAWBA)

Telephone interviews using the ASD-module of the DAWBA were used at the second stage [34] and included 15 questions about social difficulties, 14 about repetitive, restricted behaviours and interests (RRBI) and three about developmental language milestones. The same parent rated both twins during a phonecall with a single interviewer. A child received a DAWBA diagnosis of autism when the operational criteria in DSM-IV and ICD-10 were met. Asperger's diagnosis was given when parent reports indicated that all autism criteria were met but the child's early language development was not delayed and the child's intellectual ability was in the normal range. ASD (other) diagnosis was assigned if parents reported a minimum of three probable or two definite symptoms from the social difficulties domain, two probable or one definite symptom from the communication domain, and two probable or one definite symptom from the RRBI domain. The measure used in analysis was a three-category diagnosis of ASD: 0 = no ASD/controls, 1 = ASD (other), 2 = Asperger's/Autism.

Autism Diagnostic Interview (ADI-R)

The ADI-R is a well-established diagnostic tool for the assessment of autism. It comprises a semi-structured caregiver interview enquiring about current function and developmental history (93 items), carried out by a trained investigator [35]. Autism Genetics Resource Exchange (AGRE, www.agre.org) criteria were used to assign cases to one of three categories: Autism Spectrum Disorder (comprising AGRE categories Autism and 'Not Quite Autism'), 'Broad Spectrum Disorder', and 'unaffected' (see Supplementary Materials 2 for operational definitions). The measure used in

analysis was a three-category diagnosis of ASD: 0 = no ASD/controls, 1 = Broad Spectrum Disorder, 2 = Autism Spectrum Disorder.

Autism Diagnostic Observation Schedule (ADOS)

The ADOS is a well-validated, semi-structured observational assessment designed to accompany the ADI-R in ASD diagnosis [36]. The current study used recent updates to the ADOS algorithm (provided by C. Lord; Supplementary Materials 2) to yield scores for communication, social interaction and RRBIs. Clinical cut-offs were available for both ASD and autism and these diagnostic groups were combined to create one ASD category. An additional 'Broad Spectrum' category included individuals who scored just below cut-off (-2 points), to correspond to the 'Broad Spectrum' category on the ADI-R. The measure used in analysis was a three-category diagnosis of ASD: 0 = no ASD/controls, 1 = Broad Spectrum, 2 = Autism Spectrum Disorder.

Best-estimate Diagnosis (BeD)

Diagnoses were assigned, blind to zygosity and co-twin diagnostic status, following review of all available information (ADI-R, ADOS, DAWBA, clinical reports). When all available sources of information were in agreement, cases were assigned to that category. In 89 cases the diagnostic classifications across instruments were inconsistent. In these cases all available data were assessed by expert clinicians (PB +/- EC+/- SRC) and Best-estimate Diagnoses were assigned on the basis of this review. See Supplementary Materials 2 for further details. Best-estimate Diagnosis was used in analysis as a three-category measure of ASD: 0 = no ASD/controls, 1 = Broad Spectrum, 2 = ASD.

Data Analysis

Twin Correlations

Twin data analysis was performed in the Structural Equation Modeling program OpenMx [37]. Using full information maximum likelihood estimation, continuous (CAST) and ordinal measures (ASD) were analyzed jointly assuming a liability threshold model to reflect the risk for ASD [38, 39]. To get unbiased estimates, due to the selection of individuals on ASD, thresholds were fixed to z-values

corresponding to 'known' population prevalence: 1st at 5% [40, 41] discriminating between unaffected and Broad Spectrum; 2nd at 1% discriminating between Broad Spectrum and ASD. The assumption of a joint multivariate normal distribution for CAST and the three ASD diagnostic categories (Unaffected, Broad Spectrum, ASD) allowed the estimation of within and across MZ/DZ twin correlations. The MZ/DZ ratios of these correlations indicate the relative importance of genetic and environmental influences on variation within each measure and on the covariance between them, formally tested in the bivariate genetic model.

