Original contribution

Traits of ADHD and autism in girls with a twin brother - a Mendelian randomization study

Jørn Attermann[a], Carsten Obel[b], Niels Bilenberg[c],

Claudia Maria Nordenbæk[c], Axel Skytthe[d], and Jørn Olsen[a]

[a]Department of Epidemiology, and [b]Department of General Medicine, School of Public Health, Aarhus University, and [c]Child and Adolescent Psychiatric Department, and [d]The Danish Twin Register, Institute of Public Health, University of Southern Denmark, Odense, Denmark

Correspondence: Dr Jørn Attermann, Department of Epidemiology, School of Public Health, Aarhus University, Bartholins Alle 2, DK-8000 Aarhus C, Denmark.

Tel: +45 8942 6145. Email: ja@soci.au.dk

Running head: Traits of ADHD and autism in girls with a twin brother

Abstract

It has been hypothesized that prenatal exposure to testosterone may be associated with traits of attention-deficit/hyperactivity disorder (ADHD) or autism spectrum disorder (ASD). We conducted a population-based study of dizygotic female twins to elucidate this hypothesis, assuming that the sex of the co-twin influences the level of prenatal exposure to testosterone. We invited parents of 24,552 three-to-fifteen year old twins to answer questionnaires on traits of ADHD and ASD. We analyzed the data using a proportional odds model with sex of the co-twin as an instrumental variable for prenatal exposure to testosterone of female twins. We received responses for 6,339 girls from dizygotic twin pairs. Odds ratios for male versus female co-twin were 0.71 (95% confidence interval 0.61-0.81) for ADHD traits and 0.74 (0.66-0.83) for ASD traits, indicating that a twin brother reduces traits of ADHD and ASD in females.

In conclusion, we found that female twins with a twin brother scored significantly lower in parent-reported traits of ADHD and ASD than those with a twin sister. The reason for this may be parental reporting bias, or confounding by unmeasured variables, or a causal effect of an intrauterine environment modified by the sex of the co-twin in the opposite direction of what we expected.

Keywords: autism, ADHD, instrumental variable, epidemiology, twins

Introduction

Attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) are both becoming increasingly common psychiatric disorders in childhood for reasons we do not fully know. ADHD is characterized by a persistent pattern of inattention, hyperactivity and impulsivity while typical features of ASD are impaired social interaction and communication and repetitive, sterotyped behaviour and interests. In 2004 the cumulative incidence proportions of diagnosed ADHD and ASD cases in 9 year old Danish children were 35 (95% confidence interval 32-38) and 51 (47-55) per 10,000 children, respectively [5].

One proposed model for autism development is the "extreme male brain theory" which postulates that autism is a phenotype of the normal male brain where the drive to systemize is stronger than the drive to empathize [12]. This theory finds support in the higher rates of autism in males than in females with sex ratios reported from 2:1 to 16:1 [20], and in studies finding associations between autism and more common male characteristics such as left-handedness [39], lower scores in *Reading the Mind in the Eyes Test* [10], superiority in the *Embedded Figures Test* [25], larger total size of the brain, and volume differences in certain areas of the brain [11].

It has been proposed that an extreme male brain may be a result of an excess prenatal testosterone exposure [8]. Observational studies with testosterone measured in amniotic fluid from mothers undergoing amniocentesis found that elevated levels of foetal testosterone was associated with reduced eye contact in infants [29], narrower interests at 4 years of age [26], less empathy at 6-8 years [14], increased systemizing at 8 years [7], a larger number of autistic traits in toddlers [9] and in 6-10 year old [8], and male-typical play activities in 6-10 year old children [6]. In nonhuman mammals, prenatal exposure to sex hormones, in

particular androgens, has profound influence on sexually differentiated behaviour [23] and although results from animal studies need not be applicable to humans it is well known that prenatal hormone exposure programs the development of the genitals in humans [23]. Normal development of the human brain is characterized by cerebral lateralization, i.e. that one hemisphere (usually the left) specializes in verbal and reasoning skills and the other in non-verbal, emotional aspects, and this specialization is usually stronger in men than in women. Foetal testosterone may have an important impact on the lateralization by inhibiting and enhancing the development of the left and right hemispheres, respectively [22]. Girls with congenital adrenal hyperplasia are exposed to high levels of androgens and exhibit behaviour more typical of males [33]. It is, therefore, believed that a "male behaviour" connected with this disease may be caused by a prenatal exposure to abnormal high testosterone levels. Genes regulating sex hormones have also been found to be associated with traits of autism and with a diagnosis of Asperger's syndrome [13].

