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Birkbeck, University of London

Adolescent Autistic Traits and Internalising Traits:

Quantitative Genetic Investigations of Co-Occurrence Patterns

Aline D. Scherff

Thesis submitted for the degree of Doctor of Philosophy 2013

Statement of work

This thesis analyses data from the Twins Early Development Study (TEDS), headed by Prof. Robert Plomin and funded by the Medical Research Council. The quantitative genetic work throughout the thesis was carried out under the supervision of Dr. Angelica Ronald and interpretation of results was co-supervised by Prof. Tony Charman. My PhD was funded by a grant from the Bloomsbury Colleges awarded to Dr. Angelica Ronald.

TEDS data on 12- and 14- year-old twins was collected prior to the start of my PhD. I was involved in some of the age 16 year data collection, specifically in helping to prepare and collate the mailer for both cohorts. I had sole responsibility for developing hypotheses, selecting autistic and internalising trait measures, covariates and environmental variables for analysis and for conducting model-fitting using this dataset. I derived all variables, conducted all statistical analyses and wrote all of the chapters. The work presented in this thesis is original and my work, with exception to what has been acknowledged within the text. This thesis has not been submitted for any other degree at any other university.

Abstract

Autism spectrum disorders (ASD) are characterised by social-communication difficulties and non-social symptoms such as restricted and repetitive behaviours and interests. ASD characteristics can be investigated at the subclinical trait level within the general population, and these quantitative autistic traits have been shown to have a smooth distribution. Adolescence is an important developmental stage, particularly for the emergence of internalising problems. However, few studies to date have investigated the causes of co-occurring autistic traits and internalising traits during adolescence. The aim of this thesis is to explore the aetiological causes of this trait association between the ages of 12 to 16 years using a quantitative genetic approach.

This thesis employs a classic twin design and the sample came from the Twins Early Development Study (TEDS). The causes of the association between autistic and internalising traits in early adolescence are the first focus of this thesis. The analyses in Chapter 4 explore this aetiological association at ages 12-14 years, revealing a moderate phenotypic trait association and at the aetiological level moderate genetic overlap, substantial shared environmental and modest nonshared environmental overlap. Teasing apart these associations further, Chapter 5 identifies specific autistic-like behaviours by means of factor analysis. Relating these factor-derived autistic trait subdomains to the internalising trait measure demonstrated distinguishable patterns of phenotypic and aetiological associations. A factor named autistic-like 'Social Unease' showed the most phenotypic and genetic overlap with internalising traits.

Secondly, this thesis investigates in Chapter 6 the role of childhood nonshared environment on internalising and autistic traits in early adolescence using the monozygotic twin differences design. Analyses showed that birth weight, childhood hyperactivity and peer problems played a role, via the nonshared environment, in influencing individual differences in internalising and autistic traits in early adolescence.

Finally, Chapter 7 presents findings on later adolescence, at age 16 years, exploring the association of autistic traits with anxiety traits and depression traits separately and drawing on both parent and self ratings. The implications of these findings, their limitations and their contribution to the current literature are considered in the Discussion (Chapter 8).

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Published Conference Abstracts

Scherff, A.D., Charman, T., & Ronald, A. (2012). Adolescents' MZ differences on autistic traits, internalising traits and their co-occurrence: The role of early NSE trait differences. *Behavior Genetics, 42*(6), 967.

Scherff, A.D., Hallett, V., Charman, T., & Ronald, A. (2011). Association between autistic traits and internalising traits in early adolescence: A twin study. *Behavior Genetics, 41*(6), 935.

Other

Scherff, A.D. (2013). Association of Autistic Traits with Anxiety and Depression Traits in Late Adolescence in a Community-Based Twin Sample. *Poster at Bloomsbury Colleges Research Students Symposium, London*

Scherff, A.D., Charman, T., Ronald, A. (2012). Association of Internalising Traits and Autistic Traits in Adolescence in a Community-Based Twin Sample. *Poster at the International Meeting for Autism Research, Toronto*

Scherff, A.D. (2012). Autistic traits and Internalising traits: Association in Adolescence. *Talk at Bloomsbury Colleges Research Students Symposium, London*

Scherff, A.D. (2011). Association between anxiety-related behaviours and autistic traits in early adolescence: a twin study. *Talk at Bloomsbury Colleges Research Students Symposium, London*

Chapter 1 Autism Spectrum Disorders

This chapter introduces autism spectrum disorders (ASD), a group of neurodevelopmental disorders characterised by impairments in the domains of social interaction, communication and repetitive and stereotyped behaviours/ interests (RRBIs).

Historical Background illustrates the origins of the continuing efforts to relate and demarcate individual phenotypes within this spectrum. Prevalence Rates and Sex Bias *in ASD* will be discussed. Causes and Theories provides a cursory introduction which biological and cognitive factors have been implicated. Developmental Trajectory addresses findings on developmental changes in phenomenology across childhood and adolescence. Psychiatric Comorbidity of ASD and Internalising Disorders will be introduced. Quantitative Traits will argue for a role of trait-based research in understanding ASD.

1.1   Historical Background

The autistic spectrum both historically and in its current form encompasses a wide range of phenotypic presentations. Two disorders now known as Asperger's and autistic disorder fall within this spectrum, and their initial description were made independently but in close succession. Both early accounts describe the self-referential behaviour (from Latin auto meaning self) of the observed individuals, their 'early infantile autism' (Kanner, 1943) and 'autistic psychopathy' (Asperger, 1991/1944). Similarly, Asperger and Kanner both note symptomatic deficits of a behavioural, social and communicative nature. These include the need for sameness, cognitive difficulties and affective symptoms (Kanner) and social withdrawal, affective flatness and poor nonverbal communication (Asperger). The similarities in the initial naming of the observed clinical cases show an early recognition of 'autistic aloneness' (Kanner, 1943) as unique symptom and common denominator. Indeed, this was recognised by Asperger himself, stating the conditions they had described independently were 'basically different types' within a cluster of related conditions (Asperger, 1979; Miller & Ozonoff, 1997).

Simultaneously, Asperger's statement illustrates the longevity of the still continuing debate on both necessary and sufficient characteristics for the diagnosis of an autism spectrum disorder. Much thought in autism research has been devoted to demarcating inclusion as well as exclusion criteria for autistic disorder and Asperger's separately and in relation to one another. On the one hand, efforts have been made to analyse in detail,

the above cited historical accounts to identify the requisite signs and symptoms as originally described (Wing, 1981). On the other hand, an inverse approach has been applied in working back from more recent diagnostic criteria as laid out by the just-replaced Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; APA, 2000). Re-classifying Asperger's four original cases using these parameters, Miller and Ozonoff (1997) found all to be a close match to the condition of autistic rather than Asperger's disorder. They suggest that the two possible conclusions that can be drawn from this are 1) either autism and Asperger's disorder are two labels for a single condition or 2) diagnostic criteria previously have not accurately captured the condition 'Asperger's' as specified.

1.2 Diagnostic Heterogeneity

Two widely recognised manuals are available guiding clinical diagnoses of autism-associated conditions to date: The recently introduced DSM-5, compiled by the American Psychiatric Association (APA, 2013) and International Classification of Diseases (ICD-10), published by the World Health Organisation (WHO, 1992). The extent to which these two manuals differ varies by psychiatric disorder, but a review of classification differences between the previous DSM-IV and ICD-10 concluded this was not substantially impacting comparability of research derived using the respective systems (Fatemi & Clayton, 2008).

However, at the point of writing a novel challenge also lies in positioning any findings within a changing system, as the DSM-5 has just been introduced and the ICD system is due to be updated soon, both with marked changes to the Autism categories. In light of the particular endorsement of the DSM framework by the research community and this thesis' focus on observable autistic-like behaviours rather than diagnostic categories, the next paragraphs are limited to a brief summary of the previous DSM-IV ASD subtypes in order to provide a cursory introduction of the observed heterogeneity for this phenotype. The decision of focusing on this rather than the new system was based on the fact that all currently existing research and measurement scales have been within this framework. A summary of the DSM-IV diagnostic features of autistic disorder ordered by symptom groups (jointly referred to as the triad of impairments) is provided in **Error! Reference source not found.**. Table 1.2 summarises the differences in these criteria as applicable to the other three autism spectrum conditions.

The DSM-IV positions ASD as part of the category of disorders 'usually first diagnosed in infancy, childhood, or adolescence'. However, for ASD more specifically, signs are

generally noticeable early in childhood and diagnoses can be made before age 3, although the timing of identification has been found to be influenced by a number of factors such as sex and symptom severity (Begeer et al., 2013; Shattuck et al., 2009).
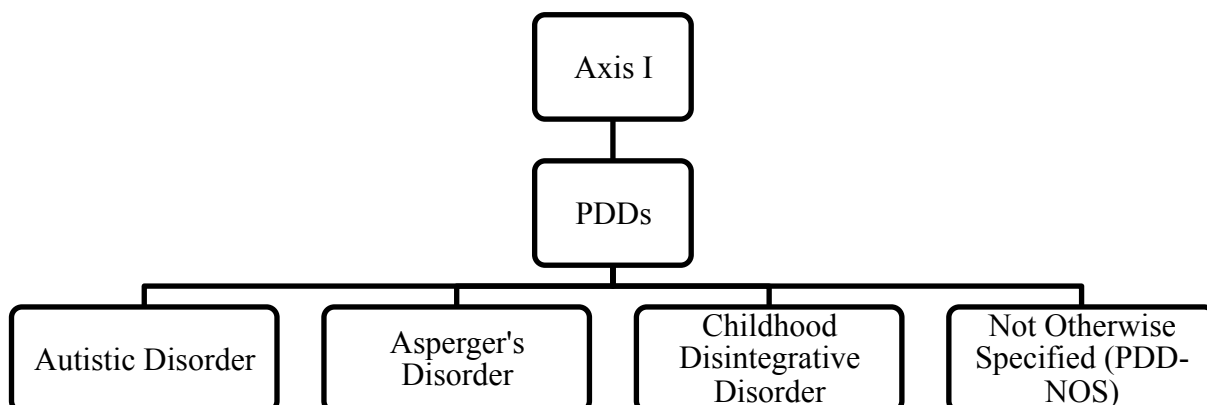
Figure 1.1 Position of disorders on the autism spectrum within the previous DSM-IV framework

ASD are located on Axis I (Figure 1.1) containing all diagnostic categories except mental retardation and personality disorder, within the group of Pervasive Developmental Disorders (PDDs). In addition to Autistic Disorder, Asperger's Disorder, Childhood Disintegrative Disorder and Pervasive Developmental Disorders-Not Otherwise Specified (PDD-NOS), the group also refers to Rett's syndrome.

Individuals with Rett's syndrome may show phenotypically similar behaviour to ASD, however these are often overcome or at least masked later in development (Kaufmann et al., 2012) and the syndrome's specific aetiology puts it closer to other genetic and neurodevelopmental disorders such as Fragile X syndrome, tuberous sclerosis and Down syndrome (Tsai, 1992). Accordingly, the DSM-5 plans has removed Rett's syndrome from the group.

In order to account for the high degree of variability of the types of symptoms, level of functioning and shared characteristics, a single Autism Spectrum Disorder category has been implemented with arrival of DSM-5. The DSM-5 classification system takes a step away from the previous developmental and conceptual differences and instead introduces 3 levels of severity of functional impairment (Appendix I and Appendix II). Another difference is the merging of the social interaction and the communication criterion into a single social-communication domain, based on an argument that deficits

in these areas are virtually inseparable and modified by contextual and environmental factors (Wilkinson, 2012). However, the transition to the DSM-5 is viewed to have only limited impact to the current thesis, which uses a trait level approach throughout and wherever specific autistic domains are used, these have been empirically derived from the sample.

Table 1.1 DSM-IV diagnostic criteria for autistic disorder

A diagnosis requires at least 6 current impairments, including 2 social items and at least 1 communication and RRBI item. Reports of delays or abnormal functioning with onset prior to age 3 years in at least 1 of social interaction, language for social communication, symbolic and imaginative play. Difficulties are not accounted better for by another disorder.

Social Difficulties:
• impairment in multiple nonverbal behaviours e.g. eye-to-eye gaze, facial expression, body postures, gestures
• lack of age-appropriate peer relationships
• lack of sharing of enjoyment, interests, or achievements
• lack of social or emotional reciprocity

Communication Difficulties:
• delay or lack of language
• where speech is present, initiation or sustaining of conversations impaired
• stereotyped or repetitive language or idiosyncratic language
• lack of make-belief play or social imitative play

Restricted Repetitive and Stereotyped Behaviours (RRBIs):
• preoccupied with stereotyped and restricted interests with abnormal intensity or focus
• inflexible adherence to specific, non-functional routines or rituals
• motor mannerisms e.g. hand flapping or twisting, complex whole-body movements
• persistent preoccupation with parts of objects

Modified from DSM-IV-TR text (APA, 2000)

Table 1.2 DSM-IV diagnostic criteria for other autism spectrum disorders

Asperger's Disorder (AS):

• Clinically significant Social Difficulties and RRBIs as in Autistic Disorder

• Significant impact on social, occupational or other important areas of functioning

• No history of general language delay

• No significant cognitive impairment

Childhood Disintegrative Disorder (CDD):

• Apparently normal development in at least the first 2 years in social, communicative and RRBI domains

• Clinically significant loss before age 10 years in at least two of expressive or receptive language, social skills or adaptive behaviour, bowel or bladder control, play, motor skills

• Abnormalities in at least two of the three autism domains

Pervasive Developmental Disorder - Not Otherwise Specified (PDD-NOS):

• Severe and pervasive impairment in development, but criteria for a specific PDD are not met

• This category includes 'atypical autism' - including presentations of late age of onset, atypical symptomatology, subthreshold symptomatology

Modified from DSM-IV-TR text (APA, 2000)

1.3   Prevalence Rates and Sex Bias

Epidemiological research shows that most individuals with ASD are diagnosed with either autistic disorder (prevalence in population: 0.2%) or PDD-NOS (0.3%; Fombonne, 2009). The prevalence of Asperger's disorder is less well established but estimated at around ¼ to ⅓ that of autistic disorder. Childhood disintegrative disorder is very rare (0.0002%). Combined, ASD are forming one of the most frequent childhood developmental disorders (>1%; Baird et al., 2006; Baron-Cohen et al., 2009; Blumberg et al., 2013; Kim et al., 2011). These figures vary considerably depending on factors such as diagnostic criteria and assessment used, however overall figures for ASD appear to be rising. This has been attributed in part to increased awareness and conceptual

broadening, though other factors that have been named are cultural and environmental reasons (Elsabbagh et al., 2012; Matson & Kozlowski, 2011; Zaroff & Uhm, 2012).

ASD are more frequently diagnosed in males with an overall ratio of 4: 1 (Fombonne, 2008). Greater differences in numbers have been reported for those with cognitive abilities in the high functioning range (6:1) and a more balanced distribution is found in those with intellectual disability (2:1; Rivet & Matson, 2011). A possible explanation for this could be that clinicians may find it difficult to recognise ASD in females with a low symptom burden. It has been noted that females meeting diagnostic criteria are much more likely to show additional problems such as lower IQ and behavioural difficulties than males (Dworzynski, Ronald, Bolton, & Happé, 2012). This could point to a better ability of females to compensate or adapt in cases where these difficulties are absent. Alternatively, male-biased diagnostic criteria that affect perception of typical and atypical behaviour in the domains of social, communication and RRBIs may lead to females being missed. Genetic factors may act as a third factor in the disparity (Constantino & Charman, 2012) and are discussed in more detail later.

1.4 Causes and Theories

Just as the conceptualisation of ASD has undergone various permutations throughout the last decades, a multitude of causes and theories have been suggested to account for ASD. Explanatory frameworks have been developed from a range of perspectives, resulting in cognitive theories and causal organic models including genetic, neural and biological factors. The heterogeneity of clinical autism phenotypes has been discussed at some length previously will not be repeated here, save to emphasise that in light of it, thinking up an all-encompassing single explanation has been challenging – as addressed in the fractionable autism triad hypothesis.

1.4.1 Fractionable Autism Triad Hypothesis

A review by Happé and Ronald (2008) argues that it could be a misconception to expect largely shared underlying causal factors to all three defining domains of ASD (see also commentary by Mandy & Skuse, 2008). They draw together evidence to suggest that each domain may have its unique *aetiology* at various levels of explanation, and those individual impairments combine to result in the distinct *presentation* of the autism phenotype.

The review includes evidence from general and clinical referral populations (Ronald, 2006; Wing & Gould, 1979) showing that the triad of symptoms tend to cluster together

in individuals, both in typically and atypically developing children. In a community sample of ~3,000 pairs of 7-9 year-old twins, the number of children at the high-scoring end of the population (95th percentile) for one domain who also scored highly on one additional domain was 1.6-3.6 times the figure expected by chance. The number of children scoring highly on all three domains was 56 times that expected by chance. This was the same sample as used in this thesis, investigated at a younger age. Despite this co-occurrence of difficulties, the extent to which domain scores co-varied was however limited to modest-to-low correlations of a magnitude of .2-.4 between social and communication domains, .3-.4 for communication-RRBI and .1-.3 for social-RRBI (Ronald et al., 2006; Ronald, Happé, & Plomin, 2005). Together this suggests that although autistic symptoms tend to co-occur, there is also evidence for the fractionation of the three aspects. A review dealing exclusively with findings on RRBIs (Leekam, Prior, & Uljarevic, 2011) suggests that for some purposes, even further differentiation of triadic aspects may be required.

Secondly, factor analytic studies were reviewed by the authors (Happé & Ronald, 2008) with respect to their ability to empirically verify whether all autistic symptoms constitute a single factor or multiple dimensions. Outcomes were split between studies finding three- to six-factor solutions and those where a large proportion of the variance was accounted for by the unrotated first principal component. Some of these differences may however be explained by their respective particular sample characteristics. After only including those studies with unrestricted variance across the ASD spectrum and a sufficient ratio of sample size to modelled items, another review (Mandy & Skuse, 2008) accepted evidence for a single factor from one study, and evidence for several factors from seven studies. Most recently, a review (Shuster et al., 2013) of all factor analytic studies on ASD *symptoms* (i.e. excluding trait studies) to date found overwhelming evidence that the combined social/communication domain is separate from the RRBI domain. More details on recent factor analytic findings on autistic *traits* are provided in Chapter 5.

The fractionation of autistic traits is also evident from family and twin studies (method and systematic review in Chapter 2 and Chapter 3). Briefly, the fractionable autism triad hypothesis states that at the genetic level, the social, communication and RRBI domains have all been shown to be substantially heritable individually (with modest nonshared environmental influence). However only a limited amount of genetic variance is shared across these, ranging from less than half in the general population to just over 50%

overlap at the 5% most impaired extreme (Ronald et al., 2006). This suggests that some genes may play a role for a range of autistic-like behaviours whereas others are particularly relevant for specific autistic symptoms.

Cognitive theories (see section 1.4.4 for a brief overview) have been both the traditional explanatory approach to ASD and highly influential. Briefly, the authors (Happé & Ronald, 2008) hold that at the cognitive level, there exist satisfactory explanatory models for each domain individually but no single theory accounts equally well for all domains. Molecular genetic and neuroimaging work suggesting fractionation is also discussed in the review but is omitted here as it is beyond the scope of this thesis. The following section (1.4.2) does however illustrate the potential complexity at this level.

1.4.2 Genetic causes

For a small proportion of 5-10% of individuals with autistic symptoms, their difficulties can be attributed to clearly defined syndromes or chromosomal abnormalities (Folstein & Rosen-Sheidley, 2001). Tuberous sclerosis, Fragile X syndrome and Rett's disorder fall into this category, and affected individuals diagnosed with these primary conditions are not usually included in aetiological studies on autism. Single gene disorders are even more rare and individually account for around 1% of ASD cases (Abrahams & Geschwind, 2008).

However, the origin of most ASD cases is thought to be non-syndromic (idiopathic) and the underlying causes are not definitively identified. At the genetic level, studies have rarely been able to single out factors with large effect sizes in explaining psychiatric disorders, and ASD is no exception. Instead, increased risk of ASD is now thought of as resulting from an accumulation of many small, common genetic variants (Klei et al., 2012). An insurmountable amount of literature has been published in the past decade since completion of the human genome project: This decade therefore opens up never seen opportunities of following up these leads and it may be hoped that further inroads in consolidating and confirming findings will soon be made. A recent review found as many as 2193 genes, 2806 single nucleotide polymorphisms and variable number tandem repeats (SNP/ VNTR), 4544 copy number variations (CNV) and 158 linkage regions associated with ASD by genome wide association studies (GWAS), genome-wide CNV studies, linkage analyses, low-scale genetic association studies, expression profiling and other low-scale experimental studies (Xu et al., 2012). In addition, epigenetic mechanisms such as maternal imprinting and gene regulation via noncoding

RNA (ribonucleic acid) could further complicate investigations (Grafodatskaya, Chung, Szatmari, & Weksberg, 2010; Hu, 2013; Skuse, 2000).

Exemplary for the many avenues that have been pursued, a particular focus in the last years has been on the role of CNVs in ASD. These insertions and deletions of portions of the genetic sequence (magnitude of >1,000 nucleotide bases) have been suggested as conferring risk of ASD in two ways – as an inherited familial load and as a result of newly occurring genetic *de novo* mutations. Therefore, CNVs could provide a mechanism by which both general vulnerability and person-specific risk is accounted for. These de novo mutations at the CNV and point mutation levels are thought to make important contributions (in up to 20% of cases; Malhotra & Sebat, 2012) to an individual's disease burden and may capture a small but significant part of the heritability of complex genetic disorders such as ASD (Ku, Vasiliou, & Cooper, 2012; Neale et al., 2012; Veltman & Brunner, 2012). A recent comprehensive review of the genetics of both syndromic and non-syndromic ASD is provided in Miles (2011).

1.4.3 Environmental causes

ASD are now thought to be among the most heritable neurodevelopmental disorders (Bailey et al., 1995; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010; see also Ronald & Hoekstra, 2011 for a comprehensive review; Taniai, Nishiyama, Miyachi, Imaeda, & Sumi, 2008). In addition, a range of environmental causes has also been proposed, falling into the two broad categories of psychological-societal and organic-toxicological. They cannot all be discussed here, however a review of early theories is provided in Rutter (1968) and recent suggestions are summarised by Herbert (2010). In short, in rare cases environmental factors have been strongly linked with autistic features, but there is no conclusive evidence to suggest any particular environmental mechanism as *causally deterministic*. Instead, a multitude of potential contributing factors (stressors) to pre/peri- and postnatal *risk* of ASD have been suggested, all needing further replication.

1.4.4 Cognitive theories

At the cognitive level, three cognitive theories of ASD have featured prominently among past decades' research. Deficits in executive function have been postulated as one such possible explanation (review in Hill, 2004). Typically functioning executive control acts as a supervisory mechanism of the brain and impairments affecting executive function could account for both social and non-social (RRBI) deficits in ASD. Secondly, the theory of mind account offers a more developmentally relevant

perspective. The main focus here is on a delayed or permanently impaired ability to 'mentalise' or 'mindread', meaning that vital milestones in introspection and attribution of beliefs, desires and intentions to others are not met (Baron-Cohen, Leslie, & Frith, 1985). This in turn is thought to result in difficulties on the social-communication domain, though the connection with savant abilities or RRBIs is less clear. Thirdly, (weak) central coherence has been proposed first as an impairment, but more recently it is viewed as a common cognitive style found in ASD (Frith, 1989; Happé, 1997). It is argued that special interests and fascination with details and parts of objects is representative of a bias for local over global processing. Thus the account benefits from incorporating observed strengths in ASD as well as the deficits, particularly in relation to the non-social domain. It appears that no single cognitive theory can satisfactorily explain all ASD symptoms. Jointly they may nevertheless posit a strong explanatory scaffold and deficits indexed by each one are consistently associated with one another (Charman et al., 2011; Pellicano, 2010).

1.5   Developmental Trajectory

Along with the other (neuro-) developmental disorders, ASD is viewed both as lifelong, stable condition, and as being subject to developmental changes. It is assumed that most fluctuations will occur in early childhood, and that only modest changes will occur upon reaching adulthood (Pellicano, 2012; Seltzer, Shattuck, Abbeduto, & Greenberg, 2004). Individuals meeting diagnostic criteria for an ASD at one time point are likely to maintain clinical status, though not necessarily present with the same subtype or the same composition of symptoms (McGovern & Sigman, 2005). Outcomes vary with a multitude of genetic, neural and cognitive factors, although regression (loss of function, e.g. language) and cognitive ability (IQ) and the potential of interventions have received a great deal of attention (Howlin, Goode, Hutton, & Rutter, 2004; Rutter, 2012).

1.6   Adolescence

Adolescence is an important time when biological, cognitive, psychological and social development and change take place. Depending on these individual aspects studied, the age range may vary to include ages 10-20 (WHO, 1992), though the widely held working definition of adolescence as the 'teen-age' years more narrowly describes the years 13 to 19. Different ranges yet may be defined with respect to early hormonal changes, legal considerations and individual differences in the onset and pace of maturation.

