No Major Effect of Twinning on Autistic Traits

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Background: It has been questioned whether the process of twinning might be a risk factor for autism spectrum conditions (ASC) and autistic traits. Aim: We sought to determine whether autistic traits and probable disorder, as measured by the Childhood Autism Spectrum Test (CAST), were more pronounced in twins compared to singletons. Samples: Data were analyzed from two large population-based samples of UK children, twins (n = 5,142 twin pairs, aged 8 years) and singletons (n = 2,805, aged 5–9 years). Results: Distributions of CAST scores in both groups were negatively skewed and scores for twins were more variable than singletons. Mean CAST total scores and standard errors (SE) were not significantly different for twins (5.1; SE 0.04) compared to singletons (4.9; SE 0.08). Moreover, contrary to expectations, the likelihood of scoring above the threshold for possible ASC was significantly lower in the twins than the singletons (OR = 0.69; P = 0.002). Subsidiary analyses of CAST scores according to sex, twin type, and subscale scores representing the subdomains of autism found a few significant differences (P < 0.01), but the effect sizes for these differences were small and none exceeded \( \eta^2 = 0.005 \). The explanation for these small differences remains obscure, but the very small effect sizes mean they are of little importance. Conclusions: Our results do not provide evidence to support twinning as a risk factor in the development of autistic traits. Autism Res 2011;4:377–382. © 2011 International Society for Autism Research, Wiley Periodicals, Inc.

Keywords: clinical psychiatry; developmental psychology; diagnosis; epidemiology; social cognition

Introduction

Twinning is a complex Happé multifactorial trait created by unique genetic and environmental influences that may affect phenotypic outcome. It has been postulated that even without the risk factors associated with being a twin, i.e. premature birth and low birth weight, twinning may incur a neurodevelopmental disadvantage among term newborns [Luu & Vohr, 2009]. This disadvantage may encompass a number of developmental conditions including those within the autism spectrum [Rutter & Redshaw, 1991; Rutter, Thorpe, Greenwood, Northstone, & Golding, 2003; Simonoff, 1992; Sutcliffe & Derom, 2006].

Autism spectrum conditions (ASC), synonymous with autism spectrum disorders (ASD), comprise autism, atypical autism, and Asperger syndrome. They occur in approximately 1 in 100 children and are a heterogeneous, behaviorally defined, group of conditions [Baird et al., 2006; Baron-Cohen, Scott et al., 2009; Fombonne, 2009]. Recent evidence suggests that the categorical conceptualization of ASC may reflect the extreme of a continuously distributed set of autistic-like traits and that mild (sub-threshold) levels of autistic behaviors might share the same genetic origins as ASC [Constantino & Todd, 2003; Ronald, Happé, Bolton et al., 2006].

There have been several studies to date that have examined the effect of twinning on the risk of categorical ASC. Initially, clinic-based studies which are prone to ascertainment bias [Visscher, 2002] reported higher than expected numbers of twins among affected sibling pairs with ASC [Betancur, Leboyer, & Gillberg, 2002; Greenberg, Hodge, Sowinski, & Nicoll, 2001]. However, these were followed by epidemiological studies that repeatedly refuted evidence in favor of twinning as a risk factor for ASC [Croen, Grether, & Selvin, 2002; Hallmayer et al., 2002; Hultman, Sjöra, & Cnattingius, 2002].

More recently, uncertainty has again been raised about whether twinning might be a risk factor for autistic traits, sub-threshold for diagnosis [Ho, Todd, & Constantino, 2005]. Ho et al. reported evidence to suggest that male twins were more at risk of having more autistic traits than their male singleton counterparts.
while Hoekstra et al. found no differences in autistic traits between twins and their nontwin siblings.

Therefore, in this study, we aimed at clarifying the role of twinning in autistic/ASC-related traits in two large epidemiological samples, powerful enough to detect small effects. Furthermore, because previous analyses of twin data using the Childhood Autism Spectrum Test (CAST) suggest that the genetic architecture may differ for different domains of the phenotype [Happe´, Ronald, & Plomin, 2006; Ronald, Happe´, Bolton et al., 2006; Ronald, Happe´, Price, Baron-Cohen, & Plomin, 2006], we wanted to examine twin-singleton differences in the component autistic trait scores as well as the total combined score.