In general, observational studies on levels of foetal testosterone are vulnerable to confounding by factors that are not registered in detail in the study. De-confounding by randomisation related to manipulating the intrauterine environment in humans is usually not possible for ethical reasons and, therefore, other design options are needed. If the presence of a male co-twin increases the level of testosterone, dizygotic twins provide a Mendelian randomisation option since the sex of each twin is independently and randomly determined at the time of conception, according to Mendel's laws on segregation and independent assortment. For a female index twin, the sex of the co-twin (Z) is, therefore, not expected to correlate with any known or unknown variable which is not a part of the causal chain from Z to traits of ADHD or ASD in the index twin (Y). Moreover, if the effect of Z on Y is mediated almost exclusively by the prenatal testosterone exposure of the index twin (X), Z could be an

instrumental variable [27] for X, since in this case, at least approximately, 1) Z is associated with X; 2) Z is independent of factors U that confound the association between X and Y; and 3) Z is independent of Y given X and U, and we have a "Mendelian randomization design". Given valid measures of ADHD and ASD, this allows us to examine the null hypothesis that prenatal exposure to testosterone is not associated with traits of ADHD or ASD by examining the association between co-twin sex and traits of ADHD or ASD.

In the present study we compared behavioural scores between dizygotic female twins with a male and a female co-twin in a population based sample of 3-15 year old twins. Scores were calculated from parental responses to items from the Child Behaviour Checklist (CBCL) [2]. We hypothesized that girls with a twin brother would have higher scores (i.e. more behavioural problems) than girls with a twin sister.

Methods

Data sources

All newborn children in Denmark are routinely registered with a unique civil-registry number in the Danish Civil Registration System (DCRS). In the years 1988-2000 13,329 twin births (26,658 individuals) were registered. As part of a larger study originating from the Danish Twin Registry [38], questionnaires were mailed in August 2003 to parents of 24,552 twins who were alive and where neither the twins nor the parents had asked for anonymity in the DCRS. Due to restrictions related to the burden of data collection it was only possible to include five items from the CBCL in the study (Table 1), all scored on a Likert scale (0 = "not true", 1 = "sometimes true", and 2 = "often true").

[place Table 1 about here]

Twin zygosity was assessed on the basis of parents' answers to four questions on likeness

which have a DNA-verified predictive value of self-reported dizygosity of 94.9% (95 % CI 92.5% - 96.7%) [15].

Eligible for the present study were female twins whose parents had returned the CBCL and zygosity questionnaires with twins being classified as dizygotic.

Statistical analysis

Items Q1 and Q2 (see Table 1) were chosen because they are core symptoms of ADHD and therefore have high face validity for ADHD-like behaviour, and we defined "ADHD score" as the sum of these two items. "Total score" was defined as the sum of the 5 items. In a recent study, sensitivity, specificity and positive predictive value for ASD of total score were 79.6% (95% confidence interval 70.8%-86.8%), 81.4% (80.7%-81.9%), and 2.7% respectively, at a cut-off score of two out of ten [31]. Since the prevalence of ASD was 0.72%, simple calculations using Bayes' formula gives a negative predictive value of 99.8%. Assuming that the risk of ASD is conditionally independent of the sex of the co-twin, given the total score, it is easy to see, again using Bayes' formula, that the relative risk of ASD for male versus female co-twin is approximately equal to the relative risk of a total score ≥ 2 for male versus female co-twin. Also, in a recent study the items Q3 and Q5 were included in a proposed scale for measuring ASD, based on CBCL [32]. Therefore, we will use total score as a proxy for ASD in the following.

Internal consistency of the ADHD and total scores were estimated by Cronbach's alpha using SAS PROC CORR. Subsequently, the two scores were analyzed separately in proportional odds models where the log odds of a high score was estimated in three models, model A (crude) with sex of co-twin as the only explanatory variable, model B with adjustment for birthweight and gestational age, and model C with further adjustment for child's age at time

of response, mother's age at birth, and mother's place of birth (in Denmark or not). By using a proportional odds model instead of a logistic regression model we avoided to specify arbitrary cut-off points for scores and obtained larger statistical power. The proportional odds model is a simple extension of the logistic regression model and odds can be interpreted like in this as, e.g., the odds of a score of 1 versus a score of 0.

