# Attention Problems and Attention-Deficit/Hyperactivity Disorder in Discordant and Concordant Monozygotic Twins: Evidence of Environmental Mediators

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**ABSTRACT**

**Objective:** To study familial and nonfamilial environmental influences on attention problems and attention-deficit/hyperactivity disorder (ADHD) in monozygotic twins discordant and concordant-high and low for these traits. **Method:** Ninety-five twin pairs from The Netherlands Twin Register were selected. Longitudinal survey data were collected at 1, 2, 3, 5, 7, 10, and 12 years from parents, twins, and teachers. Mothers participated in a structured clinical interview when twins were between 10 and 17 years of age. **Results:** Affected twins from discordant pairs scored higher than unaffected cotwins on multiple measures of attention problems, ADHD, and other behavior problems according to mother, teacher, and self. Behavioral discordance was evident at age 2 and all subsequent measurements. Compared with unaffected cotwins, affected twins had lower birth weight and delayed physical growth and motor development. Differences between discordant and concordant groups were reported for maternal smoking, sleeping in different rooms, and living with only one parent. **Conclusions:** Significant markers of ADHD are found in infancy and include low birth weight and delayed motor development. As the knowledge of specific genetic and environmental influences on ADHD increases, future studies may focus on their complex interplay. *J. Am. Acad. Child Adolesc. Psychiatry,* 2007;46(1):83–91. **Key Words:** monozygotic twins, discordant twins, attention problems, attention-deficit/hyperactivity disorder, environmental risk factors.

In the field of developmental psychopathology, there is increasing enthusiasm for the study of specific genetic and environmental influences on attention-deficit/hyperactivity disorder (ADHD; Kahn et al., 2003). This report presents a powerful method to identify environmental influences on ADHD, namely, the monozygotic (MZ) discordant twin design.

The etiology of ADHD has been studied extensively. Twin, family, and adoption studies provide evidence of significant genetic influences on ADHD (Derks and Boomsma, in press; Faraone and Doyle, 2000) with heritability estimates of at least 60%. This implies that environmental factors may explain as much as 40% of the etiology of ADHD. The search for environmental factors that contribute to the development of ADHD has yielded a number of candidates. These candidates include poor parenting strategies and family dysfunction (Biederman et al., 2002), low parental socioeconomic status (SES) and environmental deprivation (Ornoy, 2003), food additives, (Boris and Mandel, 1994), maternal smoking (Thapar et al., 2003),

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maternal alcohol consumption (Knopik et al., 2005), and traumatic brain injury (Bloom et al., 2001). The strongest evidence concerns insults that occur during the pre- and perineonatal period such as intrauterine exposure to nicotine, which has repeatedly been associated with increased (up to twofold) risk of ADHD (reviewed in Linnet et al., 2003). The relationship between maternal smoking during pregnancy and ADHD in offspring remains significant after controlling for parental ADHD status (e.g., Mick et al., 2002). Children who are born prematurely and with low birth weight are also at increased risk of developing symptoms of ADHD (for meta-analysis, see Bhutta et al., 2002). The relative value of adversity, low birth weight, exposure to intrauterine alcohol, cocaine, nicotine, lead, and viral infections have been hypothesized as potential contributors to the etiopathology of ADHD.

The interaction between environmental mediators and specific genes has been studied by Kahn et al. (2003) who reported that prenatal nicotine exposure and a particular DAT polymorphism (DAT 10 repeat) increase the risk of ADHD only when both risk factors are present. Early findings such as these have increased the need to refine both our molecular genetic and environmental assessment strategies.

A unique application of the twin design, and the focus of this report, is the investigation of specific, unique environmental influences on ADHD through the Monozygotic Twin Difference Method (Martin et al., 1997). Because MZ twins nearly always have identical genomes, most differences in their behavior must be caused by the effects of environmental influences, which may act directly on the phenotype, or, for example, through postgenomic modifications via methylation processes (Fraga et al., 2005). A group of MZ twins discordant for ADHD has previously been described in terms of clinical characteristics (Sharp et al., 2003) and brain anatomy (Castellanos et al., 2003). These MZ discordant twins demonstrated decreased familiality of ADHD in terms of lower symptom scores in fathers when compared with affected singletons. Also, affected twins had lower birth weights, were more likely to present in breech position, and had volumetric reductions in caudate nucleus compared with unaffected cotwins.

This study expands on this prior work by using prospective data to evaluate differences within MZ discordant pairs. We also compare MZ discordant pairs to MZ concordant pairs. We look at a large range of environmental mediators such as maternal smoking and alcohol use during pregnancy, duration of pregnancy, placenta sharing, birth weight and height, time in the incubator, and medical complications. Developmental processes are considered such as rate of maturation, physical health, and medical histories. Environmental factors such as sharing a bedroom or classroom and living with only one parent are also examined.