**Methods**

*The Two Epidemiological Samples*

**The twin sample.** The Twins Early Development Study (TEDS) is a longitudinal, UK population-based study of twins born between 1994 and 1996 [Oliver & Plomin, 2007; Trouton, Spinath, & Plomin, 2002]. The CAST data reported here were collected between 2002 and 2004, when the twins were aged 8 and questionnaires were returned from 6,771 families (55.0% of active contact families). The ethnicity of the responding families was Caucasian in 94%, similar to the ethnic mix in TEDS as well as the UK census data for the entire population (92%). For our present analysis, twin pairs were excluded for the following reasons (a) specific medical syndrome (not including ASD) such as those with specific chromosomal abnormalities (e.g. Down’s syndrome), genetic abnormalities (e.g. Fragile X), or brain disorders (e.g. cerebral palsy) (31); (b) unknown zygosity (177); (c) first contact data not available (199); (d) no written consent (57); (e) incorrect birth order in the age 8 questionnaire (207), and (f) specific perinatal risk factors (namely twins in special care >97 days; twins in hospital >74 days; birthweight <471 g; gestation age <27 weeks; mother’s alcohol consumption during pregnancy ≥14 units per week) (112). The overall remaining sample consisted of 6,093 twin pairs (1,023 MZM, 987, DZM, 1,142 MZF, 994 DZF, and 1,947 DZOS pairs). Of those available pairs, 951 families did not provide social class data resulting in the final analyses on 5,142 twin pairs. Therefore, 24% of responders were excluded.

It is noteworthy that the TEDS data set does not have specific information on in vitro fertilization (IVF) and therefore IVF cases are not likely to be excluded by the criteria we used.

**The nontwin (singleton) sample.** This cross-sectional epidemiological study of 5- to 9-year-old singletons attending schools in Cambridgeshire, UK, is described elsewhere [Williams et al., 2008]. Questionnaires were returned for 3,370 from a total of 11,635 families surveyed by schools (response rate = 28.7%). After exclusion criteria (chromosomal or genetic abnormalities, cerebral palsy, perinatal risk factors) were applied, data regarding 3,336 children were analyzed, and therefore 1% of responders were excluded.

**Measurement**

**Autistic traits.** The CAST is designed as a screening tool for ASC in epidemiological studies [Scott, Baron-Cohen, Bolton, & Brayne, 2002; Williams et al., 2005]. Furthermore, the CAST detects more subtle presentations of ASC and autistic traits in primary school aged children [Ronald, Happe´, Bolton et al., 2006; Ronald, Happe´, Price et al., 2006]. It is completed by an informant (usually a parent) and comprises 31 items reflecting the core domains of ASC: social reciprocity (12 items), language and communication (12 items), and RRBIs (7 items). These domain scores have been used in previous studies [Ronald, Happe´, Bolton et al., 2006; Ronald, Happe´, Price et al., 2006] and show good construct validity in that they ask about the same types of behaviors that are listed in each domain by ICD10 or DSMIV.

**Data Analyses**

Statistical analyses were conducted using STATA-10 (Stata Corporation, TX). Data were analyzed using parametric procedures such as analysis of variance (ANOVA) and linear regression. The robust cluster option was used throughout to correct standard errors to take account of the nonindependence of data when multiple family members (e.g. twins) are included [Huber, 1967]. Covariates used in the analyses were social occupational class, maternal age, and paternal age, though covarying these did not significantly change the results. The twin sample was 8 years at the time the CAST was completed and the singleton sample ranged in age from 5 to 9 years. We examined the effect of age in the singleton sample and found little correlation between CAST scores and age (Pearson Correlation = 0.001; P (two-tailed) = 0.969).

**Results**

**Primary Hypothesis: Is There a Difference in Total CAST Scores?**

The distributions of total raw CAST scores by group (twin or nontwin) are shown in Figure 1. The magnitude of skew and kurtosis in the distribution of CAST raw scores was similar for twins (1.79; 9.22) and singletons (1.99; 9.18). Although skewed, it can be seen that the whole range of scores is represented in both samples. Untransformed and transformed scores when analyzed separately gave similar results.

The twins were significantly more variable than singletons on the CAST total scores (mean standard deviation for twins = 5.14, singletons = 4.97; Levene’s test for equality of variance P <0.001, randomly selecting one twin per pair). Testing our primary hypothesis, we found no significant difference on mean CAST scores between twins and singletons (Table 1).
Secondary analyses. Secondary analyses of CAST scores were carried out because it has been suggested that the autism phenotype is fractionable and that different genetic and environmental factors influence the social, communication, and RRBI domains [Ronald, Happé, Bolton et al., 2006]. In addition, it has been proposed that intrauterine exposure to the sex hormone, testosterone, may constitute a risk factor for ASC [Baron-Cohen, Auyeung, Ashwin, & Knickmeyer, 2009]. Accordingly, CAST scores were examined by sex, twin type, and subscale scores. Small differences that reached nominal significance of \( P < 0.01 \) were found. The analyses that reached significance on mean CAST total scores and mean CAST subscale scores are highlighted in bold in Table 1. The pattern of differences was not consistent with the predictions of the fetal testosterone hypothesis.

Small differences were noted for total CAST scores in some dizygotic (DZ) twin pairs who scored higher than singletons. Higher mean scores for DZ twins was noted for opposite sex DZ pair (DZOS) boys and same sex DZ pair (DZSS) girls. DZOS boys had mean CAST scores that were 0.4 points higher than boys from DZ male pairs, 0.7 points higher than MZ male pairs, and 0.5 points higher than singleton males, and these higher scores were accounted for by ratings within the social domain. DZSS girls had mean CAST scores that were 0.3 points higher than DZOS girls, 0.4 points higher than MZ girls, and 0.6 points higher than singleton girls, and these higher scores were accounted for by ratings within the communication domain. Monozygotic (MZ) boys had small but significantly lower scores than singletons on the CAST RRBI subscale.