The assumption of proportional odds was tested by a score test using SAS PROC LOGISTIC with a dataset consisting of exactly one index twin randomly selected from each pair of twins. Proportional odds models with random effects, corresponding to twin pairs, showed non-normal distribution of best linear unbiased predictors of these. Therefore, a proportional odds model was fitted with all available data using generalized estimating equations with independent working correlation using SAS PROC GENMOD.

Ethical aspects

Approval was obtained from the Danish Data Inspection Authority. Analyses were based on encrypted data.

Results

Questionnaires were returned for 16,793 of the 24,552 twins. Of the non-responders 49.6% were girls compared to 49.4% in the responding group. Based on parents' reports on similarities in looks and behaviour 12,871 individuals were classified as dizygotic. Twenty-nine individuals were excluded because of missing information about their co-twin, leaving 12,842 individuals of whom the 6,339 girls (49.4%) were included in the analyses. Characteristics of the female twins are shown in Table 2.

[place Table 2 about here]

Answers to questions were missing for 65 girls and 157 girls with respect to ADHD and total

scores, respectively. Thus 6,274 and 6,182 female twins, respectively, were included in the statistical analyses.

Cronbach's alpha was 0.71 for both ADHD and total scores.

The distributions of scores are shown in Table 3, both for female and male twins.

[place Table 3 about here]

As expected, males were scored higher than females with 73.3% and 59.6% zero scores versus 82.3% and 65.1% zero scores, for ADHD and total scores, respectively.

In the logistic regression analyses the proportional odds assumption was found to be acceptable for both scores ($p = 0.29$ and $p=0.69$). The odds ratios for high ADHD and total scores were 0.71 (95% confidence interval 0.61-0.81) and 0.74 (0.66-0.83), respectively, adjusted for birthweight and gestational age (Table 4).

[place Table 4 about here]

Omitting adjustment, or further adjusting for child's age at response, mother's age at birth, and mother's place of birth did not change these results significantly.

Discussion

As previously shown, ADHD and total scores were generally higher for males than for females. However, girls with a twin brother scored significantly lower than girls with a twin sister, both for ADHD and total scores. That was the opposite of what we hypothesized. An important reason for the lower scores in opposite-sex than in same-sex girls may be differential parental reporting bias, where opposite-sex girls may be reported with a lower score because parents use the brother as the comparison and males generally have higher scores. We cannot correct for this possible bias, but focusing on males with a twin sister did not produce an opposite association as differential recall would predict. We have no other

explanation for the apparent “protective” effect of having a twin brother, and it could be a chance finding.

One of the strengths of our study is the population based design with many participants and without possible selection problems associated with, e.g., amniocenteses or referrals to other clinical examinations.

Our use of co-twin sex as an instrumental variable for prenatal testosterone exposure is based on three assumptions: 1) independence between co-twin sex and confounders of the association between testosterone exposure and ADHD/total scores; 2) prenatal testosterone exposure for female twins depends on the sex of the co-twin; and 3) co-twin sex and ADHD/total scores are conditionally independent, given prenatal testosterone exposure. The first assumption is likely due to Mendelian randomization. In accordance with this, adjusting for foetal growth, gestational age, child’s age at response, mother’s age at birth and mother’s place of birth did not change our results significantly.

Although there is limited evidence for this in humans, the assumption of a correlation between co-twin sex and prenatal testosterone exposure is supported by findings in mice, where exposure to testosterone is influenced by the intrauterine position of the foetus such that a male foetus has higher blood concentration of testosterone than females, and a foetus positioned between two males has a higher concentration of testosterone than a foetus of the same sex positioned between one male and one female, or between two females [41]. Transfer of testosterone between foetuses may occur via the maternal circulation or more directly between foetuses. In human pregnancies with a male foetus, testosterone has been found to cross the placenta along its gradient towards the maternal circulation and opposite for a female foetus [30], indicating that the testosterone concentration may depend on the sex of the co-twin, also in humans. An advantage of our instrumental variable for testosterone,

the co-existence of a male foetus, is its presence throughout the pregnancy where, in comparison, a biomarker study would often only provide a short term measure.