Linking childhood and adulthood, adolescence is a time of increasing demands for young people to become more self-sufficient and succeed independently of their carers' support. Thus, deficits in social skills can become both more apparent and relevant. With respect to impairments on the autistic spectrum, Rieffe et al. (2011) suggest that pre-adolescent high-functioning children with an ASD show a more fragmented emotion regulation pattern compared to typical controls, especially related to worry and rumination. This lowered resilience may in turn lead to feelings of being isolated and rejected, exacerbating the display of autistic-like behaviours. In addition, social exclusion frequently leads to risky, self-defeating and anti-social behaviour and is a risk factor for internalising behaviours including suicidal ideation for adolescents (Kirkcaldy, Siefen, Urkin, & Merrick, 2006; Peake, Dishion, Stormshak, Moore, & Pfeifer, 2013).

1.7 Psychiatric Comorbidity of ASD and Internalising Disorders

Beyond the identification of mediating factors that drive presentation of behavioural difficulties over time *within* the autistic triad, additional difficulties are ubiquitous among individuals with ASD. These co-occurring deficits commonly include epilepsy on the physiological side (Van Eeghen et al., 2013), and externalising difficulties such as Attention Deficit/ Hyperactivity Disorder (ADHD, Kadesjö & Gillberg, 2001) as well as internalising difficulties on the psychological side. Internalising difficulties and their association with ASD are the focus of this thesis and they are introduced here by reviewing psychiatric comorbidity. Later chapters will address their aetiology by summarising previous publications and finally by adding new data on adolescence.

1.7.1 Conceptualisation

Comorbidity refers to the simultaneous presentation of two distinct disorders within an individual (Bax & Gillberg, 2010). While examples of coincidental co-occurrence of a somatic disorder (e.g. pneumonia) with a psychiatric disorder (e.g. schizophrenia) are easily found, in studying psychiatric comorbidity, a degree of 'true' comorbidity is assumed. However, it should be borne in mind that it is possible for two comorbid psychiatric disorders to present phenotypically additive symptoms but show a different aetiology.

First, a causal relationship between two disorders may be implied if A causes B, or more indirectly, if C causes both A and B. However, it may be difficult to identify such perfect correlations between psychiatric disorders in clinical practice. Second, a less deterministic mechanism is presented in increased risk. In this scenario, individuals

affected by A are more likely to develop B, either generally or beyond a certain threshold (severity) of A. The concept of increased risk usefully does not preclude mutual influences of A and B. Third, in cases where risk of A and B is not unidirectional, it is more useful to conceive of C as the shared risk factors of both a genetic and environmental nature. Quantitative genetic methodology, as utilised for the analysis of the data presented in the empirical chapters, not only allows identifying the extent to which such shared factors are at work. It also makes it possible to decompose the data into aetiological factors (genetic and environmental influences) both for single disorders and on the composition of their shared variance.

1.7.2 Internalising Disorders

Internalising disorders collectively primarily refer to depression and anxiety. Conceptually, their central feature is disordered mood or emotion. This categorisation has mainly been applied in contrast to externalising disorders, signified by dysregulated behaviour (Kovacs & Devlin, 1998). A number of large-scale studies have been conducted, showing continuity of diagnoses among internalising disorders. Exemplary, the Dunedin Multidisciplinary Health and Development Study following a New Zealand birth cohort longitudinally finds that 44% of 15-year-olds with internalising disorders continued to have internalising problems at 18, while only 5% had developed externalising symptoms (Feehan, McGee, & Williams, 1993; Moffitt, Rutter, & Silva, 2001). At age 18, youths were also more likely to show the same specific internalising disorder (i.e. depression or anxiety) or meet criteria for comorbid internalising disorders than they were to have moved between single internalising disorder categories.

The two internalising disorders depression and anxiety are known to be highly comorbid: A recent study estimated current comorbid and lifetime presentation in a large clinical sample from the Netherlands (Lamers et al., 2011). Co-occurrence rates were similar for individuals with a primary depressive disorder and those with a primary anxiety disorder. The respective other disorder currently co-occurred at around 65% and lifetime co-occurrence around 75%. For both disorders, risk of developing a subsequent comorbidity was increased in individuals with earlier onset of first disorder, longer duration of symptoms and higher symptom severity.

Less unequivocal evidence has been presented with respect to the order of appearance of anxiety and depression, which may be less clear than once believed. Some findings suggest that differences can be observed in the order in which the two disorders first occur. Exemplary, in the above cited Dutch study (Lamers et al., 2011), in 57% of

comorbid cases anxiety preceded depression whereas in only 18% depression preceded anxiety. In cases where depression was the first-occurring disorder, individuals tended to be younger at first onset but symptoms of either subsequent disorder tended to be of shorter duration and fewer in numbers. Alternatively, Moffitt and colleagues present evidence suggesting different trajectories based on patterns of shared vs. specific antecedent risk factors. Specifically, comorbid major depression and generalised anxiety disorder was antedated by a broad range of highly elevated risk factors and earliest onset, most recurrence and greatest use of mental health services and medication (Moffitt et al., 2007a; Moffitt et al., 2007b). For the two disorders individually, levels of risk factors were found to be similarly high for generalised anxiety disorder, but not for major depression. While all of the investigated risk factors impacted comorbid presentation, single disorders showed differential risk factors with specific risk factors for major depression being family history of depression and low positive emotionality and specific risk factors for generalised anxiety disorder being adverse family environment and childhood behavioural problems.

Pursuing this further, literature shows that internalising disorders are not only relevant but also prevalent, both in absolute terms and with respect to other psychiatric disorders. Contrary to descriptions as '(clinical) disorders', suggesting rareness and extremeness, psychiatric difficulties of any variety are frequent. Both risk and prevalence of any single psychiatric disorder have been found to approach 50% by the age of 75 years (Kessler, Chiu, Demler, Merikangas, & Walters, 2005). Over a quarter of the general population are affected by any two or more disorders during their lifetime, and the same figure has been estimated for current comorbid presentation (Kessler et al., 2005). A comprehensive longitudinal study testing for possible changes in (co-) occurrence patterns found that levels of population caseness remained stable in the Netherlands since the mid-1990s (De Graaf, ten Have, van Gool, & van Dorsselaer, 2012).

Adult lifetime prevalence of mood disorders was estimated at around 20%, as was that of any anxiety disorder in the above Dutch sample, though an American study estimates lifetime anxiety closer to 30% (Kessler et al., 2005). Their respective 12-month prevalences were 6 and 10% (US sample: 10 and 18%). Clear sex differences were also observed with percentages of affected women around 50% higher compared to male figures. In line with previously discussed findings of anxiety more typically preceding depression than vice versa, average adolescent (ages 13-18 years) lifetime prevalence of internalising disorders is at 14% for any mood disorder and 32% for anxiety disorders

(Merikangas et al., 2010). Median ages of onset also greatly differ at 11 (anxiety) versus 30 years (depression) (Kessler et al., 2005). Early sex differences tend to be even more pronounced than in adulthood.

In summary, epidemiological studies show that anxiety and mood disorders are the overall most prevalent types of psychiatric disorders in the general population. Given this high prevalence, the high co-occurrence and the likelihood of developing the respective other disorder, internalising disorders appear as a concept of great relevance. Equally, the data discussed above shows that irrespective of great similarities, anxiety and depressive disorders also follow partially distinct developmental trajectories. Thus, there is a strong rationale for both 'lumping and splitting' (for a detailed discussion see Tandon, Cardeli, & Luby, 2009). Of particular interest for this thesis are their individual and joint associations with ASD.

1.7.3 Internalising Disorders in ASD

Psychiatric comorbidity in individuals with ASD is consistently reported as higher compared to the general population. Indeed a recent review (Kerns & Kendall, 2012) concludes that in comparison, 12-month manifestation of a second concurrent pathology in individuals with a first diagnosis of ASD is at twice the rate (i.e. ~50%). The frequency of ASD *within* other psychiatric disorders – due to low overall population prevalence of ASD – will not be addressed here and quantitative trait studies will find mention in later chapters.

Estimates how frequent anxiety co-occurs within ASD greatly vary from 11 to 84% (White, Oswald, Ollendick, & Scahill, 2009), reflecting different sampling methods. The lowest rates tend to be found in population samples using trait level measures, where most individuals will not score above the clinical cut-off on individual traits and even fewer will report difficulties on an additional psychiatrically relevant trait. Intermediate rates are found in clinical referral samples, wherein individuals who are showing signs of any psychiatric disorder are also more frequently showing signs of any further specific disorders under investigation. The highest comorbidity rates for anxiety are obtained in clinical ASD samples, where the clinical status on the first disorder is confirmed and individuals only vary on symptomatology of the second. Despite this variability and a wealth of literature, common estimates in community-based samples (i.e. samples that are neither population based, nor treatment seeking) revolve around 39% for concurrent and 50% for lifetime comorbidity (Kerns & Kendall, 2012). A recent meta-analysis of 31 eligible studies including a total of >2,000 children and

adolescents found that among co-occurring anxiety disorders in ASD, specific phobias (29.8%) were the most frequent, followed by Obsessive-Compulsive Disorders (17.4%) and Social Anxiety Disorders (16.6%)(van Steensel, Bogels, & Perrin, 2011).

Fewer studies have dealt specifically with clinical (major) depression in ASD, and prevalence rates are less well established. One review has suggested a maximum estimate of lower-by-comparison 34% (Stewart, Barnard, Pearson, Hasan, & O'Brien, 2006). This may be owed to a general focus of ASD research on child and adolescent participants, who are less likely to have experienced depressive symptoms due to age of onset differences (see above). This is illustrated by findings from a community sample of 10-14 year-olds at risk or diagnosed with an ASD, in which the number of individuals meeting DSM-IV criteria for a range of disorders was assessed, resulting in average rates of comorbid anxiety difficulties (41.9%) but very low rates of comorbid depressive disorders (1.4%; Simonoff et al., 2008).

A different interpretation is provided by Ghaziuddin, Ghaziuddin, and Greden (2002), asserting that depression may indeed be the most frequent comorbidity in ASD. Low detection rates reflect the greater difficulty for observers to recognise depression symptoms as opposed to being aware of anxiety symptoms in ASD. Comorbid depressive symptoms may be atypical or masked by ASD symptoms (Magnuson & Constantino, 2011). In aiming to distinguish common depressive symptoms – like long-term lowered mood, loss of interest and changes in sleep patterns – from developmental changes that can be accounted for within an ASD-only framework, Leyfer et al. (2006) developed an ASD-specific comorbidities questionnaire, reporting a 10% prevalence.

Standard depression inventories in ASD samples are relatively more likely to detect symptoms at the higher-functioning end of the spectrum, as illustrated by a 2% prevalence of depression in autism (Ghaziuddin, Tsai, & Ghaziuddin, 1992) compared to 30% in Asperger's syndrome (Ghaziuddin, Weidmer-Mikhail, & Ghaziuddin, 1998). Further evidence in this direction comes from a rare adult study of male and female cognitively able (mean IQ = 105) individuals with ASD, finding that 70% of participants had experienced at least one and 50% recurrent depressive episodes (Lugnegard, Hallerback, & Gillberg, 2011). In contrast, another recent study of cognitively able children and adolescents (mean IQ = 102) found a prevalence of 30% but no significant associations between either age, IQ or autism symptoms (Strang et al., 2012). In summary, individuals with ASD frequently experience additional psychiatric difficulties of which internalising disorders account for a large part.

1.8   Quantitative Traits

The above discussion illustrates the ubiquity of internalising disorders both individually and within ASD. However, at around 1% of the population, ASD themselves are relatively uncommon. This poses the challenge that although internalising disorders are relevant to ASD, recruiting sufficient numbers of individuals with both types clinical conditions can be arduous, as is establishing how the wide range of signs and symptoms of ASD specifically relate to internalising difficulties. For this reason, studying variation within the general population is a useful tool to gain greater statistical power. Moreover, trait studies add complimentary information to the knowledge obtained via clinical case studies by relating clinical constructs to patterns observed in the general population. Trait studies modelling individual differences of psychological phenotypes assume a continuous dimensional distribution of characteristics of which the clinical diagnosis is a specified cut-off point at the high extreme.

Recent years have seen a general shift in attitudes in the field toward recognising this continuity. Conceptually, autistic traits can be understood as manifestations of autistic-like behaviour, thought and emotion that are typical of the individual's personality but unlike (clinical) symptoms do not categorically imply functional impairment. Autistic trait measures tend to focus on less severe forms of difficulties on the classic three symptom domains (Social/ Communication/ RRBIs) combined with more closely theory-driven items derived from cognitive theories of ASD (Central Coherence, Theory of Mind). In extension to this, there is also strong evidence suggesting internalising traits are a valid quantitative measure of internalising disorders (Fergusson, Horwood, & Boden, 2006; Goodwin, Fergusson, & Horwood, 2004). The validity of such trait measures is discussed in greater detail in their respective empirical chapters.

Indeed, the continuous distribution of traits to clinically relevant symptoms has been so striking, that it has been argued the 'autism spectrum' can essentially be extended to an 'autism phenotype' ranging from *broad* (mostly unaffected) to *narrow* (mostly clinical) both at the phenotypic and aetiological level (Wheelwright, Auyeung, & Baron-Cohen, 2010).

1.9   Summary

This chapter has provided an overview of ASD as a heterogeneous neurodevelopmental disorder with constantly changing category boundaries. Among the psychiatric comorbidities with ASD, internalising difficulties are the most frequent. Despite this, research tends to focus on devising explanatory frameworks for single diagnostic

categories on the one hand, and exploring the causes of psychopathology as a whole on the other. The underlying aetiological patterns of specific comorbidities, such as the co-occurrence of ASD and internalising disorders, and potential changes over development, remain largely unexplored. Studies to date have mostly focused on estimating comorbid prevalence. Quantitative trait studies can supplement clinical investigations by making larger samples more easily attainable and testing concepts, exploiting the aetiological information regarding genetic and environmental effects that a twin design provides. The following chapter will introduce the quantitative genetic background and methodologies employed in such genetically sensitive analyses.

Chapter 2 Quantitative Genetics – Background and Methodology

This chapter presents the methodological and statistical concepts relevant to the empirical data presented thereafter. It briefly describes the historical background of genetically sensitive designs before giving a more in-depth overview of the parameters estimated in twin modelling.

2.1   Historical Background

Incredible progress has been made in the domain of genetics in the past 150 years. Gregor Mendel was famously the first to explain the mode of inheritance for simple traits, showing variability was maintained in the transmission of binary phenotypes through generations. At the start of the 20th century Thomas Hunt Morgan rediscovered and integrated Mendel's laws with his *Chromosome Theory of Inheritance* (Benson, 2001). This was to become the core of classical genetics, identifying chromosomes as the carriers of genetic material. However, in showing observable characteristics could be inherited, and that this occurred according to an individual's specific biological blueprint, it remained unclear how complex and continuous (quantitative) traits could be accounted for. Mid-century, this missing piece was added by Ronald Fisher (1930) and Sewall Wright (1951), arguing that simple traits essentially functioned as building blocks for complex phenotypes.

Following the Mendelian laws, simple characteristics can act additively to produce complex phenotypes whose distribution approaches a normal distribution as the number of factors involved increases. The discovery of the molecular structure of DNA by James Watson and Francis Crick in the 1950s provided a further important piece in the puzzle, describing how molecular building blocks link to form genetic sequences that could now be identified and sequenced. In the early 2000s, the Human Genome Project was declared complete (TheHumanGenomeManagementInformationSystem, 2011). Within a century, understanding of genes and heredity has progressed to having fully identified the physical makeup of this genetic material, and numerous specific functions have been localised on the genome.

In *behaviour genetics*, both *nature* (genetics) and *nurture* (environment) are studied combining strategies from psychology and genetics in order to learn more about the aetiology of phenotypes. They both build on the observation of biological function, dealing with how it is produced and trying to read its meaning. In analogy to the

century-spanning illustration given earlier, Sigmund Freud as the oft cited founding father of psychology showed an early awareness of the interrelatedness of exhibited degrees of personality traits (ex. neuroticism) and mental health issues (ex. hysteria) (Freud, 1933). Taking a quantitative approach to studying cognitive domains, Hans Eysenck lay the foundations for the Costa and McCrae (1992) *Big Five* personality inventory which is still widely used today. In a demonstration of quantitative genetic methodology applied to psychological concepts, more recent decades have shown personality dimensions to be moderately to substantially heritable (Bouchard & McGue, 2003).

2.2 The classic twin design

As early as mid-19th century, at a time before the mechanisms behind inheritance of characteristics were known, Francis Galton pioneered in formulating insights into the value of family studies, proposing mathematical solutions able to account for degrees of relatedness (Rende, Plomin, & Vandenberg, 1990). He rightly reasoned that it would thus be possible to systematically compare and contrast the effects of nature versus nurture. Within this framework, there are a number of study designs each offering a unique set of information at various levels of complexity. The following sections will introduce the methodology behind twin studies. Previous findings on autistic and internalising traits using genetically sensitive designs are reviewed in Chapter 3.

The backbone of the classic twin design is the comparison of twin similarity between monozygotic (identical) and dizygotic (fraternal) twins. Remarkably however, all early twin researchers were not aware of the crucial distinction between these two twin groups, resulting in inconsistent methodology and affecting results of many early 20th century historic case studies, an issue that continued to persist until as late as the 1950s (Rende et al., 1990).

Now known to develop from a single fertilised ovum, monozygotic (MZ) twins share all of their genetic material (disregarding spontaneously occurring mutations). Dizygotic (DZ) twins usually develop from two fertilised ova that are simultaneously implanted in the uterus. Their genetic similarity is 50% on average – equivalent to that between any first-degree full siblings. DZ twins can be either of the same (DZSS) or opposite sex (DZOS). The prevalence of each type is roughly one third. Many twin studies exclude opposite sex pairs (with the exception of some types of sex-limitation models, as discussed in section 2.2.7). If DZOS twins show lower degrees of twin similarity, this implies there are qualitative sex differences (see further details in section 2.2). These

may be due to a variety of factors such as a gendered socialisation (Pulkkinen, Vaalamo, Hietala, Kaprio, & Rose, 2003) and in utero hormonal effects (Miller, 1994), which can influence trait levels. Making a comparison between MZ (same sex by default) and DZ (DZSS) twins allows an estimate of the extent of genetic and environmental influences in a quasi-experimental setting.

2.2.1 Assumptions and considerations

There are a number of key assumptions underlying the twin method (Taylor, 2009), summarised as follows:

- Equal environments assumption (EEA)
- No Genotype-Environment effects on trait (assortative mating/ GxE correlation, passive GxC correlation, GxE interaction)
- Generalisability

2.2.1.1 Equal Environments Assumption

The equal environments assumption states that trait similarity is greater for MZ twins compared to DZ twins for genetic rather than shared environmental reasons. Put differently, the shared environments of DZ twin pairs are equivalent to the environments of MZ twin pairs. A violation of this assumption would mean that the extent of genetic influences on such traits would be systematically overestimated (Stenberg, 2013), though it has been argued that effects would be small and statistical adjustments can be made (Kendler, Neale, Kessler, Heath, & Eaves, 1993). However, a number of potential confounds invalidating this assumption have been raised and investigated. The suggested environments that differentially increase twin similarity broadly fall into two categories: biological and psychological factors. Effects of chorionicity as a pre-natal shared environment have been empirically investigated. While most MZ twins develop sharing a single placenta and chorionic membrane, a quarter of them and most DZ twins are enveloped individually. Findings suggest that monochorionicity represents a significant pre- and perinatal risk (Dube, Dodds, & Armson, 2002; Oldenburg et al., 2012). However, postnatal physiological effects are less pronounced (Hur & Shin, 2008; Trivedi et al., 2011). Chorionicity effects on psychological aspects show mixed results with small effect sizes of increased similarity for monochorionic twins on specific psychosocial characteristics reported by some (Jacobs et al., 2001; Sokol et al., 1995) but not others (Hur, Shin, & Jeong, 2007; Riese, 1999; Sokol et al., 1995).

Psychological factors challenging the equal environments assumption have been more equivocally rejected: neither differences in parental treatment, nor greater MZ visual resemblance or closer contact appear to increase twin similarity for personality or psychiatric status (Borkenau, Riemann, Angleitner, & Spinath, 2002; Hettema, Neale, & Kendler, 1995; Koenig, Jacob, Haber, & Xian, 2010). In response to the misclassification argument, errors in perception of self reported zygosity have been shown not to affect twin similarity for psychiatric disorders (Kendler et al., 1993). More recently, Visscher et al. (2006) modelled sibling similarity as a continuum of 'twinness' (i.e. as deviation from the 50% mark due to e.g. crossing-over effects and mutations). Linking genotyping data on actual gene-sharing to phenotypic similarity using maximum likelihood estimation resulted in a closely similar heritability estimate compared to twin studies. This outcome supports the validity of estimates obtained using expected proportions of genes shared, as relied upon for the classic twin design. In addition, typical mislabelling rates in twin studies are low at ~2% using questionnaire data only, and <1% when genetic information is used to supplement this (e.g. Tambs et al., 2012).

2.2.1.2 No Gene-Environment Effects

In relation to the second assumption, the classic twin design assumes that gene-environment (GxE) interactions can either be discounted or incorporated into the heritability. The presence of gene-environment effects will bias the estimates derived from twin modelling. GxE interactions can conceptually represent susceptibility, where a specific environment is required to elicit a genetic effect, or where a person's genotype determines whether a certain environment has beneficial/ detrimental effects. This mechanism has been suggested within the framework of a diathesis-stress model of co-occurring autistic traits and internalising symptoms, which were significantly associated in the presence of a high number of stressful life events, but not in their absence (Orsmond & Seltzer, 2009).

An instance of passive gene-environment correlation is present when parents' heritable characteristics influence the nature of the environment their children grow up in (e.g. anxious mothers' parenting style interacts with 5-HTTLPR genotype in its effects on child depression; Gibb, Benas, Grassia, & McGeary, 2009). The classic twin design is unable to directly test this assumption, however in non-human settings location/ conditions can be kept stable such that it is reasonable to incorporate the interaction variance into the heritability estimate. Using human data, testing GxE can be achieved

in more complex designs by combining the twin data with data from twins raised apart and those of non-biological siblings (however cf. Richardson & Norgate, 2005), but this scenario will not be addressed within the scope of this thesis.

Assortative mating is taking place when individuals select their partner in a non-random fashion based on similarity (or complementarity) for a particular trait. Its presence effects an increase in DZ similarity, which in turn produces inflated estimates of shared environmental factors. In relation to ASD, it has been suggested that individuals with high attention to detail ('hyper-systemisers') tend to connect via their preference for systemising activities (Baron-Cohen, 2006). An alternative explanation for spousal similarity however is geographic/ social stratification (Maes et al., 1998). Autistic trait similarity due to assortative mating was found by one group (Constantino & Todd, 2005; Virkud, Todd, Abbacchi, Zhang, & Constantino, 2009) though not others (Gerdts, 2012; Hoekstra, Bartels, Verweij, & Boomsma, 2007b). Similarly, outcomes for internalising disorders are mixed with no clear emerging pattern for either depression or anxiety (Desai, Schimmack, Jidkova, & Bracke, 2012; Dubuis-Stadelmann, Fenton, Ferrero, & Preisig, 2001; Low, Cui, & Merikangas, 2007; Maes et al., 1998; Mathews & Reus, 2001). The resulting bias for twin models on traits with moderate heritability (.3-.6) is nevertheless likely to be very small (Maes et al., 1998).

2.2.1.3 Generalisability of Findings

In conducting research requiring as many resources as twin studies do, it is highly desirable that findings should be generalisable to the general (singleton) population. Two main concerns here relate to very early developmental differences between twins and singletons and potential effects of mislabelling of twins on results. The argument in relation to the first point resembles that of chorionicity, as do results – sharing intrauterine space increases risk during pregnancy and at delivery, but postnatal effects are less pronounced (Knickmeyer et al., 2011; Rutter, Thorpe, Greenwood, Northstone, & Golding, 2003). Evidence has been found for early delay in language development, but these were no longer present by mid-childhood and other cognitive and psychological factors such as IQ and mental wellbeing have been found either not to differ, or to be of a small effect after correction for early risk variables such as low birth weight (Eriksen, Sundet, & Tambs, 2012; Kendler, Martin, Heath, & Eaves, 1995). Twinning itself has also been suggested as an environmental risk factor for ASD and high levels of autistic traits (Betancur, Leboyer, & Gillberg, 2002; Greenberg, Hodge, Sowinski, & Nicoll, 2001; Ho, Todd, & Constantino, 2005; Rutter, 2005) but to date

there is no consensus (Curran et al., 2011; Hallmayer et al., 2002; Visscher, 2002) on twinning rather than genetic similarity as the underlying cause. In summary, the twin method is appropriate to disentangle genetic and environmental effects, though it is important to bear in mind the just outlined possible limitations. The following sections will outline the various steps involved in analysing twin data.

2.2.2 Phenotypic analysis

Twin data is typically initially double-entered for each twin pair. Representing twin A first as twin 1, then twin 2, this procedure is repeated for their co-twin B. However, inclusion of both members of a twin pair will violate the assumption of independence of the observations (since they are related). By introducing random selection of one twin per pair for all procedures in SPSS (via a binary selection variable), twin A and B are equally likely to be selected for subsequent analysis.