The aim of this study was to identify and describe environmental mediators of attention problems (APs) and ADHD. MZ twins were selected for discordance in AP symptom scores on the Child Behavior Checklist (CBCL) and ADHD symptom scores obtained with the Diagnostic Interview Schedule for Children (DISC). Two groups of MZ concordant pairs were included: concordant for high APs/ADHD and concordant for low APs/ADHD. All twin pairs were recruited from The Netherlands Twin Register (NTR), which consists of more than 25,000 twin pairs studied prospectively since birth. Their parents and teachers participate in survey studies and provide the data for prospective analyses of AP/ADHD environmental risk factors.

## METHOD

### Subjects

Twins from the NTR (Boomsma et al., 2002) enroll in longitudinal survey studies that focus on growth, health, and the development of behavior and behavior problems. Surveys are sent to the parents when the twins are 1, 2, 3, 5, 7, 10, and 12 years old and to the teachers from age 7 onward.

Ninety-five MZ twin pairs participated in this study. They were selected from two ongoing studies of the NTR. One study (henceforth referred to as wave I) combines information from multiple informants, time points, and assessment techniques to identify heritable phenotypes for ADHD. The other study (wave II) uses magnetic resonance imaging to trace symptoms of ADHD back to abnormalities in neural structure and processing. The selection procedures employed in both waves are summarized in Figure 1.

For wave I, children were selected from birth cohorts 1989–1992 and for wave II from cohorts 1986–1994. Subjects who were likely to be MZ twins were selected among twins whose mothers had completed the NTR surveys at ages 7, 10, and/or 12 years at least at two time points. Within the remaining sample, discordant and concordant twin pairs were selected for interview participation. A twin pair was initially regarded as discordant if one twin scored high on APs measured with the CBCL ($T \geq 60$ at all available time points and $T \geq 65$ at least once). A twin pair was regarded concordant-low if both twins had low AP scores ($T \leq 55$ at all available time points) and concordant-high if both twins had high scores. Discordant and concordant pairs were matched on gender, zygosity, date of birth, maternal age, and parental SES. For wave II, matching criteria were

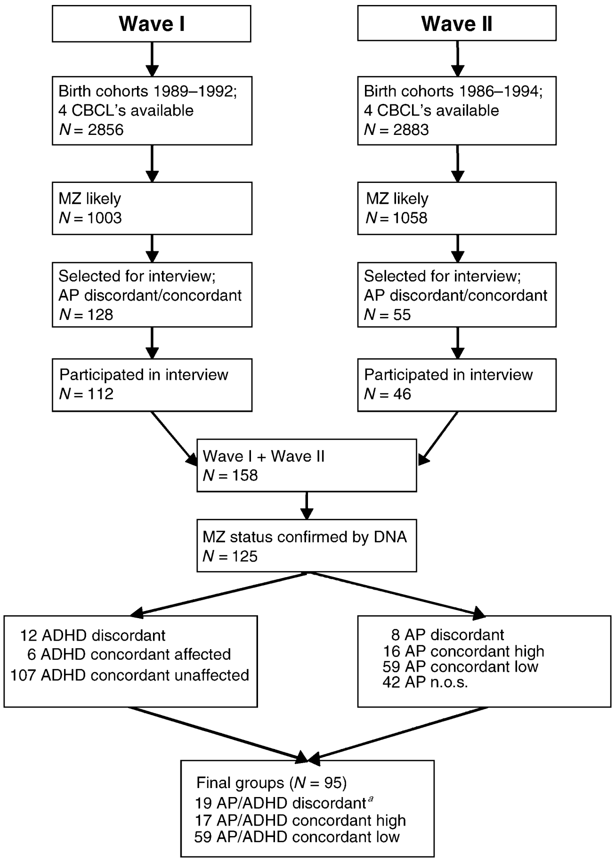


**Fig. 1** Selection of monozygotic twin pairs discordant and concordant for attention problems (AP)/attention-deficit/hyperactivity disorder (ADHD). [a] One discordant pair was excluded from the analysis because the unaffected twin used Ritalin at the time of the clinical interview. CBCL = Child Behavior Checklist; MZ = monozygotic; AP = attention problems; *N* = number of twin pairs; n.o.s. = not otherwise specified.

gender, date of birth, zygosity, handedness, and SES. Twins with severe physical or mental disabilities (e.g., autism, blindness) were excluded. No other Axis I, II, or III disorders were excluded. A total of 183 twin pairs were selected (128 in wave I and 55 in wave II), and 158 pairs (86%) successfully participated. Of the group of 158 families, 120 mothers, 104 fathers, and 135 twin pairs returned a DNA sample. Zygosity testing has been completed for 132 pairs. DNA testing revealed that 125 pairs were indeed MZ. The remaining seven pairs were DZ and were excluded from statistical analyses.