Considering the third assumption, i.e. that co-twin sex and ADHD/total scores are independent, given prenatal testosterone exposure, it is possible that environmental factors including test scoring conditions, caused by the co-twin sex, has an effect on ADHD/total scores. We are only interested in the male exposure very early in life, but "having a twin brother rather than a twin sister" remains and may influence our outcome measure. We cannot in our study correct for this.

The internal consistency of the ADHD and total scores was acceptable with Cronbach's alpha 0.71 [18].

Although ADHD and ASD, according to the DSM-IV [4], by definition cannot be diagnosed simultaneously, recent studies point towards a high co-occurrence between the two diagnoses as ADHD is found in 59-75% of children with pervasive developmental disorders, including autism, and autistic traits are overrepresented in children meeting diagnostic criteria for ADHD [35]. Both ADHD and ASD are heritable disorders [17,19,40], and may share some of the same familial or genetic causes [28,34,36]. We studied both traits of ADHD and ASD and found similar results.

The traits characterizing ASD probably reflect one end of the distribution of social-communication disability [3,24,37,42] and the same may apply to ADHD, suggesting that assessment in relation to etiological research may be better based on psychometric scales as in our study rather than dichotomous, diagnostic criteria. Furthermore, studies based on a quantitative traits approach often have higher statistical power. We used scores based on non-dichotomous Likert scales to assess traits of ADHD and ASD. We did not use register data on hospitalized ADHD or ASD, since only a minor proportion with these diseases are

hospitalised.

The CBCL has been used and validated in many different settings [1]. A study on the Social Responsiveness Scale (SRS) and CBCL found that CBCL scores explained 43% of the variance in SRS scores with the CBCL subscales *Social Problems* and *Attention Problems* accounting for the largest contribution [16].

It is possible that twins may be different from singletons with respect to the association between intrauterine testosterone exposure and developmental disorders. However, the point-prevalence of ASD in our twin-sample was 0.72% [31], and this is comparable to recent estimates in singletons of 0.6%-0.7% across different regions and countries [21].

In conclusion we found that female twins with a twin brother scored significantly lower in parent-reported traits of ADHD and ASD, measured by CBCL, than female twins with a twin sister, indicating that prenatal exposure to testosterone does not increase traits of ADHD and ASD in female twins.

The authors declare that they have no conflict of interest.

References

1. Achenbach TM, Becker A, Dopfner M, Heiervang E, Roessner V, Steinhausen HC, Rothenberger A (2008) Multicultural assessment of child and adolescent psychopathology with ASEBA and SDQ instruments: research findings, applications, and future directions. Journal of Child Psychology and Psychiatry 49:251-275

2. Achenbach TMRLA (2001) Manual for ASEBA School-Age Forms & Profiles. University of Vermont, Research Center for Children, Youth & Families, Burlington, VT

3. Allison C, Baron-Cohen S, Wheelwright S, Charman T, Richler J, Pasco G, Brayne C (2008) The Q-CHAT (Quantitative CHecklist for autism in toddlers): A normally distributed quantitative measure of autistic traits at 18-24 months of age: Preliminary report. Journal of Autism and Developmental Disorders 38:1414-1425

4. American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Publishing, Washington, DC

5. Atladottir HO, Parner ET, Schendel D, Dalsgaard S, Thomsen PH, Thorsen P (2007) Time trends in reported diagnoses of childhood neuropsychiatric disorders. Arch Pediatr Adolesc Med 161:193-198

6. Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G, Hines M (2009) Fetal Testosterone Predicts Sexually Differentiated Childhood Behavior in Girls and in Boys. Psychological Science 20:144-148

7. Auyeung B, Baron-Cohen S, Chapman E, Knickmeyer R, Taylor K, Hackett G (2006) Foetal testosterone and the child systemizing quotient. European Journal of Endocrinology 155:S123-S130

8. Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G (2009) Fetal testosterone and autistic traits. British Journal of Psychology 100:1-22

9. Auyeung B, Taylor K, Hackett G, Baron-Cohen S (2010) Foetal testosterone and autistic traits in 18 to 24-month-old children. Molecular Autism 1:11

10. Baron-Cohen S, Jolliffe T, Mortimore C, Robertson M (1997) Another advanced test of theory of mind: Evidence from very high functioning adults with autism or Asperger syndrome. Journal of Child Psychology and Psychiatry and Allied Disciplines 38:813-822

