The etiology of autistic traits in preschoolers: a population-based twin study

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Background: Autism Spectrum Disorders (ASD) are highly heritable, but the exact etiological mechanisms underlying the condition are still unclear. **Methods:** Using a multiple rater twin design in a large sample of general population preschool twins, this study aimed to (a) estimate the contribution of genetic and environmental factors to autistic traits, controlling for the possible effects of rater bias, (b) to explore possible sex differences in etiology and (c) to investigate the discordance in autistic traits in monozygotic and same-sex dizygotic twin pairs. The Netherlands Twin Register collected maternal and paternal ratings on autistic traits from a general population of 38,798 three-year-old twins. Autistic traits were assessed with the DSM-oriented Pervasive Developmental Problems scale of the Child Behavior Check List for preschoolers (1½–5 years). **Results:** Mother and fathers showed high agreement in their assessment of autistic traits ($r = .60$–$.66$). Differences between children in autistic traits were largely accounted for by genetic effects (boys: 78% and girls: 83%). Environmental effects that are unique to a child also played a modest role. Environmental effects shared by children growing up in the same family were negligible, once rater bias was controlled for. While the prevalence for clinical ASD is higher in boys than in girls, this study did not find evidence for striking differences in the etiology of autistic traits across the sexes. Even though the heritability was high, 29% of MZ twin pairs were discordant for high autistic traits (clinical range vs. normal development), suggesting that despite high genetic risk, environmental factors might lead to resilience, unaffected status in the context of genetic risk, in some children. **Conclusions:** It is important to focus future research on risk factors that might interplay with a genetic disposition for ASD, but also on protective factors that make a difference in the lives of children at genetic risk. **Keywords:** Autism spectrum disorder; heritability; child behavior checklist; twins.

Introduction

Autism Spectrum Disorders (ASD) is a neurodevelopmental disorder characterized by difficulties in social interaction and (non)verbal communication as well as by restricted and repetitive behaviors and interests (American Psychiatric Association, 2013). ASD is one of the most debilitating of the developmental disabilities in children under 5 (Baxter et al., 2015). Symptoms of ASD are typically evident before the age of 3 years and the first noticeable delays are often related to speech and language (Lord, Cook, Leventhal, & Amaral, 2000). An estimate for the global prevalence of ASD is around 1% (Baxter et al., 2015; Christensen, 2016) and the ratio in boys versus girls is 4:1 (Fombonne, 2003). A third to half of children with an ASD diagnosis have an intellectual disability (IQ < 70) (Christensen, 2016), but ASD is diagnosed along the entire range of cognitive ability.

The occurrence of an ASD diagnosis in a child is on average 20–25 times higher when a sibling is affected (Pisula & Ziegart-Sadowska, 2015; Risch et al., 2014). Relatives of individuals with ASD often show characteristics similar in kind but milder in degree than clinical autism, an observation also referred to as the broader autism phenotype (Sucksmith, Roth, & Hoekstra, 2011). Twin studies suggest that the familial risk of ASD is due to genetic factors, with high concordance rates in monozygotic (MZ) twins, which are, in general, at least double the concordance rates in dizygotic (DZ) twins (Folstein & Rutter, 1977; Ronald & Hoekstra, 2014). Recent molecular studies confirm the influence of genetic factors (Robinson et al., 2015).

It has been proposed that clinical ASD, like many other disorders, represents the extreme end of a constellation of continuously distributed traits that can also be observed in the general population (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Constantino, 2011). Heritability estimates of autistic traits in the general population of school-aged children, as rated by parents and teachers, vary between 60% and 90%. Most studies found that the environment shared between family members (the 'common environment') had no or only a negligible influence on individual differences in autistic traits [though see (Hallmayer et al., 2011) for an exception, and (Tick, Bolton, Happé, Rutter, & Rijsdijk, 2016) for an explanation for these findings].

Less is known about the heritability of autism in preschoolers. Two studies reported the heritability of autistic traits in preschoolers and found estimates of around 40% (Edelson & Saudino, 2009; Stilp,

Gernsbacher, Schweigert, Arneson, & Goldsmith, 2010). In contrast to school-aged children, both studies suggested that common environmental factors played a significant role. One study from the Wisconsin Twin panel found a heritability of 44%(CI: 0%–90%) and common environmental effects accounted for 32% (CI: 0%–76%) of the variance in a sample of 1,211 2 to 3-year-old twin pairs using the Modified Checklist for Autism in Toddlers. However, when using a stricter cut-off for the ASD category the estimate of the genetic effects increased to 74% (CI: 3%–99%) and the influence of the common environment decreased to 19% (CI: 0%–75%) (Stilp et al., 2010). In a sample of 313 2-year-old same-sex twin pairs from the Boston University Twin Project heritability was reported at 38% (CI: 14%–59%) and common environment (21%: CI: 4%–40%) had a modest influence on autistic traits measured with the Child Behavior Check List (Edelson & Saudino, 2009).

