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# Autism Spectrum Disorders and Autistic Traits: A Decade of New Twin Studies

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**Researchers continue to pursue a better understanding of the symptoms, comorbidities, and causes of autism spectrum disorders. In this article we review more than 30 twin studies of autism spectrum disorders (ASDs) and autistic traits published in the last decade that have contributed to this endeavor. These twin studies have reported on the heritability of autism spectrum disorders and autistic traits in different populations and using different measurement and age groups. These studies have also stimulated debate and new hypotheses regarding why ASDs show substantial symptom heterogeneity, and what causes their comorbidity with intellectual disability, language delay, and other psychiatric disorders such as ADHD. These studies also reveal that the etiology of autism and autistic traits assessed in the general population is more similar than different, which contributes to the question of where the boundary lies between autism and typical development. Recent findings regarding molecular genetic and environmental causes of autism are discussed in the relation to these twin studies. Lastly, methodological assumptions of the twin design are given consideration, as well as issues of measurement. Future research directions are suggested to ensure that this decade is as productive as the last in attempting to disentangle the causes of autism spectrum disorders.** © 2011 Wiley-Liss, Inc.

**Key words:** autism; twins; genetics; comorbidity

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## INTRODUCTION

Four twin studies of autistic disorder between 1977 and the late 1990s revolutionized the way we understand autism: by demonstrating that autism is highly heritable, findings from twin studies hushed the "nurture" proponents (at the time, this included those who thought a "cold" style of parenting caused autism [Bettelheim, 1967]), and heralded the start of a multi-million dollar genetics research area. In the last decade, over 30 twin studies of autism spectrum disorders and dimensional assessments of autistic traits have been published.

In this review, we describe how the well-documented original twin studies of narrowly defined autism have been succeeded by twin studies of autism spectrum disorders (ASD; the broader category of conditions that includes autistic disorder as well as Asperger syndrome and Pervasive developmental disorder not otherwise specified; PDD NOS), and by a new wave of twin studies exploring the etiology of dimensional assessments of autistic traits in the general population. We discuss how this literature contributes to our understanding of the dimensional nature of autistic behaviors and how findings from twin studies relate to specific genetic and environmental causes of ASD and autistic traits. It is not within the scope of this review to include a systematic account of molecular genetic findings in ASD; the reader is directed elsewhere [Abrahams and Geschwind, 2008; Freitag et al., 2010]. Furthermore, we consider how twin research has provided evidence for etiological heterogeneity in autistic symptoms, and what it has added to our understanding of the overlap between autism with intellectual disability, language development and other psychiatric conditions. Finally, after considering the limitations, assumptions and measurement considerations inherent in these twin studies, we provide suggestions for future research directions.

## THE HERITABILITY OF AUTISM, AUTISM SPECTRUM DISORDERS, THE BROADER AUTISM PHENOTYPE

It is well-established that twin studies of narrowly defined autism reported monozygotic (MZ) twin pairs to be more similar than dizygotic (DZ) twins in their concordance for autism [Folstein and

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**TABLE I. Twin Studies of Strictly Defined Autism and Autism Spectrum Disorders (Presented Chronologically)**

| | Sample and measures | | | | | |
|---|---|---|---|---|---|---|
| **Refs.** | **Sample ascertainment** | **N pairs, cases; IQ** | **Age, sex** | **Diagnosis** | **Results, concordances** | **Conclusions** |
| Folstein and Rutter [1977] | Systematic attempt to identify all twins with autism in UK via letters to psychiatrists, twin registers and autism society | 21 pairs (11MZ, 10 DZSS), 25 cases; 48% with IQ < 50 | 5–23 years; 3.2:1 | Criteria outlined by Kanner [1943] and Rutter Folstein & Rutter [1977] | Autism: MZ: 36%, DZ: 0%. BAP: MZ: 82%, DZ: 10%. Biological hazards surrounding birth process did not explain concordance rates. In 12 of the 17 discordant pairs, one twin had experienced biological hazard—always the twin with autism diagnosis | Autism shows genetic influence. Genetic influences may be linked with a broader range of impairments. Concordances not completely explained by biological hazards in the perinatal period, but they appeared to play a contributory role |
| Ritvo et al. [1985] | Via advert in autism society newsletter | 40 pairs (23 MZ, 10 DZSS, 7 DZOS), 66 cases | 3–31 years; 3.1:1 | DSM III | Autism: MZ: 96%, DZ: 24% | Strong genetic influence on autism |
| Steffenburg et al. [1989] | Systematic attempt to identify all twins with autism in Denmark, Finland, Iceland, Norway, and Sweden via letters to child psychiatrists, twin registers and autism society. | 21 pairs: 11 MZ, 10 DZSS, 1 triplet set, 34 cases; 50% with IQ < 50 | 2–23 years; 1.6:1 | DSM-III-R | Autism: MZ: 91% (plus one set of identical triplets), DZ: 0%. BAP: MZ: 91%, DZ: 30%. In the discordant pairs, always twin with autism who had more peri-natal stress | Similar conclusions to Folstein and Rutter [1977, above], except that this study did not find evidence that the broader definition of impairments was more heritable than autism |
| Bailey et al. [1995] | Folstein and Rutter's [1977] sample were contacted and reassessed, and additional twins were identified using same methods | 44 sets of twins and triplets (25 MZ, 20 DZSS, 2 triplet sets), 59 cases; 36.4% nonverbal IQ - < 50; 65.5% verbal IQ <30 | NA; 3.4:1 | ICD-10 | Autism: MZ: 60%, DZ: 0%. BAP: MZ: 92%, DZ: 10%. Environmental causes of brain damage did not explain concordance rates. In discordant pairs, twin with autism experienced more biological disadvantage. Liability threshold modeling produced broad heritability estimates of 91–93% | Replicated Folstein and Rutter's [1977] findings with larger sample including the original sample. Derived specific heritability estimate |
| Taniai et al. [2008] | Via child screening system in specific regions of Nagoya city, Japan as well as referrals from nurseries, hospitals and clinics | 45 twin pairs (19 MZ, 14 DZSS, 12 DZOS); 46.5% IQ < 70 | 3- to 6-year-olds; 3:1 | Case vignettes | ASD: MZ: 95%, DZ: 31%. Continuous Childhood Autism Rating Scale scores showed heritability of 73% for males and 87% for females and modest nonshared environment (13–17%). No evidence for the existence of sex-specific genetic influences | First twin study to provide MZ and DZ concordances for ASD. Reported high heritability for autistic symptoms assessed quantitatively in a clinically ascertained ASD sample |

(*Continued*)

TABLE I. (*Continued*)

| | Sample and measures | | | | | |
|---|---|---|---|---|---|---|
| **Refs.** | **Sample ascertainment** | **N pairs, cases; IQ** | **Age, sex** | **Diagnosis** | **Results, concordances** | **Conclusions** |
| Rosenberg et al. [2009] | Voluntary Interactive Autism Network (IAN) online database for US residents | 277 twin pairs (67 MZ, 120 DZSS; 90 DZOS); 23% with intellectual disability | Age 18 or less (mean 7.7 years) | Diagnostic information supplied by families | ASD: MZ: 88%, DZ: 31%. Severity concordance within ASD pairs: MZ: 96%, DZ: 81% (severity concordance defined as both twins had autism and/or PDD-NOS [PDD-NOS considered by authors as milder form of autism and as such grouped together] or both twins had Asperger syndrome [considered by authors as markedly different from PDD-NOS or autism], otherwise twins considered discordant). Parent-reported ASD diagnoses showed good agreement with SCQ and SRS questionnaires | Largest twin study of ASD showed high heritability of all ASD. First study to rely on parent-reported diagnostic information |
| Lichtenstein et al. [in press] | Identified from the Child and Adolescent Twin Study in Sweden (CATSS), part of the Swedish Twin Registry | 117 twin pairs (29 MZ, 48 DZSS; 40 DZOS); 128 cases, 34% with learning disorders | Age 9 or 12, 4:1 | ASD diagnosis on basis of parent interview on Autism—Tics, AD/HD, and other Comorbidities inventory (A-TAC) | ASD: MZ: 39% (47% for males only, not enough data for females only), DZ: 15% (14% for males, 20% for females). Liability threshold models estimated heritability of ASD at 80% and nonshared environmental influences explained remaining 20% of variance. Did not discriminate between different types of ASD diagnoses | Largest representative twin study of ASD. Inclusion of model-fitting provided specific estimates of genetic and environmental influences. Parent report measure has good reliability and validity information but was not suitable for discriminating ASD subtypes |