A further standard procedure (McGue & Bouchard, 1984) is to regress out mean effects for age and sex in advance of model-fitting – (same-sex) twins are perfectly correlated for both. Uncorrected data would artificially inflate twin correlations and estimates of shared environment (Eaves, Eysenck, & Martin, 1989).

2.2.3 The univariate model

Univariate trait analysis presents aetiological influences on a single trait. Univariate twin correlations model twin 1 x twin 2 on the same phenotype using intraclass correlations. These are used rather than interclass Pearson correlations, as there is no meaningful conceptual difference between the labels 'twin1' and 'twin 2', and pairs are considered to be unordered (see double entry/ random selection). Phenotypic variance is decomposed into various components, comparing MZ (r_{MZ}) and DZ (r_{DZ}) correlations using Falconer's formulae (Falconer & MacKay, 1996).

Additive genetic influences (A): Additive genetic effects are suggested by $r_{MZ} > r_{DZ}$. This accounts for the fact that MZ twins share all, and DZ twins on average half of their genetic material.

$$A \; = \; 2 \, (r_{MZ} - r_{DZ})$$

Dominant genetic influences (D): If $r_{DZ} < .5\ r_{MZ}$, genetic dominance effects (D) – indicating genetic effects via interactions between alleles at the same locus – are suggested. For an interaction to take place, at least two alleles are involved and both twins must present with the same combination of alleles, thus dominant genetic relatedness is assumed to be 1 within MZ pairs and $.5^2 = .25$ within DZ pairs.

$$D\ =\ 2\ (r_{MZ} - 2r_{DZ})$$

Shared environmental influences (C): Shared environmental effects are environmental factors that make children growing up in the same family similar. The a priori assumption is that neither twin type is more or less susceptible to environmental influences than the other. These effects are present to the extent that twins exceed the similarity that would be expected genetically. Shared environmental influences are indicated by $r_{DZ} > .5\ r_{MZ}$.

$$C\ =\ r_{MZ} - A$$

Nonshared environmental influences (E): Nonshared environmental effects are environmental influences that make children growing up in the same family different. E is not fixed and includes the measurement error. With both A and C increasing MZ similarity (since MZ twins share all of their genetic material and shared environmental factors are entirely in common by definition), $r_{MZ} < 1$ is suggestive of nonshared environmental influences.

$$E\ = 1 - r_{MZ}$$

As a caveat, some of the limitations of the classic twin design (discussed in greater detail in later chapters) should be mentioned here. First, shared environmental effects and dominance effects show confounding effects and both cannot be represented simultaneously in the classic twin design (i.e. either ACE *or* ADE are modelled). Second, untestable for the same reasons are whether very low DZ correlations could be better accounted for by epistasis (non-allelic gene interaction), sibling interaction effects (extreme behaviour of one twin results in low ratings of the other twin's behaviour) or contrast effects (differences in phenotypic variance by twin type).

Phenotypic correlation

Univariate twin correlations

Cross-twin cross-trait correlation

MZ = monozygotic twins
DZ = dizygotic twins

Partitioned (co-)variance

Parameter estimates of a², c², e²

Parameter estimates of r_g, r_c, r_e

a² = additive genes
c² = shared environment
e² = nonshared environment
r_g = genetic correlation
r_c = shared environmental correlation
r_e = nonshared environmental correlation

Figure 2.1 Summary of correlation types and derived model parameters

2.2.4 Bivariate analyses

In addition to univariate genetic and environmental parameters, twin model-fitting can estimate the degree of genetic and environmental influences shared across two traits, ranging from no (0) to complete (1) overlap. In this analysis, twins' similarity is estimated across traits. These cross-twin cross-trait (CTCT) correlations associate twin 1 on trait 1 x twin 2 on trait 2.

Overlap in additive genetic influences is estimated by the *genetic correlation (r_g)*. Shared environmental overlap is indicated by the *shared environmental correlation (r_c)* and nonshared environmental overlap by *nonshared environmental correlation (r_e)*. In the ADE model, overlap in dominance effects is expressed by the *non-additive genetic correlation (r_d)*. Finally, *bivariate heritability* provides a reading of the extent to which genetic and environmental factors mediate the phenotypic correlation between two traits and is obtained by representing their proportions with respect to the total phenotypic correlation. Bivariate shared and nonshared environment are calculated by substituting the respective environmental parameter estimates.

$$Biv\ h^2\ =\ \surd(\ a^2{}_{trait1}) \times r_g \times \surd(\ a^2{}_{trait2})$$

To further illustrate how these statistics relate to the study of comorbidity, decomposing the CTCT into *genetic and environmental correlations* answers the question of the *degree of overlap*: 'how much of their individual aetiological explanation is shared across phenotypes'? For example, are all of the genetic effects influencing autistic traits also affecting internalising traits? Interpretation of *bivariate heritability* in contrast in this context would be informative of the *overall importance* of the genetic influences on the overlap between autistic and internalising traits – 'how much of their phenotypic overlap is explained by genetic and environmental influences'?

2.2.5 Structural Equation Model-Fitting (SEM)
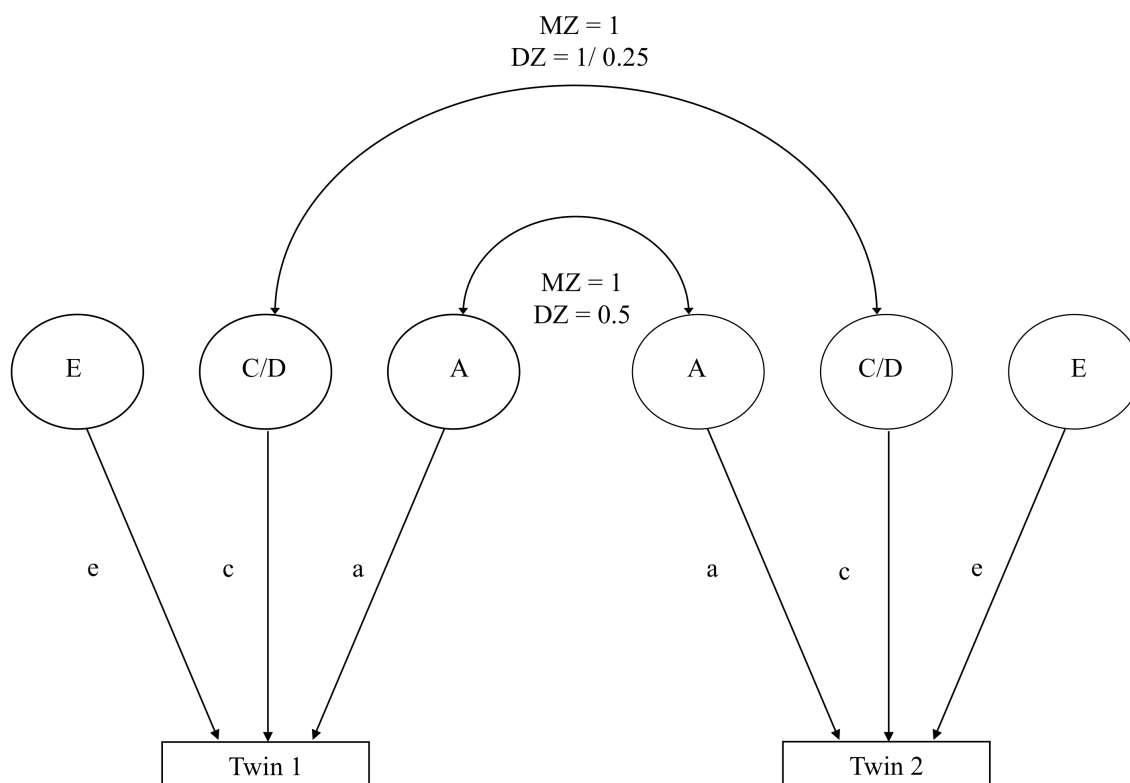
Figure 2.2 Path diagram of a univariate ACE/ ADE model

Structural equation modelling (SEM) is a statistical technique originally developed by Sewall Wright (1921). Its main application is to explore causal relationships using a combination of statistical data and qualitative causal assumptions. While a quick approximation to this using twin data can be achieved using Falconer's formulae, SEM offers a systematic way to compare alternative hypotheses by operationalising predicted

associations between concepts and finally to select the best-fitting model. It is thus able to provide estimates of the magnitude of genetic and environmental influences as well as testing different hypothesised models.

Conventionally, SEM results are displayed using path diagrams, whose elements are an exact representation of the assumed dependencies among the modelled set of variables.

Rectangular box: Represents an observed variable. In the univariate model (**Error! eference source not found.**), these are twin 1 and twin 2's recorded scores. In bivariate models (**Error! Reference source not found.**, Figure 2.4), results are typically shown by phenotype (data from all twins is used for estimation).

Circles: Show latent variables. These represent the hypothesised conceptual associations on the observed measures. In order to achieve an unambiguous result, in SEM, estimated parameters must be limited to a number that is justified by the data points available. These are the phenotypic trait variance, the MZ and the DZ covariance. Using these, the model is only *identified* (i.e. providing a single solution) modelling either C or D.

Single-headed arrows: Indicate a causal relationship.

Double-headed arrows: Indicate correlation without causation.

Expected correlations: are set at the hypothesised default relationship of MZ to DZ twins as discussed above – A = 1, .5 (MZ/ DZ); C = 1; E = not applicable.

Path-tracing rules: The expected correlation between two variables in a path diagram can be estimated by tracing all paths connecting those variables, following three main rules.

- The same variable cannot be passed through twice;
- there must be no more than one double headed arrow in each chain of paths;
- a path cannot be traced forward and then backwards within the same chain.
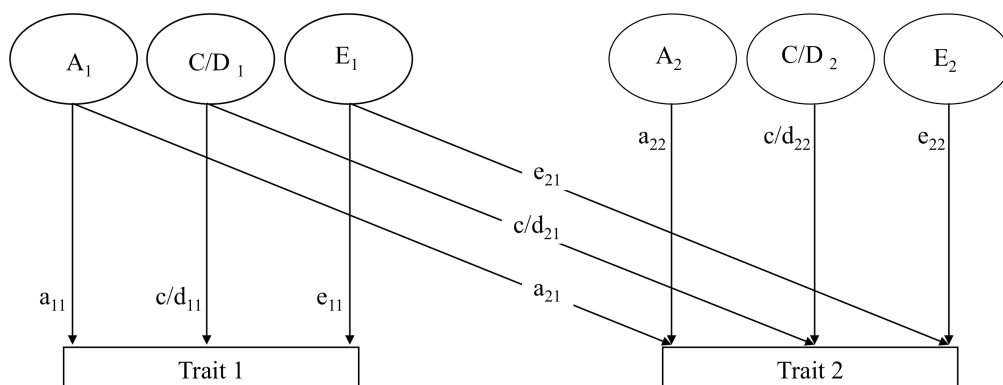
Figure 2.3 Cholesky decomposition of a bivariate model

Cholesky decomposition: the Cholesky decomposition is used during optimisation of SEM bivariate models. It partitions variance and orders causal pathways in so that the first latent factor (separately for A, C/D and E) influences all observed traits, the second influences all but the first and in a multivariate model this pattern would be continued.

Correlated factors solution: For ease of interpretation, results are typically presented as the mathematically equivalent correlated factor solution (Loehlin, 1996).
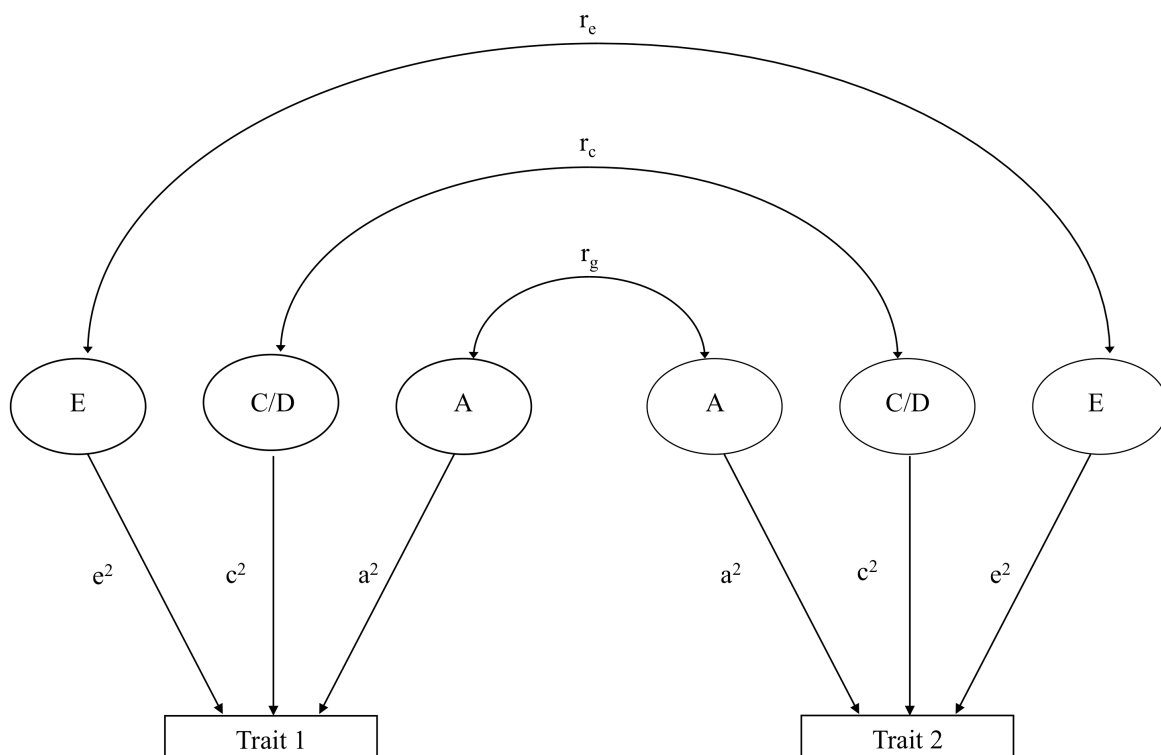
Figure 2.4 The correlated factors solution

2.2.6 Parameter estimation using Mx

Mx is a software package (Neale, Boker, Xie, & Maes, 2003) specifically designed for analysis of twin data, using scripts and calculations based on matrix algebra and with final parameter estimates resulting from iterative permutations. The scripting process will not be tackled here, except to mention some important additional modelling assumptions that Mx makes: It is assumed that the total of all genetic and environmental influences are the same for all twins. Wherever datapoints are missing, this occurs randomly rather than systematically. Raw scores of the twin variables are approximating a normal distribution or skew is sufficiently corrected in appropriate transformations.

The following sections describe the types of models and relevant statistics that Mx provides that are relevant to this thesis and that allow to select the most parsimonious model of the co-occurrence of autistic and internalising traits.

2.2.7 Model testing and fit statistics

Parsimony refers to a principle of interpreting data – when testing hypotheses, if explanations are equally reasonable, the one making less new assumptions (leaving more *degrees of freedom (df)* to vary) is more parsimonious (since it introduces less possibilities for error).

Saturated model: The (phenotypic) saturated model acts as a reference model, accounting for all of the variance. It models as many parameters as observations (df=0, it is *just-identified*). As such, it does not make any restrictions or assumptions, however it has no predictive value. Constrained saturated models are used as initial reference points in twin modelling to reflect some fundamental assumptions about similarities in means and variances for twins. Full ACE/ ADE models are tested in reference to these.

-2LL: Minus twice the log likelihood is a fit function distributed equivalently to chi-squared. Mx uses maximum likelihood estimation to determine the fit of the hypothesised models. The -2LL is informative about relative fit rather than making any statements on whether any model is a good fit for the data.

Akaike's Information Criterion (AIC): The Akaike information criterion is a fit index presented in addition to the chi-square test. It penalises for complexity of the model, with lower values indicating a better fit compared to the reference model.

$$AIC \ = \ -2LL \ - \ 2df$$

Testing submodels: The conceptual basis of testing ACE/ ADE models on twin data has been outlined above. *Nested models,* which are simpler models derived from these initial models (making less assumptions of the data) are then tested in comparison to the fit statistics of the full models. Submodels incrementally drop (set to 0) parameters. With reference to the classic full ACE/ ADE models, AE, CE/ DE and E only submodels can be tested. Setting the E parameter to 0 is not possible since it is defined to contain the error term. Obtaining p-values in excess of .05 (i.e. nonsignificant) indicates that the respective model is not a significantly worse fit and should be preferred because it offers a more parsimonious interpretation of the data than the full model. Once the best-fitting model is selected, SEM is also informative of the magnitude of each of the included influences (parameters). If the confidence interval on any estimate overlaps with 0, this indicates that although the parameter is important for the overall structure of the model, there are great levels of variance on this parameter that make it impossible to distinguish it from zero (non-significance).

Sex differences: In addition to testing aetiological influences on the overall population, it may be of interest to check for sex differences on psychiatrically relevant traits. *Scalar* sex differences denote differences in phenotypic variance between males and females. *Qualitative* sex differences imply a differential aetiology for across the sexes for the investigated traits. The calculation of these compares DZSS and DZOS twin similarity. DZOS twins are parameterised allowing for lower overlap on genetic and environmental influences (r_g <.5 or r_c <1). *Quantitative* sex differences assumes the same causal factors behind male and female phenotypic presentation of a trait, but the magnitude of their effect varies by sex.

2.2.8 MZ differences design

The aim of the MZ differences design is to identify and estimate the magnitude of environments serving as unique contributors, decreasing twin similarity (Pike, Reiss, Hetherington, & Plomin, 1996b). Within the classic twin design, MZ twins are modelled to have a complete overlap on both genetic and shared environmental influences. Thus, any differences on phenotypic expression of a trait are necessarily due to nonshared (idiosyncratic) environmental factors, which includes measurement error. It is possible to take these relative difference scores both on outcome measures (e.g. autistic traits) and environmental measures (e.g. birth complications, school performance). By correlating these, inferences about the nonshared environmental

(NSE) contributions to twin pair discrepancy can be made. The procedure is utilised and will be explained in more detail in Chapter 6.

2.3 Summary

This chapter set out to introduce some of the historical background leading to the current interest in twin data. Key concepts such as the basic assumptions of the twin design were outlined and statistical relationships between twin types highlighted, before giving a short overview on SEM model fitting and a number of related research designs. By summarising existing behaviour genetic literature on ASD and internalising disorders, the following chapter will bring together the phenotypes introduced in Chapter 1 and the quantitative genetic methodology of Chapter 2, demonstrating its application in implementing the behavioural-genetic analysis possible within a twin sample such as TEDS.

Chapter 3 Behaviour genetic studies of autistic and internalising traits

This chapter summarises previous behaviour genetic literature on the ASD and internalising phenotypes, drawing on family and adoption. Relevant early and recent findings from clinical twin studies will also be introduced, though individual results using quantitative trait measures will receive more attention in relation to the empirical findings in the following chapters. At the end of this chapter, the specific research questions for this thesis will be outlined.

3.1   Scope and limitations of family and adoption studies

Family studies are useful in allowing estimates of transgenerational risk to be made. A particular strength of this design is its obvious relevance on the individual level, specifically in helping to faster identify risk of potential mental health issues from family history; and in informing further research, such as continuing to search for both general and specific risk factors. However, family studies are limited to showing *how much* but not *why* family members resemble each other. In families where biological siblings are reared together, their similarity is a combination of both genetic factors and their shared environment. For instance, all siblings tend to receive similar parenting. Further, cohort effects may contribute to differences between siblings.

Adoption studies in contrast allow the separating out of genetic from environmental influences. By comparing variously the similarity of adopted children with their adoptive and biological relations, inferences about heritability and environmental contributions can be made. Greater correlations on a trait with the adoptive family indicate environmental aetiology, and genetic influences are demonstrated by the respective overlap with biological relatives. However, heritability estimates may be inflated by prenatal factors, while matching children with adoptive parents of similar background *(selective placement)* confounds environmental estimates.

Adoptive parents are also known to exceed population average with respect to socioeconomic status (SES), education levels, parental age and mental health (Brodzinsky & Palacios, 2005). It is unsurprising that biological parents who were in the position to give up their children for adoption are comparing unfavourably on all of these measures. The question to which extent biological ties are important to parental investment has also been matter of intense debate but the aforementioned advantageous

characteristics in adoptive families may serve to counteract or at least mask a lack of genetic predisposition (Hamilton, Cheng, & Powell, 2007).

However, a further known issue with adoption studies is also the overall poorer outcomes of adoptees. Unlike their well-adjusted adoptive parents, by the time adoptees reach adolescence, they show significantly worse outcomes both psychologically and behaviourally compared to non-adoptees (Miller, Fan, Christensen, Grotevant, & Dulmen, 2003). Both groups were equally likely to score within the mid-range, but a 3:1 odds ratio toward having been adopted was found among the 5% worse-adjusted adolescents. Adopted children are also more at risk from prenatal parental substance and alcohol abuse, which is known to have a variety of direct and indirect adverse effects (Moe, 2002). This highlights a challenge for adoption studies to incorporate as much as possible data on biological parents in order to be able to meaningfully interpret any aetiological (dis-)similarities found. Another important issue is that of the generalisability of findings from adoption and blended family studies to the general population. For children not adopted at the time of birth, time spent in their biological home is a further complicating factor. A clearer picture of trait aetiology may thus emerge from twin studies (for underlying assumptions and considerations of twin studies refer to 2.2.1).

3.2 Behaviour genetic findings on ASD

In a seminal case-control study on sibling concordance for ASD, 2.9% were concordant for autistic disorder (Bolton et al., 1994), translating into sibling prevalence rates double that of the general population (Rutter, 2000). This concordance increased to up to 20% concordance when including the entire clinical ASD spectrum. Similarly, a Danish population study (Lauritsen, Pedersen, & Mortensen, 2005) including ~950,000 children found a 22-fold relative risk of autism for individuals with a sibling also diagnosed with autism, compared to individuals without a sibling history of autism. In the presence of a history of broader autism diagnoses in a sibling, a 13-fold increase of risk of autism for the second sibling was observed. These figures translated into 3% and 2% absolute risk, in line with the above earlier findings. The familiality of ASD also extends to the Broader Autism Phenotype (BAP), the sub-clinical manifestation of traits characteristic for clinically diagnosed autism, which has consistently been reported in parents of children with ASD (early studies reviewed in Bailey, Palferman, Heavey, & Le Couteur, 1998; recent findings reviewed in Sucksmith, Roth, & Hoekstra, 2011).

However, in discussing the familiality of ASD, its symptoms and traits, a distinction that has been suggested with respect to aetiology and outcomes in this context is between simplex (one individual in the family affected) and multiplex (two or more individuals affected) autism. In a study measuring quantitative autistic traits in siblings of known ASD cases (Virkud et al., 2009), both typically developing multiplex siblings and fathers showed a highly significant shift in their distribution toward the high end of the scale compared to their simplex equivalents. Parents of all families also tended to resemble one another with respect to autistic trait levels, and it was argued this aggregation may in turn make clinical ASD cases in future offspring generations more likely. Another study (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010) found that in ASD families with multiple children, 11% had more than one child with a clinical ASD diagnosis, and an additional 20% of presumed-unaffected siblings had a history of language delay. Within this language delay group, 54% showed autistic-like speech. However, when grouped into single and multiple incidence categories, unaffected simplex siblings showed a relative absence of quantitative autistic traits relative to unaffected multiplex siblings.

In the first twin study on autism, Folstein and Rutter (1977) reported 36% MZ concordance compared to 0% DZ concordance rates for autism in 21 twin pairs. Twin similarity increased to 82% (MZ) and 10% (DZ) respectively when comparing similarity on associated language and cognitive impairments. In both cases, the observed differences in concordances suggested high heritability of both the narrow definition of clinical autism and the broader ASD category. A number of other twin studies with at least one diagnosed proband in a twin pair have also been conducted, consistently reporting large genetic and low nonshared environmental effects, and potentially genetic dominance as indicated by low DZ concordance (Bailey et al., 1995; LeCouteur et al., 1996; Ritvo, Freeman, Masonbrothers, Mo, & Ritvo, 1985; Rosenberg et al., 2009; Steffenburg et al., 1989; Taniai et al., 2008).

Recently, one study that has received a great deal of attention is that of Hallmayer et al. (2011), re-investigating twin similarity across childhood in a clinical sample (N = 192 twin pairs) using DSM-IV criteria. For autism, concordances of .58-.60 (MZ) and .21-.27 (DZ) were found, and for ASD, they were .50-.77 (MZ) and .31-.36 (DZ). These rates translated into a substantial genetic component (37% autism, 38% ASD) but the relatively greater DZ similarity also emphasises a role for shared environmental factors (55% autism, 58% ASD); the contribution of nonshared environment was low (8%

autism, 4% ASD). This finding of shared environment using a clinical sample is novel. However, one limitation in interpreting this potentially paradigm-shifting finding is that shared environmental effects are difficult to detect in small samples, and a possible explanation for the differences in findings can be seen in the use of less than 50 twin pairs in all but two of comparable clinical studies (Hallmayer et al., 2011; Rosenberg et al., 2009). Further to this, clinical samples have included wide age ranges, ignoring potential changes in the contribution of individual aetiological components. Thus future research will have to show to which extent these results are effects of model-selection (ACE vs. AE) or reflect true aetiological patterns.