In general, mothers and fathers show a relatively high agreement in their ratings of problem behaviors in their offspring ($r = .60$) (Achenbach, McConaughy, & Howell, 1987). Rater disagreement can be due to rater bias (e.g. a parent's skewed perception of problem behavior) or caused by real behavioral differences, because the parents may observe and interact with their child in distinct situations. Using the agreement between parents to determine the underlying causes of autistic traits will result in more reliable estimates of heritability as it controls for the possible effects of rater bias. A twin study looking at the etiological overlap between autistic traits as rated by parents, teachers and children in 9-year-olds concluded that genetic and environmental effects were not entirely correlated and there were some rater specific genetic and environmental effects (Ronald, Happé, & Plomin, 2008).

A relatively unexplored topic in autism research is sex differences in the genetic etiology of autism. The high ratio of boys versus girls with ASD raises the question whether the underlying etiology in autistic traits is different for boys and girls. It could be that different genes or different environments contribute to individual differences in ASD or that the same genes and environments play a role, but to a different extent. Including opposite-sex twin pairs form the key to investigate these differences in etiology (Eaves, Last, Young, & Martin, 1978; Vink et al., 2012). If different genes are expressed in boys and girls, it is expected that children of an opposite-sex twin pair resemble each other to a different extent than children of a same-sex twin pair. In general population twin samples of school-aged children, no or only small qualitative and quantitative differences between boys and girls in the genetic etiology of autistic traits were observed (Constantino & Todd, 2003; Ronald, Happé, & Plomin, 2005; Ronald, Larsson, Anckarsäter, & Lichtenstein, 2011).

Advancing our understanding of the origins of individual differences in autistic traits in preschoolers is important, as this is the age at which an ASD can first be reliably diagnosed (Chawarska, Klin, Paul, & Volkmar, 2007). Moreover, evidence suggests that early interventions from the preschool age onwards are likely to be most effective, as environmental effects might have a larger impact when brain plasticity is maximal (Dawson et al., 2010). Although genetic effects play a substantial role in ASD, concordance rate of MZ twins is not 100% (Folstein & Rutter, 1977; Ronald & Hoekstra, 2014). An increasing amount of evidence is pointing to the critical role of the environment in the emergence of the disorder (Mandy & Lai, 2016). Discordance might be due to adverse environmental factors triggering the genetic risk for ASD or it could be that protective environmental factors prevent a child at risk from developing ASD (resilience) (Masten, 2001). Identifying adverse and/or protective environmental factors is important to provide more insight into why some children develop ASD.

Using a multiple rater twin design in a large sample of general population preschool twins, this study aims to (a) estimate the contribution of genetic and environmental factors to autistic traits, controlling for the possible effects of rater bias, (b) explore possible sex differences in etiology of autistic traits and (c) to investigate the discordance in autistic traits in MZ and same-sex DZ twin pairs.

Methods
Participants

The Netherlands Twin Register (NTR) was established around 1987 by the Department of Biological Psychology at the Vrije Universiteit Amsterdam and registers approximately 40% of all multiple births in the Netherlands. The NTR sample is population-based and is considered to be generally representative of the twin population in the Netherlands (van Beijsterveldt et al., 2013). The parents of twins participate in longitudinal data collection and the survey sent to mothers and fathers of twins at age 3 includes the Child Behavior Check List (CBCL) 1.5–5 (Achenbach & Rescorla, 2000). Informed consent has been obtained for all participants and data collection was approved by the medical ethical review committee of the VU Medical Centre Amsterdam (IRB00002991). Maternal and/or paternal ratings, for the vast majority from biological parents, on autistic traits were available for 38,798 3-year-olds ($M = 3.3$ years, $SD = .3$) from birth cohorts 1989–2010. For several cohorts (1989–1990) surveys were collected only from mothers (due to financial constraints). Most of the children had both a maternal and a paternal rating on autistic traits (63.8%) while for the remaining children only one rating on autistic traits was available (mother: 33.7%; father: 2.5%). The response rate was 63.4% in mothers and 43.2% in fathers.