Percentages refer to calculated pairwise concordance rates unless otherwise stated. Ratio of males to females presented in Age and Sex column. All study samples are independent with exception of [1977] and Bailey et al. [1995]. NA, Information not available; MZ, monozygotic twins; DZ, dizygotic twins; DZSS, same-sex DZ twins; DZOS, opposite-sex DZ twins; ASD, autism spectrum disorders; BAP, broader autism phenotype; PDDNOS, pervasive developmental disorder not otherwise specified; SCQ, social communication questionnaire; SRS, social responsiveness scale.

Rutter, 1977; Ritvo et al., 1985; Steffenburg et al., 1989; Bailey et al., 1995]. Table I outlines the twin studies of narrowly defined autism and ASD. In the original Folstein and Rutter [1977] study, MZ twins, who share all of their genes, were 36% concordant—that is, in just over a third of pairs both twins had autism. In DZ twins, who share on average half their DNA, there was 0% concordance—that is, all twin pairs were discordant for diagnosis: one had autism, the other did not. The concordance rates were not found to be explainable by biological hazards associated with the twins' birth. Model-fitting in a later paper estimated the heritability of autistic disorder as 91–93% [Bailey et al., 1995].

It was also found that when criteria were widened to include individuals who show some but not all of the features of autism, this "broader autism phenotype" [BAP, as described by Folstein and Rutter, 1977] the MZ concordance increased to 92% and the DZ concordance increased to 10%, respectively [Bailey et al., 1995] (see Table I).

More recently, two twin studies of the broader group of all autism spectrum disorders have reported high MZ concordances (88–95%) and DZ concordances of 31% [Taniai et al., 2008; Rosenberg et al., 2009]. These DZ concordances for ASD are notable for being higher than in any previous twin studies of autism, whereas the MZ concordances are similar to those reported in some of the previous studies. The first ASD twin study employed a sample of children with ASD who were diagnosed using case vignettes in Japan [Taniai et al., 2008]. Using the Childhood Autism Rating Scale (CARS) as a quantitative assessment of autistic symptoms, they reported heritability estimates of 73% for males and 87% for females. Some questions remain regarding the comparability of the diagnoses made by case vignettes in Japan to the standard Western diagnostic instruments. The second ASD twin study relied on parent-report of ASD diagnoses through a US-based voluntary online register [Rosenberg et al., 2009]. This is a less systematic or reliable ascertainment method than employed in the previous twin studies but has the advantage of giving a large sample size (with 277 twin pairs it is the largest twin study of ASD published so far). The twin concordances from this second ASD twin study (MZ: 88%, DZ: 31%) are highly similar to those from the first ASD study. Finally, the third and most recent twin study of ASD includes the largest representative sample employed to date and reports both concordances and model-fitting analyses [Lichtenstein et al., in press]. The concordances for all ASD (the measure did not distinguish different types of ASD) were 39% for MZ twins and 15% for DZ twins; liability model analyses suggested a heritability of 80%, thus again indicating strong genetic influences on ASD.

In sum, since the original twin studies showed the high heritability of autistic disorder, three new studies have reported high heritability for ASD. The median values for MZ and DZ concordances, were 76% and 0%, respectively, from the four original studies of narrowly defined autism, and 88% and 31% from the three new studies of the broader ASD group. It is likely that some researchers will be keen to see further twin studies published that use more conventional in-person psychiatric assessments of ASD, such as the Autism Diagnostic Observational Scale (ADOS) and Autism Diagnostic Interview-Revised (ADI-R). Nevertheless these three new studies provide valuable data on the etiology of ASD for the time being.

## AUTISTIC TRAITS IN COMMUNITY SAMPLES

Relatives of individuals with ASD show elevated levels of autistic traits [e.g., Bishop et al., 2006; Constantino et al., 2006] suggesting that subclinical autistic traits share familial influences with diagnosed ASD. Furthermore, autistic traits measured in the general population show a smooth distribution throughout the normal range to the clinical extreme [e.g., Skuse et al., 2005; Hoekstra et al., 2008]. Finally, common genetic variants that are, by definition, present in a significant proportion of the general population, are thought to play a role in the etiology of autism [e.g., Campbell et al., 2006; Alarcón et al., 2008; Chakrabarti et al., 2009; Wang et al., 2009; Ronald et al., 2010a; Anney et al., 2010]. For these reasons it is thought that understanding the etiology of individual differences in autistic traits in the general population will aid our understanding of the causes of autism. There are several methodological advantages that general population samples bring to research on the etiology of autism, such as substantially more power to conduct model-fitting analyses, the derivation of specific parameter estimates and the potential to test more complex multivariate hypotheses.

Table II describes twin studies of autistic traits assessed in general population samples. These studies report that autistic traits, as assessed using quantitative scales such as the Childhood Autism Spectrum Test [CAST; Williams et al., 2008], Autism-spectrum Quotient [AQ; Baron-Cohen et al., 2001], and Social Responsiveness Scale [SRS; Constantino, 2002], show a smooth distribution in community samples, and heritability estimates range from 36% to 87% in twin samples ranging from age 2 to age 18. The general trend is for heritability to vary between 60% and 90% for parent- and teacher-rated autistic traits in middle childhood and older [Constantino and Todd, 2000, 2005; Ronald et al., 2005, 2006a, 2008a; Skuse et al., 2005], with self-report assessments of autistic traits giving more moderate heritability estimates [36–57%; Hoekstra et al., 2007a; Ronald et al., 2008a]. The two twin studies of early childhood, on 2-year-olds, also reported moderate heritabilities (40% and 44%) of parent-rated autistic traits [Edelson and Saudino, 2009; Stilp et al., 2010], suggesting that heritability of autistic traits may increase with age.

Shared environmental influences are environmental influences that are common to both twins and make children growing up in the same family similar. Some studies in middle-to-late childhood report modest shared environmental influences ranging from 10% to 32% [Constantino and Todd, 2000, 2003, 2005; Ronald et al., 2008a], but the majority find no significant effects (see Table II). All studies report moderate influences of the nonshared environment, defined as environmental influences that make children growing up in the same family different, and which by default include measurement error in their term.