Closely similar twin correlations (age 8 years: .79 MZ, .21-.39 DZ) have been reported for autistic traits in the same twins (TEDS sample) as studied in this thesis (Hallett, Ronald, & Happé, 2009a; Hallett, Ronald, Rijsdijk, & Happé, 2010). Heritability was estimated at 67-70% at age 8, not including shared environmental effects. At age 12, small shared environmental effects were detected (.04-.19) and heritability was similar (.32-.41) to that derived from the clinical sample above (Hallmayer et al., 2011).

Shared environmental effects have also been reported studying twins' autistic traits across childhood in the general population (Constantino & Todd, 2003), finding 39-51% heritability, and 25-43% shared environmental effects. In a Swedish population sample of 9-12 year old twins (Lichtenstein et al., 2010), genetic effects accounted for 80% of similarity on autistic traits, and shared environmental influences were not detected. In summary, literature suggests a high MZ and roughly halved DZ twin similarity on the ASD phenotype and autistic traits, resulting in high heritability. Lower though substantial heritability and shared environmental effects are reported in a minority of studies.

3.3   Behaviour genetic findings on Internalising Disorders

In typical families, the transmission rate of internalising disorders is well established (Singer, 2006; Tambs, 1991). A large-scale study on familial aggregation asking participants about family history of psychiatric disorders showed that parents of individuals having any psychiatric diagnosis had an odds ratio of 2.7 for lifetime major depression and 3.1 for generalised anxiety disorder compared to relatives of individuals who did not report mental health issues (Kendler, Davis, & Kessler, 1997). Familial aggregation with parent and offspring sharing a specific diagnosis were at odds ratios of 1.9 (depression) and 1.8 (anxiety) respectively. These figures suggest that there may be familial general vulnerability factors and disorder-specific characteristics that are passed

on through the generations. In addition, underlying genetic factors have been demonstrated by the finding that biological parents frequently share a diagnosis of depression with their adopted-away offspring, but adoptive parents of depressed adoptees were no more likely than controls to have a mood disorder (Wender et al., 1986).

Twin studies on internalising disorders show average heritabilities at the lower boundary of what has been reported for ASD (Gregory & Eley, 2011; Lau & Eley, 2010). Reviews on anxiety disorders (Hettema, Neale, & Kendler, 2001) and (unipolar) depression (Sullivan, Neale, & Kendler, 2000) among non-ASD families have found 32% and 37% heritability respectively, with the remaining variance due to nonshared environment.

In TEDS, heritability of internalising traits was similar (age 8: .45, age 12: .40), though small shared environmental effects were also found at both ages (.18 and .07 respectively; Hallett et al., 2010). Types of anxiety traits have been studied in TEDS individually at age 4, finding moderate heritability (.39-.44) and shared environmental effects (.14-.36; Eley et al., 2003). Depression traits were studied individually at age 8 and 10, finding low genetic influences (age 8: .11, age 10: .06), modest shared environmental effects (age 8: .16, age 10: .24) and a large part was accounted for by nonshared environmental factors (age 8: .73, age 12: .70, Gregory, Rijsdijk, Dahl, McGuffin, & Eley, 2006).

A finding highlighting the role for both shared environment and genetic factors is shown in the fact that in anxious mothers, ability to express warmth and positivity in child interactions is diminished and is a salient predictor of child anxiety (Whaley, Pinto, & Sigman, 1999). Beyond the creation of a less supportive family environment, a potential underlying genetic mechanism for this is that children's serotonin transporter genotype (5-HTTLPR) moderates intergenerational transmission of both anxiety and depression (Gibb, Benas, Grassia, & McGeary, 2009) and the susceptibility to stressful life events (Kumsta et al., 2010). While it is important to be aware of interactions of genotype and environmental events, as discussed in Chapter 2, within the classic twin design, this cannot be directly tested.

3.4 Findings on co-occurring ASD and Internalising Disorders

Elevated rates of psychiatric disorders have been shown in first-degree relatives of individuals with ASD (Bolton, Pickles, Murphy, & Rutter, 1998; Daniels et al., 2008;

DeLong, 2004; Fairthorne, Langridge, Boruke, & Leonard, 2013; Jokiranta et al., 2013; Lauritsen et al., 2005; Mazefsky, Conner, & Oswald, 2010; Mazefsky, Folstein, & Lainhart, 2008; Micali, Chakrabarti, & Fombonne, 2004; Mouridsen, Rich, Isager, & Nedergaard, 2007; Piven et al., 1991; Piven & Palmer, 1999). Among their mental health difficulties, both depression and anxiety are particularly prevalent in parents and siblings, affecting 20-71% (depression) and 8-29% (anxiety) (Bolton et al., 1998; Mazefsky et al., 2008; Piven & Palmer, 1999). Most parents show an onset of these disorders prior to the birth of their child with an ASD, such that a purely stress-related explanation is unlikely (Piven et al., 1991).

Other family studies have addressed the association of psychiatric symptoms in family members on the one hand with the comorbidity within probands with ASD on the other (Ghaziuddin & Greden, 1998; Mazefsky et al., 2010; Mazefsky et al., 2008). Children in the comorbid ASD + depression group were more likely to have family members with depression (77%) than children with ASD only (30%). In an adult sample, a considerable number (88%) of ASD probands had an internalising disorder, and 60% had at least one parent with depression. Particularly probands with a maternal history of depression tended to also show this condition (odds ratio of 20). Of note, no association was found for comorbid anxiety disorders, which were the most prevalent comorbidity in the probands, but only observed in few parents. However, as discussed in Chapter 1, anxiety disorders tend to occur earlier in life and could have been subject to parental underreports to a greater extent than depression (Simon & Vonkorff, 1995).

In addition, family studies have explored if position on the broader autism phenotype (BAP) and levels of internalising traits are associated in parents of children diagnosed with ASD (Bolte, Knecht, & Poustka, 2007; Bolton et al., 1998; Murphy et al., 2000). These studies report in agreement that parents of probands showed overall elevated levels of internalising traits. However, these were not associated with parents' autistic-like behaviours. An interesting result of one study (Bolton et al., 1998) also suggests that female relatives were more liable to affective symptoms, while male relatives showed more BAP symptoms, i.e. the lack of association is due to a sex-specific distribution of psychiatric traits in relatives. A different interpretation was suggested by another study, finding relatively greater reserved/ schizoid tendencies in parents of simplex families, while multiplex parents scored highest on depression, though there was no significant difference between these groups and the control group of parents learning disabled children (Bolte et al., 2007)

As will be discussed in Chapter 4, only a limited number of quantitative twin studies using large samples have been investigating autistic and internalising traits and the aetiology of their co-occurrence across childhood, and few have specifically focused on pre-adolescence, but none has studied adolescence. Chapter 6 aims to identify childhood environments relevant for trait differences in adolescent twins. Developmental stability of the two phenotypes will be addressed in Chapter 7. As briefly raised previously in the section Fractionable Autism Triad Hypothesis, the three domains within the ASD symptomatology tend to cluster but also show a degree of independence; such heterogeneity has also been reported for types of internalising disorders, and currently known aetiological (dis-) similarities of both will be discussed in Chapter 5. Implications of the reported aetiologies and overlap on traits will be discussed in the general discussion.

3.5 Research questions

This chapter has presented evidence on the heritability of ASD and internalising disorders from family studies and drawing on using the methodology of twin designs. In summary, ASD has been shown substantially heritable and internalising disorders moderately heritable, with variable findings on the role of shared environmental effects and a greater role of nonshared environmental influences on internalising than autistic behaviours. Aetiological findings from existing studies on their overlap are presented in relation to the novel findings in the following chapter. However, all such previous work has either targeted early to mid-childhood development or combined child and adult data, leaving a dearth of specific knowledge on these trait aetiologies in adolescence.

This thesis will aim to add to the understanding of this important developmental period, as outlined below. Each of these research questions is described in more detail in their respective chapters.

I. **What are the phenotypic and aetiological associations between autistic and internalising traits in adolescence?**

Data from the Twins Early Development Study (TEDS) will be used to investigate univariate trait aetiology and bivariate aetiological associations in Chapter 4, using a general population sample of 12-14 year old twins.

II. **Are there specific types of autistic-like behaviours particularly relevant to internalising difficulties?**

Chapter 5 uses exploratory factor analysis (EFA) to derive subdomains of autistic behaviours. Twin modelling on bivariate subdomain and internalising trait pairings is carried out to investigate where the greatest phenotypic similarities lie, and to investigate their individual and shared aetiologies.

III. Are early differences in autistic and internalising traits indicative of adolescent trait level differences?

The analyses in Chapter 6 will draw on nonshared environmental (NSE) trait differences in monozygotic (MZ) twins to identify childhood environments contributing to twin differences, and to answer the question of the extent to which early twin differences in autistic and internalising traits, and other select environmental factors are predictive of twin differences in early adolescence.

IV. How (dis-) similar are the phenotypic and aetiological associations for autistic traits with anxiety traits to those with depression traits?

Twins' trait associations between autistic traits and internalising difficulties will be looked at in greater detail at age 16 by investigating separately anxiety traits and depression traits (Chapter 7).

Chapter 4 Investigating the association of autistic and internalising traits in early adolescence in a population-based twin sample

As outlined in Chapter 1, comorbid presentation of internalising disorders within ASD is prevalent. Results from family studies suggest a role for causal influences in their transmission, and an overview of aetiological findings from twin studies on ASD and internalising disorders individually has been given in Chapter 3. First, the current chapter reviews existing findings of previous twin studies on this bivariate aetiological overlap (both clinical and population-based). Second, the data presented thereafter is the first to specifically address the shared aetiology of autistic and internalising traits in adolescence.

Note: The data presented in Chapter 4 and Chapter 5 have been published in *Scherff, A., Taylor, M., Eley, T.C., Happé, F., Charman, T., & Ronald, A. (2013). What causes Internalising Traits and Autistic Traits to co-occur in Adolescence? A community-based twin study. Journal of Abnormal Child Psychology. (doi: 10.1007/s10802-013-9796-y)*

4.1   Background

4.1.1 Findings on internalising difficulties in clinical ASD in TEDS

One previous study of internalising difficulties in ASD has been carried out using the Twins Early Development Study sample (TEDS, i.e. same sample as in this thesis; Hallett, 2010). Diagnostic status on ASD was assessed using the gold-standard diagnostic tools the Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000) and the Autism Diagnostic Interview – Revised (ADI-R, Lord, Rutter, & Lecouteur, 1994). Internalising difficulties were assessed using parent and self report on 36 items each on the Revised Child Anxiety and Depression Scale (RCADS, Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000) and self report on 13 items on the Mood and Feelings Questionnaire (MFQ, Angold et al., 1995). Phenotypic correlations between diagnosed ASD and internalising difficulties were moderate (r = .32 with anxiety; r = .26 with depression).

Probands (N = 118), typically developing controls (N = 110) and low IQ controls (N = 24) were compared in a liability threshold model that also included ascertainment correction to adjust for the non-random sampling. Thus, parameters were set a priori to test for additive genetic and nonshared environmental effects only by investigating a high (a^2 = .90, e^2 = .10) and medium heritability (a^2 = .70, e^2 = .30) scenario, and ASD prevalence was set to 1%.

For internalising difficulties, a range of genetic models was tested, and those excluding shared environmental effects were found to provide the best fit. The same extent of genetic influences (both $a^2 = .86$) and unique environment (both $e^2 = .14$) was detected on both depression and total anxiety scale.

Genetic correlations between trait pairs were moderate, but a substantial nonshared environmental correlation was only found for the association of ASD with depression traits (anxiety traits : $r_g = .40$, $r_e = -.04$; depression traits: $r_g = .25$, $r_e = .52$). Bivariate heritability accounted for all of the phenotypic similarity with anxiety (Biv $h^2 = .33$, 100%), and a high proportion of phenotypic covariance with depression (Biv $h^2 = .22$, 85%).

4.1.2 Findings on co-occurring autistic and internalising traits in adulthood

In addition to the above clinical study, a number of trait studies have studied specifically the association of autistic and internalising traits in twins, described in the following. As part of STAGE (Study of Twin Adults: Genes and Environment), self report on autistic, anxiety and depression traits was collected from ~18,000 Swedish adult twins (Lundström et al., 2011). Autistic and anxiety traits were assessed on the Autism-Tics, ADHD, and other Co-morbidities inventory (A-TAC, Hansson et al., 2005), using 12 items for autistic and six for anxiety traits. Depressive traits were recorded on eleven items on the Iowa version of the Center for Epidemiologic Studies Depression Scale (CES-D, Carpenter et al., 1998).

Phenotypic correlations of autistic with anxiety traits were moderate ($r = .18$) and the association with depression traits was close to double ($r = .32$). Classing participants into six categories according to their autistic trait scores showed that those in the clinical ASD band had an odds ratio of 21.2 compared to those in the lowest trait band for being at risk of co-occurring anxiety problems, and 11.7 for depression. This risk increased monotonically across autistic trait categories.

At the aetiological level, their results showed autistic traits to be moderately heritable ($a^2 = .32$), as was depression ($a^2 = .36$). Heritability was lower for anxiety ($a^2 = .13$). Although all best-fitting models included shared environmental influences, these were negligible (all point estimates $c^2 = .00$), as suggested by DZ twin similarity close to half those of MZ twins. Nonshared environmental influences were the largest aetiological factor (autistic traits: $e^2 = .68$; anxiety: $e^2 = .87$; depression: $e^2 = .64$).

At the bivariate level, most of the trait similarity of co-occurring autistic, anxiety and depression traits was contributed by genetic factors. Genetic correlations revealed substantial trait overlap (both with anxiety and depression: $r_g = .51$, $r_e = .10$), and genetic factors accounted for most of the phenotypic covariance with autistic traits (anxiety: Biv $h^2 = .10$, 56%; depression: Biv $h^2 = .18$, 56%;), and the remainder by nonshared environmental factors, while overlap on shared environmental influences was statistically non-significant.

4.1.3 Findings on co-occurring autistic and internalising traits in childhood

4.1.3.1 Mixed Mid-Childhood cohorts

Lundström et al. (2011) also analysed parent report on ~11,000 twins from two combined child cohorts (aged either 9 or 12 years) assessed as part of CATSS (Child and Adolescent Twin Study in Sweden). As in the adult sample, autistic and anxiety traits were assessed on the A-TAC. No child depression trait measure was taken. Associations of autistic and anxiety traits at the phenotypic level were twice that in the above adult sample (r = .36). Like adults, risk of co-occurrence increased across risk bands; children in the clinical ASD band in relation to those in the lowest band had an odds ratio of 21.6 for co-occurring anxiety problems.

Model-fitting showed autistic traits to be substantially heritable ($a^2 = .71$, $e^2 = .29$). In line with previous studies, this figure was lower for anxiety – in children, genetic and nonshared environmental influences were equally important ($a^2 = .51$, $e^2 = .49$). Again, shared environmental influences (and overlap) were fitted but were indistinguishable from zero. The genetic correlation was equivalent to that found in adulthood, however, the nonshared environmental correlation was increased (anxiety: $r_g = .53$, $r_e = .49$). Most of the phenotypic overlap was of genetic aetiology (Biv $h^2 = .33$, 92%).

4.1.3.2 In Mid-Childhood in TEDS

The second set of trait studies was conducted using the same twin participants as in the following analyses, assessed at younger ages (Hallett et al., 2009a; Hallett et al., 2010). In contrast to the Swedish group, Hallett and colleagues were able to investigate trait associations separately for parent report of their twins when aged 8 and 12 years, however, internalising traits were measured on an omnibus scale. The sample (TEDS), as well as the autistic trait measure Childhood Autism Spectrum Test (CAST, Scott,

Baron-Cohen, Bolton, & Brayne, 2002) and internalising trait measure (SDQ, Goodman, 1997) are described in more detail in the method section below.

Phenotypic correlations between autistic and internalising traits at 8 years were moderate (r = .26). The univariate heritabilities resemble those outlined in the above mixed cohorts in that autistic traits were more heritable (a^2 = .65) than internalising traits (a^2 = .50). However in distinction, overall twin similarity was greater in this UK sample; in addition to twin similarity due to additive genetic effects, the TEDS data suggested significant shared environmental influences. Thus, there was a relatively lower degree of nonshared environmental influences on each trait (autistic traits: c^2 = .15, e^2 = .21; internalising: c^2 = .28, e^2 = .22).

Although the genetic correlation between traits was only modest (r_g = .12), more than half of phenotypic overlap between autistic and internalising traits was driven by genetic factors (Biv h^2 = .15, 58%). Shared environmental influences remained significantly different from zero at the bivariate level and showed high overlap, while nonshared environmental influences showed low overlap (r_c = .96; r_e = .07).

At age 12, the magnitude of the phenotypic trait associations remained the same (males: r = .29; females: r = .26). Univariate model-fitting indicated quantitative sex differences on (univariate) autistic traits, but not on internalising traits. As a result, the aetiological components of internalising matched the 8-year findings (a^2 = .46-.49, c^2 = .08-.13, e^2 = .41-.42), but autistic trait aetiology varied by sex (males: a^2 = .65, c^2 = .07, e^2 = .29; females: a^2 = .52, c^2 = .21, e^2 = .27). Specifically, female twins' autistic trait similarity implicated shared environmental effects to a greater extent than in males.

The best-fitting bivariate model at 12 years was a full ACE model. This included a moderate shared environmental correlation point estimate (r_c = .22), however, its confidence interval overlapped with 0. Significant modest genetic and nonshared environmental correlations were found (r_g = .17, r_e = .14), and bivariate heritability was moderate (Biv h^2 = .09, 35%).

4.1.4 Summary
One previous twin study each has addressed internalising traits in clinical ASD, depression and anxiety traits individually in relation to autistic traits in adults, anxiety (only) and autistic traits in a mixed sample of 9 and 12 year olds, and the association of internalising traits with autistic traits separately for age 8 and 12.

In agreement with results from the family studies presented in Chapter 3, internalising traits were moderately heritable and autistic traits substantially heritable. Figures for internalising heritabilities were at or below the lower boundary of autistic traits. Patterns are also beginning to emerge from bivariate analysis, though not it should be noted that given the paucity of specific published research, these inferences must be made with caution: Genetic correlations ranged from modest (~.10) to substantial overlap (~.50). There is also evidence for modest (~.10) nonshared environmental correlations, though some exceptions (with no clear pattern) have found greater figures (~.50). Bivariate heritability appears high (90%) from studies fitting AE models and more moderate (~35-60%) wherever ACE models were fitted.

4.1.4.1 Shared Environment

An ongoing debate in twin research is the relative absence of reports of significant shared environmental influences on traits (Hopper, 2000). In the classic twin design this is conceptually due to confounding between genetic and shared environmental effects, the design's low power to detect the latter, and frequent discarding of the shared environmental effects for better parsimony (Lopes, Andrew, Carbonaro, Spector, & Hammond, 2009). Family designs have suggested a role for characteristics shared within families, and twin designs have attributed a substantial part to genetic factors. In twin-only studies, increasing the sample size and retaining the shared environment in the model were suggested by Lopes et al. (2009), simultaneously warning that beyond N = 2,000 this strategy is unlikely to significantly increase point effects but rather to serve to reduce confidence intervals.

Above trait studies should have been powerful enough to find detectable shared environmental effects (within the other limitations of the classic twin design) given their large sample sizes (i.e. excluding the clinical study - however see Hallmayer et al., 2011 for a univariate clinical ASD study reporting shared environmental influences). With caution, a possible emergent pattern could be increasing shared environmental overlap between autistic and internalising traits with age: none were found at age 8, the combined 9 and 12 year cohorts included shared environmental overlap in the final model but point estimates equalled zero, and the age 12 point estimate was modest but confidence intervals included zero.

At a univariate level, drawing on early childhood findings, a review of twin studies on autistic traits has previously suggested heritability estimates increase with development (Ronald & Hoekstra, 2011). The question whether heritability and shared environment

are increasing or decreasing during childhood and adolescence has also been of interest for other phenotypes (e.g. cognitive ability; Haworth, Dale, & Plomin, 2009).

The only adult results available did not indicate greater than childhood shared environmental influences. Of note, dissimilar to the childhood measures, adult measures were collected using self report (i.e. two twins responding vs. one parent), which may explain the lower adult similarity ratings. The lower partitionable covariance could have affected modelling outcomes, and indeed heritability estimates from self report have been demonstrated as comparatively lower (Hoekstra, Bartels, Hudziak, van Beijsterveldt, & Boomsma, 2007a; Ronald, Happé, & Plomin, 2008).

4.1.4.2 Sex differences

Results from the age 12 cohort in TEDS indicated significant quantitative sex differences on (univariate) autistic traits but not on internalising traits or the trait overlap (Hallett et al., 2010). Such sex differences had not been found previously at age 8. Sex limitation models were not tested in either the child or the adult Swedish sample (Lundström et al., 2011). However, as discussed in Chapter 1, internalising disorders are more prevalent in females, and this difference becomes more pronounced across development (Hankin et al., 1998). Chapter 3 also reviewed evidence that particularly maternal (but not paternal) depression was associated with depressive symptoms in ASD probands, though no further division into male and female probands was made.

4.1.4.3 Adolescence

Previous twin studies have omitted the important developmental period of adolescence (including participants up until the age of 12 years, and again from 18 years). However, while children's autistic trait levels are relatively stable across development (Robinson et al., 2011b), adolescence poses heightened demands on individuals to manage their lives more and more independently, e.g. performing well at school, forming and maintaining friendships, and dealing with stressful life events. Both autistic symptoms and internalising problems impact on successfully dealing with the responsibility of increased independence (Orsmond, Wyngaarden Krauss, & Seltzer, 2004). In addition to these psychosocial factors, adolescents undergo a range of neurobiological changes that affect behavioural tendencies (Sturman & Moghaddam, 2011). Developmental changes on internalising and autistic-like behaviours will receive further discussion in Chapter 7.

4.1.5 Objectives

The present twin study extends research on the phenotypic and aetiological relationship between internalising and autistic traits into early adolescence. In light of the patterns that have emerged from previous research, solutions both in- and excluding shared environmental influences, and quantitative sex differences on genetic and environmental influences for males and females were tested during model-fitting.

4.2 Methods

4.2.1 Twins Early Development Study (TEDS)

4.2.1.1 General Aim

The Twins Early Development Study (TEDS) is a large community-based sample following monozygotic and dizygotic twins born in England and Wales in 1994-1996 longitudinally (an up-to-date introduction can be found in Haworth, Davis, & Plomin, 2013). The study has collected a multitude of behavioural, developmental and cognitive measures that have been used to study many areas of typical and atypical child development, ranging from academic achievement to behaviour problems. In addition to studying these characteristics at a phenotypic level, due to their twin status, data from participants has allowed insights on quantitative genetic and environmental influences and most recently molecular genetic contributors to these traits.

4.2.1.2 Participants

Originally, 16,810 twin pairs and their families were recruited by identifying multiple births from birth records (via the Office for National Statistics, ONS). The ratio of male to female twin participants (close to 1:1) and that of each zygosity groups (⅓ MZ, ⅓ DZSS, ⅓ DZOS) match the expected distribution of twin births in the United Kingdom. The composition of the sample also reflects general population characteristics with respect to ethnicity, and the proportions have remained fairly stable throughout (TEDS: ~92% White; UK: 93%, Walker, Maher, Coulthard, Goddard, & Thomas, 2001). As of 2012, over 10,000 pairs remain enrolled as participants. Twins have been assessed at ages 2, 3, 4, 7, 9, 10, 12, 14 and 16 years. Informed consent has been obtained at each stage. Families were assigned to one of four cohorts based on twin birth dates (Cohort 1: Jan-94 to Aug-94; Cohort 2: Sep-94 to Aug-95; Cohort 3&4: Sep-95 to Dec-96). Not all cohorts were contacted for all studies. A total of 8,697 families returned data at least once during adolescence (ages 12, 14, 16) with an overall response rate of 74.1%.

4.2.1.3 Exclusions

Families were excluded if severe pre- or postnatal complications or severe medical conditions were reported or if first contact data, consent or sex and zygosity data were missing. Excluded conditions included Down's syndrome, cerebral palsy, spina bifida, brain haemorrhage and visual or hearing impairments. Individuals with a known diagnosis of ASD were <u>not</u> excluded. Diagnoses were made using subsamples of TEDS at three different time points and have included screening on the Social Communications Questionnaire (SCQ, Rutter, Bailey, & Lord, 2003), the Development and Well Being Assessment (DAWBA, Goodman, Ford, Richards, Gatward, & Meltzer, 2000) and home visits for face-to-face twin testing and parent questionnaires within the Social Relationships Study (SRS, description in Hallett, 2010). Zygosity was determined using parent ratings of physical similarity (Price & Jaffee, 2008), which are shown to agree in over 95% with DNA test results (Price et al., 2000). This has been supplemented by select DNA data on 7,000 pairs and full genome-wide DNA data on 3,500 individuals.