The sample consisted of 3,123 MZ male twin pairs (MZm), 3,366 DZ male twin pairs (DZm), 3,435 MZ female twin pairs (MZf), 3,090 DZ female twin pairs (DZf) and 6,404 DZ twin pairs of opposite sex (DOS). Twin pairs for which zygosity was unknown were excluded from the analyses ($N = 11$). For same-sex pairs, the determination of zygosity status was based on blood markers ($N = 223$) or DNA polymorphisms ($N = 1,578$). For the remaining same-sex pairs, a discriminant analysis

using the survey items from the longitudinal parental reports on resemblance in appearance and confusion of the twins by parents and others was applied to establish zygosity (Rietveld et al., 2000).

Measures

The Child Behavior Check List (CBCL) for preschoolers (1½– 5 years) assesses behavior at home (Achenbach & Rescorla, 2000). Mothers and fathers are asked to indicate for 99 problem items whether a child displayed a certain type of behavior currently or in the prior 2 months. The CBCL includes the DSM-oriented scale Pervasive Developmental Problems (PDP), consisting of 13 items which psychiatrists and psychologists indicated to be in line with the DSM-IV diagnostic categories of Asperger's disorder and autistic disorder relevant to preschoolers (American Psychiatric Association, 1994). Items (e.g. 'Disturbed by any change in routine' and 'Shows little affection toward people') are scored on a 3-point scale from 0 (not true or never) to 2 (completely true or very often). The 1-week test-retest reliability of the PDP scale is 0.86 and the internal reliability is 0.80 (Achenbach & Rescorla, 2000). In our sample the internal reliability was slightly lower for both mother ($\alpha = 0.69$) and father ($\alpha = 0.68$) ratings.

The PDP scale has been shown to be able to reliably distinguish preschoolers subsequently diagnosed with ASD from children with typical development (sensitivity = 0.98; specificity = 0.91). The proposed cut-off score for identifying children with ASD is a T score of 70 (sum score 9 or higher) (clinical range) while a score between 65 and 70 is considered to be indicative of an ASD (sum score of 7 or 8) (subclinical range) (Achenbach & Rescorla, 2000; Narzisi et al., 2013).

Statistical analyses

Sum scores for the PDP scale were computed when subjects had no or at most one missing item on the scale. A missing item was imputed by the averaged item score of the scale for that child. Twin correlations were estimated by maximum likelihood estimation (MLR) in Mplus (Muthén & Muthén, 2010), in a saturated model which also included estimation of means and variances for boys and for girls for mother and father ratings. We tested for mean differences between MZ and DZ twins, between boys and girls and between mother and father ratings. Twin correlations were estimated for the five different zygosity-by-sex groups (MZm, DZm, MZf, DZf and DOS). Twin correlations, separately for mother and father ratings, the correlations between mother and father ratings and the cross-correlations (father rating of twin 1 with mother rating of twin 2 and vice versa) were estimated for the five sex-by-zygosity groups.

Twin studies, which capitalize on the difference in genetic relatedness between MZ and DZ twins, can be used to explore the underlying etiology of autistic traits in the general population. For an extended description of the twin method and its assumptions, see for example (Knopik, Neiderhiser, DeFries, & Plomin, 2016; Posthuma et al., 2003). The variance in traits such as ratings of autism can be decomposed into variance due to additive genetic effects (A), dominant genetic effects (D) or common environment (C) and unique environment (E). Additive genetic effects are the sum of all allelic effects across multiple loci. Dominant genetic effects result from interactions between alleles. Common environmental effects are influences that are shared between twins who grow up in the same family and enhance their similarity beyond the similarity due to shared genes. Unique environmental effects are influences that are not shared between twins and make children less similar. The unique environmental effects can also include measurement error.