In sum, twin studies of autistic traits have been important in supporting the notion of autism as a continuously distributed trait, a position that has been championed by a number of autism researchers [Baron-Cohen et al., 2001; Constantino and Todd, 2003; Skuse et al., 2005; Ronald et al., 2006a; Allison et al., 2008;

**TABLE II. Twin Studies of Autistic Traits**

| | Sample and Measures | | | | | |
|---|---|---|---|---|---|---|
| **Study** | **Sample** | **N pairs** | **Age, sex** | **Measure** | **Results** | **Conclusions** |
| Constantino and Todd [2000] | Community sample, Missouri twin study | 232 pairs (98 MZ, 134 DZ) | 7–15 years; all male | SRS: 65-items. Parent-report | Twin correlations: MZM 0.73; DZM 0.37. Strong additive genetic influence (76%), moderate nonshared environmental influence (24%). No significant shared environmental or nonadditive genetic influence | Autistic traits are highly heritable in males |
| Constantino and Todd [2003] | Community sample, Missouri twin study | 788 pairs (268 MZ, 270 DZSS, 250 DZOS) | 7–15 years; 43.7% male | SRS. Parent-report | Twin correlations: MZM: 0.73; DZM: 0.37. MZF: 0.79; DZF: 0.63; DZOS: 0.59. Modest genetic influences (48%) and significant moderate shared and nonshared environmental influences (32% and 20%, respectively) | Autistic traits for both males and females show moderate heritability (48%). Unlike the previous study, significant shared environmental influences were found |
| Constantino and Todd [2005] | Community sample, Missouri twin study | 285 pairs (89 MZF, 69 DZF, 127 DZOS) | 8–17 years; 22.3% male (from male twins in DZOS pairs). Parents: aged 30–55, 50% male | SRS child and adult versions; maternal report of twins and spousal report of parents | For combined parent and child samples: high heritability (87% males, 73% females), modest shared environment (12% males, 10% females) and nonshared environment (0% males, 17% females), assortative mating estimate = 0.29. Significant parent-offspring intraclass correlations were also reported | Autistic traits are highly heritable in children and adults. Evidence of assortative mating. Conclusions based on largely female twin sample |
| Ronald et al. [2005] | Representative UK sample, Twins Early Development Study (TEDS) | 3138 pairs with teacher data; 3,996 pairs with parent data | Age 7; 48% male | DSM-IV based social and non-social questionnaires, parent and teacher report | High heritability of parent- and teacher-rated social and nonsocial autistic traits (62–76%), modest nonshared environment (25–38%). Modest genetic overlap between social and nonsocial autistic traits (genetic correlation = 0.07–0.40) and modest nonshared environmental overlap (nonshared environment correlation = −0.02–0.18) | First twin study of social and nonsocial components separately showed they are both individually heritable but show limited genetic overlap |
| Skuse et al. [2005]; see also Scourfield et al. [1999] | Representative UK sample, Cardiff Study of All Wales and North of England Twins | 670 pairs (278 MZ, 180 same-sex DZ and 198 DZOS) | 5- to 17-year-olds (M = 10.6 years), 48% male | Social and Communication Disorders Checklist, parent-report | Twin correlations: MZ: 0.73; DZM: 0.38. Heritability: 74%, non-shared environmental influence: 26% | Social cognitive skills show high heritability and no shared environmental influence |

(*Continued*)

TABLE II. (*Continued*)

| Study | Sample and Measures: Sample | N pairs | Age, sex | Measure | Results | Conclusions |
|---|---|---|---|---|---|---|
| Ronald et al. [2006a] | Representative UK sample, TEDS | 3,419 pairs; sample included representative proportion of children with ASD | Age 8; 49% male | CAST, parent-report | High heritability for autistic traits in whole sample (81–86%) as well as for extreme autistic traits using >85%, >90%, >95% and >98% cut-offs, using both DeFries Fulker analyses (group heritability = 64–73%) and liability threshold models (heritability = 86–92%). Autistic trait subscales (social impairments, communication impairments, RRBIs) all show high heritability individually. No evidence for shared environmental influences. Nonshared environment modest but significant (14–19%). Multivariate models indicated modest genetic overlap between subscales (genetic correlations = 0.18–0.50) | Large twin study of autistic traits confirms their high heritability in general population and in extreme groups. Evidence for limited genetic overlap between individual autistic traits |
| Hoekstra et al. [2007] | Representative Dutch sample, subsample of the Netherlands Twin Register | 380 twin pairs, 94 siblings, 128 parents of twins | Twins: 18 years; Siblings: range 10–35 years, average 18 years. 47% male | Dutch AQ, self-report | Twin correlations: MZM 0.59, DZM 0.36, MZF 0.51, DZF 0.43, DZOS: 0.35, all twin-sibling pairs: 0.28. Substantial heritability (57%) and moderate nonshared environmental influences (43%) on self-reported autistic traits in late adolescence. No evidence for different genetic influences on males and females | First twin study of late adolescence confirms substantial heritability in this age group. No evidence for assortative mating for autistic traits |
| Ronald et al. [2008a] | Representative UK sample, TEDS | 2,586 pairs with teacher data; 3,259 pairs with parent data; 3,109 pairs with self report data | Age 9; 49% male | Abbreviated CAST, parent-, teacher- and self-report | Correlations between raters were significant but moderate (r = 0.16–0.33). High heritability for parent ratings (82–87%), moderate for teacher (69%), modest for child self-report (36–47%). Shared environment influences found only for male self report data (18%). Genetic overlap was significant but moderate across all raters (average genetic correlation between raters = 0.40) | Heritability estimates differ depending on type of rater. Different raters pick up on partly different genetic phenotypes |

(*Continued*)

TABLE II. (*Continued*)

| | Sample and Measures | | | | | |
|---|---|---|---|---|---|---|
| **Study** | **Sample** | **N pairs** | **Age, sex** | **Measure** | **Results** | **Conclusions** |
| Edelson et al. [2009] | Community sample, Boston University Twin Project | 313 pairs, 145 MZ, 168 DZ | Age 2; 53% male | Child Behavior Check List (CBCL) Pervasive Developmental Problems scale, parent-report | Twin correlations: MZ: 0.58, DZ: 0.38. Moderate heritability (40%), significant shared environment (20%), nonshared environment (40%) | First twin study of autistic traits in young children. Moderate heritability and significant shared and nonshared environmental influences in this age group |
| Stilp et al. [2010] | Representative US sample, Wisconsin Twin Panel | 1,211 pairs (414 MZ, 410 same-sex DZ, 387 DZOS) | Age 2–3, 50% male | Eight items similar to items from Modified Checklist for Autism in Toddlers (M-CHAT), parent report | Twin correlations: MZM 0.62, DZM 0.25, MZF 0.53, DZF 0.34, DZOS: 0.44. Using categorical data, liability threshold models estimated heritability at 44%, shared environment as 32% and non-shared environment as 24%; but with a more extreme threshold, these values were 74%, 19%, and 7%, respectively | Autistic behaviors in toddlers (such as a lack of pointing, looking and imitating) show moderate genetic influence and significant shared and nonshared environmental influences |
| Ronald et al. [2010] | Representative Swedish sample, CATSS | 6,223 pairs (1,788 MZ, 1,728 DZSS, 2,024 DZOS, 683 exclusions/ missing data) | Two independent samples of twins, one aged 9 years, one aged 12 years; 51% male | Autism symptom items from the Autism—Tics, AD/HD, and other Comorbidities inventory (A-TAC), parent report | Autism symptoms divided into three subscales based on factor analysis of items. Heritabilities of three autism symptoms 49–76%; remaining variance explained by nonshared environment. Multivariate common pathway model fit the three autism symptoms best, showing common genetic and nonshared environmental influences on each symptom domain, but also symptom-specific genetic and nonshared environmental influences that could not be dropped from the model. Similar results across gender and age | The core symptoms of autism, when assessed in the general population, show modest overlap and have partly separate genetic influences |

Studies that used the same sample (as noted above) are not independent. MZM, monozygotic males; DZM, dizygotic males; MZF, MZ females; DZF, DZ females; DZOS, DZ opposite-sex pairs; SRS, social responsiveness scale; CAST, Childhood Autism Spectrum Test; AQ, Autism-spectrum Quotient; TEDS, Twins Early Development Study; CATSS, Child and Adolescent Twin Study in Sweden. RRBIs, restricted repetitive behaviors and interests.