11. Baron-Cohen S, Knickmeyer RC, Belmonte MK (2005) Sex differences in the brain: implications for explaining autism. Science 310:819-823

12. Baron-Cohen S (2010) Empathizing, systemizing, and the extreme male brain theory of autism. Prog Brain Res 186:167-175

13. Chakrabarti B, Dudbridge F, Kent L, Wheelwright S, Hill-Cawthorne G, Allison C, Banerjee-Basu S, Baron-Cohen S (2009) Genes Related to Sex Steroids, Neural Growth, and Social-Emotional Behavior are Associated with Autistic Traits, Empathy, and Asperger Syndrome. Autism Research 2:157-177

14. Chapman E, Baron-Cohen S, Auyeung B, Knickmeyer R, Taylor K, Hackett G (2006) Fetal testosterone and empathy: Evidence from the Empathy Quotient (EQ) and the "Reading the Mind in the Eyes" Test. Social Neuroscience 1:135-148

15. Christiansen L, Frederiksen H, Schousboe K, Skytthe A, von Wurmb-Schwark N, Christensen K, Kyvik K (2003) Age- and sex-differences in the validity of questionnaire-based zygosity in twins. Twin Research 6:275-278

16. Constantino JN, Hudziak JJ, Todd RD (2003) Deficits in reciprocal social behavior in male twins: Evidence for a genetically independent domain of psychopathology. Journal of the American Academy of Child and Adolescent Psychiatry 42:458-467

17. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, Sklar P (2005) Molecular genetics of attention-deficit/hyperactivity disorder. Biological Psychiatry 57:1313-1323

18. Fitzpatrick R, Davey C, Buxton MJ, Jones DR (1998) Evaluating patient-based outcome measures for use in clinical trials. Health technology assessment (Winchester, England) 2:i-74

19. Folstein S, Rutter M (1977) Genetic Influences and Infantile-Autism. Nature 265:726-728

20. Fombonne E (2005) The changing epidemiology of autism. Journal of Applied Research in Intellectual Disabilities 18:281-294

21. Fombonne E (2009) Epidemiology of pervasive developmental disorders. Pediatr Res 65:591-598

22. Geschwind N, Galaburda AM (1985) Cerebral Lateralization - Biological Mechanisms, Associations, and Pathology. 1. A Hypothesis and A Program for Research. Archives of Neurology 42:428-459

23. Hines M (2004) Brain gender. Oxford University Press, New York

24. Hoekstra RA, Bartels M, Verweij CJH, Boomsma DI (2007) Heritability of autistic traits in the general population. Archives of Pediatrics & Adolescent Medicine 161:372-377

25. Jolliffe T, Baron-Cohen S (1997) Are people with autism and Asperger syndrome faster than normal on the embedded figures test? Journal of Child Psychology and Psychiatry and Allied Disciplines 38:527-534

26. Knickmeyer R, Baron-Cohen S, Raggatt P, Taylor K (2005) Foetal testosterone, social

relationships, and restricted interests in children. Journal of Child Psychology and Psychiatry 46:198-210

27. Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Smith GD (2008) Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Statistics in Medicine 27:1133-1163

28. Lionel AC, Crosbie J, Barbosa N, Goodale T, Thiruvahindrapuram B, Rickaby J, Gazzellone M, Carson AR, Howe JL, Wang Z, Wei J, Stewart AFR, Roberts R, McPherson R, Fiebig A, Franke A, Schreiber S, Zwaigenbaum L, Fernandez BA, Roberts W, Arnold PD, Szatmari P, Marshall CR, Schachar R, Scherer SW (2011) Rare Copy Number Variation Discovery and Cross-Disorder Comparisons Identify Risk Genes for ADHD. Science Translational Medicine 3:95ra75

29. Lutchmaya S, Baron-Cohen S, Raggatt P (2002) Foetal testosterone and eye contact in 12-month-old human infants. Infant Behavior and Development 25:327-335

30. Meulenberg PMM, Hofman JA (1991) Maternal Testosterone and Fetal Sex. Journal of Steroid Biochemistry and Molecular Biology 39:51-54