4.2.1.4 Measures

Testing has included mainly paper-based questionnaires but also telephone interviews and web-based testing for cognitive assessments and clinical assessments in select subsamples, such as specific spin-off studies. Multiple informants have been included in the data collection, though emphasis in early years has been on parent report, supplemented by teacher report at ages 7-14. Finally, on reaching the adolescent and young adult years, twins' self report has become a central component of the study.

Included in TEDS were measures that were economising the time requirement on participants and could test concepts within only few items whilst being highly descriptive of (in-)appropriate-for-age behavioural presentation. Twins have been chronologically assigned to four cohorts and not all measures have been collected for each cohort. It should be noted therefore that the specific questions investigated within this thesis and the level of details at which they can be scrutinised is in part determined by the available measures and number of completed questionnaires at each age.

4.2.1.5 Current sample

Studying internalising behaviours *within* autistic traits, young adolescents from TEDS were included in the current sample if autistic trait data was non-missing. At both ages studied in this chapter, all cohorts were contacted.

Table 4.1 TEDS study data returns at ages 12 and 14 years

Cohort	1	2	3 & 4	Total
Pairs included at birth	3,746	5,564	7,400	16,810
Age 12: Contacted	2,189	2,697	3,553	8,439
Age 12: Valid Returns	1,392	1,961	2,523	5,876
(Percentage)	(64%)	(73%)	(71%)	(70%)
Age 14: Contacted	2,487	3,946	4,685	11,118
Age 14: Valid Returns	1,146	1,261	966	3,373
(Percentage)	(46%)	(32%)	(21%)	(31%)

Note: At age 14, return rates are lower than at any previous and later time point in TEDS. This was due to low funds affecting the resources available to devote to postal reminders and telephone calls. In addition, online questionnaires were trialled that year, causing additional problems and leading to decreased returns.

16, 810 twin pairs (N=33,620 twins) were originally recruited to TEDS

11,118 twin pairs (N=22,236) were retained in TEDS at age 14

3,313 twin pairs had autistic trait data associated with *both* twins at age 14

164 twins met exclusion criteria, 362 pairs had no internalising trait data at age 12

2,869 twin pairs (N=5,738) were included in the final sample
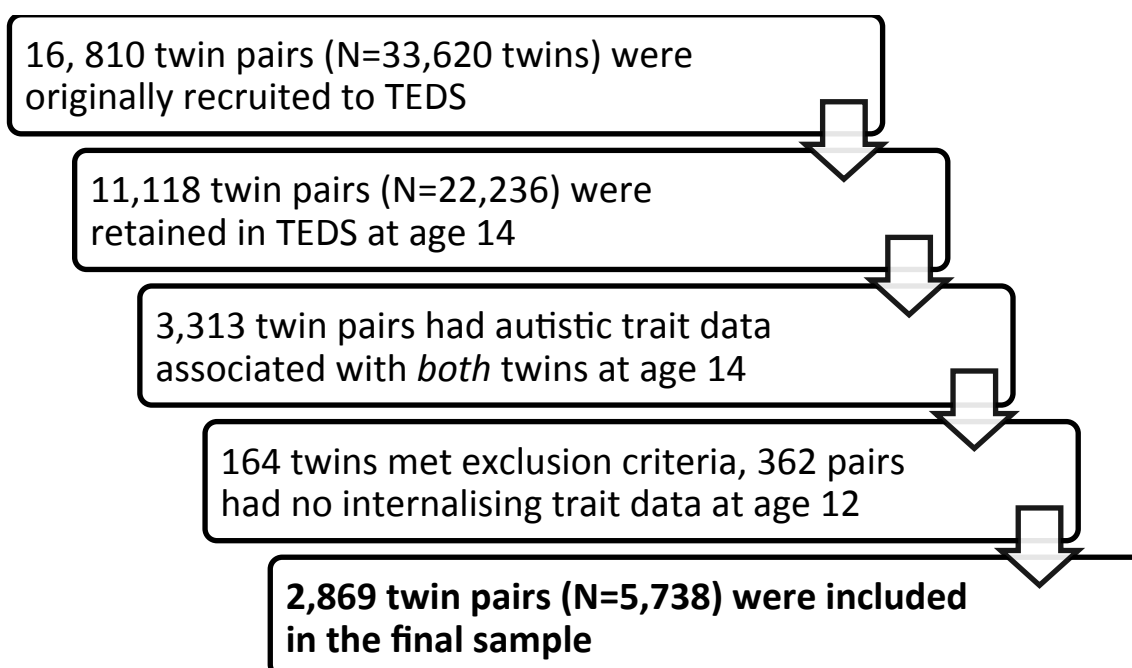
Figure 4.1 Selection of twins at ages 12/ 14

Exclusions were made using the criteria outlined above. Thereafter, 3,232 parent questionnaires on autistic traits at age 14 years (mean = 14.18, SD = 0.50) remained in the dataset. All parents informed on both twins. Of these, 2,869 parent reports on internalising traits at age 12 years (mean = 11.41, SD = 0.63) were available. Bivariate analyses were based on 485 MZM, 431 DZM, 607 MZF, 508 DZF and 838 DZOS pairs.

4.2.1.5.1 Representativeness of sample for adolescent internalising and autistic traits

As previously discussed, the representativeness of the sample with respect to ethnicity, twin type and sex ratio in TEDS throughout childhood and adolescence has been tested and confirmed (Haworth et al., 2013). In addition, the distribution of socio-economic characteristics within the sample including parental education level and employment status remained stable. With respect specifically to the phenotypes studied in this thesis, for autistic traits previously at ages 9 and 12 years (Hallett, 2010; Ronald et al., 2008), valid twin data of those who participated compared to those who have been invited but did not respond have been shown to be representative of both the whole TEDS sample and the general UK population. Similarly, no systematic differences between either the UK population or the full TEDS sample were found for internalising traits at ages 9 and 12 years (Hallett, 2010). This pattern also held true in the adolescent sample (at ages 12, 14, 16 years) studied in this thesis. In line with its conception as a personality-related trait measure, the autistic scales show a smooth distribution of scores. As expected, the internalising scales more closely mapping psychiatric symptoms show a long tail toward the high-scoring end of the spectrum.

4.2.2 Measures

4.2.2.1 Internalising Traits

Internalising traits at age 12 years were assessed using the parent reported 'emotional symptoms' subscale on the Strengths and Difficulties Questionnaire (SDQ, (Goodman, 1997). The SDQ is a brief behavioural screening questionnaire designed to capture a variety of aspects known to hinder (hyperactivity, emotional symptoms, conduct problems, and peer problems scales) and help (prosocial scale) psychosocial development of youths aged 4-16 years.

The SDQ has been subject to multiple studies evaluating its validity, with favourable results. Test-retest stability is satisfactory both for the SDQ overall (r = .88, within 2 months) and for the emotional symptoms subscale (r = .76, Muris, Meesters, & van den

Berg, 2003). Despite its brevity, the SDQ compares favourably to the widely used in-depth screen Child Behaviour Checklist (CBCL)(Achenbach, 1991) in distinguishing clinical symptoms as measured on the ICD-10 (Goodman & Scott, 1999; WHO, 1992). The correlation of emotional symptoms with CBCL internalising scores is good (r = .70, Muris et al., 2003).

Similarly, correlations with the Revised Children's Manifest Anxiety Scale (RCMAS, Reynolds & Richmond, 1985) both on total anxiety score (r = .73) and anxiety subscales (r = .43-.68) indicate that the SDQ emotional symptoms scale is not only appropriate to measure overall internalising difficulties, but also captures aspects of individual internalising disorders. In TEDS, reported SDQ emotional symptoms at age 12 were moderately correlated (r = .41, p <.01) with same-age depression symptoms on the Mood and Feelings Questionnaire (MFQ, Angold et al., 1995; described in greater detail in Chapter 7), in support of its construct validity.

The emotional symptoms subscale consists of five items scored on a 3-point Likert scale (0, not true; 1, somewhat true; and 2, certainly true). The items included on this scale are: '*Often complains of headaches, stomach ache or sickness*'; '*Many worries, often seems worried*'; '*Often unhappy, down-hearted or tearful*'; '*Nervous or clingy in new situations, easily loses confidence*'; and '*Many fears, easily scared*'. The responses to the items showed good internal consistency in the current TEDS sample at age 12 years (Cronbach's α = .67). This is similar to TEDS data at age 8 (Cronbach's α = .63), and the internal consistency found in a large community sample of 9-15 year-olds (Cronbach's α = .70, Muris et al., 2003).

4.2.2.2 Autistic Traits

Autistic traits were assessed using Autism Spectrum Quotient adolescent version (AQ, Baron-Cohen, Hoekstra, Knickmeyer, & Wheelwright, 2006), a measure of quantitative autistic traits. Being closely similar to the self report adult version, the adolescent questionnaire records parent ratings on their 10-16 year-olds. Due to their great similarity, studies using any AQ version (child/ adolescent/ adult) are presented here in support of the AQ's validity.

The AQ has been shown to exhibit good test-retest reliability and validity. Scores in the general population remain stable (r = .78, within 1-6 months; Hoekstra, Bartels, Cath, & Boomsma, 2008) and this has also been shown in a child sample with greater proportions of clinical cases (r = .85, within 3 months; Auyeung, Baron-Cohen,

Wheelwright, & Allison, 2008). Score distribution and cut-offs have been replicated cross-culturally (Wakabayashi, Baron-Cohen, Wheelwright, & Tojo, 2006), and the test authors have suggested that it may have acceptable properties to be used as a screening instrument in clinical practice, demonstrating it correctly identified 83% of Asperger cases (Woodbury-Smith, Robinson, Wheelwright, & Baron-Cohen, 2005).

Another quantitative measure is the Childhood Autism Spectrum Test (CAST, Scott et al., 2002). Unlike the AQ, its items more closely follow the autistic triad of symptoms and subscale scores for social difficulties, communication difficulties and non-social behaviours can be obtained. A recent review concludes that the CAST is relatively more effective to identify individuals with autistic-like difficulties across the entire ASD spectrum compared to other studied measures in childhood (Fernandopulle, 2011). AQ scores of 14-year-olds in the current sample show a significant positive correlation ($r = .54$, $p < .01$) with TEDS data on the CAST collected 24 months apart (at age 12), supporting the construct validity of the AQ (see also Appendix III).

The AQ consists of 50 items with which respondents can 'definitely agree', 'slightly agree', 'slightly disagree' or 'definitely disagree'. Half of the statements are reverse items. The original publication suggests binary scoring, however, many studies using the AQ have since adopted a four-point Likert scale (0-3). Of note, the AQ-child (Auyeung et al., 2008), a more recent adaptation of the AQ adolescent version to children aged 4-11 years, developed by the same group as the AQ adult and adolescent version, already suggests the use of this extended scale. Allowing this greater range of possible scores may increase sensitivity in studies – such as the current study – using non-clinical samples.

Of the original 50 items making up the AQ, only 38 were included in TEDS due to space restrictions. However, a prior case of an abridged version of the AQ similar to the present version (AQ-short, Hoekstra et al., 2011; described in greater detail in Chapter 5) shows fewer items can reliably measure autistic traits with good validity. Indeed, most recently it has been demonstrated that 10 'best items' can reliably be used to flag up cases that are likely assigned clinical status in further assessment (AQ-10, Allison, Auyeung, & Baron-Cohen, 2012). In the current sample, reliability of the 38-item AQ was good (Cronbach's $\alpha = .81$). As discussed above and in light of the shortened measure, a 4-point scoring system was adopted (range 0-114). Example items can be seen in Chapter 5 (Table 5.1).

4.3   Analysis

Total scores of autistic trait and internalising trait scales were created using pro-rated scores. In order to receive a total, participants had to have more than 50% valid items.

$$Pro-rated\ subscale\ score\ = \frac{(Sum\ of\ item\ scores) \times (N\ of\ items\ in\ scale)}{(N\ of\ items\ answered)}$$

Data preparation included normalisation of scales. The parent reported internalising trait scale was positively skewed (skewness = 1.33) and was log-transformed. The AQ had only modest skew (<1) and was not transformed. In line with standard behaviour genetic procedures, age of the twins at testing and sex were regressed out of all scores, and the residual scores were used in all ensuing model fitting in the structural equation modelling package Mx. Phenotypic correlations between traits, intraclass twin correlations on single traits and cross-twin cross-trait (CTCT) were obtained giving an initial overview of the aetiological structure of the data and suggesting likely models which were parameterised during univariate and bivariate model-fitting.

Specifically, pairwise intraclass twin correlations showed the association between twins 1 and 2 for univariate internalising traits and univariate autistic traits. Using these correlations, it was possible to identify the presence of dominance ($2r_{DZ} < r_{MZ}$) or shared environmental ($2r_{DZ} > r_{MZ}$) effects and sex effects ($r_{males} \neq r_{females}$) within the data. The presence of genetic influences was suggested by $r_{MZ} > r_{DZ}$ and nonshared environmental influences were involved since all ICCs <1. Univariate model-fitting was used to obtain more accurate parameter estimates and to test for aetiological sex differences. Initially, a full model including A, C, and E parameters was tested. Then, nested models incrementally dropping parameters were compared, containing A and E parameters, then E only. These models were compared using the log likelihood fit statistic and Akaike's Information Criterion (AIC), with lower values reflecting a better fit. As described in Chapter 2, by specifying separate matrices for male and female MZ and DZ twins, quantitative sex differences were also modelled.

Bivariate analyses were carried out to determine the aetiological association between autistic and internalising traits. First, phenotypic correlations were obtained to determine the extent of trait overlap within the same person. This trait overlap was studied across twins using CTCT by associating twin 1's autistic traits with twin 2's internalising traits. A bivariate Cholesky decomposition was carried out, as described in section 2.2.5.

4.4 Results

The descriptive statistics for the internalising traits and autistic traits are presented in Table 4.3. The results showed that for internalising traits, females scored higher than males ($F_{1, 2868}$ = 11.62, p <.001). There was no significant effect of zygosity on mean scores ($F_{2, 2868}$ = 0.14, p = .87) and no significant sex-by-zygosity interaction ($F_{2, 2868}$ = 0.17, p = .84).

Autistic trait scores were significantly higher for males than for females ($F_{1, 3232}$ = 83.83, p <.001). Zygosity effects were non-significant ($F_{2, 3232}$ = 1.30, p = .27), but there was a significant sex-by-zygosity interaction ($F_{2, 3232}$ = 6.95, p <.002), in which monozygotic males (MZM) scored lower than males in dizygotic opposite-sex pairs (DZOM).

Figure 4.2 Phenotypic correlations of age 12 internalising traits and age 14 autistic traits, and the proportions of correlations accounted for by genetic, shared and nonshared environmental effects
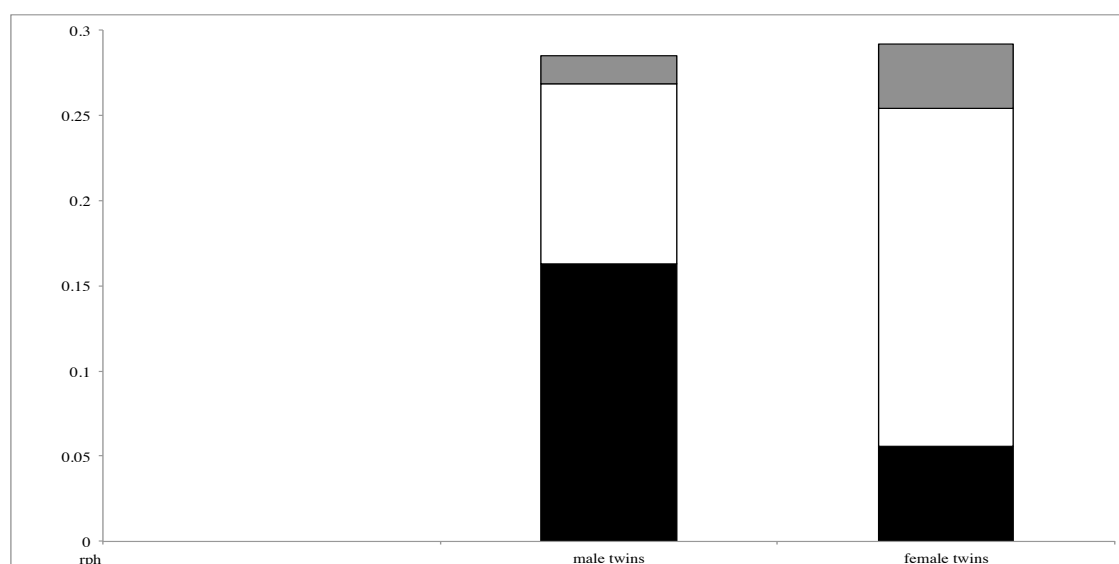
Table 4.2 Legend for Figure 4.2

		Males	Females
	r_{PH} (95% CI)	.29 (.26-.37)	.30 (.26-.36)
■	Genetic factors	.16 (55%)	.06 (20%)
□	Shared environment	.11 (38%)	.20 (67%)
▣	Nonshared environment	.02 (7%)	.04 (13%)

Note: Aetiological factors in the table summatively show the phenotypic trait covariance with internalising traits. *E.g. Males r_{ph} = .16 + .11 + .02 = .29*

Table 4.3 Descriptive statistics: age 12 internalising traits and age 14 autistic traits

| Trait (measure) | No. of items | Mean Score (SD) | | | | | | | ANOVA | | | |
		Whole Sample	MZM	MZF	DZM	DZF	DZOM	DZOF	Sex	Zyg	Sex-Zyg	R^2
Internalising Traits (SDQ)	5	1.70 (1.86)	1.52 (1.71)	1.81 (1.95)	1.61 (1.83)	1.80 (1.87)	1.58 (1.87)	1.81 (1.95)	<.001***	.87	.84	.004
Autistic Traits (AQ)	38	21.04 (9.03)	21.55 (8.95)	20.02 (8.73)	22.69 (9.25)	19.94 (8.45)	23.50 (9.71)	20.02 (8.73)	<.001***	.27	<.05*	.029

ANOVA = analysis of variance; DZF = dizygotic females; DZM = dizygotic males; DZOF = females in dizygotic opposite- sex pairs; MZF = monozygotic females; MZM = monozygotic males; DZOM = males in dizygotic opposite-sex pairs; Zyg = zygosity. AQ = Autism Spectrum Quotient; SDQ = Emotional symptoms subscale of the Strengths and Difficulties Questionnaire. *p <.05; **p <.01; ***p <.001

4.4.1 Twin correlations

Phenotypic correlations (see Figure 4.2) of autistic and internalising traits were r = .29 (95% CI: .26-.37) for males and r = .30 (95% CI: .26-.36) for females. The amount of variance in the sample explained by the overlap of the two traits was 9%.

Table 4.4 presents the intraclass twin correlations and CTCT. Genetic influences appeared to be involved because MZ twins showed a greater similarity on both internalising and autistic traits than did DZ twins ($r_{MZ} > r_{DZ}$). Correlations of DZ twins exceeded that expected due to their relative genetic similarity compared to MZ twins ($r_{DZ} > .5\ r_{MZ}$), suggesting a role for shared environment. Nonshared environment was indicated by less than perfect correlations ($r_{MZ} < 1$). Overall, these patterns were consistent with an ACE model for both internalising and autistic traits. Female DZ correlations were more similar to their MZ counterparts than for males, indicating less genetic overlap between these traits for females. The CTCT correlations suggest genetic overlap between the traits in males, while the high levels of female DZ CTCT correlations with respect to their MZ counterparts suggest the involvement of shared environment on the trait overlap. Again, less than complete overlap suggested a role for nonshared environment.

Table 4.4 Intraclass and CTCT correlations of age 12 internalising traits and age 14 autistic traits

	Internalising Traits	N	Autistic Traits	N	CTCT
MZM	.61 (.56-.67)	485	.93 (.91-.94)	355	.31 (.21-.40)
DZM	.37 (.29-.45)	431	.62 (.55-.68)	344	.19 (.08-.29)
MZF	.61 (.55-.65)	607	.90 (.88-.91)	486	.24 (.15-.33)
DZF	.33 (.25-.40)	508	.70 (.65-.75)	405	.30 (.21-.40)
DZOS	.37 (.31-.43)	838	.57 (.52-.62)	668	.23 (.15-.31)

Note: 95% confidence intervals shown in parenthesis. CTCT = Cross-twin cross-trait correlations. DZF = DZ females; DZM = DZ males; DZOS = DZ opposite-sex twin pairs; MZF = MZ females; MZM = MZ males.

Table 4.5 Fit statistics of univariate models of age 12 internalising traits

	Overall Fit of Model					relative Fit of Model			
	−2LL	χ²	df	p	AIC	χ²	df	p	AIC
Saturated	10885.961								
ACE	10886.834	0.873	6	.99	-11.127				
AE	10888.844	2.883	7	.90	-11.117	2.01	1	.16	0.01
CE	10962.015	76.054	7	.001	62.054	75.181	1	.001	73.181
E	11512.084	626.123	8	.001	610.123	625.25	2	.001	621.25
Saturated (Sex Limitation)	10882.635								
ACE (sex)	**10885.166**	**2.531**	**9**	**.98**	**-15.469**				

Note: Values of the nested models are compared with the saturated model. -2LL = log likelihood fit statistic; χ² = likelihood ratio χ² test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit). Best-fitting model shown in bold.

Table 4.6 Fit statistics of univariate models of age 14 autistic traits

	Overall Fit of Model					Relative Fit of Model			
	$-2LL$	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	6962.26								
ACE	6971.642	9.382	6	.15	-2.618				
AE	7046.006	83.746	7	.001	69.746	74.364	1	.001	72.364
CE	7322.278	360.018	7	.001	346.018	350.636	1	.001	348.636
E	8905.68	1943.42	8	.001	1927.42	1934.038	2	.001	1930.038
Saturated (Sex Limitation)	6941.934								
ACE (sex)	**6956.37**	**14.436**	**9**	**.11**	**-3.564**				

Note: Values of the nested models are compared with the saturated model. -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit). Best-fitting model shown in bold.

4.4.2 Univariate model-fitting

Univariate fit statistics are shown in Table 4.5 and Table 4.6. The best fitting univariate models for autistic traits was an ACE model, both for the whole sample and including quantitative sex differences, as indicated by the low AIC value. For internalising traits, AIC values on the whole sample were very similar for the ACE and AE model (with p-values giving preference to the AE due to greater parsimony). However, results from the sex limitation model reveal sex-specific quantitative aetiology to be the best fit, including shared environmental influences.

4.4.3 Bivariate model-fitting

Bivariate fit statistics can be seen in Table 4.7. The lowest AIC value was found for the full ACE sex limitation model, including quantitative sex differences on genetic, shared environmental and nonshared environmental influences. Figure 4.3 shows parameter estimates from the bivariate sex limitation model, presented as correlated factors solution.

Results show that half of the twin similarity on internalising traits was due to genetic influences (males: $a^2 = .49$, females: $a^2 = .52$). There was a low degree of shared environmental influences (males: $c^2 = .12$, females: $c^2 = .08$), consistent with the similar AIC values for the ACE and AE models. Nonshared environmental influences on internalising traits were moderate (males: $e^2 = .39$, females: $e^2 = .40$).

While overall twin similarity on autistic traits was similar, a greater part of this was accounted for by genetic influences in males (males: $a^2 = .60$, females $a^2 = .42$). In females, shared environmental influences had as much an effect on phenotypic trait levels as did heritability (males: $c^2 = .33$, females: $c^2 = .49$). As a result of high phenotypic similarity in twins, nonshared environmental influences were low (males: $e^2 = .07$, females: $e^2 = .09$).

The genetic correlation between internalising traits and total autistic traits was modest in females and moderate in males (males: $r_g = .30$, females: $r_g = .12$). Nonshared environmental correlation was modest (males: $r_e = .10$, females: $r_e = .20$). The shared environmental correlation was high (males: $r_c = .53$, females: $r_c = 1$), however only confidence intervals for females were different from zero.

Aetiological components are displayed as proportions of the phenotypic trait overlap in Figure 4.2. The genetic contribution to the observed correlation was 55% in males and 20% in females. The females' phenotypic correlation was driven by a high proportion of

shared environmental factors (females: 67%, males: 38%). The proportion of the phenotypic correlation explained by nonshared environment was low for both sexes (males: 7%, females: 13%).