**TABLE III. Impact of Genetic and Environmental Factors on Twin Similarity**

| Type of causal factor | Example in ASD literature | Impact on twin similarity | Interpretation |
|---|---|---|---|
| Common additive SNP or CNV | rs7794745 within contactin-associated protein-like 2 (CNTNAP2) [Arking et al., 2008] | MZ twins share all these SNPs, DZ twins share on average 50%. These SNPs will make MZr on average twice DZr | These effects contribute to the heritability estimate |
| Common nonadditive SNP | Interaction of haplotypes (groups of SNPs) between candidate genes in the serotonin metabolic and neurotransmission pathways [Coutinho et al., 2007] | If nonadditive SNPs are at the same locus ("dominance"), MZ twins share all these dominant SNPs, fraternal twins share on average 25%. These genetic polymorphisms will make MZr on average four times DZr. If the nonadditive SNPs are at different loci ("epistasis"), MZ twins share all these SNPs, fraternal twins share less than 25%. MZr will be greater than four times DZr | Nonadditive genetic influences contribute to the "broad sense" heritability estimate, which by definition is heritability that includes both additive and dominant genetic effects. Epistasis is not modeled in classic twin designs but is suggested if the magnitude of the MZr is more than four times the DZr |
| De novo mutation or CNV | De novo CNV located at 2q37.2–2q37.3 that involves a loss of 50 genes. This is an example of one of 17 de novo mutations identified in subjects with simplex ASD [Sebat et al., 2007] | MZ twins will share all these mutations if they occur in the germline. Therefore these mutations will act to increase MZr. DZ twins will share 0% of de novo mutations and therefore will not alter DZr | De novo mutations are genetic but not inherited. Heritability is expected to be inflated by de novo mutations. The implication is that the percent variance explained by heritability in twin models will be larger than the amount of variance that can be explained by *inherited* genetic variance from parents to offspring if denovo mutations are involved |
| Chromosomal (cytogenetic) abnormality | Chromosome 15q duplication [Cook et al., 1997] | MZ twins will share all these mutations if they occur in the germline. DZ twins will share 0% of de novo mutations. Most chromosomal anomalies occur as an accident in the egg or sperm, and are therefore not inherited. The anomaly is present in every cell of the body. Some anomalies, however, can happen after conception, resulting in mosaicism (where some cells have the anomaly and some do not) | For not inherited, de novo chromosome anomalies, see "de novo mutation" above. For inherited chromosome anomalies, it would be expected that these would be inherited as per common SNPs |
| Maternal prenatal exposure, e.g., smoking | | MZ and DZ twins both share prenatal exposures, therefore such an environmental effect will act to increase similarity of both MZr and DZr. If the environmental effect interacts with the genes in each child (e.g., if the environmental effect is more or less detrimental in a child with certain genetic polymorphisms), then gene–environment interactions will occur (see Box 1). Maternal behavior, which will impact on the prenatal environment, is partly driven by the maternal genotype, which is highly correlated with the child's genotype (gene–environment correlation—see Box 1). Therefore a prenatal environment cannot be assumed to be independent of the child's genotype | On the surface, prenatal environments should act to increase shared environment estimates because both MZr and DZr are increased. However, they may also increase the heritability estimate or shared or nonshared environmental estimates (see Box 1). |
| Postnatal birth complication/ exposure e.g. maternal depression, intensive care treatment | | Postnatal environments could act to increase both MZr and DZr if they are shared environments that act to increase sibling similarity (e.g., maternal depression). If they act to decrease sibling similarity they are defined as nonshared environment, and will decrease both MZr and DZr (e.g., one twin requires intensive care treatment). Like prenatal environment, the effects of postnatal environment could interact or correlate with the child's genotype (see Box 1). | Postnatal environments could contribute to the shared environment, nonshared environment or heritability (see Box 1). The MZ Differences design can be used to identify nonshared environmental effects independent of DNA code (see text) |

SNP, single nucleotide polymorphism; CNV, copy number variation; MZr, similarity between MZ twins in a pair; DZr, similarity between DZ twins in a pair.

Hoekstra et al., 2008]. Twin research has demonstrated the magnitude of the role of both genetic and environmental influences on autistic traits across development, both measured in the general population and in the extremes of this population. Similar heritability estimates for autism and autistic traits do not prove that the same genetic influences are involved. The Defries Fulker extremes analyses presented in Ronald et al. [2006a; see Table II] suggested that there was a genetic link between impairments at the quantitative extreme of the distribution of autistic traits and variation in the general population. Definitive proof will come when genetic variants associated with diagnosed autism are found to also be associated with normal variation in autistic traits.

## TWIN STUDIES AND GENETIC AND ENVIRONMENTAL RISK FACTORS

While this review does not aim to include a review of molecular genetic or environmental research into ASD [please see reviews by Abrahams and Geschwind, 2008; Freitag et al., 2010], in order to build a coherent picture of the etiology of ASD, it is vital to consider how to relate twin study findings to discoveries regarding specific genetic and environmental risk factors.

Table III outlines the main categories of genetic variants that have been associated with ASD, as well as two of the most salient categories of ''environmental'' factors for ASD, prenatal maternal exposures and postnatal birth complications or exposures. For each category of risk factor, Table III outlines how these would potentially impact twin correlations and how we can interpret these findings. It is probable that several, or even all, of the processes described in Table III might be operating simultaneously in the etiological process(es) of autism, making interpretation all the more complex.

It is thought that common single nucleotide polymorphisms (SNPs) or copy number variants (CNVs), whether operating additively or nonadditively, will explain part of the heritability estimate in ASD or autistic traits. In contrast, de novo mutations and cytogenetic abnormalities that occur in the germline and are not inherited from parent to offspring will inflate MZ similarity. Thus some genetic risk factors for ASD and autistic traits may be inherited and heritable, such as common SNPs, and others are expected to be highly heritable (in that they contribute to the heritability estimate) but not inherited, such as de novo mutations [Beaudet, 2007]. As we learn more about the frequency and penetrance of rare de novo mutations associated with ASD, it will be vital to consider how to interpret twin data in light of these findings.

The causality of de novo mutations identified in individuals with ASD still needs to be established; de novo CNVs also occur in controls [see Pinto et al., 2010]. Also, it is not known if de novo CNVs associated with autism are themselves causal, or if the de novo CNV duplicates or deletes a specific (inherited) gene that is the causal polymorphism.

Twin studies are useful for identifying the degree of environmental influences and whether the environmental influences are shared or nonshared. Once environmental risk factors are identified, however, delineating their mode of action can be challenging, as described in Table III. Environmental variables are not always independent of genetic effects, the so-called ''nature of nurture'' [Plomin and Bergeman, 1991]. Box 1 outlines gene–environment interaction and gene–environment correlation, two concepts in behavior genetics that are likely to be important in understanding the etiology of ASD.

For many of the twin concordances and twin correlations of autism/ASD and autistic traits, presented in Tables I and II respectively, DZ similarity is less than half the MZ similarity, which is the pattern that would be expected from nonadditive genetic effects, de novo mutations and chromosomal abnormalities.

**BOX 1.**

Apart from direct effects of genes, shared environment and non-shared environment, these effects can also correlate or interact with each other. This box serves to explain how gene–environment correlations or interactions can impact on the pattern of findings in twin studies.

Exposure to environmental effects may depend on an individual's genotype. For example, people with a genetic make-up predisposing to athletic talent may be selected to use prestigious training facilities that will help to further advance their running abilities. In this instance, the genetic and environmental influences on running ability are correlated. In twin studies, a correlation between genes and non-shared environment will inflate the MZ correlation to a greater extent than the DZ correlation, resulting in an overestimation of genetic effects. A correlation between genes and shared environmental influences will inflate both the MZ and DZ correlations, resulting in an overestimation on the shared environment effects. In extended twin designs (in which data are collected from multiple relatives, including the parents, spouses or children of these twins) the effect of gene–environment correlations can be tested directly [see e.g., Keller et al., 2009].