A psychometric rater model was used to decompose the variance in the behavior that both parents assessed similarly

and on the behavior that parents rated differently (Bartels et al., 2007; Hewitt, Silberg, Neale, Eaves, & Erickson, 1992). The shared behavioral view represents a more reliable measure of autistic traits as it is based on the agreement between two parents resulting in more reliable estimates of the genetic and environmental effects. In addition, parent-specific effects denote disagreement between mothers and fathers. Parental disagreement could be due to mothers and fathers assessing a unique and different aspect of the child's behavior reflected in the genetic effects. Disagreement between parents can also be due to rater bias, which could for example comprise a certain view on autism, and this will end up in the common environmental effects. The view of a rater would bias the ratings of both type of twins in the same way, and thereby artificially inflates the estimate of the influence of the common environment. Mother and father ratings are not expected to show the same bias in their ratings, making it parent specific. Thus, if an influence of the common environment is found on the shared parental view, one can be confident the effect is not due to bias (Saudino, 2005). In the psychometric model, qualitative sex differences were tested by constraining the genetic correlation in DOS twins to 0.5 and quantitative sex differences were tested by constraining the magnitude of genetic and environmental influences to be equal across sex. Next, the significance of additive genetic, dominant genetic, and common environmental effects was tested.

The difference in model fit between the nested models was assessed with a log-likelihood ratio test (LRT) which calculates the difference in −2log-likelihood (−2LL) between the models and evaluates the chi square-statistic using the difference in degrees of freedom between the models. To correct for multiple testing, the Bonferroni method was used and a p-value of <.004 (.05/12) was considered significant.

Results

Table 1 gives the prevalence of the different item responses for all items of the CBCL PDP scale, separately for mother and father ratings. Figure 1 displays the distribution of the sum scores on the PDP scale, separately for mother and father ratings and for boys and girls. Significance testing in the saturated model revealed that sum scores for autistic traits did not differ between first and second born twins (χ^2 (8) = 19.2, p = .014). There were mean differences between MZ and DZ twins (χ^2 (8) = 89.5, p < .001), between boys and girls (χ^2 (8) = 270.7, p < .001) and across parents (χ^2 (6) = 60.3, p < .001). MZ twins scored higher than DZ twins on the PDP scale. Boys showed more autistic traits than girls and mothers reported more autistic traits than fathers (see Table 2). The agreement between parents ranged from .60 to .66, indicating that mothers and fathers agree quite strongly in their assessment of each child's autistic traits. Correlations between twins and between parental ratings for the five zygosity-by-sex groups are reported in Table 3. All MZ correlations were higher than DZ correlations, suggesting an important role for genetic effects.

The psychometric rater model indicated that mothers and fathers hold a largely shared view on autistic traits. The correlation between the genetic effects in opposite-sex twin pairs could be constrained to be 0.5 (χ^2 (1) = 1.6, p = .211; model 3 in Table 4), suggesting absence of qualitative sex differences,

Table 1 Items. Prevalence of the item responses on the DSM-oriented scale pervasive developmental problems (CBCL 1½–5)

	Mother ratings				Father ratings			
	N	Never	Sometimes	Often	N	Never	Sometimes	Often
Afraid to try new	37,992	54.2	41.4	4.5	26,245	58.9	37.2	3.9
Avoids eye contact	37,967	68.2	28.3	3.5	26,229	68.1	28.7	3.2
Cannot stand things out of place	37,938	75.3	19.4	5.3	26,234	74.7	20.0	5.3
Disturbed by change	37,869	59.5	34.1	6.4	26,188	60.5	34.1	5.5
Does not answer	37,718	52.5	41.8	5.7	26,070	55.8	39.3	4.9
Does not get along with peers	37,935	90.2	9.1	0.7	26,237	91.7	7.9	0.5
Rocks head, body	37,864	94.4	2.8	2.3	26,147	95.0	3.2	1.8
Unresponsive to affection	37,892	94.1	4.8	1.1	26,172	93.6	5.1	1.3
Little affection	35,715	94.3	5.2	0.5	26,164	93.6	5.9	0.5
Speech problem	37,548	74.2	17.6	8.2	25,915	77.4	16.1	6.5
Strange behavior	37,301	94.4	3.9	1.7	25,863	95.8	3.1	1.1
Upset by new	37,678	73.2	22.7	4.1	26,091	75.7	21.3	3.0
Withdrawn	37,934	92.5	6.8	0.7	26,183	93.0	6.3	0.7
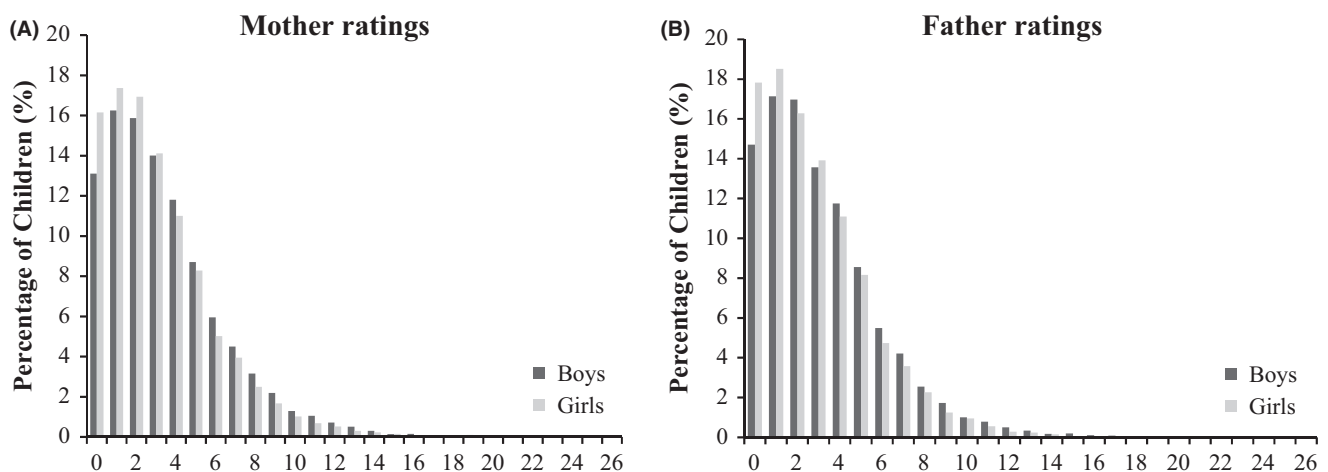
Figure 1 Autistic traits. Distribution of the Pervasive Developmental Problems (CBCL 1.5–5) sum scores as rated by mothers (A) and fathers (B)