The mothers of the twins were interviewed with the DISC (see below), and the diagnostic data were used to determine the twins' ADHD status (affected/unaffected; based on type A criteria of the *DSM-IV*: six or more/fewer than six symptoms). By combining ADHD status with AP status (high/low) derived from the CBCL, three groups were defined: discordant MZ twins (ADHD discordant and/or AP discordant; *n* = 19 pairs); concordant-high MZ twins (ADHD concordant affected provided that a twin pair is not AP discordant and/or AP concordant-high provided that a twin pair is not ADHD discordant; *n* = 17 pairs); and concordant-low MZ twins (ADHD concordant unaffected and AP concordant-low; *n* = 59 pairs). This classification excluded 29 MZ twin pairs who were ADHD concordant unaffected and APs not otherwise specified (one twin had AP scores that were neither high nor low).

The study procedures conformed to the guidelines of our local ethics committee. Mothers provided written informed consent in advance of participation.

## Measures

*Clinical Interview.* The mother completed a clinical interview, administered over the telephone by trained medical students and tape recorded in most cases. The interview was based on the Dutch version of the DISC-IV Parent Version (Ferdinand and Van der Ende, 1998). This is a highly structured interview designed for 6- to 17-year-old children. Diagnostic criteria are based on the type A criteria of the *DSM-IV* (American Psychiatric Association, 1994). The interview also contained questions about the use of psychotherapeutic drugs and the home environment. The 11 items belonging to the AP subscale of the CBCL were also administered.

*Postinterview Questionnaires to Mothers and Twins.* After the clinical interview, mothers completed the CBCL (CBCL/4–18; Achenbach, 1991a) and the Strength and Weaknesses of ADHD Symptoms and Normal Behavior scale (SWAN; Swanson et al., 2001). With the mother's permission, all twins ages 11 years or older were invited to complete the Youth Self-Report (YSR; Achenbach, 1991b).

*DNA Zygosity Testing.* A few months after the clinical interview, all of the families were asked to provide buccal samples for DNA extraction. Zygosity testing included polymerase chain reaction of 11 highly polymorphic genetic markers.

*Longitudinal NTR Surveys.* Prospective data were obtained from the longitudinal NTR surveys. A few months of the twins' birth mothers reported characteristics of the pregnancy, birth, and postnatal period. At age 2, the NTR survey assessed twins' behavioral characteristics, medical history, motor development, and physical growth. At age 3, the survey included the CBCL (CBCL/2–3; Achenbach, 1992), in addition to questions about the twins' medical history and physical growth. At age 5, the survey included 42 items from the Devereux Child Behavior Rating Scale (van Beijsterveldt et al., 2004; Spivack and Spotts, 1966). Questions about day care, school, home environment, medical history, growth, and language and motor development were also included. At ages 7, 10, and 12, the surveys included the CBCL/4–18. Information was also collected about physical and mental disabilities, weight, height, school situation, medical conditions, and use of medication or whether they were receiving health care.

Parental SES was assessed at ages 7 and 10 years and was based on a full description of the occupation of the parents. The level of occupation was coded according to the system used by Statistics Netherlands (Centraal Bureau voor de Statistiek, 1993) into five levels based on the mental complexity of the work and ranged from low skilled (1) to academic work (5). According to Statistics Netherlands, the percentages of families in each of the classes were 7%, 25%, 40%, 20%, and 8%, respectively. The mean SES in the general population was 2.97. For this report, the most recent data available for parental SES were used. When the occupation information was present for both parents, the highest level was taken.

From age 7 onward, teacher data were collected once permission to approach the teacher was obtained from the parents. In the NTR, 80.1% of the parents provided consent to approach the teachers. The response rates of the teachers were 77%, 75%, and 71% at ages 7, 10, and 12 years, respectively. The Attention Problem scale (20 items) derived from the Teacher Report Form (Achenbach, 1991b) at age 12 was analyzed.

### Statistical Analyses

*Within-Pairs Analyses.* Analyses of child-specific variables (e.g., birth weight) were restricted to discordant pairs. Paired differences (i.e., affected twin versus unaffected cotwin) were tested for significance with *t* tests for two related samples (continuous data), Wilcoxon signed rank test (ordinal data), and McNemar $\chi^2$ tests for matched pairs (nominal data). One-tailed probabilities were used as the affected twins were expected to show more adversity than their unaffected cotwins.

*Between-Pairs Analyses.* Variables that reflect correspondence between members of a twin pair (e.g., same or different schools) were compared across the three groups of twins. Variables that reflect characteristics that are shared by both members of a twin pair (e.g., maternal smoking) were tested with one-way analyses of variance (continuous data), Kruskal-Wallis tests (ordinal data), and $\chi^2$ tests (nominal data). Post hoc comparisons included the $\chi^2$ test (nominal data) and the Mann-Whitney *U* test (ordinal data).

Two-tailed probabilities were used because we had no clear expectation regarding the direction of effects. Because of the small sample size, α was set at .10 to ensure sufficient statistical power and to minimize the risk of making type II errors.