Table 4.7 Fit statistics of bivariate models of age 12 internalising traits and age 14 autistic traits

	Overall Fit of Model					Relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	17648.722								
ACE	17664.959	16.237	17	.51	-17.763				
Dropped r_c	17681.41	32.688	18	.02	-3.312	16.45	1	.001	14.45
Dropped r_g	17672.411	23.689	18	.17	-12.311	5.452	1	.006	4.452
Dropped r_g and r_c	17803.389	154.667	19	.001	116.667	138.43	2	.001	134.43
Saturated (Sex Limitation)	17611.927								
ACE (sex)	**17642.120**	**30.193**	**34**	**.66**	**-37.807**		**11**		

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the ACE Cholesky and submodels (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; E = nonshared environmental influences. Best-fitting model shown in bold.

r_G
M: 0.30 (0.10-0.49)
F: 0.12 (-0.09-0.30)*

r_C
M: 0.53 (-0.06-1)*
F: 1 (0.53-1)

A   C   A   C

M: 0.49 (0.32-0.64)   M: 0.12 (0-0.28)   M: 0.60 (0.49-0.72)   M: 0.33 (0.20-0.44)
F: 0.52 (0.38-0.60)   F: 0.08 (0.02-0.21)   F: 0.42 (0.33-0.51)   F: 0.49 (0.39-0.57)

Internalising Traits   Autistic Traits

M: 0.39 (0.34-0.44)   M: 0.07 (0.06-0.09)
F: 0.40 (0.35-0.45)   F: 0.09 (0.09-0.11)

E   E

r_E
M: 0.10 (-0.01-0.21)*
F: 0.20 (0.11-0.29)
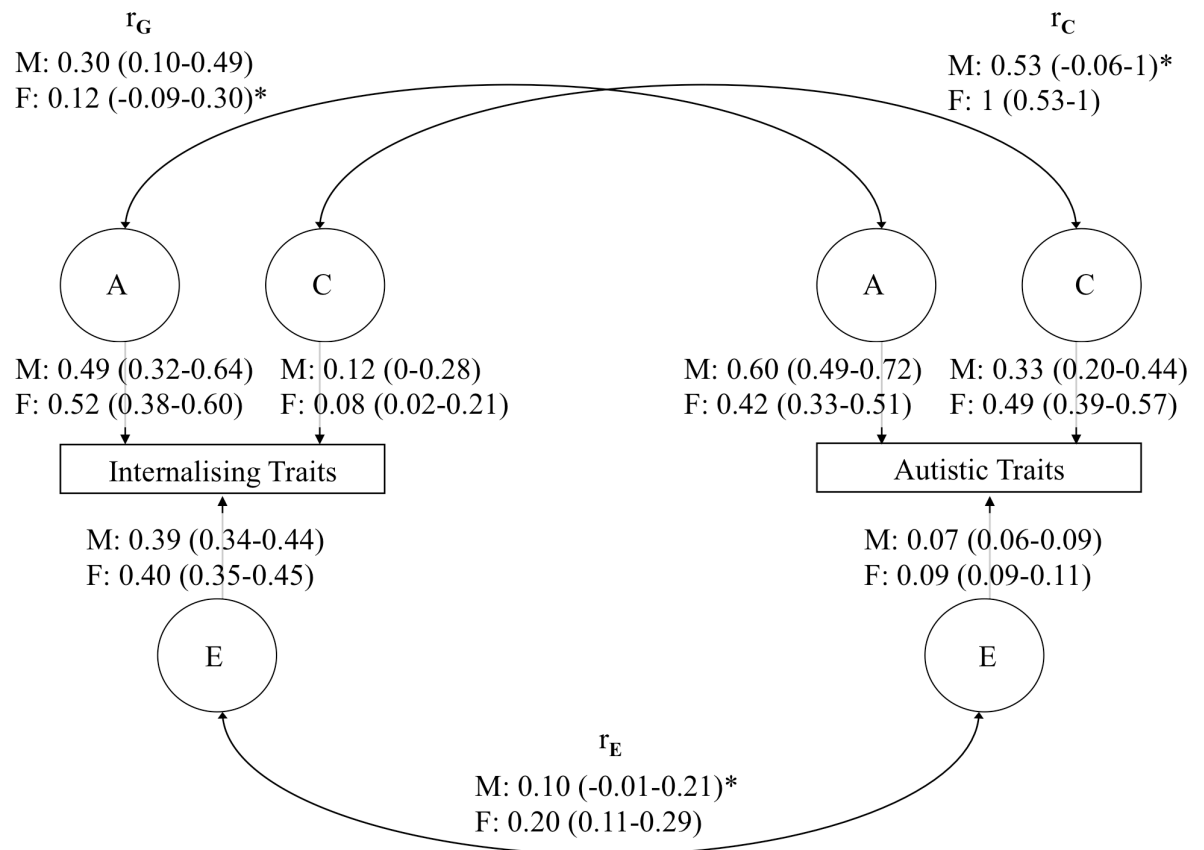
Figure 4.3 Path diagram showing results from the correlated factors solution of age 12 internalising traits and age 14 autistic traits sex limitation bivariate model.

Note: F = females; M = males; r_G = genetic correlation; r_C = shared environmental correlation; r_E = nonshared environmental correlation; *nonsignificant path (confidence intervals overlapping with 0).

4.5   Discussion

The present study examined within a community sample of 12- to 14-year-old twins the patterns of co-occurring internalising and autistic traits and the degree to which they can be attributed to genetic and environmental factors. This is the first twin study to address phenotypic and aetiological overlap of these traits during early adolescence.

4.5.1 Internalising traits at age 12

Internalising traits were moderately heritable (~50%), and this result is similar to findings in the same (overlapping) sample in mid-childhood (Hallett et al., 2010). For internalising traits, shared environmental influences were low (halved from age 8, Hallett et al., 2009a), while nonshared environmental influences were moderate (double

that of age 8). One consideration that may be relevant and needs investigation with respect to the above findings of greater nonshared environmental influences on internalising traits is, that it is also important to acknowledge that along with adolescents' growing independence, there may be increasing potential for factors other than their family to become influential.

4.5.2 Nonshared environment

One hypothesis for greater adolescent nonshared environmental influences on internalising traits might be that feelings of anxiety and depression with age are increasingly elicited by experiences distinct from day-to-day risk factors present within the family/ faced by the other twin. However, which specific factors these are, is difficult to predict and warrants further research. Importantly, these factors can include a wide range of environments ranging from twin-specific events (e.g. accidents) to the same environments (e.g. divorce) being perceived differently by each twin. To name an example, having different sets of friends may provide unique experiences that decrease twin similarity. However it may also make them *more similar* in other respects, such as providing opportunities for both to take on a leadership role. Conversely, having the same set of friends likely increases time and experiences shared, however twins may feel a greater need to establish their unique identity among peers, thus changing *how* they experience the shared time.

The effect of a number of environmental factors in terms of their association with nonshared environmental influences has been tested previously on other phenotypes (reviewed in Plomin, 2011). Psychosocial contributors identified by identical twin differences studies include influences of differential parental treatment and classroom experiences on adjustment (Oliver, Pike, & Plomin, 2008; Pike, McGuire, Hetherington, Reiss, & Plomin, 1996a), and school experience on achievement (Asbury, Almeida, Hibel, Harlaar, & Plomin, 2008). Similarly, multivariate genetic analyses including additional family members have studied effects of parenting characteristics on offspring psychological outcomes (e.g. Jaffee et al., 2004), reporting some small nonshared environmental influences (independent of genetics and shared environment, Plomin, 2011). Chapter 6 is setting out to identify environments important for nonshared environmental (idiosyncratic) influences *on internalising and autistic traits in adolescence* by means of a monozygotic twin differences design.

4.5.3 Autistic traits at age 14

Autistic traits showed an overall similar degree of heritability at age 14 as previously at age 12, but sex differences in the heritability estimate were more pronounced (males >females). Twin similarity on autistic traits was significantly greater in adolescence than late childhood (age 12: $r_{MZ} = .78$, $r_{DZ} = .34$-$.51$; age 14: $r_{MZ} = .90$-$.93$, $r_{DZ} = .62$-$.70$), and this was mainly due to the presence of moderate shared environmental influences. Compared to the parameter estimates at 12 years, shared environment was more than doubled in females (from 21%), and close to five times (from low 7%) as large in males. Low significant nonshared environmental influences were found.

4.5.4 Shared environment and sex differences

As discussed in this chapter's introduction, arguments have been made both for increasing genetic effects on autistic traits over the childhood years and/ or for a greater contribution of shared environment. To iterate, the relatively higher shared environmental component on autistic traits must be interpreted with care as even moderate levels are difficult to reliably detect (Burt, 2009 – note also the wide confidence interval on the shared environmental correlation between traits). Results from this study do however support an increase in adolescence of both genetic and shared environmental influences. In addition, the sex differences hypothesised initially were confirmed by this study's results. Interestingly, the question of which aetiological factor underlies twin similarity and that of emerging sex differences appear interrelated.

Specifically, the point estimate *in males* suggest marginally elevated heritability of autistic traits from age 12 to age 14. With respect to the male confidence interval, at least half and up to 70% of twin covariance on this trait was genetic. *In females*, age 14 point heritability was .10 lower than age 12. The lower boundary of the confidence interval suggests genetic influences of around one third, and the upper boundary a maximum of one half. Although there was marginal overlap on confidence intervals between sexes, it appeared that by early adolescence genetic effects play a greater role in male phenotypic presentation of autistic traits.

For shared environmental influences, greater effects were observed in both sexes in adolescence. However, two interpretations of this are possible. On the one hand, point estimates show that shared environment accounts for a third of the phenotypic variance in males and half of the variance in females, suggesting that for autistic trait shared environment is more important in the latter. On the other hand, the relative difference of male shared environment to female shared environment was greater at age 12 than at

age 14 (1:3 compared to 2:3), which may raise timing-related questions about common environments acting on autistic traits experienced later by males than females.

The best-fitting model for internalising traits at age 12 included sex differences, but as visible from univariate twin correlations, these were minimal. Quantitative sex differences were not included in Hallett et al. (2010)'s final model on the same measure. A possible explanation lies in the differences in modelling strategies, resulting in diverging decisions. First, twins in the current study were included on the basis that their parents had provided data on autistic traits at age 14, excluding ~800 twin pairs included at age 12. Second, fit comparisons in Hallett et al. (2010) also included other considerations such as estimation of longitudinal effects (from age 8).

4.5.5 Patterns of trait overlap in early adolescence

Internalising traits were significantly associated with autistic traits, and the magnitude of this association resembled that of previous twin trait studies. The genetic overlap between autistic traits and internalising traits showed a pattern of being higher for males than females (r_g = .30 and .12, respectively), although confidence intervals overlapped. The female point estimate was thus consistent with previous estimates at the lower end, while the genetic correlation for males was mid-range of the reviewed prior studies on autistic and internalising traits. Bivariate heritability of females was below that found in previous ACE models, while that of males was within the upper limits of previous results. Taken together this suggests that in males, in the majority genetic influences act on the co-occurrence of internalising traits and autistic traits in early adolescence, and a moderate amount of genes are shared across traits.

Shared environmental point estimates showed full overlap between autistic traits and internalising traits in females and substantial overlap in males, though confidence intervals of males included zero. Whether this may indicate sex-specific vulnerabilities (Constantino & Charman, 2012) and/ or coping strategies (Dworzynski et al., 2012) that transfer across traits and could be utilised in interventions warrants further research. This component could also reflect wider shared family characteristics (e.g. family members on the broader autism phenotype, BAP) that mediate phenotypic outcomes. Nevertheless, this significant shared environmental overlap in females would suggest that identifying and addressing shared (e.g. family) risk environments on one trait could have an important role in also reducing girls' difficulties on the other trait. Nonshared environmental influences were mostly independent between autistic traits and

internalising traits, meaning different unique experiences are relevant in decreasing twin similarity on each trait.

4.5.6 Limitations

The discussion has aimed to relate the new findings on early adolescence to the preliminary patterns established using all relevant previous work, but in addition has focused in more detail on the most comparable results derived from TEDS. In making these inferences, certain caveats must be considered.

The analyses in this chapter benefited from the use of a large sample and the incorporation of the same internalising measure and twin sample as previously investigated in two studies in relation to co-occurring internalising and autistic traits. However, in comparing the mid-childhood results to the current study on early adolescence, a limitation is the use of different measures for autistic traits, and the utilised measures being taken two years apart. Thus, longitudinal analyses of the examined traits across childhood and adolescence while of interest would be challenging practically.

A further limitation of this study relates to the question whether parent ratings represent an accurate picture of their youths' behaviour. In analysing parent report only, the study did not address effects of rater bias and correlated measurement error. Beyond statistical biases, conceptually, selecting the appropriate informant is particularly relevant in relation to internalising traits, which may not be easily observed. Indeed, evidence in relation to the emotional symptoms subscale of the SDQ suggests that at ages 10-19, self report is superior to parent ratings in its discriminative ability, and this finding is specific to internalising difficulties measured on this instrument (Van Roy, Veenstra, & Clench-Aas, 2008). Notwithstanding, parent report is widely used in childhood research such that similar biases are likely to affect a large proportion of studies. In aiming to extend research from mid-childhood into early adolescence, continuing to use the same rater may thus allow for more direct comparisons to be made. It should be noted that concerns have been raised whether individuals with clinical ASD have the introspective capacity to report internalising difficulties (Mazefsky, Kao, & Oswald, 2011), though others have demonstrated an unimpaired ability of ASD probands to report at least on their primary (i.e. ASD) symptomatology using trait measures (Armstrong & Iarocci, 2013). Little is known whether the tendency to under-report is specific to clinical groups or incremental with autistic traits levels. Using parent compared and self report

should therefore be viewed as complimentary approaches with their individual (dis-) merits (both are used in Chapter 7).

Another criticism may be the use of the emotional symptoms scale of the SDQ as the internalising trait measure. Although it has been shown to carry appropriate validity (see Method section) as shorthand for depressive and anxiety traits, including only five items provided limited coverage of these difficulties. Regarding the naming of the internalising measure, a wide array of symptoms is conceptually encompassed by 'internalising' including, but not exclusive to those of anxiety and depression. On the SDQ emotional symptoms scale relate, four of the five items relate to anxiety and depression. The rationale in naming the scale 'internalising traits' is that on the one hand it is more descriptive than 'emotional symptoms' and on the other hand the concept of internalising is the narrowest befitting both the anxiety and depression aspect. Beyond this, there is a history of describing the SDQ emotional symptoms scale as 'internalising' scale both in previous studies on the current sample (e.g. Hallett et al., 2009a; Hallett et al., 2010) and by other groups using different samples (e.g. Tiffin, Arnott, Moore, & Summerbell, 2011).

Finally, while one previous study has investigated the association of clinical ASD and internalising traits, clinical diagnoses of anxiety disorders are not available in the TEDS sample. As such, unfortunately it would not be possible to conduct an equivalent analysis in twins diagnosed for both these conditions. However, at the population level and particularly with respect to comparing results with the two previous twin studies using the Swedish samples, reports on separate trait measures can add to current knowledge on co-occurring autistic and anxiety/ depression traits in adolescence (see Chapter 7).

Conversely, it is important to consider the heterogeneity within the range of typical autistic-like behaviours to determine whether any particular autistic subdomains are associated more strongly with internalising traits, and if such differences are reflected in their aetiological overlap. The following chapter will aim to identify specific subdomains of autistic behaviours in the population in order to investigate this question.

Chapter 5 Disentangling the overlap between adolescent autistic and internalising traits: The role of autistic sub-domains

While the previous chapter has investigated the overall aetiological pattern, this chapter takes a more in-depth look at early adolescence, aiming to identify which specific autistic-like behaviours are most relevant to internalising difficulties.

Note: The data presented in Chapter 4 and Chapter 5 have been published in *Scherff, A., Taylor, M., Eley, T.C., Happé, F., Charman, T., & Ronald, A. (2013). What causes Internalising Traits and Autistic Traits to co-occur in Adolescence? A community-based twin study. Journal of Abnormal Child Psychology. (doi: 10.1007/s10802-013-9796-y)*

5.1 Background

Recently, Happé and Ronald (2008) have proposed the 'fractionable' autism triad hypothesis, suggesting that largely independent genetic effects cause each of the three core features within the triad of autistic impairments (Happé, Ronald, & Plomin, 2006; Robinson et al., 2012; Ronald et al., 2006; Ronald et al., 2005; Ronald, Larsson, Anckarsäter, & Lichtenstein, 2011). In addition, at the trait level, overall differences in population frequency for each of the subdomains have been demonstrated (Lundström et al., 2011): In this large child sample, lack of flexibility was most frequent, followed by communication problems. Social interaction problems were least frequent. These findings advocate the investigation of specific autistic subdomains in addition to measures of total autistic traits in research on causal processes.

5.1.1 Association of specific internalising with specific autistic-like behaviours

To date, one quantitative genetic study has related autistic to internalising subdomains in childhood. Hallett, Ronald, Rijsdijk, and Happé (2012) investigated three subdomains of autistic traits in relation to four subtypes of internalising traits in the same sample as used in the present study, but assessed at age 7-8 years old. They found both phenotypic and genetic associations to be strongest for autistic communication impairments and repetitive/ restrictive behaviours with generalised anxiety and negative affect, (males: r = .21-.36, r_g = .21-.30; females: r = .16-.33, r_g = .18-.24). Less phenotypic and genetic overlap was reported between internalising traits and autistic social difficulties (males: r = .02-.14, r_g = .08-.19; females: r = .01-.07, r_g = .03-.08). They also reported modest quantitative sex differences on the parameter estimates in these findings on 7-8-year-olds. The inter-relations among autistic subdomains, and their relationship with

internalising traits have not been previously examined in a population-based twin sample in early adolescence.

Previous studies took a top-down approach to measuring trait levels along the classic three symptom domains on the CAST (Ronald et al., 2006). A complimentary approach is the bottom-up analysis of items based on factor analysis to investigate the co-occurrence patterns *as they are in the data* and which may cater more specifically to personality level expressions of traits. In light of the lack of specific research on adolescent internalising and autistic traits, conducting a factor analysis is hoped to provide a flexible approach that allows new patterns to emerge.

5.1.2 Findings from Factor Analyses of the Autism Spectrum Quotient

A number of factor analyses of the adolescent autistic traits measure in TEDS (i.e. the AQ) have been conducted previously, most of which serve one of three objectives – validation of the AQ instrument and structure (Auyeung et al., 2008; Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Hoekstra et al., 2008; Hoekstra et al., 2011; Hurst, Mitchell, Kimbrel, Kwapil, & Nelson-Gray, 2007; Stewart & Austin, 2009; White, Bray, & Ollendick, 2012) or delineation of the broader autism phenotype and the relationship with personality domains (Austin, 2005; Wakabayashi, Baron-Cohen, & Wheelwright, 2006).

The developers have created the original questionnaire to assess five areas on ten items each: social skills, attention switching, attention to detail, communication and imagination. The results of a factor analysis found by the same group using the AQ-child (sample size N ~1000, Auyeung et al., 2008) provided support for all subsequent structural interpretations made by other groups. Initially, their principal component analysis extracted five factors with Eigenvalues >1, confirming the design. The corresponding scree plot showed a slope that would have supported single factor, two and four factor solutions. The final solution chosen by the test authors included four factors: With respect to the original design, items on three factors (social skills, attention to detail, imagination) remained mostly unchanged while the new strongest factor ('mind-reading') was mostly merged items from the former communication and attention switching scales.

5.1.2.1 Non-hierarchical models

In the first published factor analysis of the AQ, Austin (2005) found evidence for either one general factor or alternatively three domains that resembled the classic social, non-

social and communication distinction, but did not find a distinct domain for imagination. It is however not clear to what extent these results are a product of comparatively low sample size (N = 200) relative to the items included in the factor analysis (Costello and Osborne (2005) recommend at least 10:1 as a rule of thumb, i.e. N = 500 for AQ). In a later study by the same group using an overlapping larger sample (N = 536, Stewart & Austin, 2009), the resulting factor number changed to 4 and the item structure resembled that of Auyeung et al. (2008). Similarly, a final four-factor solution (social difficulties, restricted interests and attention to details and patterns, theory of mind deficits and preference for routine) was arrived at in White et al. (2012). In distinction to this, Hurst et al. (2007) replicated the initial three factor solution of Austin (2005) as the best fit in a large sample (N = 1,005).

5.1.2.2 Higher order factor models

The most comprehensive factor analysis on the AQ to date has comprised four independent samples (Dutch student sample N = 1,263; Dutch general population sample N = 1,121; English student sample N = 1,838; English ASD sample N = 274), and conducted both exploratory (EFA) and confirmatory (CFA) factor analyses (Hoekstra et al., 2008; Hoekstra et al., 2011). Investigated models included the developer 5-factor model, a single factor solution and a model derived from EFA. The best fit provided a model that maintained the five conceptual domains proposed by Baron-Cohen et al. (2001), but these were subordinate in a two-factor hierarchical model. Four domains loaded onto the higher order social interaction domain (social skill, attention switching, communication, imagination), while a single domain (attention to detail) was contained in a non-social factor.

5.1.2.3 Replication of previous factor models

In an attempt to consolidate previous findings, a recent study (Kloosterman, Keefer, Kelley, Summerfeldt, & Parker, 2011) systematically tested using confirmatory factor analysis (cf. (White et al., 2012) for exploratory approach) all 2, 3, 4 and 5 factor solutions in their sample (N = 522), replicating the precise factor structures of all AQ factor analyses previously published (i.e. excluding Hoekstra et al., 2011). They found that none of the tested model was satisfactory in cross-validation. Reducing the number of items by almost half (down to 28) greatly improved both variance explained by the model (almost double at 45%) and increased the proportion of item loadings meeting the cross-validation criterion (from 58-60% to 71%). The structure of the shortened AQ revealed a five-factor model conceptually close to the domains proposed in the five

factor models outlined above (social skills, communication/ mindreading, restrictive/ repetitive behaviour, imagination, attention to detail). A caveat here, again, is the lowered subject to item ratio in the split samples (test sample N = 300 and confirmatory sample N = 222). Nevertheless, the one factor analysis not included in this systematic replication independently created and validated their own 28-item version (with 20 overlapping items), also arriving at 5 subdomains (see previous paragraph, Hoekstra et al., 2011).

5.1.3 Objectives
In summary, all factor analyses support the differentiation of one non-social and at least one social factor for the study of specific autistic subdomains. With the fractionality of autistic traits in mind, the aim of the present study was to make aetiological inferences about the association between internalising traits and specific autistic traits in adolescence by using subdomains derived from a factor analysis.

5.2 Methods

5.2.1 Sample
Data from the same sample of 2,869 MZ and DZ twin pairs were analysed as in the previous chapter, described in section 4.2.1.5.

4.2.2 Measures
The same measures as described in the previous chapter were used. These were parent report on both the emotional symptoms scale of the SDQ for internalising traits (see section 4.2.2.1) and the Autism Spectrum Quotient (AQ) for autistic traits (section 4.2.2.2).

5.2.1.1 Item selection for Factor Analysis
As discussed above, a number of studies have carried out factor analyses on the AQ. A decision was taken to select those items for exploratory factor analysis (EFA) that matched those used in the study drawing on the largest prior sample – Hoekstra et al. (2011) AQ-short (using a 28-item version) – to ensure close similarity with a published measure. Of their items, 24 AQ items were available among the 38 collected for TEDS. Correlation between total scores using 38 items and 24 items was r = .94, p <.001. Removing items from the scale did not affect internal consistency (24-item Cronbach's α = .78).

5.3 Results

5.3.1 Results from Factor Analysis

5.3.1.1 Factor structure

Exploratory factor analysis was carried out in SPSS using principal components analysis (PCA) with varimax rotation. As previous publications on the AQ have presented solutions with 1-5 factors, solutions were explored with varying factor numbers. The variance explained by resulting factor structures was similar across solutions, as were inferences drawn from visual inspection of the scree plot. Item loading matrices showed the 5-factor solution to provide the phenotypically clearest structure.

Eigenvalues and variance accounted for by factors 1 through 5 were 4.12/ 11.77%, 2.62/ 9.95%, 1.79/ 9.81%, 1.34/ 9.10% and 1.10/ 7.04% respectively. Total variance explained by the five factors was 47.67%. Items were counted as loading onto a factor if their factor loadings were .40 or higher. After application of this criterion, two items loaded on more than one factor and were allocated to the factor they loaded on most strongly. Factors, items and their respective loadings are shown in Table 5.1. Phenotypic correlations of the derived autistic trait subdomains with the overall autistic trait measure are included in Table 5.2.

5.3.1.2 Creation of Autistic trait subscales

The five resulting factors were named: a) Attention to Details/ Special Interests (item numbers from original published AQ: 6, 9, 19, 23, 41), b) Social Unease (2, 11, 22, 25, 34, 46, 47), c) Poor Mentalising (10, 20, 36, 42, 45), d) Solitariness (1, 13, 15), and e) Poor Imagination (3, 4, 8, 50). Scales for subsequent twin modelling were derived by averaging scores of all items, using mean replacement to account for accidental omission of single items. Twins' data were coded as missing whenever less than 50% of the items had been answered. The five subscales had only modest skew (<1) and were not transformed.