Table 2 Descriptives. Means, standard deviations (SD) and sample size of the sum scores on the DSM-oriented scale pervasive developmental problems (CBCL 1½–5)

	Mother ratings			Father ratings		
	N	Mean	SD	N	Mean	SD
MZm	6,089	3.45	3.0	4,251	3.19	2.8
DZm	6,551	3.44	3.0	4,413	3.20	2.9
MZf	6,699	3.28	2.8	4,664	3.09	2.7
DZf	6,039	3.21	2.8	4,053	3.01	2.7
DOSm	6,211	3.53	3.0	4,181	3.36	2.9
DOSf	6,217	2.71	2.6	4,164	2.61	2.5

MZm = monozygotic boys; DZm = dizygotic boys; MZf = monozygotic girls; DZf = dizygotic girls; DOSm = dizygotic of opposite sex boys; DOSf = dizygotic of opposite sex girls.

that is, the expression of the same genes in boys and girls. The path loadings of the latent A, C or D, and E factors could not be constrained to be equal in boys and girls (χ^2 (8) = 77.6, $p < .001$; model 4 in Table 4), suggesting presence of quantitative sex differences. Next, the parameter estimates of the shared and specific genetic and environmental effects were

dropped from the model (models 5–8 in Table 4). All parameters were found to be significant (leading to a significant deterioration of model fit).

The standardized parameter estimates and their 95% confidence intervals for the best-fitting model are displayed in Figure 2. Most of the total variance in autistic traits rated by mothers and fathers was shared by both raters [boys (mothers: 62%; fathers: 61%); girls (mothers: 64%; fathers: 65%)], indicating that mothers and fathers observe and report similar behavior in their children. Additive (boys: 53%; girls: 67%) as well as dominant (boys: 25%; girls: 16%) genetic effects had an influence on the shared behavioral view on autistic traits resulting in a high broad sense heritability, both in boys (78%) and girls (83%). Additive genetic effects also had some, although much lower, influence on the specific behavioral view of mothers (boys: 17%; girls: 15%) and fathers (boys: 12%; girls: 9%). Common environmental effects had no influence on the shared behavioral view on autistic traits and a small influence on the specific behavioral view of mothers (boys: 8%; girls: 8%) and fathers (boys: 16%; girls:

Table 3 Correlations (95% confidence intervals). Cross-rater within-twin, within-rater cross-twin and cross-rater cross-twin correlations for the DSM-oriented scale pervasive developmental problems (CBCL 1½–5)