The Bivariate Genetic Model

Using biometrical genetic theory, the (co)variance of CAST and each ASD diagnosis was modeled as the effects of A (additive genetic), C (shared environment) and E (non-shared environment + measurement error) factors [42]. As the order of traits is immaterial, we interpreted the standardized solution in which the paths from the A_1 factor to CAST and the A_2 factor to ASD are the square roots of their respective heritabilities and the correlation between A_1 and A_2 the genetic correlation between them (r_a) [43, 44]. The same logic applies to non-shared environmental effects (Figure 2). Shared environmental factors were modelled on ASD only, they do not influence the variance of CAST [45], and therefore cannot explain the covariance with ASD. In addition to the standardized path estimates, we calculated the phenotypic correlation (r_{ph}) due to A (r_{ph_a}) as $\sqrt{h_1^2} * r_a * \sqrt{h_2^2}$ and due to E (r_{ph_e}) as $\sqrt{e_1^2} * r_e * \sqrt{e_2^2}$, which can be expressed as proportions of r_{ph} [46, 47].

Results

Probandwise concordance rates

Probandwise concordance rates were calculated by: 2*(concordant N pairs) / 2*(concordant N pairs) + discordant pairs (Table 1). These express the probability that the co-twin of a proband (affected twin) is also affected, and are commonly used as an index of twin resemblance. The high MZ (.62-.94) and low DZ concordances (.05-.61) suggest substantial genetic influence. For example, MZ concordances are .87 for ASD and .94 for Broad Spectrum (BeD), in contrast to .22 and .46, respectively for DZ concordances. However, concordance rates cannot be used to estimate genetic and environmental parameters as they do not take population prevalence rates into account.

**********INSERT TABLE 1 ABOUT HERE**********

Diagnostic Agreement

Agreement of classification of individuals into the three categories (unaffected, Broad Spectrum, ASD) for different diagnostic measures was calculated by means of weighted Kappa coefficients in Stata (w2). These values (-1 to 1) represent the observed agreement between two diagnostic tests relative to the expected agreement between tests occurring by chance alone [48]. There was moderate Kappa agreement for DAWBA and ADOS (.58), substantial agreement for DAWBA and ADI-R and DAWBA and BeD (both .72), for ADI-R and ADOS (.67), for ADOS and BeD (.79), and almost perfect agreement for ADI-R and BeD (.91).

Twin Correlations

The 2:1 MZ/DZ ratio of the cross-twin within-trait correlations for ADOS and BeD suggest a significant contribution of genetic effects, with the remainder explained by non-shared environmental effects (Table 2). This is not the case for DAWBA, where the DZ correlation is less than half that of the MZ pairs, pointing to non-additive genetic effects. For ADI-R the DZ correlation is higher than half the MZ correlation, indicating genetic and shared environmental effects. The MZ/DZ ratio of the

cross-twin cross-trait correlations for CAST and each diagnosis indicate mainly genetic and non-shared environmental influences on their overlap.

**********INSERT TABLE 2 ABOUT HERE**********

The Bivariate Genetic Model

Table 3 reports the standardized results of the bivariate ACE models. Variance in CAST (age and sex regressed) was due to genetic influences (78%, 95% CI: 76–79%) and non-shared environmental effects (22%, 95% CI: 21-23%), as reported previously [30]. Genetic influences were significant for all clinical measures with the highest heritability reported for BeD (95%, 95% CI: 74-98%) and shared environments significantly explaining the variance of ADI-R only (30%, 95% CI: 08-47%). The correlations between CAST and each of the ASD variables (r_{ph}, column 7) is the sum of the paths via the A and E factors connecting the two variables (r_{ph_a} and r_{ph_e}, column 8-9). The phenotypic correlations were moderate to high (.52-.65) and genetic factors accounting for 77-100% of the covariance. The genetic correlations, i.e. the extent to which the same genetic factors influence CAST and clinical measures independent of their heritabilities, are substantial (.52-.89). The remainder of the covariance was explained by non-shared environmental factors (r_{ph_e}), although non-significant for the overlap between CAST and ADI-R or ADOS. Figure 2 is the path diagram with standardized estimates of the reduced bivariate A(C)E model for CAST and BeD, the best diagnostic estimate of ASD. The findings indicate strong and overlapping genetic influences on both dimensional and categorical measures.