Gene–environment interaction is present when the effect of an environment on the outcome depends on the genotype of an individual (or, equivalently, when the effect of an individual's genotype is dependent on the environment the person is exposed to). Consider for instance the consequences of adverse prenatal or perinatal events, such as maternal smoking or birth complications. If the effects of these adverse events are more or less detrimental depending on the child's genotype (e.g., the effects are especially pronounced if the child is a carrier of a particular risk allele), there is evidence for gene–environment interaction. If the environmental exposure is shared between the twins, the interaction between genes and shared environment will mimic genetic effects, and the effects of the shared environment will be underestimated. If the environmental effects are non-shared, the gene–environment interaction results in a decrease of both the MZ and DZ correlation and mimics the effect of non-shared environment, and genetic effects are underestimated.

## "MZ DIFFERENCES" DESIGN

Twin studies of autism, ASD and autistic traits consistently demonstrate that nonshared environment plays a small but potentially important causal role. MZ twins are not 100% similar in terms of autism, ASD, BAP, or autistic traits. The most effective way to identify nonshared environmental influences is to employ an MZ differences design. Because MZ twins are genetically identical at the DNA sequence level [but may show differences in gene expression due to for example differences in DNA methylation levels; Jirtle and Skinner, 2007], any differences between two identical twins are due to nonshared environment.

Nonshared environmental influences are defined as environmental influences that make children growing up in the same family different, and can include epigenetic processes, gene expression, some de novo mutations, illnesses, intra- and extra-uterine environment and measurement error. As such interpretations of nonshared environmental effects should always be considered in light of this definition.

A handful of studies have used structural MRI methods to report brain differences between MZ twins discordant for a narrow definition of autism.[1] Fourteen MZ pairs, nine of whom were clinically discordant for strictly defined autism, were examined and some neuroanatomical differences associated with this discordance (such as cerebellar volume) were reported. There was however also strong concordance across these pairs, for example, in cerebral gray and white volumes [Kates et al., 2004]. Recently specific brain regions including the prefrontal cortex, amygdala and hippocampus were examined, again finding that the degree of within-pair neuroanatomic concordance varied by brain region [Mitchell et al., 2009]. The same sample has also been used to explore gyrification (cortical folding) patterns [Kates et al., 2009]. Further research that attempts to replicate these interesting findings is needed.

One of the most well replicated associations of putative risk factors with ASD is with perinatal obstetric complications [Kolevzon et al., 2007; Ronald et al., 2010c]. Perinatal obstetric complications could be a result of pre-existing genetic abnormalities in individuals who later develop ASD, they could be a causal environmental risk factor, or both. To address whether perinatal obstetric complications could be an environmental risk factor for autistic traits, MZ twins who were discordant for postnatal birth complications (e.g., one twin had been in intensive care, the other had not) were compared on their later autistic trait scores. In some cases, significant correlations were observed between the two "difference" scores, that is, the twin with more postnatal birth complications had more autistic traits at a later age compared to their co-twin [Ronald et al., 2010c]. This finding does not rule out that *some* postnatal birth complications associated with autism or autistic traits could be due to genetic factors, but, if replicated, suggests that postnatal complications, regardless of DNA, can have a causative influence on a child's later autistic traits. This would fit with the predictions from twin studies, which consistently find evidence for nonshared environmental effects on autism and autistic traits.

[1]Case studies of single twin pairs with ASD have been omitted from this review. Although case studies are useful at a descriptive level, statistical results cannot be derived from individual pairs.

Finally, a sample of three MZ pairs discordant for an autism diagnosis (one twin in each pair had autism, the other had some autistic traits and was described as "not quite autistic" or "broad spectrum") have been studied in relation to their gene expression profiles [Hu et al., 2006; Sarachana et al., 2010] and their methylation profiles [Nguyen et al., 2010]. Both gene expression and epigenetic changes can occur as a result of genetic or environmental influences. The combination of phenotypically discordant genetically identical MZ twins and gene expression or epigenetic profiling allows for the discovery the biological mechanisms underlying nonshared environmental influences on autism (because DNA code is controlled for in the MZ differences design). This is a promising field for further research.

## MULTIVARIATE TWIN STUDIES OF AUTISM AND AUTISTIC TRAITS

Univariate twin studies have provided insight into the genetic and environmental influences on autism, ASD, and autistic traits. The multivariate extension of the twin design can unravel the etiology of the overlap between different conditions or traits. Multivariate analyses permit the estimation of the genetic (or environmental) correlation between different traits. A genetic correlation reflects the extent to which trait or disorder X and trait or disorder Y are affected by the same set of genes. A genetic correlation of 1.0 suggests complete genetic overlap between the two traits, a genetic correlation of 0.0 indicates that the traits are affected by two entirely separate sets of genetic influences. In the following sections, we consider how twin research has provided evidence for etiological heterogeneity in autistic symptoms, and what it has added to our understanding of the overlap between autism with intellectual disability, language development and other psychiatric conditions.

## DEGREE OF GENETIC AND ENVIRONMENTAL OVERLAP BETWEEN DIFFERENT AUTISTIC SYMPTOMS

ASDs are characterized by a triad of symptoms in the domains of social impairments, communication impairments and restrictive repetitive behaviors and interests (RRBIs). Several groups of researchers have suggested that autism is best understood as a disorder of "multiple deficits" [Wing and Wing, 1971; Bishop, 1989; Goodman, 1989; Happé et al., 2006; Mandy and Skuse, 2008] while other researchers argue that autistic symptoms together represent a single underlying dimension [e.g., Constantino et al., 2004]. Understanding which of these models is most accurate has many implications, for example, for how best to define ASD subtypes, for understanding familial risk, and for designing management and treatment options.

It is notable that the autism phenotype "splinters" among family members who share proportions of the proband's genetic make-up. That is, relatives often show mild versions of just part of the autism phenotype, for example, social impairments, without communication difficulties or RRBIs. Thus family studies suggest that different causative factors influence the three components [e.g., Bolton et al., 1994]. While the majority of factor analytic studies support the

notion of two, three, or more dimensions underlying autistic symptoms [see reviews by Happé and Ronald, 2008; Mandy and Skuse, 2008], a minority of studies report a single dimension underlying autistic symptoms [e.g., Constantino et al., 2004].

Three twin studies from a large general population twin sample have reported that the three sets of autistic symptoms are all highly heritable individually but are caused by largely different sets of genetic influences, when assessed in the general population in middle childhood, both dimensionally [Ronald et al., 2005, 2006a] and at the impaired 95% extreme [Ronald et al., 2006b]. The genetic correlations were all modest to moderate in these studies [Ronald et al., 2005, 2006a]. This finding has been replicated across two other samples [Edelson et al., 2009; Ronald et al., in press]. Using a sample of twins with ASD who had been diagnosed using a parent interview, a similar modest degree of genetic overlap between the different ASD symptoms has been reported [Dworzynski et al., 2009]. In another study of twins diagnosed with ASD, social dysfunction and nonverbal communication symptoms were reported to show a modest degree of common genetic influences [Mazefsky et al., 2008]. The comparison of symptom profiles within MZ pairs who are concordant for ASD is another potentially informative approach. However the two studies of this kind so far have presented contradictory findings and the small sample sizes mean that statistical comparisons between twin similarity estimates are limited [Le Couteur et al., 1996; Kolevzon et al., 2004].

The implication of these multivariate twin studies of autism symptoms and autistic traits is that the autism ''triad,'' that is, the three core sets of symptoms that define ASD, may be largely fractionable, and causal explanations should be sought for each symptom group separately, rather than for autism as a whole [Happé et al., 2006; Happé and Ronald, 2008]. Molecular genetic research has begun to explore the possibility of symptom-specific genetic influences in autism using candidate gene studies, linkage, and genome-wide association [Brune et al., 2006; Alarcón et al., 2008; Ronald et al., 2010a]. Studying subphenotypes, or endophenotypes that are relevant to autism, may aid identifying genes associated with specific heritable facets of the condition.