	Cross-rater Within-twin correlations	Within- rater (mother) Cross-twin correlations	Within- rater (father) Cross-twin correlations	Cross- rater Cross-twin correlations
MZm	.64 (.61–.68)/.63 (.59–.67)	.74 (.71–.77)	.77 (.74–.80)	.51 (.47–.56)/.50 (.46–.54)
DZm	.66 (.63–.70)/.65 (.62–.69)	.37 (.33–.41)	.41 (.36–.46)	.23 (.18–.28)/.21 (.17–.26)
MZf	.64 (.61–.68)/.61 (.58–.65)	.74 (.72–.77)	.77 (.75–.80)	.51 (.48–.55)/.51 (.47–.55)
DZf	.62 (.59–.66)/.60 (.57–.64)	.38 (.34–.42)	.43 (.39–.48)	.22 (.17–.27)/.19 (.15–.24)
DOS	.66 (.63–.68)/.60 (.58–.63)	.39 (.36–.42)	.43 (.40–.46)	.24 (.20–.27)/.23 (.20–.26)

MZm = monozygotic male twin pairs; DZm dizygotic male twin pairs; MZf = monozygotic female twin pairs; DZf = dizygotic female twin pairs; DOS = dizygotic of opposite sex twin pairs.

Table 4 Model fitting. Multivariate results for the DSM-oriented scale Pervasive Developmental Problems (CBCL 1½–5) as rated by mothers and fathers

	ep	−2LL	AIC	BIC	Compared to model	$\Delta\chi^2$	Δdf	p
0 Saturated model	70	143,128.5	286,397.0	286,948.2	–	–	–	–
1 ACE model	31	143,282.0	286,626.1	286,870.2	0	153.5	39	<.001
2 ADE model	31	143,269.1	286,600.2	286,844.3	0	140.6	39	<.001
3 No qualitative sex differences[a]	30	143,270.6	286,601.3	286,837.5	2	1.6	1	.211
4 No quantitative sex differences	22	143,348.2	286,740.4	286,913.7	3	77.6	8	<.001
5 No shared A	28	143,350.0	286,756.0	286,976.5	3	79.8	2	<.001
6 No shared D	28	143,282.6	286,621.2	286,841.7	3	11.9	2	.003
7 No specific A	26	143,389.0	286,830.1	287,034.8	3	118.3	4	<.001
8 No specific C	26	143,409.6	286,871.3	287,076.0	3	138.9	4	<.001

ep = estimated parameters; −2LL = −2log likelihood; df = degrees of freedom; AIC = Akaike information criterion; BIC = Bayesian information criterion.
[a]Best fitting model.

15%).This suggests that there are no aspects of the common environment that have a true effect, independent from rater bias, on individual differences in autistic traits. Unique environmental effects were present in the shared behavioral view (boys: 22%; girls: 18%) and also had a small influence on the specific behavioral view of mothers (boys: 14%; girls: 14%) and fathers (boys: 11%; girls: 11%). While there were significant quantitative sex differences in the etiology of autistic traits, the differences were only modest.

There were 123 (3.0%) MZ twin pairs and 140 (3.7%) DZ same-sex twin pairs where one or both of the children classified in the clinical range for ASD according to both parents. For MZ twin pairs belonging to this subsample, both children were classified as in the clinical range for ASD (concordant) in 40% of these twin pairs, while in 31% the co-twin scored in the subclinical range. A total of 36 children (29%) with the same genetic make-up as their 'affected' co-twin scored in the range of normal development (discordant). For DZ same-sex twin pairs the concordance was 9%, in 11% of the twin pairs the co-twin scored in the subclinical range and 80% of the twin pairs were discordant. MZ twin pairs discordant (clinical vs. normal range) for ASD did not differ in a number of environmental risk factors, that is, birth weight (μ_{ASD} = 2,347 g, μ_{NoASD} = 2,344 g, $t(35)$ = .03, p = .975), presence of birth complications (ASD = 34.6%, NoASD = 38.5%, t (25) = .23, p = .820) and time

spent in an incubator (ASD = 70.6%, NoASD = 61.8%, t (33) = .45, p = .657).