**********INSERT FIGURE 2 ABOUT HERE**********

**********INSERT TABLE 3 ABOUT HERE**********

Discussion

Key findings

The current study examined the genetic and environmental contributions to ASD in a large systematically screened population-based twin sample, and the genetic/environmental overlap between a continuous measure of autistic-like traits and categorical diagnostic assessments. Our study was novel in including subclinical high trait and selected low risk twins as well as those with diagnosed ASD in order to capture the full range of liability. The probandwise concordance rates and liability threshold model analyses reassert the importance of genetic factors in the etiology of ASD. Analyses partitioning liability into genetic, shared and non-shared environmental components indicated that the majority of liability could be attributed to additive genetic influences and a smaller proportion attributed to non-shared environmental influences. This held across a number of different measures. There was very little evidence for shared environmental effects overall, contra Hallmayer et al's findings [15]; although the wide CIs in their results for A and C overlap with some of the present estimates. In our study, only the ADI-R parent-reported developmental history measure showed significant shared environment effects. Since the ADI-R was completed by the same parent for both twins, the estimated influence of shared environment may be inflated by rater bias. However, the wide confidence intervals (.08-.47) warrant caution in interpretation.

Our findings also confirm that the heritability of the liability to ASD is high when incorporating subclinical high trait score cases into the model, extending support for the notion that the genetic liability to autism confers a risk for a 'broader' autism phenotype. Indeed, the relationship between CAST and the diagnostic assessments indicated a substantial genetic correlation as well as a significant correlation in the non-shared environmental factors that influence variations in both traits. This indicates common etiological underpinnings for individual differences in autistic traits across the whole spectrum and in our three clinically meaningful categories (ASD, subclinical high trait and low risk/trait). This provides support for examining broader autistic traits in the general population as a complementary strategy for identifying the genetic risk factors for ASD [49-51]. Our findings are

broadly in line with those of recent twin and family studies, pointing towards strong genetic effects in ASD and no strong influence from shared environmental factors. The strengths of the current study add validity to these conclusions, as previous research has often lacked the rigor and systematic approach to sample selection employed here. The population-based sampling in the current study, the two stage systematic screening methods employed and the inclusion of sub-clinical individuals ensured the capture of a more complete picture of genetic risk (additive and non-additive) to ASD than in previous studies. A novel contribution is the strong evidence that the same genetic influences are largely responsible for the overlap between dimensional trait measures and categorical diagnostic constructs of ASD. Additionally, this is one of the largest screened population-based twin studies yet reported.

The limitations of this study include the fact that a minority of the potentially eligible twin pairs did not enroll in the study. Secondly, although being one of the largest twin studies, the sample size was insufficient to allow any meaningful analyses of the basis for sex differences in ASD. In addition, twin study methodologies assume that the environments of MZ and DZ [52] twins are equal, and that twins are not at especially high risk for the disorder under investigation. The available evidence indicates that both these assumptions are justified in this study [53]. Another issue is that genetic modeling assumes that there are no gene-environment interactions or correlations; if these exist, the estimates of both environmental and genetic effects may be inflated [54]. Heritability estimates are also population specific and depend on the dynamic interaction with the current environment. Our analysis took a liability threshold approach but clearly other types of analyses (e.g. continuous data modeling, DeFries-Fulker quantile regression) are possible and may be warranted by future developments in the molecular genetics of ASD. Recent findings lend support to a polygenic trait approach [19].

Conclusion

The current study brings together the strengths of previous studies and provides a more complete picture than any of them individually, by being nationally representative and incorporating

dimensional as well as categorical measures using a systematic repeated screening methodology. We conclude that liability to ASD and a more broadly defined high autism trait phenotype in UK twins aged 8+ years derives from substantial genetic and moderate non-shared environmental influences. Genetic influences on diagnosed ASD are shared with those on autistic traits in the general population.

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Table 1 MZ and DZ Probandwise Concordance Rates across MZ and DZ affected twins (including same and opposite sex twins).

ASD [a]	MZ twin pairs [c] Discordant/Concordant	MZ Probandwise Concordance Rate	DZ twin pairs [c] Discordant/Concordant	DZ Probandwise Concordance Rate
DAWBA	12/15	.71	74/2	.05
ADI-R	15/12	.62	80/8	.17
ADOS	8/12	.75	57/9	.40
Best-estimate Diagnosis	8/17	.87	77/11	.22
ASD+Broad Spectrum [b]				
DAWBA	16/24	.75	118/5	.08
ADI-R	4/24	.92	54/43	.61
ADOS	7/16	.82	56/18	.39
Best-estimate Diagnosis	3/24	.94	70/30	.46

[a] ASD rates reflect twins included in category 2 only.