## RESULTS

### General Characteristics

We identified 19 MZ twin pairs discordant for APd (Table 1). This group consisted of nine male and 10 female pairs who were between 11 and 15 years old (mean 13.36, SD 1.54) at the time of the clinical interview. Eight pairs were AP discordant and 11 pairs were ADHD discordant. Of the pairs who met type A criteria for ADHD, none had received prior treatment except in one family. The discordant twins had few nonpsychiatric medical conditions. However, in one set of twins (no. 3), the affected twin was developmentally retarded and suffered from dyspraxia. Both twins from pair no. 6 had diabetes, and both members of pair no. 15 were born with craniofacial anomalies (affected twin: cleft lip; unaffected cotwin: cleft lip, jaw, and palate). Parental SES was rated 4 in the majority (58%) of the MZ discordant twin pairs, with the remaining pairs rated 2 (21%) or 3 (21%). Average maternal age at time of birth was 30.05 years (SD = 4.00), which is similar to the average age of 29.97 in the NTR 1986–1992 cohorts.

There were 17 MZ twin pairs concordant-high for AP (7 male and 10 female pairs; mean age 13.07 years, SD 1.98). The female majority in the concordant affected pairs is the result of the selection procedure that was based on *T* scores that were

**TABLE 1**
Demographics and ADHD Symptom Classification of MZ Twins Discordant for APs/ADHD

| Twin Pair No. | Age, y[a] | Sex | AP Scores Affected/Unaffected | ADHD Affected/Unaffected | ADHD Subtype Affected |
|---|---|---|---|---|---|
| 1 | 15.77 | M | High/low | No/no | — |
| 2 | 15.41 | F | High/low | No/no | — |
| 3 | 15.37 | M | High/low | No/no | — |
| 4 | 15.05 | M | High/low | No/no | — |
| 5 | 14.88 | F | High/low | No/no | — |
| 6 | 14.98 | M | High/high | Yes/no | IA |
| 7 | 13.96 | F | High/low | No/no | — |
| 8 | 12.99 | F | ~/~ | Yes/no | H/I |
| 9 | 13.00 | F | ~/~ | Yes/no | IA |
| 10 | 13.94 | M | High/high | Yes/no | C |
| 11 | 12.95 | F | High/high | Yes/no | IA |
| 12 | 12.08 | M | High/~ | Yes/no | H/I |
| 13 | 12.01 | F | High/~ | Yes/no | H/I |
| 14 | 11.58 | M | High/high | Yes/no | IA |
| 15 | 11.52 | M | High/low | No/no | — |
| 16 | 11.38 | F | ~/~ | Yes/no | IA |
| 17 | 12.79 | F | High/low | No/no | — |
| 18 | 11.03 | F | High/~ | Yes/no | H/I |
| 19 | 13.08 | M | High/~ | Yes/no | IA |

*Note:* ADHD = attention-deficit/hyperactivity disorder; MZ = monozygotic; AP = attention problems measured with the Child Behavior Checklist; M = male; F = female; AP scores high = *T* score ≥60 at all available time points and ≥65 at least once; AP scores low = *T* score ≤55 at all available time points; AP scores ~ = *T* scores fall in neither high nor low status; ADHD yes = twin meets *DSM-IV* type A criteria; ADHD no = twin does not meet *DSM-IV* type A criteria; IA = inattentive subtype; H/I = hyperactive/impulsive subtype; C = combined subtype.

[a] Age at the time of the clinical interview.

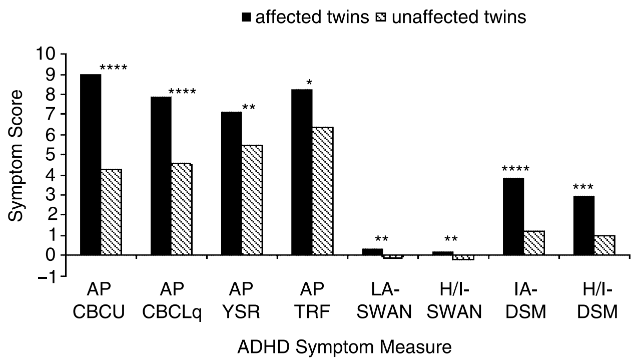


**Fig. 2** ADHD symptom ratings in monozygotic twins discordant for AP/ADHD. AP = attention problems (Child Behavior Checklist); IA = symptoms of inattention; H/I = symptoms of hyperactivity/impulsivity; CBCL = Child Behavior Checklist; YSR = Youth Self-Report; TRF = Teacher Report Form; SWAN = Strengths and Weaknesses of ADHD–Symptoms and Normal Behavior scale; i = ratings obtained during the clinical interview; q = ratings obtained with questionnaire after the clinical interview. *$p < .10$; **$p < .05$; ***$p < .01$; ****$p > .005$; *****$p < .001$. Scales differ in range (AP CBCLi, AP CBCLq, AP YSR and AP TRF: 0–22; IA-SWAN and H/I-SWAN: −3 to 3; IA-DSM and H/I-DSM: 0–9). Figure is based on data from 19, 17, 15, 11, 16, 16, 19, and 19 (for symptom measures left to right) complete twin pairs.

computed separately in boys and girls. The group of concordant-high twin pairs includes 11 pairs who are AP concordant, 5 pairs who are ADHD concordant, and 1 pair that is both AP-and ADHD concordant. Of the 34 concordant-high children, 12 children met criteria for ADHD. Four children (both twins in one pair and the first born twin in two pairs) used Ritalin at the time of the interview. Of the concordant-high twin pairs, 71% has a parental SES of 3, with the remaining pairs rated as 1 (6%), 4 (18%), and 5 (6%). Maternal age at time of birth is 28.16 years on average (SD 3.45).