Discussion

Mothers and fathers showed a high agreement in their ratings of autistic traits as assessed with the PDP scale of the Child Behavior Checklist for preschoolers (Achenbach & Rescorla, 2000). In this large study of nearly 19,000 twin pairs, heritability estimates of autistic traits in 3-year-olds were high in both boys (78%) and girls (83%). The high broad heritability of autistic traits in toddlerhood is in line with previous studies on autistic traits in childhood, adolescence and adults and studies in clinical samples (Ronald & Hoekstra, 2014). The only two studies that also looked at autistic traits in very young children reported lower heritability estimates and a stronger influence of the common environment. The present study also found a small influence of these common environmental effects, but only on the parent-specific view on autistic traits and not on the shared behavioral view. The absence of common environmental effects with an influence on the shared behavioral view indicates that there are no 'true' common environmental factors that are independent of rater bias. Not taking into account the possibility of rater bias will result in an overestimation of common environmental effects.
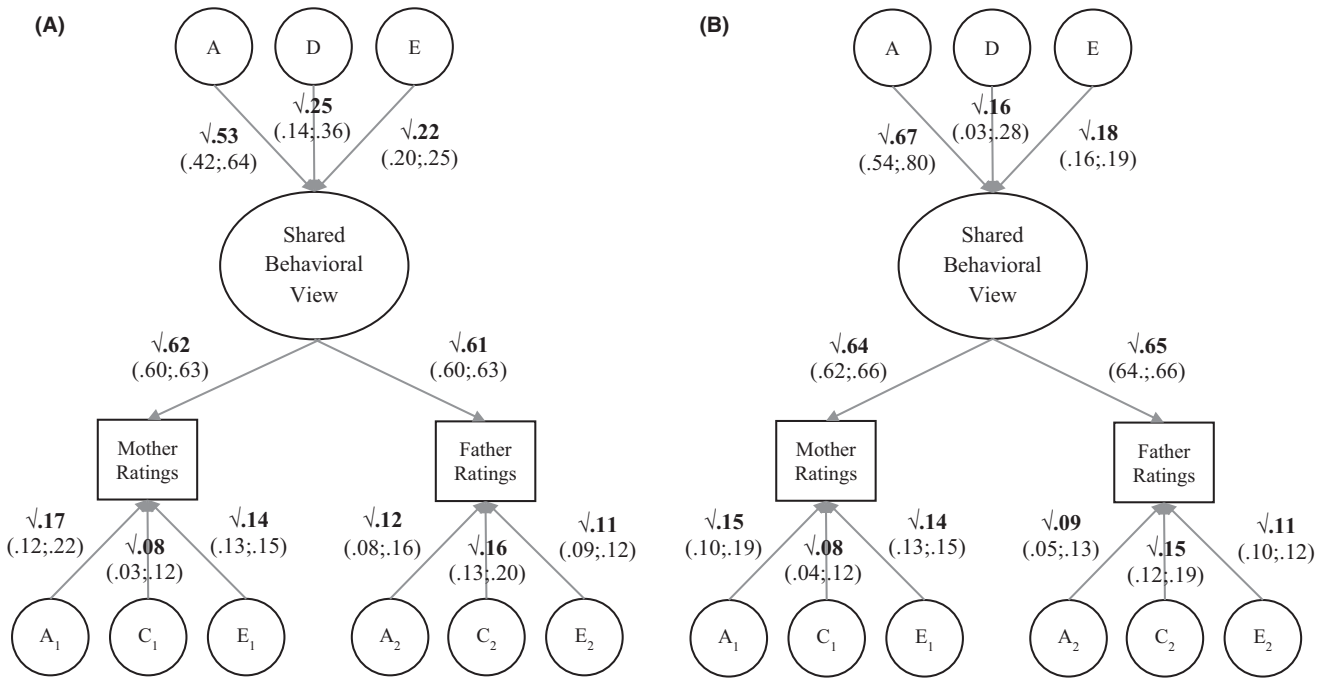
Figure 2 Parameter estimates. Standardized parameter estimates (95% CI's) of the best fitting model, separately for boys (A) and girls (B)

A consistent high heritability from early childhood into adulthood fits with research showing that ASD is usually a lifelong disorder with relatively stable characteristics (Turner, Stone, Pozdol, & Coonrod, 2006; Robinson et al., 2011). Genetic studies have shown that the stability in autistic traits could mainly be explained by an overlap in the genes that affected the behavior of the child at all ages while the influence of the environment shared between twins on the development of autistic traits was almost non-existent (Holmboe et al., 2014).