## AUTISM AND INTELLECTUAL DISABILITY

Intellectual disability ($IQ \leq 70$) is common in ASD. However people with ASD are found along the entire spectrum of intellectual ability and prevalence estimates of intellectual disability in ASD vary widely between studies [Chakrabarti and Fombonne, 2005; Fombonne, 2006]. Twin studies can help to elucidate whether autism and intellectual disability share common etiological influences. So far, three studies from two different research groups have explored the genetic overlap between autistic traits and intellectual abilities. Nishiyama et al. [2009] examined the genetic correlation between IQ and autistic traits in 45 young twin pairs in which at least one twin had an ASD diagnosis. A very strong negative genetic correlation ($r_g = -0.95$) was reported, suggesting that the genes affecting the risk for autism and the genes influencing IQ largely overlap, acting to increase risk for autism and decrease propensity for intellectual development. Due to the small sample size the confidence intervals (CI) varied widely (the 95% CI was between −1.00 and −0.60). Moreover, the authors put forward that the genetic correlation they reported may be inflated because of the inclusion of severely intellectually disabled children who only had a mild degree of autism and had received a PDD NOS diagnosis.

It has been suggested that the association between intellectual disability and autism may be inflated in clinical samples, since the probability of clinical ascertainment is greatly increased in individuals expressing both conditions [Skuse, 2007]. These possible effects of clinical ascertainment bias [Boomsma et al., 2002] can be avoided by studying the association between autistic traits and IQ in the general population. A recent general population twin study [Hoekstra et al., 2009] examined the extent to which extreme autistic traits (defined by a score in the top 5% of the population on a measure of autistic traits) were related to intellectual difficulties (defined by a score in the bottom 5% on measures of intelligence and academic achievement). Both extreme traits showed only a modest degree of genetic overlap; this was true for both parent-rated and teacher-rated autistic traits, and for both poor academic achievement and low IQ-scores (genetic correlations between 0.04 and 0.44). Furthermore, the longitudinal association between autistic traits and IQ was explored using data from the whole population sample [Hoekstra et al., 2010]. A stable set of genetic influences could explain the stability of autistic traits over time (at ages 8, 9, and 12 years), whilst another set of genetic influences explained the stability in IQ scores over time (ages 7, 9, and 12 years). The genetic overlap between these two sets of genetic influences was only modest (genetic correlation = −0.27, 95% CI −0.34 to −0.22) and was mainly accounted for by pragmatic communication difficulties characteristic of autism. This study was limited in that it included few cases with severe or profound intellectual disabilities, as it was drawn from a population-based sample. It may be that genetic influences involved in causing autism in people with severe intellectual impairment are somewhat distinct from the genetic influences causing autism in people with normal or near-normal intelligence, and that the genetic influences causing autism in the severely intellectually impaired also impact on IQ. Although further studies are needed in this area, this is one hypothetical scenario that would reconcile the different results in these studies [Hoekstra et al., 2009, 2010; Nishiyama et al., 2009].

Results from molecular genetic studies have provided clues to the genes involved in the overlap between autism and intellectual disability [Pinto et al., 2010], most notably genes linked to synaptic changes [Persico and Bourgeron, 2006]. However, the most recent twin studies suggest that there is also substantial genetic specificity. Unraveling the genetic and biological pathways that can result in autism with intact general cognitive abilities remains a great challenge; the recent results suggest that this avenue should be explored. In this light the results from a linkage study [Liu et al., 2008] are of interest. No significant linkage peaks were detected in the full sample of autism families, but stratifying the sample as a function of normal cognitive ability ($IQ \geq 70$) resulted in significant linkage on chromosome 15q13.3–q14. Future studies are needed to replicate this finding and to elucidate whether this region can indeed provide a first clue on genetic influences associated with autism that spare intellectual functioning.

**TABLE IV. Twin Studies of Psychiatric Comorbidity in Autism and Autistic Traits**

| Study | Comorbid trait/disorder | Sample description, age and size | Measures | Key findings |
|---|---|---|---|---|
| Constantino et al. [2003] | ADHD | Community sample (Missouri Twin Project), 7- to 15-year-old; N = 219 male twin pairs | CBCL attention problems subscale and Social Responsiveness Scale, parent-report | All CBCL subscales explained 43% of variance in autistic traits. Attention problems explained the most variance, but despite this significant overlap, some genetic influences remained specific to autistic traits. Genetic correlation was not reported |
| Reiersen et al. [2008] | ADHD | Community sample (subsample of Australian Twin Register), 18–33 years old; N = 674 twins (275 complete pairs) | Abbreviated Social Responsiveness Scale and DSM-IV ADHD items, self-report | Genetic correlation between autistic traits and ADHD behaviors = 0.72. Substantial genetic overlap between adult self-reported autistic and ADHD traits |
| Ronald et al. [2008b] | ADHD | Population-based sample (TEDS), including children with suspected ASD and ADHD. Eight- to nine-year-olds; N = 6,771 pairs | Conners DSM IV subscales, parent-report; Strengths and Difficulties Subscale, teacher-report; Childhood Asperger Syndrome Test, parent- and teacher-report | Genetic correlations between autistic traits and ADHD behaviors = 0.54–0.57 (depending on sex and rater) in general population. In diagnosed children, genetic correlation = 0.62. Substantial genetic overlap between autistic traits and ADHD traits, and between ASD and ADHD diagnoses, in middle childhood |
| Ronald et al. [2010] | ADHD | Community sample (Boston University Twin Project), 2-year-olds; N = 312 pairs | CBCL Pervasive Developmental Problems and ADHD subscales, parent-report | Genetic correlation between autistic traits and ADHD behaviors = 0.27 |
| Lichtenstein et al. [in press] | ADHD, Developmental Coordination disorder, Tic disorder, learning disorders | Population-based sample (CATSS) screened for disorders, 9- and 12-year-olds, original N = 8,429 pairs | Autism—Tics, AD/HD, and other Comorbidities inventory (A-TAC), parent report used to identified individuals with neuropsychiatric disorders | High genetic correlations reported between ASD and all neuropsychiatric disorders studied (ADHD, Developmental Coordination disorder, Tic disorder, learning disorders). Highest genetic overlap was observed between ASD and ADHD, with over three-quarters of the variance attributable to genetic influences on ASD shared with ADHD, and a genetic correlation of 0.87 |

(*Continued*)

**TABLE IV. (*Continued*)**

| Study | Comorbid trait/disorder | Sample description, age and size | Measures | Key findings |
|---|---|---|---|---|
| Hallett et al. [2009] | Anxiety-related behaviors | Population-based sample (TEDS), 8–9 years old, N = 3,233 twin pairs | Childhood Asperger Syndrome Test; anxiety-related items, based on Anxiety Related Behaviors Questionnaire, parent-report | Genetic correlation between autistic traits and anxiety-related behaviors = 0.12–0.19; shared environmental correlation = 0.96–1.00 |
| Hallett et al. [2010] | Anxiety-related behaviors | Population-based sample (TEDS), 8-year-old followed longitudinally to age 12, N = 5,876–7,834 twin pairs | Childhood Asperger Syndrome Test; anxiety-related items, based on Anxiety Related Behaviors Questionnaire, parent-report | Longitudinal cross-lag associations were explored within a twin model. An asymmetric bidirectional association between autistic-like and internalizing traits across ages 8 and 12 was found, suggesting some "phenotypic causality." Both traits were moderately to highly heritable, but were largely independent with regard to their genetic overlap. Autistic-like communication difficulties made the most significant contribution to later internalizing traits |
| Jones et al. [2009] | Psychopathic traits | Community sample (subsample of TEDS), 9-year-olds; N = 642 pairs. | Antisocial Process Screening Device and Childhood Asperger Syndrome Test, parent-report | Genetic correlation between autistic traits and psychopathic traits = 0.43 |
| Hoekstra et al. [2007] | Withdrawn behavior and social problems | Community sample (subsample of Netherlands Twin Register), 18-year-olds; N = 424 pairs + 206 non-twin siblings | Youth Self Report; Autism Spectrum Quotient, self-report | Withdrawn behavior and social problem subscales were the most important predictors of autistic traits in the Youth Self Report measure. Genetic correlation between autistic traits and social problems = 0.71; genetic correlation between autistic traits and withdrawn behavior = 0.56 |

Studies that used the same sample (as noted above) are not independent. TEDS, Twins Early Development Study; CATSS, Child and Adolescent Twin Study in Sweden. ADHD, Attention deficit hyperactivity disorder; CBCL, Childhood behavior checklist.