There were 59 MZ twin pairs concordant-low for APs (30 male and 29 female; age, mean 13.01 years, SD 1.41). In this group, most twins have a parental SES of either 3 (37%) or 4 (36%), with the remaining pairs rated as 2 (7%) or 5 (20%). Maternal age is 30.06 years on average (SD 3.66).

The three groups of MZ twins did not differ significantly in terms of age, sex, or maternal age. A significant group difference was found in parental SES (Kruskal-Wallis $\chi^2 = 5.00$; $p_{\text{two-tailed}} = .082$). Parental SES is significantly higher among concordant-low twins than among concordant-high twins (Mann-Whitney $U = 342.50$; $p_{\text{two-tailed}} = .034$). The parental SES of discordant twins lies between that of the two other groups, but differs significantly from neither.

### Current Behavior Problems

Affected members of the MZ discordant twin pairs score higher than their unaffected cotwins on all measures of ADHD-related symptoms (Fig. 2). The difference within discordant pairs in ADHD-related symptoms is paralleled by differences in multiple other problem behaviors. Compared with their unaffected cotwins, affected twins report more rule-breaking behavior ($\text{mean}_{\text{aff}}$ 3.40, $\text{SD}_{\text{aff}}$ 1.30; $\text{mean}_{\text{unaff}}$ 2.80, $\text{SD}_{\text{unaff}}$ 1.74; $t(14) = 2.07$, $p_{\text{one-tailed}} = .029$), have more social problems ($\text{mean}_{\text{aff}}$ 3.67, $\text{SD}_{\text{aff}}$ 2.72; $\text{mean}_{\text{unaff}}$ 2.47, $\text{SD}_{\text{unaff}}$ 2.07; $t(14) = 1.75$, $p_{\text{one-tailed}} = .051$), and are more aggressive ($\text{mean}_{\text{aff}}$ 10.93, $\text{SD}_{\text{aff}}$ 4.54; $\text{mean}_{\text{unaff}}$ 7.67, $\text{SD}_{\text{unaff}}$ 4.45; $t(14) = 2.53$, $p_{\text{one-tailed}} = .012$), withdrawn ($\text{mean}_{\text{aff}}$ 3.40, $\text{SD}_{\text{aff}}$ 2.69; $\text{mean}_{\text{unaff}}$ 2.33, $\text{SD}_{\text{unaff}}$ 2.53; $t(14) = 2.62$, $p_{\text{one-tailed}} = .010$), and anxious/depressed ($\text{mean}_{\text{aff}}$ 8.20, $\text{SD}_{\text{aff}}$ 5.51; $\text{mean}_{\text{unaff}}$ 5.27, $\text{SD}_{\text{unaff}}$ 4.62; $t(14) = 2.62$, $p_{\text{one-tailed}} = .073$). The maternal CBCL ratings yield significant differences on aggressive behavior ($\text{mean}_{\text{aff}}$ 11.41, $\text{SD}_{\text{aff}}$ 7.64; $\text{mean}_{\text{unaff}}$ 7.76, $\text{SD}_{\text{unaff}}$ 7.21; $t(16) = 2.29$, $p_{\text{one-tailed}} = .018$) and social problems ($\text{mean}_{\text{aff}}$ 3.06, $\text{SD}_{\text{aff}}$ 3.17; $\text{mean}_{\text{unaff}}$ 1.82, $\text{SD}_{\text{unaff}}$ 1.94; $t(16) = 2.11$, $p_{\text{one-tailed}} = .025$). Psychiatric diagnoses other than ADHD are also found exclusively in affected children, in pair no. 12 (social phobia and oppositional disorder), and in pair no. 19 (specific phobia for blood and wounds).

### Development

The longitudinal data show that the differences in ADHD symptomatology were present at an early age and were relatively stable throughout development (Fig. 3).

Also with regard to other behavior problems, differences within discordant pairs have been present since toddlerhood. Figure 4 shows the differences in aggressive behavior and social problems because these are most prominent and also most persistent across time. The earliest reports of the twins' behavioral characteristics were collected at age 2 and concern sleep, crying, and amount of distress from disturbances in the daily routine. During the first 18 months of life, affected twins cried significantly more often (Wilcoxon $Z = -1.41$, $p_{\text{one-tailed}} = .079$) than unaffected cotwins.