An ASD is four times more prevalent in boys than girls (Fombonne, 2003) and there are also some differences in how ASD manifests across sex (Lai, Lombardo, Auyeung, Chakrabarti, & Baron-Cohen, 2015; Rubenstein, Wiggins, & Lee, 2015). One possible explanation for the difference in prevalence might be different genetic pathways underlying the disorder in boys and girls. This study found no evidence for the existence of differences in genes responsible for individual differences in autistic traits (qualitative sex differences), but did find some differences in the magnitude of the contribution of genes to variation in autistic traits (quantitative sex differences). However, these quantitative sex differences were rather subtle and only significant because of the large sample size. Sex differences in the etiology of autistic traits thus appear relatively minor. This does not exclude the possibility that there are sex differences in the etiology of clinical ASD. The finding of negligible sex differences in our general population twin study suggests that the influence of common genetic variants on ASD is likely to be similar in boys and girls. However, there may be differences in the influence of rare gene

variants. Girls had a threefold increase in deleterious autosomal copy number variants as compared to boys with ASD, but were less likely to develop diagnosable neurodevelopmental problems, suggesting that girls are better able to overcome genetic mutations (Jacquemont et al., 2014).

A strength of this study is the availability of both a mother report as well as a father report for a very large number of preschoolers. The large sample size ensured that the study had adequate power to detect sex differences in the etiology of autistic traits. Knowledge about the etiology of ASD in preschoolers is important as early intervention in ASD has been shown to have positive long-term outcomes (Boyd, Odom, Humphreys, & Sam, 2010; Bradshaw, Steiner, Gengoux, & Koegel, 2015). Interventions targeting ASD include speech and language therapy, sensory integration and, applied behavior analysis (Green et al., 2006; Love, Carr, Almason, & Petursdottir, 2009). Evidence for the existence of critical periods of development and higher plasticity of the brain in toddlerhood might explain the successes of these interventions (Dawson, 2008). Treatment in a sensitive period, when neural plasticity is heightened, might alter the brain to a more normal developmental trajectory.

A limitation of this study is that we did not consider gene-environment interaction or correlation (Purcell, 2002). Gene-environment (GxE) interaction holds that the adverse effect of an environmental factor depends on the genotype of the child and vice versa the effect of the genetic make-up of a child might depend on the environment to which he or she is exposed. When GxE interaction is not taken into account this will overestimate the influence of the

unique environmental effects. As we find high heritability and modest effects of unique environment, effects of GxE are unlikely to be large. The effect of gene-environment correlation depends on whether environmental effects are shared by children from the same family or not. When rGE is present, the estimate for the influence of the genetic effects is overestimated, whereas rGC will act like the common environment (Purcell, 2002).

Even though genetic factors play a major role in the etiology of autistic traits the concordance rate for an ASD diagnosis, based on a cut-off on the measure for autistic traits, was not 100% in genetically identical twins. The results of this study are in keeping with the consistent evidence for a modest, but potentially crucial role for environmental effects unique to each twin, although there may also be rare monozygotic pairs who are discordant for post-twinning de novo CNVs (Ehli et al., 2012). In our study more than one-quarter of the children at genetic risk for ASD, i.e. with an identical co-twin scoring in the clinical range of autistic traits, scored in the range of normal development. Either adverse environmental risk factors might have triggered ASD or protective environmental risk factors might have prevented a child from developing ASD. The discordance between the identical twin pairs was not explained by differences in birth weight, birth complications or time in incubator. In an earlier study from the Netherlands twin Register, birth weight was observed to have a causal influence on attention problems and explain discordance in monozygotic twin pairs (Groen-Blokhuis, Middeldorp, van Beijsterveldt, & Boomsma, 2011). Despite the substantial comorbidity of ADHD and autism (Ghirardi et al., 2017) the role of low birth weight in these disorders thus seems to differ. Other, risk factors that have been found in relation to ASD include parental age, prenatal maternal smoking, medication use and obesity (Gardener, Spiegelman, & Buka, 2011; Mandy & Lai, 2016). As these exposures are shared by twins, they are unlikely to explain discordance in monozygotic twins. On the other hand, much less is known about protective environmental factors in the light of ASD. When children show a normal development in face of genetic risk for ASD they are described as resilient (Masten, 2001). It is therefore important to focus future research not only on risk factors that might interplay with a genetic disposition for ASD, but also on protective factors that make a difference in the lives of children at genetic risk.

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Key points

- Autistic traits, as measured more reliably with multiple informants, are as heritable in early childhood as has been previously reported for older children and adults.
- There were only very small differences in the underlying etiology of autistic traits in boys and girls.
- Environmental effects that are unique to a child also play a modest role, but the family environment seems to be less important.

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