Table 5.1 Factor loadings and items for age 14 autistic trait subdomains

Item	AQ Item N	Reversed	Attention to Details/ Special Interests	Social Unease	Poor Mentalising	Solitariness	Poor Imagination
% of Variance			11.77	9.95	9.81	9.10	7.04
Eigenvalues			4.12	2.62	1.79	1.34	1.10
S/he is fascinated by dates	9		.73				
S/he is fascinated by numbers	19		.73				
S/he notices patterns in things all the time	23		.72				
S/he usually notices car number plates or similar strings of information	6		.68				
S/he likes to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.)	41		.57				
New situations make him/her anxious	46			.66			
S/he enjoys meeting new people	47	x		.64			
S/he enjoys doing things spontaneously	34	x		.59			

Item	AQ Item N	Reversed	Attention to Details/ Special Interests	Social Unease	Poor Mentalising	Solitariness	Poor Imagination
S/he finds social situations easy	11	x		.56			
It does not upset him/her if his/her daily routine is disturbed	25	x		.52			
S/he prefers to do things the same way over and over again	2			.48			
S/he finds it hard to make new friends	22			.47			
S/he finds it difficult to work out people/s intentions	45				.78		
S/he finds it difficult to imagine what it would be like to be someone else	42				.64		
When s/he is reading a story, s/he finds it difficult to work out the characters/ intentions	20				.55		
S/he finds it easy to work out what someone is thinking or feeling just by looking at their face	36	x			.46		
In a social group, s/he can easily keep track of several different people's conversations	10	x			.42		

Item	AQ Item N	Reversed	Attention to Details/ Special Interests	Social Unease	Poor Mentalising	Solitariness	Poor Imagination
S/he prefers to do things with others rather than on her/his own	1	x				.67	
S/he finds her/himself drawn more strongly to people than to things	15	x				.62	
S/he would rather go to a library than a party	13					.43	
If s/he tries to imagine something, s/he finds it very easy to create a picture in my mind	3	x					.70
When s/he is reading a story, s/he can easily imagine what characters might look like	8	x					.70
S/he finds it very easy to play games with children that involve pretending	50	x					.43
S/he frequently gets so strongly absorbed in one thing that s/he loses sight of other things	4						-.42
Number of items			5	7	5	3	4
Reliability (Cronbach's Alpha)			.75	.72	.64	.46	.30

Table 5.2 Phenotypic correlations of age 14 total autistic traits and autistic trait subdomains

	Pearson Correlation				
	Autistic Traits	Attention to Details/ Special Interests	Social Unease	Poor Mentalising	Solitariness
Attention to Details/ Special Interests	.54				
Social Unease	.80	.20			
Poor Mentalising	.66	.08	.41		
Solitariness	.48	.15	.34	.12	
Poor Imagination	.54	.03 (ns)	.30	.45	.08

Note: All significant at p <.01; ns = non-significant.

5.3.2 Phenotypic results

5.3.2.1 Descriptives

The descriptive statistics for the autistic trait subdomains are presented in Table 5.3. Means for internalising traits have been presented previously in Table 4.3

Four autistic trait subdomains showed sex effects with males scoring higher than females on all except one subdomain (Attention to Details/ Special Interests: $F_{1, 3228}$ = 54.85, Poor Mentalising: $F_{1, 3214}$ = 55.47, Solitariness: $F_{1, 3222}$ = 19.90, Poor Imagination: $F_{1, 3232}$ = 125.17, all p <.001; Social Unease $F_{1, 3223}$ = 1.50, p = .22). Zygosity effects were only present on Attention to Details/ Special Interests with MZ scoring higher than DZ (Attention to Details/ Special Interests: $F_{1, 3228}$ = 6.65, p <.001; Social Unease $F_{1, 3223}$ = 0.62, Poor Mentalising: $F_{1, 3214}$ = 0.78, Solitariness: $F_{1, 3222}$ = 0.93, Poor Imagination: $F_{1, 3232}$ = 1.5, all p >.05). Sex-by-zygosity interactions were present on four subdomains (Attention to Details/ Special Interests: $F_{1, 3228}$ = 3.15, Social Unease $F_{1, 3223}$ = 4.36, Poor Mentalising: $F_{1, 3214}$ = 4.16, Solitariness: $F_{1, 3222}$ = 4.96, all p <.05; Poor Imagination: $F_{1, 3232}$ = 125.17, p = .23). DZOM scored higher than MZM on Attention to Details/ Special Interests and Social Unease, and monozygotic females (MZF) scored higher than dizygotic females (DZF) on Poor Mentalising and Solitariness.

5.3.2.2 Phenotypic correlations

Figure 5.1 and Table 5.4 present the phenotypic correlations of internalising traits with individual subdomains. Although phenotypic overlap was modest for some of the subdomains, all associations were significant (whole sample all p <.001, split by sex all p <.05). The amount of variance explained by the association of Social Unease with internalising traits was substantial (12%) and higher than that of the overall measure of autistic traits (9%) and accounted for twice the combined variance of all other subdomains (6%).

5.3.2.3 Intraclass twin correlations

Table 5.5 presents the intraclass twin correlations for all traits. Univariate twin correlations on internalising traits were equivalent to those presented in Chapter 4. All autistic trait subdomains showed genetic effects because r_{MZ} >r_{DZ}. Nonshared environmental influences were present on all subdomains as r_{MZ} <1. On four of the five subdomains, r_{DZ} >.5 r_{MZ}, indicating the presence of shared environmental influences; the exception was Solitariness, for which low DZ correlations (r_{DZ} <.5 r_{MZ}) of male twins suggested the presence of dominance.

Table 5.3 Descriptive statistics: age 14 autistic trait subdomains

Autistic trait subdomain (Range)	Mean Score (SD)							ANOVA			
	Whole Sample	MZM	MZF	DZM	DZF	DZOM	DZOF	Sex	Zyg	Sex-Zyg	R^2
Attention to Details/ Special Interests (0-15)	3.84 (3.36)	3.94 (3.40)	3.28 (3.15)	4.26 (3.47)	3.60 (3.22)	4.77 (3.73)	3.28 (3.15)	<.001***	<.001***	<.05*	.02
Social Unease (0-21)	6.27 (3.95)	6.04 (4.00)	6.31 (3.90)	6.33 (4.04)	6.28 (3.77)	6.72 (4.14)	6.31 (3.90)	.22	.54	<.05*	.003
Poor Mentalising (0-15)	4.43 (2.76)	4.65 (2.64)	4.31 (2.67)	4.97 (2.95)	4.03 (2.56)	4.81 (2.86)	4.31 (2.67)	<.01***	.46	<.05*	.02
Solitariness (0-9)	2.74 (1.92)	2.87 (1.87)	2.74 (1.86)	2.78 (1.93)	2.61 (2.00)	3.04 (1.95)	2.74 (1.86)	<.001***	.40	<.05*	.01
Poor Imagination (0-12)	3.76 (2.12)	4.05 (2.12)	3.39 (2.03)	4.34 (2.14)	3.40 (1.96)	4.21 (2.24)	3.39 (2.03)	<.001***	.22	.23	.04

*p <.05; **p <.01; ***p <.001
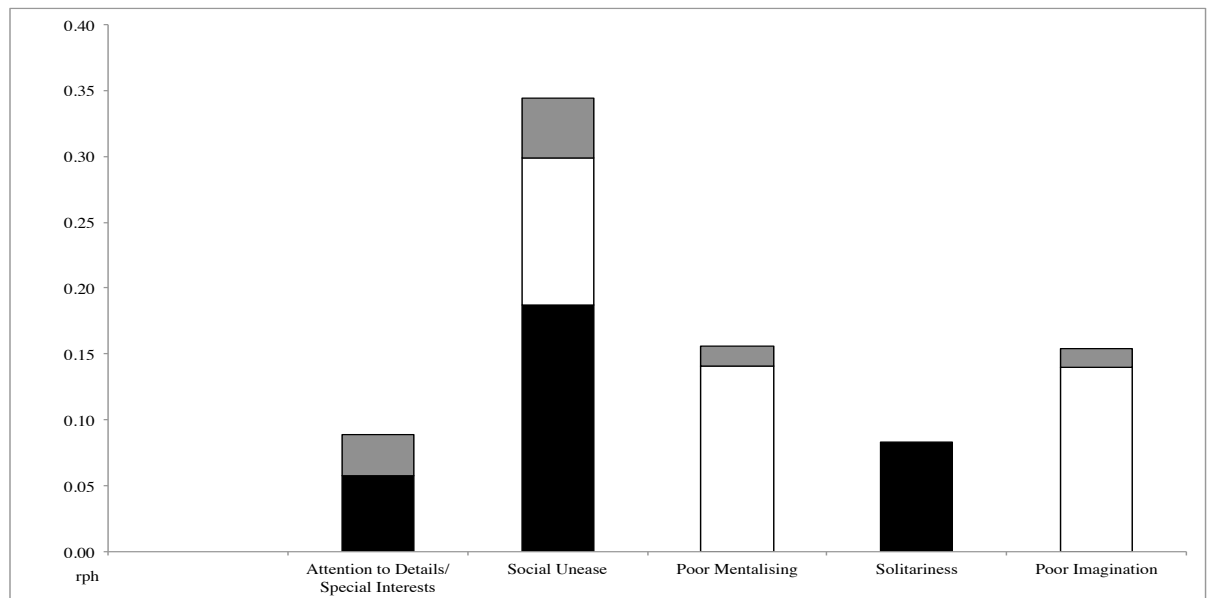
Figure 5.1 Phenotypic correlations of age 12 internalising traits and age 14 autistic trait subdomains, and the proportions of correlations accounted for by genetic, shared and nonshared environmental effects

Table 5.4 Legend for Figure 5.1

	Attention to Details/ Special Interests	Social Unease	Poor Mentalising	Solitariness	Poor Imagination
r_{PH} (95% CI)	.09 (.04-.11)	.35 (.33-.39)	.15 (.15-.24)	.08 (.05-.12)	.15 (.09-.16)
■ Genetic factors	.06 (67%)	.19 (54%)	-	.08 (100%)	-
☐ Shared environment	-	.11 (31%)	.14 (93%)	- (dominance)	.14 (93%)
▨ Nonshared environment	.03 (33%)	.05 (14%)	.01 (7%)	-	.01 (7%)

Note: Aetiological factors in the table summatively show the phenotypic trait covariance with internalising traits. *E.g. Social Unease r_{ph} = .19 + .11 + .05 = .35*

Table 5.5 Intraclass and CTCT correlations of age 12 internalising traits and age 14 autistic trait subdomains

	Internalising Traits	N		Attention to Details/ Special Interests	N	CTCT	Social Unease	N	CTCT
MZM	.61 (.56-.67)	485		.96 (.95-.96)	355	.10 (-.02-.20)	.92 (.91-.94)	355	.31 (.21-.41)
DZM	.37 (.29-.45)	431		.77 (.72-.81)	344	-.01 (-.12-.10)	.58 (.51-.65)	344	.17 (.06-.28)
MZF	.61 (.55-.65)	607		.93 (.92-.94)	486	.05 (-.04-.15)	.88 (.85-.90)	486	.33 (.24-.41)
DZF	.33 (.25-.40)	508		.80 (.76-.83)	405	.10 (-.01-.20)	.66 (.61-.72)	405	.36 (.27-.45)
DZOS	.37 (.31-.43)	838		.66 (.61-.70)	668	.05 (-.03-.13)	.57 (.52-.62)	668	.26 (.19-.34)
	Poor Mentalising	N	CTCT	Solitariness	N	CTCT	Poor Imagination	N	CTCT
MZM	.92 (.90-.93)	354	.23 (.12-.33)	.85 (.82-.88)	352	.14 (.03-.25)	.90 (.88-.92)	352	.18 (.07-.28)
DZM	.52 (.44-.59)	343	.19 (.08-.30)	.38 (.29-.47)	344	.07 (-.05-.18)	.67 (.61-.73)	344	.15 (.04-.26)
MZF	.89 (.87-.91)	483	.14 (.05-.23)	.80 (.77-.83)	485	.02 (-.08-.11)	.88 (.86-.90)	485	.11 (.01-.20)
DZF	.64 (.58-.69)	405	.18 (.08-.28)	.47 (.39-.54)	400	.13 (.03-.23)	.68 (.62-.73)	404	.15 (.04-.25)
DZOS	.52 (.46-.57)	668	.19 (.11-.26)	.31 (.24-.38)	664	.10 (.02-.18)	.57 (.52-.62)	666	.12 (.04-.19)

Note: 95% confidence intervals shown in parenthesis. CTCT = Cross-twin cross-trait correlations with internalising traits. DZF = DZ females; DZM = DZ males; DZOS = DZ opposite-sex twin pairs; MZF = MZ females; MZM = MZ males.

5.3.2.4 Cross-twin cross-trait correlations

The CTCT of all subdomains with internalising traits are shown in Table 5.5. In line with phenotypic associations of internalising traits with individual subdomains on the whole sample, CTCT with Social Unease were moderate and CTCT with Poor Mentalising and Poor Imagination more modest. While CTCT with Attention to Details/ Special Interests and with Solitariness were low, not differentiating by sex (i.e. modelling all MZ vs. DZSS – shown in Appendix IV) increased trait covariance to levels (~.15) viewed as appropriate to proceed with twin modelling.

5.3.3 Model-fitting results

Depending on patterns suggested by univariate twin correlations and CTCT, univariate and bivariate Cholesky decompositions into either ACE (Attention to Details/ Special Interests; Social Unease; Poor Mentalising; Poor Imagination) or ADE (Solitariness) models were carried out, and nested models tested. Univariate models tested were ACE/ADE, AE, CE, E. Bivariate models tested were full ACE, dropped r_g, dropped r_c/ r_d, dropped r_g + dropped r_c/ r_d. Best-fitting models were selected on the basis of the lowest AIC value. Fit statistics for best-fitting univariate models are presented in Appendix V.

5.3.3.1 Internalising traits

The AIC value for the full ACE Cholesky model (AIC_{ACE} = -11.127) was minimally lower than that of the nested AE model (AIC_{AE} = -11.117), indicating an overall better fit of the ACE model to the data. In relation to the relative fit of the nested model, given such minimal differences, dropping shared environment was not a significantly worse fit compared to the ACE model. Drawing on the findings in Chapter 4 (i.e. ACE as the best fit in the sex limitation model and a particular interest in the role of shared environment in adolescence), a decision was made to retain C for greater consistency.

Accordingly, the resulting parameter estimates of internalising traits on the overall sample corresponded with those reported on the split by sex sample in the previous chapter. Genetic influences and nonshared environmental influences were substantial, and shared environmental influences were low (a^2 = .52, c^2 = .09, e^2 = .39).

5.3.3.2 Autistic trait subdomains

Best-fitting univariate models for the domains Attention to Details/ Special Interests, Social Unease, Poor Imagination and Poor Mentalising were full ACE models, and an ADE model provided the best fit for Solitariness. Additive genetic effects ranged from

moderate to high for the autistic trait subdomains, with Attention to Details/ Special Interests showing the lowest heritability (a^2 = .33) and Solitariness the highest heritability (a^2 = .78; Poor Imagination: a^2 = .42; Social Unease: a^2 = .52; Poor Mentalising: a^2 = .69). Shared environmental influences were moderate for four domains on the ACE model (Poor Mentalising: c^2 = .22; Social Unease: c^2 = .37; Poor Imagination: c^2 = .47; Attention to Details/ Special Interests: c^2 = .61). For Solitariness, there was a small effect of genetic dominance (d^2 = .05). There were low nonshared environmental influences on all autistic trait subscales (e^2 = .06-.16).

5.3.3.3 Co-occurring internalising traits and autistic trait subdomains

Figure 5.2 shows parameter estimates of the best-fitting models of internalising traits and autistic trait subdomains, and Figure 5.1 shows them as proportions phenotypic covariance. Fit statistics of bivariate models are on internalising traits with the Attention to Details/ Special Interests domain are shown in Table 5.6, with Social Unease in Table 5.7, with Poor Mentalising in Table 5.8, with Solitariness in Table 5.9 and with Poor Imagination in Table 5.10.

As the main aim of the model-fitting analyses was to obtain an overall sense of co-occurrence patterns between specific domains of autistic-like behaviours and internalising traits, bivariate models with the five subdomains on the whole sample were deemed a sufficiently detailed level of analysis. Quantitative sex differences were not modelled, as the previous chapter has demonstrated them to be modest, resulting in twin covariances split by sex that were too low to proceed with twin-modelling.

Bivariate models included genetic, shared and nonshared environmental correlations between internalising traits and Attention to Details/ Special Interests, Social Unease, Poor Imagination and Poor Mentalising. No correlation parameter was fitted between shared environment on internalising traits (c^2) and dominance effects (d^2) on the Solitariness domain (genetic and nonshared environmental correlations were included).

Genetic correlations of internalising traits with individual autistic trait subdomains could be dropped from the model for Poor Mentalising and Poor Imagination without a significant deterioration in fit. Solitariness (r_g = .13) and Attention to Details/ Special Interests (r_g = .14) showed a modest genetic overlap with internalising traits and the genetic overlap with internalising traits was moderate for Social Unease (r_g = .36). The shared environmental correlation was dropped for Attention to Details/ Special Interests. Shared environmental correlations were moderate for Social Unease with

internalising traits (r_c = .61) and Poor Imagination with internalising traits (r_c = .68) and high for Poor Mentalising with internalising traits (r_c = 1). Nonshared environmental correlations were modest for internalising traits with Poor Imagination, Poor Mentalising, and Social Unease (r_e = .07-.22) and showed confidence intervals overlapping with 0 for Attention to Details/ Special Interests and Solitariness. Bivariate heritabilities and values for bivariate shared and nonshared environment are shown in Table 5.2. Bivariate statistics are similar for males' co-occurring internalising with total autistic traits (Biv h^2 = .16, Biv c^2 = .11, Biv e^2 = .19) and Social Unease (Biv h^2 = .19, Biv c^2 = .11, Biv e^2 = .05). Females' phenotypic correlations of internalising and total autistic traits were mostly accounted for by bivariate shared environment (Biv h^2 = .06, Biv c^2 = .20, Biv e^2 = .04), as was the case for internalising traits with Poor Mentalising and Poor Imagination (both Biv c^2 = .14, Biv e^2 = .01). Mainly genetic factors accounted for the modest association of internalising traits and Attention to Details/ Special Interests (Biv h^2 = .06, Biv e^2 = .03) and wholly for Solitariness (Biv h^2 = .08).

Table 5.6 Attention to details/ special interests: fit statistics of bivariate models of age 12 internalising traits and age 14 autistic traits subdomain

	Overall Fit of Model					Relative Fit of Model			
	−2LL	χ²	df	p	AIC	χ²	df	p	AIC
Saturated	17189.46								
ACE	17202.436	12.976	17	.74	-21.024				
Dropped r$_c$	**17202.633**	**13.173**	**18**	**.78**	**-22.827**	**0.198**	**1**	**.66**	**-1.802**
Dropped r$_g$	17204.932	15.472	18	.63	-20.528	2.496	1	.11	0.496
Dropped r$_g$ and r$_c$	17211.401	21.941	19	.29	-16.059	8.965	2	.01	4.965

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ² = likelihood ratio χ² test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences

Table 5.7 Social unease: fit statistics of bivariate models of age 12 internalising traits and age 14 autistic traits subdomain

	Overall Fit of Model					Relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	17813.679								
ACE	**17853.965**	**40.286**	**17**	**.001**	**6.286**				
Dropped r_c	17861.091	47.412	18	.001	11.412	7.126	1	.008	5.126
Dropped r_g	17876.903	63.224	18	.001	27.224	22.938	1	.001	20.938
Dropped r_g and r_c	18037.476	223.797	19	.001	185.797	183.511	2	.001	179.511

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences

Table 5.8 Poor mentalising: fit statistics of bivariate models of age 12 internalising traits and age 14 autistic traits subdomain

	Overall Fit of Model					Relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	17967.204								
ACE	17984.775	17.571	17	.42	-16.429				
Dropped r_c	17997.558	30.354	18	.03	-5.646	12.783	1	.001	10.783
Dropped r_g	**17985.574**	**18.37**	**18**	**.43**	**-17.63**	**0.799**	**1**	**.27**	**-1.201**
Dropped r_g and r_c	18049.116	81.912	19	.001	43.912	64.341	2	.001	60.341

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences

Table 5.9 Solitariness: fit statistics of bivariate models of age 12 internalising traits and age 14 autistic traits subdomain

	Overall Fit of Model					Relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	18706.08								
ACE/ADE	18720.571	14.491	17	.63	-19.509				
Dropped r_d	**18720.927**	**14.847**	**18**	**.67**	**-21.153**	**0.356**	**1**	**0.55**	**-1.644**
Dropped r_g	18725.54	19.46	18	.36	-16.54	4.969	1	.03	2.969
Dropped r_g and r_c	18737.525	31.445	19	.04	-6.555	16.954	2	.001	12.954

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences

Table 5.10 Poor imagination: fit statistics of bivariate models of age 12 internalising traits and age 14 autistic traits subdomain

	Overall Fit of Model					Relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	17975.873								
ACE	17966.998	21.125	17	.22	-12.875				
Dropped r_c	18016.83	40.957	18	.001	4.957	19.832	1	.001	17.832
Dropped r_g	**17998.465**	**22.592**	**18**	**.21**	**-13.408**	**1.466**	**1**	**.23**	**-0.534**
Dropped r_g and r_c	18035.783	59.91	19	.001	21.91	38.784	2	.001	34.784

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences
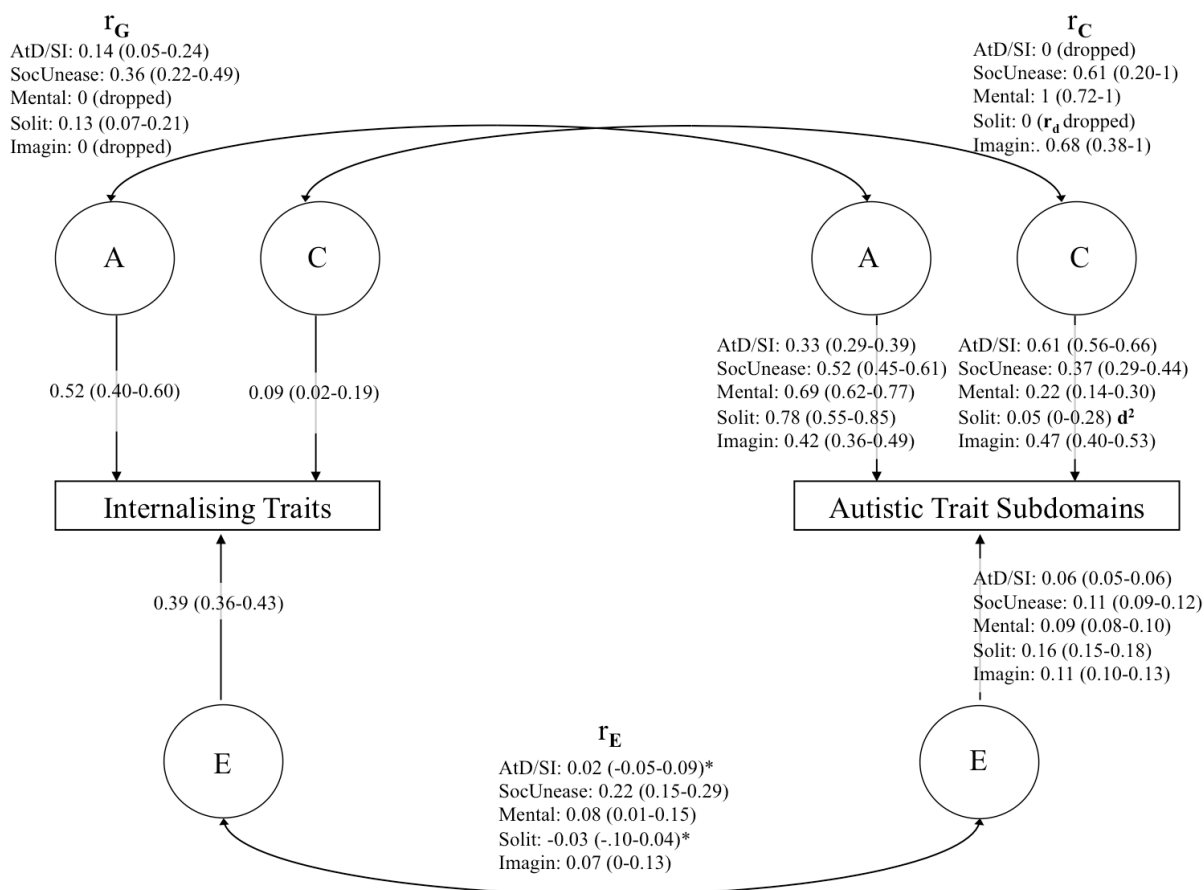
Figure 5.2 Path diagram showing results from the correlated factors solution of age 12 internalising traits and age 14 autistic trait subdomains bivariate models.

Note: AtD/SI = Attention to Details/ Special Interests; SocUnease = Social Unease; Mental = Poor Mentalising; Solit = Solitariness; Imagin = Poor Imagination; r_G = genetic correlation; r_C = shared environmental correlation; r_E = nonshared environmental correlation; *nonsignificant path (confidence intervals overlapping with 0). Solitariness model includes D = dominance effects and r_D = non-additive genetic correlation

5.4 Discussion

Following on from the previous chapter, the causes of autistic and internalising traits and their association in adolescence were further explored by studying the aetiological association between internalising traits and the five factor analysis-derived autistic trait subdomains.

5.4.1 Factor-derived autistic trait subdomains

In the current analysis, the overall autistic trait measure (AQ) was represented by five separable subdomains, which were empirically derived and conceptually represented Attention to Details/ Special Interests, Social Unease, Poor Mentalising, Solitariness and Poor Imagination. As discussed at the beginning of this chapter, previous factor analyses showed little agreement on factors found, such that the present study was carried out without strong predictions as to expected factor numbers and item loadings.