The affected twins also lagged behind their unaffected cotwins in early motor development. They were slightly but significantly older when they learned how to roll over ($\text{mean}_{\text{aff}}$ 6.88 months, $\text{SD}_{\text{aff}}$ 2.26; $\text{mean}_{\text{unaff}}$ 6.38

months, $SD_{unaff}$ 1.65; $t(15) = 1.44$, $p_{one\text{-}tailed} = .086$) and to sit upright ($mean_{aff}$ 9.47 months, $SD_{aff}$ 2.97; $mean_{unaff}$ 8.89, $SD_{unaff}$ 2.23; $t(17) = 2.08$, $p_{one\text{-}tailed} = .027$). The passing of later milestones in motor development was not significantly different within discordant pairs.

We found evidence of a growth discrepancy between affected and unaffected twins during the first 2 years of life. Affected twins weighed less than their unaffected cotwins around ages 6 months ($mean_{aff}$ 6,589.44 g, $SD_{aff}$ 1,126.76; $mean_{unaff}$ 6,910.28 g, $SD_{unaff}$ 837.02; $t(17) = -1.74$, $p_{one\text{-}tailed} = .050$), 1 year ($mean_{aff}$ 9,099.72 g, $SD_{aff}$ 1,539.82; $mean_{unaff}$ 9,375.56 g, $SD_{unaff}$ 1,226.16; $t(17) = -1.38$, $p_{one\text{-}tailed} = .093$), and 2 years ($mean_{aff}$ 11,967.69 g, $SD_{aff}$ 2,690.85; $mean_{unaff}$ 12,536.92 g, $SD_{unaff}$ 2,493.12; $t(12) = -1.65$, $p_{one\text{-}tailed} = .062$). Similarly, affected twins were of shorter statue at 6 months ($mean_{aff}$ 64.33 cm, $SD_{aff}$ 4.28; $mean_{unaff}$ 65.14 g, $SD_{unaff}$ 3.04; $t(15) = -1.61$, $p_{one\text{-}tailed} = .065$), 1 year ($mean_{aff}$ 74.38 cm, $SD_{aff}$ 3.71; $mean_{unaff}$ 75.04 cm, $SD_{unaff}$ 3.34; $t(15) = -1.41$, $p_{one\text{-}tailed} = .089$), and 2 years ($mean_{aff}$ 86.54 cm, $SD_{aff}$ 6.33; $mean_{unaff}$ 87.96 cm, $SD_{unaff}$ 5.76; $t(12) = -2.03$, $p = .033$). At age 3, neither weight ($mean_{aff}$ 13,970.00 g, $SD_{aff}$ 2,802.40; $mean_{unaff}$ 14,290.00 g, $SD_{unaff}$ 2,147.58) nor height ($mean_{aff}$ 96.05 cm, $SD_{aff}$ 5.79; $mean_{unaff}$ 96.00 cm, $SD_{unaff}$ 5.27) differed within discordant pairs.

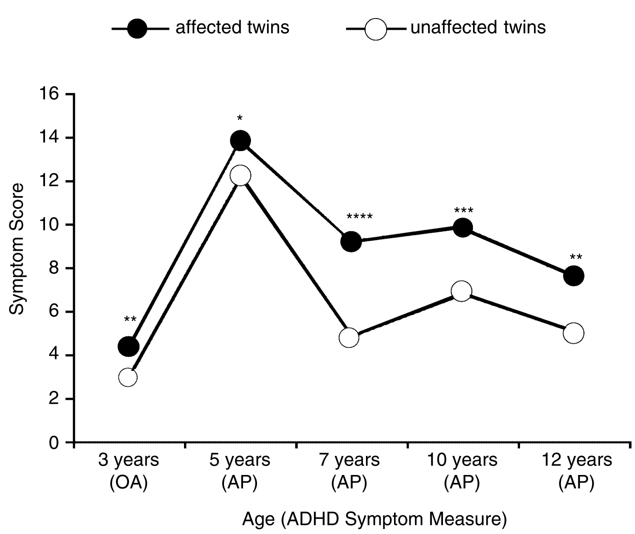


**Fig. 3** Development of ADHD symptoms in monozygotic twins discordant for AP/ADHD. OA = overactivity (Child Behavior Checklist); AP = attention problems (Devereux Child Behavior Rating Scale at age 5; Child Behavior Checklist at ages 7, 10, and 12); $^{*}p < .10$; $^{**}p < .05$; $^{***}p < .01$; $^{****}p > .005$. Scales differ in range (OA age 3: 0–10; AP age 5: 0–25; AP ages 7, 10, and 12: 0–22). Figure is based on data from 15, 17, 17, 18, and 15 (for ages left to right) complete twin pairs.

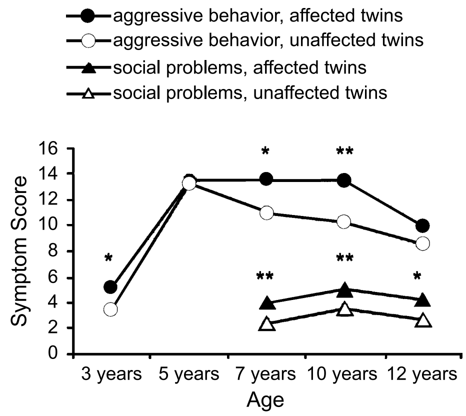


**Fig. 4** Development of additional behavior problems in monozygotic twins discordant for AP/ADHD. AP = attention problems; $^{*}p < .10$; $^{**}p < .05$. Scales differ in range (aggressive behavior age 3: 0–18; aggressive behavior age 5: 0–35; aggressive behavior ages 7, 10, and 12: 0–40; social problems ages 7, 10, and 12: 0–16). Social problems were not assessed at ages 3 and 5. Figure is based on data from 15, 17, 17, 18, and 15 (for ages left to right) complete twin pairs.