## AUTISM AND EARLY LANGUAGE PROBLEMS

Delays in the development of speech and language are the most common early signs of autism recognized by parents [De Giacomo and Fombonne, 1998]. A significant proportion of children with autistic disorder do not develop any useful language. In contrast, children with Asperger syndrome do not show any significant general language delay [American Psychiatric Association, 2000], illustrating the large variability in these problems within the ASD population as a whole. Twin studies have demonstrated a moderate to high heritability for language [see Stromswold, 2001 for a review] and specific language impairment [Bishop, 2002].

Similar to the studies into the overlap between autism and IQ, twin studies can shed a light on the genetic correlation between language delay and autism. Dworzynski et al. [2007, 2008] studied the association between early language (at ages 2, 3, and 4 years) and subsequent autistic traits at age 8 in a general population sample. Early language problems (indexed by language scores in the bottom 5% of the population) were only modestly related to later autistic traits, most notably autistic pragmatic communication problems. This phenotypic correlation was entirely explained by genetic influences; the genetic correlation between extreme autistic traits and early language problems was modest (genetic correlation = 0.33) [Dworzynski et al., 2008]. Analyses using the data from the whole sample reported a modest to moderate overlap between the genetic influences on language delay and the genetic effects on autistic traits [Dworzynski et al., 2007]. Further twin studies on the association between language development and ASD or autistic traits measured contemporaneously are now needed.

Molecular genetic studies have made some exciting discoveries of potential vulnerability genes common to both language and autism. A linkage study in 153 families affected with autism [Alarcón et al., 2002] suggested a quantitative trait locus for the language endophenotype "age at first word" on chromosome 7q. Although two later studies could not replicate this linkage peak [Alarcón et al., 2005; Spence et al., 2006], subsequent association and gene expression analyses implicated the CNTNAP2 gene in this region as a susceptibility gene for autism [Alarcón et al., 2008].

## AUTISM AND OTHER PSYCHIATRIC CONDITIONS

Comorbidity is rife throughout child psychiatric disorders, and autism is no exception. For example, between 24% and 59% of individuals with autism are thought to have an anxiety disorder [Weisbrot et al., 2005], and 28% meet criteria for ADHD [Simonoff et al., 2008]. Twin studies of autistic traits have developed some interesting hypotheses concerning the causes of this comorbidity.

Table IV outlines twin studies of psychiatric comorbidity in ASD and autistic traits. As shown in the table, significant genetic overlap has been reported between autistic traits and ADHD behaviors in the general population [Constantino et al., 2003; Reiersen et al., 2008; Ronald et al., 2008b, 2010b], as well as between children who appear to meet diagnostic criteria for ASD and ADHD according to parent report [Ronald et al., 2008b; Lichtenstein et al., in press]. The genetic correlations between autistic traits and ADHD behaviors reported in these studies were all substantial ($r_g$ between 0.54 and 0.87), apart from a more modest estimate ($r_g = 0.27$) found in young twins [Ronald et al., 2010b].

Multivariate twin models on autistic traits and anxiety have been reported. Rather than genetic influences playing a major role in their overlap (as appeared to be the case between ASD and ADHD), autistic traits and anxiety-related behaviors appear to co-occur in middle childhood mainly because of a combination of common environmental influences and phenotypic interaction over time [Hallett et al., 2009, 2010]. As shown in Table IV, genetic correlations between autistic traits and anxiety-related behaviors in middle childhood were low (0.12–0.19) suggesting that these types of psychopathology co-occur for reasons other than shared genetic pathways. In the only twin study of comorbid mental health problems in autistic traits in late adolescence [Hoekstra et al., 2007b], autistic traits were found to be significantly related to withdrawn behavioral problems and social problems. Autistic traits and anxiety/depressive behaviors also correlated modestly, but this correlation ceased to be significant after the effects of social and withdrawn behavioral problems were taken into account in the regression model. Substantial genetic overlap between both withdrawn behaviors and social problems with autistic traits was found. While further research is needed, one tentative hypothesis based on existing data is that anxiety-related behaviors co-occur with autistic traits in childhood due to environmental influences or an interaction between the two sets of behaviors, whilst the co-occurrence of anxiety and autistic traits in late adolescence is more likely to reflect an underlying genetic vulnerability.

Lastly, a new avenue of research has begun to explore whether there are overlapping genetic and environmental influences between autistic traits and less common psychiatric conditions such as psychopathic tendencies [Jones et al., 2009], tic disorder and developmental coordination disorder [Lichtenstein et al., in press].

## ASSUMPTIONS AND LIMITATIONS OF THE TWIN DESIGN

### Generalizability of Twin Studies

Like any other design, findings from classical twin studies need to be interpreted in the light of potential limitations. Firstly, since twins are often born 3–4 weeks premature and are lighter at birth than the average singleton, one could question whether twins are representative of the general population.

Some studies have suggested that the process of twinning may be a risk factor for the development of autism [Betancur et al., 2002; Greenberg et al., 2001 but see Visscher, 2002]. However, large population-based studies do not support these findings [Croen et al., 2002; Hallmayer et al., 2002; Hultman et al., 2002]. One study reported preliminary evidence that male twins may show slightly more autistic traits compared to male singletons [Ho et al., 2005]. Singletons and twins came from two different samples in this study, and the two samples were not matched for age, IQ, or social economic status. In a twin family study that also included the siblings of the twin pairs, and as such controlled for possible confounding effects of social economic status or parental education, mean self-reported autistic trait scores were found to be similar in twins and non-twin siblings [Hoekstra et al., 2007a].

In another study, there were no significant twin-sibling mean differences on measures of social impairments or RRBIs for teacher or parent-rated data in 7-year-olds, with the exception of parent ratings of DZ twins, who showed significantly higher social impairments [Ronald, 2006]. As such, two out of three of these studies suggested, for the most part, that level of autistic traits is unrelated to being born a twin or singleton.

The issue of generalizability *across* twin studies is also worth considering. Twin studies tend to be large longitudinal cohort studies on which a lot of measures are included. Samples are not all independent, with a large number of the twin study findings described here stemming from, in particular, the UK Twins Early Development Study, the Netherlands Twin Register, the Child and Adolescent Twin Study in Sweden, the US Missouri twin study, and the Autism Genetic Resource Exchange.

Finally, a minority of MZ twin pairs experience in utero twin-to-twin transfusion syndrome which involves disproportionate blood supply between the twins. This syndrome can lead to a variety of complications and is likely to result in birth weight differences between twins in a pair. This syndrome is twin-specific and therefore findings from twin studies that are due to the effects of twin-to-twin transfusion syndrome are not generalizable to singleton populations.