31. Nordenbaek C, Kyvik KO, Skytthe A, Bilenberg N (2011) Efficiency of a small size screening instrument in identifying children with autism spectrum disorders in a large population of twins. Epidemiology Research InternationalArticle ID 412150 doi:10.1155/2011/412150

32. Ooi YP, Rescorla L, Ang RP, Woo B, Fung DSS (2011) Identification of Autism Spectrum Disorders Using the Child Behavior Checklist in Singapore. Journal of Autism and Developmental Disorders 41:1147-1156

33. Pasterski VL, Geffner ME, Brain C, Hindmarsh P, Brook C, Hines M (2005) Prenatal hormones and postnatal socialization by parents as determinants of male-typical toy play in girls with congenital adrenal hyperplasia. Child Development 76:264-278

34. Reiersen AM, Constantino JN, Grimmer M, Martin NG, Todd RD (2008) Evidence for Shared Genetic Influences on Self-Reported ADHD and Autistic Symptoms in Young Adult Australian Twins. Twin Research and Human Genetics 11:579-585

35. Reiersen AM, Constantino JN, Volk HE, Todd RD (2007) Autistic traits in a population-based ADHD twin sample. Journal of Child Psychology and Psychiatry 48:464-472

36. Rommelse NNJ, Franke B, Geurts HM, Hartman CA, Buitelaar JK (2010) Shared heritability of attention-deficit/hyperactivity disorder and autism spectrum disorder. European Child & Adolescent Psychiatry 19:281-295

37. Rutter M (2005) Aetiology of autism: findings and questions. Journal of Intellectual Disability Research 49:231-238

38. Skytthe A, Kyvik K, Holm NV, Vaupel JW, Christensen K (2002) The Danish Twin

Registry: 127 birth cohorts of twins. Twin Res 5:352-357

39. Soper HV, Satz P, Orsini DL, Henry RR, Zvi JC, Schulman M (1986) Handedness Patterns in Autism Suggest Subtypes. Journal of Autism and Developmental Disorders 16:155-167

40. Steffenburg S, Gillberg C, Hellgren L, Andersson L, Gillberg IC, Jakobsson G, Bohman M (1989) A Twin Study of Autism in Denmark, Finland, Iceland, Norway and Sweden. Journal of Child Psychology and Psychiatry and Allied Disciplines 30:405-416

41. vom Saal FS (1989) Sexual-Differentiation in Litter-Bearing Mammals - Influence of Sex of Adjacent Fetuses Inutero. Journal of Animal Science 67:1824-1840

42. Wing L (1988) The autistic continuum. *Aspects of autism: Biological research*. Gaskell/Royal College of Psychiatrists, London,

Tables

Table 1. Statements about children's behaviour in the parental questionnaire (English version)

Item number	Text	CBCL item number	CBCL subscale
Q1	Cannot sit still, is restless or hyperactive.	10	ADHD problems
Q2	Cannot concentrate, cannot pay attention for long.	8	ADHD problems
Q3	Exhibits strange behaviour.	84	Thought problems
Q4	Exhibits a sudden change in mood or feelings.	87	Social problems
Q5	Cannot get along with others.	25	Aggressive behavior

Table 2. Characteristics of the female twins in the study

Characteristics	*N* (%)					
	All (*N* = 6,339)		With female co-twin (*N* = 3,412)		With male co-twin (*N* = 2,927)	
Birthweight						
≤ 1,999 g	882	(13.9)	486	(14.2)	396	(13.5)
2,000 – 2,499 g	1,653	(26.1)	892	(26.1)	761	(26.0)
2,500 – 2,999 g	2,358	(37.2)	1,221	(35.8)	1,137	(38.9)
3,000 – 3,499 g	1,073	(16.9)	556	(16.3)	517	(17.7)
≥ 3,500 g	168	(2.7)	96	(2.8)	72	(2.5)
Data missing	205	(3.2)	161	(4.7)	44	(1.5)
Gestational age						
≤ 36 wk	2,224	(35.0)	1,182	(34.6)	1,042	(35.6)
37 - 39 wk	3,829	(60.4)	2,069	(60.6)	1,760	(60.1)
≥ 40 wk	264	(4.2)	150	(4.4)	114	(3.9)
Data missing	22	(0.4)	11	(0.3)	11	(0.4)
Apgar score						
0 - 5	51	(0.8)	25	(0.7)	26	(0.9)
6 -10	6,075	(95.8)	3,213	(94.2)	2,862	(97.8)
Data missing	213	(3.4)	174	(5.1)	39	(1.3)
Parity						
0	1,977	(31.2)	1,082	(31.7)	895	(30.6)
1	2,550	(40.2)	1,379	(40.4)	1,171	(40.0)
≥ 2	1,812	(28.6)	951	(27.9)	861	(29.4)
Age						
≤ 4 y	1,460	(23.0)	752	(22.0)	708	(24.2)
5 - 9 y	2,817	(44.4)	1,466	(43.0)	1,351	(46.2)
≥ 10 y	2,062	(32.5)	1,194	(35.0)	868	(29.7)