### Further Characteristics of Discordant Twins

In discordant pairs, affected twins were about equally often the first born (nine pairs) as the second born (10 pairs). The average birth weight of affected twins was significantly lower (mean 2,421.84, SD 542.23) than the average birth weight of unaffected cotwins (mean 2,591.05, SD 517.22): $t(18) = -1.99$, $p_{one\text{-}tailed} = .031$. No difference was found in the twins' height at birth.

The incidence of neonatal medical complications was similar in affected and unaffected twins. However, when both twins were in the incubator (12 pairs), the duration of treatment was significantly longer for the affected ($mean_{aff}$ 12.51 days, $SD_{aff}$ 16.02) than for the unaffected ($mean_{unaff}$ 8.84 days, $SD_{unaff}$ 13.66) twin ($t(11) = 1.62$, $p_{one\text{-}tailed} = .067$).

Except for the initial differences in maturation, both members of most discordant pairs have been in good physical health. Their medical histories are dominated by harmless injuries (e.g., cuts, wounds) and common diseases of childhood (e.g., ear infections, chickenpox). The only severe condition reported is the occurrence of meningitis at age 5 in one of the affected twins (pair no. 5). Within pairs, there are no significant differences in the number of general practitioner consults, hospitalization, anaesthetization, or drug treatments.

### Environmental Mediators of Behavioral Discordance

A significant difference was found in the frequency with which mothers in the discordant, concordant-high, and concordant-low groups reported smoking during pregnancy ($\chi^2_2$ = 6.25, $p_{\text{two-tailed}}$ = .044). Maternal smoking was more common in the group of concordant-high twins (6/17, 35%) than in the group of concordant-low twins (6/59, 10%): $\chi^2_1$ = 6.27, $p_{\text{two-tailed}}$ = .012). Discordant twins (maternal smoking reported in 4/19, 21% of pairs) did not differ from the concordant groups on this measure. No group differences were found in other pregnancy characteristics (i.e., pregnancy duration, maternal alcohol consumption, proportion of twins sharing the placenta).

During the first 2 years of life, discordant twins slept in the same room in a majority of cases (17/18, 94%). A similar pattern is found among concordant-high twins (17/17, 100%) and concordant-low twins (54/57, 95%), and there is no significant difference across groups. Sleeping in separate rooms becomes more common at later ages, and significant group differences appear both at age 2 to 4 years ($\chi^2_2$ = 5.16, $p_{\text{two-tailed}}$ = .076) and age 4 to 6 years ($\chi^2_2$ = 4.79, $p_{\text{two-tailed}}$ = .091). Sleeping in separate rooms was significantly more common in discordant pairs than in concordant-low pairs both at age 2 to 4 years ($n_{\text{disc}}$ = 4/18, 22%; $n_{\text{conc-low}}$ = 3/57, 5%; $\chi^2_1$ = 4.65, $p_{\text{two-tailed}}$ = .031 and age 4 to 6 years ($n_{\text{disc}}$ = 6/17, 35%; $n_{\text{conc-low}}$ = 7/57, 12%; $\chi^2_1$ = 4.79, $p_{\text{two-tailed}}$ = .029). No group differences were found in the proportion of twins pairs who were in the same class and/or at the same school at age 5. A significant difference was found in the proportion of twins who lived with both parents at age 5 ($\chi^2_2$ = 9.10, $p_{\text{two-tailed}}$ = .011). Post hoc analyses revealed that living with only one parent was significantly more common among concordant-high twins ($n_{\text{conc-high}}$ = 3/17, 18%) than among discordant twins ($n_{\text{disc}}$ = 0/18, 0%; $\chi^2_1$ = 3.47, $p_{\text{two-tailed}}$ = .062) or concordant-low twins ($n_{\text{conc-low}}$ = 1/58, 2%; $\chi^2_1$ = 6.60, $p_{\text{two-tailed}}$ = .010).

## DISCUSSION

One of the promises of the genomic era in medicine is that through the discovery of genes involved in the etiopathology of common diseases, such as ADHD, advances will be made regarding diagnosis, prognosis, and treatment. Although multiple genes of small effect have been identified, principally through candidate gene approaches, none have yielded the kind of information that has led to changes in the way this disorder is assessed or treated. An equally important approach to developing new assessment and treatment is the study of environmental mediators of complex illness. We have tested whether environmental contributors either put children at risk of or protect them from developing ADHD.