[b] ASD+Broad Spectrum rates reflect pairs in which a child was either included in category 1 or 2.

[c] Number of Discordant and Concordant pairs included in the calculation.

Table 2 MZ and DZ within-trait and cross-trait twin correlations (95% CI) based on 4 bivariate analyses of CAST with each diagnostic ASD measure.

	r_{MZ} [a]	r_{DZ} [a]	cross-twin cross-trait (MZ) [b]	cross-twin cross-trait (DZ) [b]
CAST	**.79** (.77-.80) [c]	**.31** (.28-.33) [c]	n/a	n/a
DAWBA	**.82** (.67-.90)	.22 (.00-.42)	**.40** (.34-.45)	-.01 (.00-.07)
ADI-R	**.87** (.77-.93)	**.65** (.55-.73)	**.60** (.54-.65)	**.40** (.35-.45)
ADOS	**.77** (.62-.87)	**.40** (.26-.63)	**.56** (.49-.61)	**.30** (.23-.37)
Best-estimate Diagnosis	**.99** (.98-.99)	**.53** (.41-.63)	**.61** (.57-.66)	**.37** (.31-.42)

[a] Maximum likelihood within-trait twin correlations (r_{MZ} & r_{DZ}, including same and opposite sex DZ pairs) estimated in a model with the two thresholds on the liability to ASD set to population values of Broad Spectrum (5%) and ASD (1%) prevalence.

[b] Maximum likelihood cross-twin cross-CAST correlation, obtained for each diagnostic variable and CAST separately.

[c] For CAST, 4 sets of correlations are available as 4 bivariate analyses were performed; here only one is given (the other three were of similar value and with overlapping 95% Confidence Intervals).

Significant estimates (i.e. 95% Confidence Intervals not spanning zero) are given in **bold**.

Table 3 Standardized Estimates (95% CI) of the reduced A(C)E bivariate models of CAST and each of the 4 clinical measures of ASD.

	h^2	c^2	e^2	r_a[a]	r_e[a]	r_{ph}[b]	r_{ph_a}[c]	r_{ph_e}[c]
CAST	**.78** (.77-.79)	.00 (.00-.00)	**.22** (.21-.23)	-	-	-	-	-
DAWBA	**.78** (.48-.87)	.00 (.00-.00)	**.22** (.13-.36)	**.52** (.47-.67)	**.48** (.32-.64)	**.52** (.48-.55)	**.40** (77%)	**.12** (23%)
ADI-R	**.56** (.37-.82)	**.30** (.08-.47)	**.14** (.07-.47)	**.89** (.70-.99)	.19 (.00-.41)	**.61** (.56-.66)	**.58** (≈100%)	.03 (≈0%)
ADOS	**.76** (.41-.86)	.00 (.00-.30)	**.24** (.14-.39)	**.73** (.63-.99)	-.02 (.00-.15)	**.54** (.51-.60)	**.56** (≈100%)	-.02 (≈0%)
Best-estimate Diagnosis	**.95** (.74-.98)	.00 (.00-.26)	**.05** (.02-.17)	**.70** (.63-.80)	**.48** (.16-.84)	**.65** (.24-.67)	**.60** (92%)	**.05** (8%)

Thresholds on the ASD liability were fixed at 5% (Broad Spectrum) and 1% (ASD); the estimates for CAST across the four models were of similar value and with overlapping 95% Confidence Intervals;

[a] r_a, r_e - genetic and non-shared environmental correlations between CAST and ASD measure;

[b] r_{ph} - phenotypic correlation between CAST and ASD measures;

[c] r_{ph_a}, r_{ph_e} - part of the phenotypic correlation due to genetic and unique environmental influences, respectively; values in parentheses are percentages of r_{ph}. Proportions cannot be calculated for ADOS due to the opposite signs of r_{ph_a} and r_{ph_e} , but if we disregard the non-significant contributions of r_{ph_e} for both the CAST-ADOS and CAST-ADI-R relationships, shared genetic effects explain nearly all of the observed correlations.