### The Equal Environments Assumption

The equal environment assumption has been tested at length [e.g., Kendler et al., 1993; Derks et al., 2006] for different phenotypes and seems tenable for most. In brief, this assumption is that the environment that is shared between the siblings is similar for both MZ and DZ twins. If this assumption is violated, for instance because MZ twins experience more similar environments than DZ twins, this would result in an overestimation of the genetic influences on autism or autistic traits.

### Assortative Mating

Finally, the classical twin design assumes random partner selection, that is, that partners do not actively or passively select each other based on their phenotype. Positive assortative mating (a positive correlation between partners' phenotypes) leads to a greater resemblance in DZ twins and non-twin siblings, whilst MZ resemblance remains unaltered, resulting in attenuated heritability estimates. Five studies to date have examined assortative mating for autistic traits in the general population or in clinical samples, with contrasting results. Constantino and Todd [2005] found a spousal correlation of 0.38 for autistic traits as measured using the SRS in the general population. Two subsequent studies using the SRS in parents of a child with autism found spousal correlations of respectively 0.26 [Virkud et al., 2009] and 0.34 [Schwichtenberg et al., 2010]. In contrast, Hoekstra et al. [2007a] and Pollmann et al. [2010] found near-zero partner correlations in general population samples using the full-scale AQ and the AQ-short. The latter two studies relied on self-report, whilst the studies using the SRS asked spouses to rate each other's autistic traits. Shared beliefs or perceptions about the couple's relationship may have inflated the spousal correlation in these studies. In contrast to the resemblance on the AQ-short ($r = 0.03$), Pollmann et al. [2010] did find significant spousal correlations for relationship satisfaction ($r = 0.32$), relationship intimacy ($r = 0.28$) and partner trust ($r = 0.21$), strengthening the idea that the studies using spousal report may have mainly picked up shared beliefs about the relationship quality, rather than resemblance for autistic traits per se. An alternative explanation for these conflicting findings would be that self-report assessment of autistic traits, as employed by Hoekstra et al. [2007a] and Pollmann et al. [2010], may underestimate assortative mating. Various twin registers around the world have now started to include data of siblings, spouses, and children of twins, so that many more family relationships can be modeled in the future. In the so-called extended twin family designs [see e.g. Eaves, 2009; Maes et al., 2009] it will be possible to test directly the possible effects of assortative mating.

## MEASUREMENT CONSIDERATIONS

When interpreting findings from twin studies it is important to consider the measurements used to assess the phenotype under study. Diagnostic measurement of autism and ASD has changed over time (see Table I): first, the psychiatric definitions of autism and ASD have evolved since the first twin study of autism in 1977 (and are due to change again with the advent of DSM-V), and second, more recent studies, unlike the older studies of autism, have used parental interview methods to obtain ASD diagnostic information. The most commonly used measures of autistic traits, such as the AQ, the SRS, the CAST and the Child Behavior Checklist Pervasive Developmental Problems subscale, differ in their psychometric properties (such as response format, factor structure, age appropriateness, and relative focus on social-communication versus restricted repetitive autistic symptoms). Results from different studies may be partly due to differing measurement tools.

Since measurement error is reflected in the nonshared environmental influences, a phenotype can only be as heritable as the reliability of the tool used to assess the trait. In other words, an unreliable measure will never show high heritability. For this reason, apparent age-related changes in heritability could be due to differences in measurement accuracy in early and later childhood.

The use of clinical or continuously distributed assessment tools may also affect the results. The structural equation modeling technique employed in twin modeling assumes a normal data distribution. Clinical assessment tools are usually less sensitive in picking up the variation at the unimpaired end of the scale, resulting in skewed distributions in general population samples, which may in turn lead to a bias in the heritability estimates [Derks et al., 2004].

Twin study findings may also be influenced by the informant of the behavior under study. Different raters provide different perspectives on behavior [see e.g. Constantino et al., 2007; Ronald et al., 2008a] and these different perspectives lead to different heritability estimates. Apart from real differences in behaviors picked up by different raters, the unique perception of an informant may also be influenced by rater bias. Rater bias may arise if the respondent holds on to particular normative standards, has a specific response style, or a stereotypical view of the behavior under study. When the same informant reports on the behavior of both twins, rater bias may lead

to an overestimation of shared environmental effects due to correlated rater bias across the twin pair [Bartels et al., 2007].

Lastly, sample size is an important factor when interpreting twin study findings. Twin studies require large sample sizes [Posthuma and Boomsma, 2000] to detect modest effects of genes and shared environment (nonshared environments are always specified as these effects include measurement error). Likewise, large sample sizes are needed to detect subtle gender differences in the influence of genes and environment [Polderman et al., 2006]. Lack of these effects in studies with small or moderate sample sizes may be due to lack of power.

## CONCLUSIONS

Our understanding of the causes of autism, broader ASD, and autistic traits is continually evolving through new discoveries and it is argued that the last decade of twin studies has added considerably to this research field. This literature provides new evidence regarding the dimensional nature of autistic behaviors, the etiological heterogeneity of autistic symptoms, and why ASD and autistic traits co-occur with intellectual disability, language delay and other psychiatric disorders. Although more research is needed in this area, the findings reviewed here have provided specific and testable hypotheses for molecular genetic autism research. Example hypotheses include that a substantial proportion of genetic risk factors associated with ADHD will also be associated with risk for ASD, that different genetic causal pathways will be associated with different types of autistic symptoms, and that the genetic causes of autism are largely distinct from the genetic causes of intellectual disability. Heterogeneity reported in clinics in terms of the range in presentation of ASD symptoms and variation in intellectual functioning is supported by the findings reported above, namely, that different symptoms within ASD may have partly different underlying causes, and that ASD symptoms may be partly genetically independent of intelligence. Given this evidence from twin studies, we should expect many children to display part of the autism phenotype and for ASD to occur regardless of the intellectual ability of an individual.

## FUTURE DIRECTIONS

Despite the considerable impact of the last decade of twin studies on ASD and autistic traits, further research is needed to settle existing contradictory findings and to address so far unresearched questions. For example, twin studies of psychiatric comorbidity could explore the degree to which genes and environment explain co-occurrence of other so far neglected comorbid symptoms such as conduct problems, sleep problems, antisocial behavior, and depression. Further work could teach us more about developmental change and continuity in genetic and environmental influences on ASD and autistic traits, particularly in early childhood, for which there are only two cross-sectional twin study of autistic traits to date [Edelson and Saudino, 2009; Stilp et al., 2010], and adulthood, for which no peer reviewed papers have been published yet. One longitudinal analysis, albeit with limited power due to a small sample (95 male twin pairs), suggests that change over time in autistic traits from early childhood to adolescence is explained by mostly genetic, and to a lesser extent, nonshared environmental influences [Constantino et al., 2009].

The types of measures used to assess features of autism need to be further developed. Age-appropriate measures that reliably capture autistic traits at different time points in life are necessary to conduct reliable longitudinal analyses. Moreover, the comparability of measures of dimensional autistic traits with measures used in clinical samples is an important consideration. Novel approaches to measurement were employed in a recent study that related autistic traits to lab measures of orientation and engagement in 2-year-olds [Edelson and Saudino, 2009], and two studies of older children that have employed cognitive assessments of theory of mind [Ronald et al., 2006] and emotion attribution [Jones et al., 2009] in relation to autistic traits. Further studies including cognitive phenotypes related to autism are needed to examine the association between specific cognitive abilities and autistic traits. Such studies will also be instrumental in integrating psychological and biological explanations of autism. Moreover, studies focusing on special abilities [Vital et al., 2009] can teach us more about the association between autism and special talent.

We can look forward to findings reported from several new systematic twin studies of ASD currently underway [Goldsmith, 2009]. These developments give hope for continued progress in understanding the causes of ASD in the next 10 years.

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