We chose a study population of MZ twins discordant, concordant-high, and concordant-low for AP/ADHD to evaluate and identify the contributions of environmental mediators. Our results show that affected children from discordant pairs have higher symptom ratings than unaffected cotwins on multiple measures (CBCL, YSR, Teacher Report Form, SWAN, *DSM*/DISC-P) of inattention, hyperactivity, and impulsivity, as well as on other behavior problems, including social problems, rule-breaking behavior, aggression, withdrawn behavior, and anxiety/depression. Although affected children have high symptom ratings, comorbidity with psychiatric disorders was low, probably because we did not study a clinically referred sample.

Because MZ discordant twins have identical genomes, the differences between affected and unaffected members are the result of the environment. Our findings are in accordance with those of Sharp et al. (2003) who also reported cross-rater agreement in symptom severity and substantial comorbidity in a sample of MZ twins discordant for ADHD. The longitudinal data in our study showed that the different behavioral profiles of MZ discordant twins are of early onset and are relatively consistent across time. In infancy, the affected twins already showed signs of greater behavioral imbalance (e.g., frequent crying). At age 3, they were rated as more overactive. APs were more prominent in affected twins at all ages, whereas persistent symptoms of aggressive behavior and social problems emerged at ages 3 and 7, respectively.

At the core of our analyses were attempts to identify conditions predictive of current AP/ADHD status. In MZ discordant pairs, affected twins experienced more adversity in infancy (lower birth weight and more time spent in the incubator) and were disadvantaged in terms of maturation (delayed physical growth and slower acquisition of motor skills). Low birth weight and prematurity are among the most frequently suggested risk factors for ADHD (Bhutta et al., 2002). Our results not only back up these suggestions but also indicate the truly environmental origin of these risk factors. The MZ discordant twins described by Sharp et al. (2003) and Castellanos et al. (2003) also provide evidence of the impact of neonatal stressors, namely, low birth weight and breech position, and suggest they have an effect through interfering with the formation of striatal circuits. Similarly, Asbury et al. (2006) report a significant correlation between MZ differences in birth weight and MZ differences in hyperactivity symptoms at age 7.

The inclusion of MZ concordant twins provided reference values for exposure to shared environmental risk factors and for the degree of differential experiences. Group comparisons revealed that maternal smoking during pregnancy and living with only one parent was more common among MZ twins concordant-high for APs/ADHD, although the former was significantly more common only when compared to concordant-low twins. Another finding was that discordant twins more often had separate bedrooms during their preschool years, although this may be a consequence of the discordance rather than a cause.

### Clinical Implications

We used a phenotyping strategy that applied each of the prevailing paradigms of phenotype definition (*DSM-IV* interview–based symptom scores, parent reports, teacher reports, and self-reports). In the MZ discordant twins, the affected member of the twin was rated as higher by all informants, on all measures, for symptoms of inattention, hyperactivity from the ADHD stable of symptoms, and also on rule breaking, social problems, aggression, anxious/depression, and withdrawn behavior. This was true even when self-report data (YSR) were analyzed. These data support that children with identical DNA can be remarkably and consistently different. In fact, the profile that emerges of the affected children goes well beyond simple ADHD and includes children who are deviant in attention, aggression, anxious/depression, and rule-breaking behavior. We describe key developmental periods during which symptomatic findings may be evident and predictive. For example, using assessment approaches such as those discussed above, these children are identified as behaviorally different at age 3. These differences are stable and persistent, remaining evident at age 12. There are, however, other markers that discriminate at an early age, but do not persist. These include low birth weight, which has long been a ubiquitous marker of a difficult intrauterine environment. The MZ discordant affected twins weighed significantly less at birth than their unaffected cotwin. After age 3, weight disparity is not useful as a marker because the difference disappeared by the third year of life. Another example of a developmental window is the achievement of developmental landmarks. MZ discordant affected children were behind in the achievement of their early landmarks, but not later landmarks. This work is a small step toward identifying environmental influences. The MZ discordant design is ideal for considering environmental mediators of complex traits as well as providing an elegant approach for the future studies of epigenetic modifications.

### Limitations

One limitation of our study is the sample size, even though the selection of twin pairs was from a large register. This may be seen as inevitable, given the strong genetic influences on APs and ADHD. Although we chose a liberal threshold, effects of small or even moderate size could have been overlooked. Furthermore, the MZ discordant twins may not be representative of the general population of children with attention problems and ADHD, although we excluded the most atypical cases (i.e., twins with severe physical or mental disabilities) from participation.

We are not able to specifically identify at this point which environmental insults are so robust that they may not require genetic predisposition to put a child at risk of ADHD. However, we have identified a strategy that may allow a more refined study of maternal health behavior as it relates to child outcomes. For example, maternal smoking was more common in MZ pairs concordant for APs/ADHD than in the other groups, providing at least secondary evidence that smoking, in addition to a common genetic risk, leads to increased rates of ADHD in offspring. Finally, we did not use *DSM-IV* age at onset or impairment criteria for ADHD diagnosis to improve comparability with CBCL ratings for which no such criteria are available.

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