Mother's age						
≤ 24 y	432	(6.8)	244	(7.2)	188	(6.4)
25 - 34 y	4,703	(74.2)	2,562	(75.1)	2,141	(73.2)
≥ 35 y	1,204	(19.0)	606	(17.8)	598	(20.4)
Father's age						
≤ 24 y	168	(2.7)	112	(3.3)	56	(1.9)
25 - 34 y	3,351	(52.9)	1,814	(53.2)	1,537	(52.5)
≥ 35 y	2,138	(33.7)	1,089	(31.9)	1,049	(35.8)
Data missing	682	(10.8)	397	(11.6)	285	(9.7)
Mother born in DK						
Yes	5,900	(93.1)	3,166	(92.8)	2,734	(93.4)
No	439	(6.9)	246	(7.2)	193	(6.6)

Table 3. Distribution of ADHD and total scores by sex of the twins

Sex of index-twin	Sex of co-twin	*N* (%)							
		ADHD score							
		0	1	2	3	4			Total
Male	Male	2,594	489	306	84	61			3,534
		(73.4)	(13.8)	(8.7)	(2.4)	(1.7)			(100.0)
	Female	2,116	381	248	81	64			2,890
		(73.2)	(13.2)	(8.6)	(2.8)	(2.2)			(100.0)
	Total	4,710	870	554	165	125			6,424
		(73.3)	(13.5)	(8.6)	(2.6)	(1.9)			(100.0)
Female	Male	2,466	242	116	43	26			2,893
		(85.2)	(8.4)	(4.0)	(1.5)	(0.9)			(100.0)
	Female	2,699	401	196	53	32			3,381
		(79.8)	(11.9)	(5.8)	(1.6)	(1.0)			(100.0)
	Total	5,165	643	312	96	58			6,274
		(82.3)	(10.2)	(5.0)	(1.5)	(0.9)			(100.0)
		Total score							
		0	1	2	3	4	5	6-10	Total
Male	Male	2,028	700	331	205	77	42	82	3,465
		(58.5)	(20.2)	(9.6)	(5.9)	(2.2)	(1.2)	(2.4)	(100.0)
	Female	1,736	509	266	128	78	59	76	2,852
		(60.9)	(17.9)	(9.3)	(4.5)	(2.7)	(2.1)	(2.7)	(100.0)
	Total	3,764	1,209	597	333	155	101	158	6,317
		(59.6)	(19.1)	(9.5)	(5.3)	(2.5)	(1.6)	(2.5)	(100.0)
Female	Male	1,958	534	196	77	45	14	28	2,852

	(68.7)	(18.7)	(6.9)	(2.7)	(1.6)	(0.5)	(1.0)	(100.0)
Female	2,064	666	332	130	68	27	43	3,330
	(62.0)	(20.0)	(10.0)	(3.9)	(2.0)	(0.8)	(1.3)	(100.0)
Total	4,022	1200	528	207	113	41	71	6,182
	(65.1)	(19.4)	(8.5)	(3.4)	(1.8)	(0.7)	(1.2)	(100.0)

Table 4. Odds ratios with 95% confidence intervals for ADHD and total scores in females with a male versus a female co-twin

	Model A[a]	Model B[b]	Model C[c]
ADHD score	0.69 [0.60-0.80]	0.71 [0.61-0.81]	0.71 [0.61-0.82]
Total score	0.73 [0.65-0.82]	0.74 [0.66-0.83]	0.74 [0.66-0.83]

a: Model A: No adjustment (crude OR).

b: Model B: Adjusted for birthweight and gestational age.

c: Model C: Adjusted for birthweight, gestational age, child's age, mother's age at birth, and mother's place of birth.