The effect sizes for all these differences found in the secondary analyses were very small with the largest effect size, \( \eta^2 = 0.002 \), being for mean total CAST scores in DZOS. None of the effect sizes for the other significant differences exceeded \( \eta^2 = 0.005 \).

Secondary Hypothesis: Do the Groups Differ in the Number of Children Meeting the Screening Cut-Off?

The likelihood of being above the CAST screening cutoff (\( \geq 15 \)) was significantly predicted by group membership and gender (Wald \( (2) = 99.14, P < 0.0001 \)). Contrary to expectation, being a twin significantly decreased the odds of being above the screening cutoff (OR = 0.69, \( P = 0.002, \text{CI} 0.54–0.87 \)). As expected boys were over three times more likely to score above the CAST screening cutoff than girls (OR = 3.42, \( P < 0.001, \text{CI} 2.66–4.40 \)).

Discussion

The results of these analyses using two large population-based data sets show that there was little difference between twins and singletons on CAST scores for dimensional ratings of autistic traits. Our large sample allowed us to examine differences emerging within classes of twins and across different types of autistic traits. We found differences that were principally accounted for by DZ rather than MZ twin scores; findings that could reflect contrast effects in the twins due to either rater bias or sibling interaction effects on the scores, as suggested by previous modeling in the twin data set [Ronald, Happé, Bolton et al., 2006].

The data sets we used are not directly comparable to those used in previous reports comparing parent-rated dimensional autistic traits in twins and singletons in population-based samples [Ho et al., 2005]. We used
Table 1. Mean Scores (Standard Errors) for CAST Total Score and Subscale Scores

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<tr>
<th></th>
<th>Total CAST</th>
<th>Social Communication</th>
<th>Attention-to-Detail</th>
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<tr>
<td>All twins</td>
<td>5.8 (0.06)</td>
<td>4.4** (0.09)</td>
<td>5.1 (0.04)</td>
<td>2.0 (0.03)</td>
<td>4.8 (0.09)</td>
<td>5.1**</td>
<td>6.2** (0.11)</td>
<td>4.3 (0.07)</td>
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<td>MZ</td>
<td>5.5 (0.12)</td>
<td>4.2 (0.09)</td>
<td>4.8 (0.09)</td>
<td>1.8 (0.07)</td>
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<td>D2SS</td>
<td>5.8 (0.11)</td>
<td>4.6** (0.09)</td>
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<tr>
<td>D2OS</td>
<td>6.2** (0.10)</td>
<td>4.0** (0.09)</td>
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Note: MZ, monozygotic; DZ, dizygotic; SD, standard deviation.

**P < 0.01; **P < 0.05; ***P < 0.001. Bold numbers highlight significant differences between twin groups and singletons.

Minor obstetric complications have been consistently reported to be more frequent in autistic probands and their families. Therefore, the fact that twins are prone to pregnancy and birth complications may be relevant. Obstetric complications have been examined in both twin studies [Bailey et al., 1995] and family studies [Bolton et al., 1994] of autism and autistic traits, although the causes underlying their association are unclear [Ronald, Happe, Dworzynski, Bolton, & Plomin, 2010].

Reports of a link between fetal testosterone (fT) and sexually dimorphic aspects of brain development found in autism [Giedd et al., 1996; Harden, 2000] have led researchers to examine the possibility of a link between autistic traits and fT levels [Auyeung et al., 2009; Baron-Cohen, Knickmeyer, & Belmonte, 2005]. One such published study examined the relationship between fT and scores on two parent report questionnaires of autistic traits (CAST and the Child Autism Spectrum Quotient...
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(AQ-Child) in a modest sample (N = 235) of children aged 6–10 years. FT levels were positively associated with scores on both questionnaires consistent with the hypothesis that greater prenatal androgen exposure is related to children exhibiting more autistic traits [Auyeung et al., 2009]. In our study, we found that opposite sex twin pair girls had similar or even slightly lower scores than girls from DZ same sex pairs. The results therefore are not consistent with the hypothesis that exposure to FT from the male co twin might increase the risk for autistic traits.

Limitations of our study include the fact that the two samples we investigated may not be directly comparable. The data from two separate studies were opportunistically compared by us. The age range of the singleton was greater than for twins. The twin study was a longitudinal study and there may have been attrition of families experiencing more behavioral difficulties with their children. However, the number of children with ASC in the twin sample is representative of the general population. Different selection factors for obtaining consent may have applied to the two studies. Despite the limitations, the findings we report here indicate that twinning is not a major risk factor for autistic traits or probable autism spectrum disorder and therefore that findings from twins can be extrapolated to nontwin populations.

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