Attention to Details/ Special Interests, the first factor with the highest Eigenvalue, contained identical items to the 'numbers' factor of a previous factor analysis (Hoekstra et al., 2011). However, this previous choice of name did not easily map onto behaviours relevant for ASD diagnoses. Therefore the label of this subdomain was changed to 'Attention to Details/ Special Interests', which was seen as both a good fit of the items contained, and a better match with respect to terms used to describe autism symptoms. The items on this domain (e.g. S/he notices patterns in things all the time; S/he likes to collect information about categories of things) appear to be tapping subclinical behaviours relevant to RRBIs seen in ASD. A hypothesis linking the AQ's exemplar of a special interest on this factor scale to RRBIs in the clinical ASD population has been posited by Baron-Cohen's 'enhanced systemising' within the 'extreme male brain theory' (Baron-Cohen, 2010). In support of the validity of this AQ domain, it showed significant correlations with the RRBI subscale from a different measure of autistic traits (CAST) demonstrated in TEDS in mid-childhood (e.g. in Hallett et al., 2012).

The name of the second factor, Social Unease, was thought to reflect the fact that all items on the factor are either clearly addressing social flexibility or have the potential to act as social indicators. Factor four, Solitariness, contained items asking about individuals' tendency to avoid social situations and their need for aloneness. Factor three, Poor Mentalising, was descriptive of adolescents' ability to understand others people's beliefs and desires and intentions (theory of mind), while factor five, Poor Imagination, indicated whether they found it easy to attribute mental states to fictional characters and enjoyed pretending.

As Appendix III shows, correlations of AQ factors with CAST domains support the validity of the AQ subdomains in that the social/communication AQ subdomains show significant positive correlations with the CAST social-communication subscale, as would be expected, and significantly lower correlations with the CAST RRBI subscale. Further, the non-social AQ domain, now called 'Attention to Details/ Special Interests', shows a significant positive correlation with the CAST RRBI subscale, and significantly lower correlations with the CAST social-communication subscales, as would be expected. These correlations are based on cross-age comparisons because the CAST data were available at ages 8 and 12 years in the sample, and the AQ was collected at age 14 years. This will have the effect of dampening slightly the magnitude of the correlations between CAST and AQ, but despite this limitation these cross-age data provide support for the AQ domains' construct validity.

5.4.2 Association of adolescent internalising traits and autistic subdomains

Attention to Details/ Special Interests, Poor Imagination and Social Unease were moderately heritable, whilst Poor Mentalising and Solitariness more highly heritable. Solitariness also showed low dominance effects. Attention to Details/ Special Interests showed the highest degree of shared environmental influences, which were more moderate on the remaining three scales. Nonshared environmental influences were low for all subdomains. Interestingly, the lowest heritability and highest shared environmental estimate was observed for Attention to Details/ Special Interests, indicating a that shared environmental characteristics explain most variance in adolescents' mathematical, collector and special interest activities.

Genetic correlations for internalising traits with Poor Mentalising and with Poor Imagination were non-significant, suggesting that these traits are genetically independent. Genetic correlations between internalising traits and Attention to Details/ Special Interests and Solitariness were low, and moderate between internalising traits and Social Unease. The non-additive genetic correlation between internalising traits and Solitariness, and the shared-environmental correlation between internalising traits and Attention to Details/ Special Interests, could be dropped. The three other domains showed high shared environmental overlap. However, as raised in the previous chapter, this particular statistic must be interpreted with caution. Nonshared environmental correlations were low with the exception of Social Unease, which was moderate.

Nevertheless, the nonshared environmental overlap that was observed raises the question which specific nonshared environmental variables might explain this overlap.

5.4.2.1 Social Unease

Social Unease showed comparatively higher phenotypic and genetic correlations with internalising traits than the other AQ subdomains. In early adolescence, individuals who felt more uncomfortable in social situations also more frequently had anxious-depressed feelings, and this was partially due to the same genetic influences. The Social Unease scale contained two items that could be interpreted as also relating to internalising traits ('S/he finds social situations easy (reversed)' and 'New situations make him/her anxious'). In order to test whether these two items were driving the association between Social Unease and internalising traits, correlations and models were re-run between internalising traits and Social Unease after excluding these two items from the latter scale. Excluding these two items from the scale did not change results and the phenotypic correlation with internalising traits and the bivariate parameter estimates were similar as for the full Social Unease scale. Thus, Social Unease does not seem to be driven by social avoidance, but relates to one's ability to appropriately engage and feel comfortable in a social setting.

5.4.3 Limitations

As discussed above, in interpreting the results, subscales appeared to demonstrate good face validity in that it was possible to identify descriptive names for each one from phenotypic examination of the items. However, a number of issues resulting from the particular factor structure need to be raised. The number of items contained on the subdomains ranged from three to seven, resulting in different possible variances for each scale. Moreover, only the first two domains showed satisfactory reliability (>.70). In the split by sex sample, covariances of some subdomains with internalising traits were low, making twin models including quantitative sex differences not feasible.

Another limitation of this study is that it did not distinguish twins with respect to their clinical status, and it was possible that probands could have shown response patterns differentially affecting the factor domains. However, in basing all analyses on individual differences in trait levels, it is nevertheless desirable to use the full spectrum of phenotypic presentations. Past analyses in TEDS have shown that twin analysis results on autistic traits are consistent whether or not ASD cases are included in the sample e.g. Ronald et al. (2006). Excluding suspected ASD cases from the current sample did not significantly alter the phenotypic and twin correlations. Including

suspected cases is not limited to publications on the TEDS sample but instead is fairly common place within twin studies of autistic traits (see also Lundström et al., 2011). For these reasons, the suspected ASD cases were included on all analyses.

5.4.4 Conclusions and future directions

The results presented here open up several avenues for follow-up studies. First, no data is presently available as to what extent the AQ *subdomains* (e.g. as suggested by the test authors or derived by any of the previous factor analyses) correlate with *clinical* assessments of autism symptoms.

Second, while this study was able to explore autistic trait subdomains in relation to internalising traits, internalising trait subdomains could not be investigated because of the brief measure used. Nevertheless, some inferences can be made drawing on the age 8 findings in Hallett et al. (2012), reporting the greatest involvement of the internalising domains of negative affect and generalised anxiety with autistic trait domains of communication and RRBIs. Therefore, it would be interesting for future to research to explore whether these specific aspects of internalising difficulties continue to be most highly associated with autistic traits in adolescence, and particularly with Social Unease.

Third, how do associations at the extreme relate to clinical comorbidity? One hypothesis of interest is that individuals at the high end of the spectrum of autistic traits who show high degrees of Social Unease are at higher risk of developing internalising problems than those with similar autistic trait levels scoring highly on other subdomains. Relatedly, an important issue is to what extent anxiety and ASD overlap in clinical populations is similar to of anxiety and autistic trait overlap in the general population. Wood and Gadow (2010) propose that investigations should consider effects of both ASD-related stressors contributing to anxiety, and ASD symptom severity being mediated by anxiety, as well as measurement issues. However, the present study helps to address the question of to what extent autistic traits and internalising traits represent two separable phenotypes by providing information about their overlap in the general population.

Finally, the low-to-moderate univariate nonshared environmental influences found and the significant degree of overlap in nonshared environmental influences between internalising traits and Social Unease, demonstrate that individual twins in each pair are affected by twin-specific factors increasing or decreasing trait levels in adolescence. This is further explored in the following chapter.

Chapter 6 Examining the role of nonshared environment on autistic traits and internalising traits across development

While the two previous chapters have explored twin similarity, this chapter focuses on what can be learned about adolescent autistic traits and internalising traits studying twin differences. Focusing on nonshared environment, the aims of the following analyses are twofold. First, this study asks how do differences in autistic and internalising trait manifestations in mid-childhood relate to adolescent trait differences on these traits. Second, the question whether there are significant associations between trait differences on specific childhood environments and adolescent autistic and internalising trait differences is explored.

6.1 Background

6.1.1 Findings on the magnitude of Nonshared Environment in autistic and internalising traits and their co-occurrence

As previously discussed, both autistic traits and ASD are familial and subject to substantial causal genetic influences. Moreover, shared environmental factors serve to further increase twin similarity. However, studies also consistently report a degree of independence between MZ twins, finding differences in phenotypic outcomes. Within the classic twin design, MZ dissimilarities are used to obtain the nonshared environmental parameter estimate (see Chapter 2; n.b. – e^2 is inclusive of the error term). Findings from previous twin studies suggest a small but potentially important causal role of nonshared environment on outcomes on the autism and internalising phenotypes.

Existing twin studies on autistic and internalising traits have been reviewed extensively in Chapter 4 and only their findings relating to the extent of nonshared environmental influences are reiterated here. Results showed that in mid-childhood (Hallett et al., 2010), autistic traits demonstrated a moderate degree of nonshared environmental influences (age 8: $e^2 = .21$; age 12: $e^2 = .27$), and this figure was similar in a cross-childhood cohort combining 9 and 12 year-olds ($e^2 = .29$; Lundström et al., 2011). In comparison, the results on early adolescence autistic traits presented above in Chapter 4 suggest nonshared environmental influences have only a small role to play at age 14 ($e^2 = .07-.09$). Contrary to this, data from an adult sample (using self report) arrived at

much more substantial parameter estimates for nonshared environment (e^2 = .68, Lundström et al., 2011).

As was found in Chapter 5, specific autistic trait subdomains varied in the composition of their aetiological factors. With respect to nonshared environment, such influences ranged from being as modest as on the overall adolescent autistic trait scale (i.e. Attention to Details/ Special Interests: e^2 = .06) to more closely resembling the moderate mid-childhood estimates (Solitariness: e^2 = .16).

Nonshared environmental factors appeared to play a relatively greater role for the internalising phenotype, as there were lower degrees of twin similarity on these traits. For internalising traits, moderate nonshared environmental effects (age 8: e^2 = .22; age 12: e^2 = .41) were reported in mid-childhood and early adolescence (Hallett et al., 2010). More specifically for anxiety, estimates were substantial across mid-childhood (e^2 = .49, Lundström et al., 2011). Results from the adult sample suggest large nonshared environmental influences in adulthood (anxiety: e^2 = .87; depression: e^2 = .64, Lundström et al., 2011).

All studies find significant overlap of nonshared environmental factors between internalising and autistic traits. The highest nonshared environmental correlation between traits was reported for the mixed age child cohort (autistic traits – anxiety: re = .49, Lundström et al., 2011). Other results from specific developmental time points suggest lower overlap (autistic traits – internalising; age 8: re = .07; age 12: .14; age 12/14: re = .10-.20, Hallett et al., 2010; adolescent estimates from Chapter 4), as does the adult data (re = .10, Lundström et al., 2011). Similarly to the univariate findings on specific autistic trait subdomains in adolescence, Chapter 5 also showed that nonshared environmental overlap between individual subdomains and internalising traits varied from including confidence intervals across zero (Attention to Details/ Special Interests: r_e = .02; Solitariness: r_e = -.03) to showing significant and moderate overlap (Social Unease: r_e = .16).

6.1.2 The MZ differences design

Within this design, relative within twin-pair differences on one phenotype, correlated to such differences on another, are informative of the extent to which the two are related for reasons that are not shared with the other twin (Pike et al., 1996b). As MZ twins reared together share all of their genetic and shared environmental aetiology, the obtained correlations give a direct estimate of nonshared environmental effects (NSE).

For instance, choosing one phenotype to represent an environmental risk factor that precedes the second, psychopathological or personality trait, a significant association between the two suggests potential causal NSE effects of the former on the latter.

6.1.2.1 Sources of nonshared environmental effects

In essence, any phenotype that can be assessed for twins separately can be studied for its NSE effects. This includes (but is not limited to) twin-specific events (e.g. accidents), but also refers to the differential effects of shared experiences (e.g. parental divorce) on twins' phenotypes. A recent review (Plomin, 2011) summarising the advances made in identifying sources of differential experiences in the past three decades concludes that a) nearly all child-specific measures of the family environment show some differences between children growing up in the same family and b) much less is known about specific sources of nonshared experience outside the family.

6.1.3 Previous findings on specific nonshared environmental associations and causal effects

Existing MZ differences studies have uncovered specific environmental effects that individually typically explain 1-5% of total variance in phenotypic outcomes (Plomin, 2011; Turkheimer & Waldron, 2000). Since these effects are calculated as the squared correlations of specific nonshared environments with behavioural outcome variables, obtained NSE differences correlations were generally within the range of .10-.25. Plomin (2011) suggests that these effects are largely independent and known specific environments in the study of a variety of psychological traits typically add up to account for 13% of the total trait variance. He further argues that this means that about a quarter of idiosyncratic effects have already been identified if nonshared environment accounts for an average of 40% of trait variance on psychological traits. This latter assumption is consistent with the magnitude of e^2 on internalising traits at age 12 (see Chapter 4).

A number of NSE differences have been studied (both in MZ differences studies and in more complex full multivariate genetic designs) and the following variables have been identified as important (Plomin, 2011; Turkheimer & Waldron, 2000) for various psychiatric traits and behavioural problems. The greatest attention has been given to parenting and effects on adjustment. Significant effects on psychosocial outcomes were found for parental discipline (Asbury, Dunn, Pike, & Plomin, 2003), parental (and particularly maternal) negativity (Asbury et al., 2003; Beaver, 2008; Burt, McGue, & Iacono, 2009; Larsson, Viding, Rijsdijk, & Plomin, 2008; Mullineaux, Deater-Deckard, Petrill, & Thompson, 2009; Pike et al., 1996a) and physical and emotional abuse (Jaffee

et al., 2004; Mullineaux et al., 2009; Narusyte, Andershed, Neiderhiser, & Lichtenstein, 2007; Viding, Fontaine, Oliver, & Plomin, 2009), differential parent-child relationship (Burt, McGue, Iacono, & Krueger, 2006; Deater-Deckard et al., 2001). Further, perceptions of the classroom have been studied in relation to academic achievement (Asbury et al., 2008; Oliver et al., 2008). Recently, research has also made inroads into using the MZ differences design for the study of epigenetic effects, CNVs, and methylation (Bruder et al., 2008; Kaminsky et al., 2008; Mill et al., 2006; Poulsen, Esteller, Vaag, & Fraga, 2007), though the present study will not address such potential differences.

6.1.3.1 NSE difference studies on internalising

A number of previous studies provide results more closely related to internalising behaviour and deserve individual mention. First, 3 ½ year-olds according to one study (Deater-Deckard et al., 2001) received differential maternal treatment, and the twin who received more supportive and less punitive forms of parenting was also higher in positive mood and lower in negative mood (r = .38). Second, another study found moderate NSE difference correlations between birth weight and negative parental feelings with internalising traits at age 4 in TEDS (r = .19, Asbury et al., 2003). Third, Mullineaux et al. (2009) report that 4-8 year-old twins who were subject to more maternal negativity and less maternal warmth showed less positive social engagement (including positive affect; r = .28-.59), although no significant associations were found directly between maternal characteristics and child internalising behaviours. Fourth, in a sample of 10-18 year-old twins, MZ twin differences in depressive symptoms were moderately correlated with MZ twin differences in both maternal (r = .23) and paternal (r = .25) negativity (Pike et al., 1996b).

6.1.3.2 NSE difference studies on the autism phenotype

In contrast to the findings discussed above, existing MZ difference studies on the autism phenotype have not been primarily concerned with family environment, focusing instead on structural brain changes, potential biomarkers and important factors around birth. Research has been carried out both on twins discordant for clinical ASD; one study applied the MZ differences design to differential autistic trait levels in the general population.

First, one group has studied the differences in brain structure (Kates et al., 2004; Kates, Ikuta, & Burnette, 2009; Mitchell et al., 2009) showing that both concordant for ASD and discordant pairs demonstrated high degrees of similarity in the neuroanatomical

features studied. Significant differences were also found comparing affected twins compared to typically developing controls. A second group has been interested in gene expression and methylation profiles and biomarkers (Hu, Frank, Heine, Lee, & Quackenbush, 2006; Nguyen, Rauch, Pfeifer, & Hu, 2010; Sarachana, Zhou, Chen, Manji, & Hu, 2010). Third, most recently a study using the TEDS sample reported methylation differences in MZ twins discordant for ASD and autism-related traits using genome-wide methylation profiling (Wong et al., 2013).

Fourth, the NSE effect of pre/perinatal and neonatal complications on later NSE differences in autistic-like features at ages 7 and 8 years were studied in ~2,000 twin pairs in TEDS (Ronald, Happé, Dworzynski, Bolton, & Plomin, 2010). At age 7, social, but not non-social NSE autistic trait differences were significantly associated with NSE differences in pre/perinatal problems and neonatal problems (both r = .05). The same was true of lower birth weight (r = .07-.10), more time spent in hospital (r = .04-.05) and more days in special care (r = .05). At age 8, NSE differences in total autistic traits were partially predicted by NSE pre/perinatal (r = .05) and neonatal problem differences (r = .06). Twins with greater autistic-like social difficulties were more often the ones having spent more days in special care (r = .05), and autistic-like communication was associated with pre/perinatal and neonatal problems (both r = .06). Again, differential non-social autistic-like behaviours showed no significant association with any of the investigated NSE differences.

6.1.4 Objectives and selection of NSE differences measures
As just reviewed, candidate variables for MZ difference studies on internalising traits have focused on parental treatment before or during mid-childhood, while those on autistic traits have studied genetic, biological and medical factors, which may indicate a more general preference of research to focus on social vs. biological explanations for the respective phenotypes.

Initially, variables for the current study were selected exploring any previously implicated NSE variable from 6.1.3.1 and 6.1.3.2 for their (continued) effect on internalising and autistic trait differences in adolescence. In addition, the current study aimed to overcome the bias in variable selection for NSE differences by also testing more psychosocial concepts in association with autistic trait differences and exploring biological concepts in relation to internalising trait differences. The inclusion of such prior NSE variables was determined by their availability in TEDS. Second, additional

NSE variables were selected as factors of interest; the rational behind each of the NSE variables used in this chapter's analyses is introduced in the following section.

6.1.4.1 NSE differences in early trait manifestations

Autistic trait levels have been shown as phenotypically stable across development in the general population (Robinson et al., 2011b; Whitehouse, Hickey, & Ronald, 2011). The aetiology is similar in individuals with non-clinical trait levels and at the quantitative extreme (Robinson et al., 2011a). However, little is known to what extent earlier autistic traits are associated with later traits via NSE.

In contrast, it appears that internalising traits show an overall lower degree of phenotypic trait level stability. To the extent that stability does exist, this has been attributed by multiple studies to genetic causes (Bayer, Hastings, Sanson, Ukoumunne, & Rubin, 2010; Haberstick, Schmitz, Young, & Hewitt, 2005; Hoekstra et al., 2007a; Trzaskowski, Zavos, Haworth, Plomin, & Eley, 2012). More specifically in a study on genetic and environmental contributions to stability and change in children's internalising traits, half of the genetic influences were stable across time in early childhood (4-7 years), but this figure was much more modest ($r^2 = 4\%$) for common nonshared environmental influences (van der Valk, van den Oord, Verhulst, & Boomsma, 2003). This would suggest that the following analyses are likely to find significant modest-to moderate ($r \sim \sqrt{.04} = .20$) NSE correlations between earlier and later internalising trait differences.

The longitudinal association on internalising and autistic traits has also been studied in mid-childhood (ages 8-12 years), replicating moderate overall phenotypic trait stability (b = .37) for internalising traits and substantial phenotypic autistic trait stability (b = .51-.56; Hallett et al., 2010). In addition, the study also showed significant but low (b = .06) cross-trait effects of earlier internalising traits on later autistic traits, and reversely, earlier autistic on later internalising traits showed a stronger, modest effect (b = .10-.15). This suggests that at the aetiological level there could also be small significant longitudinal NSE effects between the two traits. The first part of the current study is to study how NSE differences in internalising traits (at ages 2, 3, 4, 7, 9 years) and autistic traits (at ages 8, 9 years) are correlated with NSE on adolescent internalising traits (at age 12 years) and autistic traits (at age 14 years).

6.1.4.2 NSE differences in externalising traits

Externalising traits including hyperactivity and conduct problems have been conceptualised as clearly behaviourally distinct to internalising traits and are thus of particular interest for their association with internalising traits. Further, externalising disorders are also of great relevance to the study of autistic traits since ADHD is the second most common comorbidity within ASD after internalising disorders (e.g. (Simonoff et al., 2008). Mixed results have been reported as to the significance of longitudinal effects of externalising on later internalising disorders throughout childhood at the phenotypic level (Fischer, Rolf, Hasazi, & Cummings, 1984; Somersalo, Solantaus, & Almqvist, 1999). In a recent longitudinal study (Taylor et al., 2013) on the association of ADHD and autistic traits in mid-childhood (8-12 years), earlier ADHD had a greater effect on later autistic trait presentation than vice versa. It is hypothesised that the current study will find significant NSE difference associations of externalising traits (conduct problems and hyperactivity at ages 4, 7, 9, 12 years) with adolescent internalising traits (at age 12 years) and autistic traits (at age 14 years).

6.1.4.3 NSE differences in psychosocial development variables

Differences in parental negativity and parental discipline have been shown to have NSE effects on a range of phenotypes including early childhood internalising (Asbury et al., 2003), but this has not been tested in relation to internalising in adolescence or autistic traits at any time. The question, which other *specific* environmental factors may contribute to children's and adolescents' psychological adjustment and psychosocial development brings to mind a wide range of possible factors. Recently, combining previous existing instruments on environment relevant to psychosocial development and personal growth including those used in behaviour genetic work, a study has identified six relevant types of such environments: Parental warmth and support, school discipline, sibling warmth, family openness, family conflicts, and peer relations and support (Persson, 2011). As best available proxies in TEDS, peer problems and prosocial behaviour (at ages 4, 7, 9, 12 years), as well as overall academic liking and overall perceived academic ability (at ages 9, 12 years) were chosen and their NSE difference associations with adolescent internalising traits (at age 12 years) and autistic traits (at age 14 years) tested.

6.1.4.4 NSE differences in pre/perinatal, neonatal problems and birth weight

As discussed above, MZ differences in pre/perinatal and neonatal problems as well as differences in birth weight have been found to show NSE effects on mid-childhood autistic trait differences (Ronald et al., 2010), and the current study will test for their continued effect in adolescence (at age 14 years), as well as on adolescent internalising trait differences (at age 12 years).

6.1.4.5 Summary

The present study investigates firstly, whether the twin with greater internalising trait levels in earlier childhood is also the twin with greater difficulties on the same trait in early adolescence. The same question is asked about autistic traits. Further, it is investigated whether such NSE difference associations exist across time and across traits. The second question that the following analyses are striving to address is which of the potentially influential childhood environments serves to make twins different on autistic traits and internalising traits in early adolescence using the MZ twin differences design. Small significant effects on trait pairs via NSE are predicted.

6.2 Methods

6.2.1 Sample

This study uses the same sample and exclusion criteria as described in 4.2.1.5. Briefly, twins were excluded for severe medical conditions and chromosomal abnormalities. Further, parent report data on 14-year autistic traits and 12-year internalising traits had to be available. Having satisfied these conditions, in the following analyses, only data of MZ twins were used (N = 1,225 twins, ~600 pairs).

6.2.2 Measures

Childhood measures were selected among available measures in TEDS, including early manifestations of internalising and autistic traits, externalising traits, peer problems, prosocial behaviour, academic liking and academic perceived ability, pre/perinatal, neonatal problems and birth weight. Whenever available, parent report on measures was preferred in order to obtain within-rater associations with the parent rated adolescent trait measures. Where necessary, scales were transformed for normalisation and mean effects of sex and age regressed out as laid out in Chapter 2.

6.2.2.1 Childhood and adolescent internalising traits

Ages 4, 7, 9 years and early adolescent internalising traits at age 12 were measured on the emotional symptoms subscale of the SDQ, the measure used in the previous chapters (described in 4.2.2.1). Childhood internalising traits at ages 2 and 3 years were assessed using items of the anxiety subscale of the Preschool Behaviour Questionnaire (Behar, 1977) and the Revised Rutter Parent Scale for Preschool Children (RRPSPC; (Behar & Stringfield, 1974). The six items respectively ask if the child worries, is solitary, miserable, afraid of new things, cries easily and stares blankly. They are scored on a 3-point Likert scale (0-2, does not apply/ applies sometimes/ frequently applies). The questionnaire is an extension of the Children's Behaviour Questionnaire (Rutter, 1967) into early childhood, a measure which is also a common predecessor with the structurally and conceptually closely similar SDQ.

6.2.2.2 Childhood and adolescent autistic traits

At ages 8 and 9 years, autistic traits were assessed on the CAST using the total score of 31 questions, with response format yes (1) or no (0). At age 14 years, the 24-item version of the AQ was used (cf. Chapter 5). Both measures have been described previously in (see 4.2.2.2).

6.2.2.3 Childhood externalising traits

Data on conduct problems and hyperactivity was available as part of the SDQ measure at ages 4, 7, 9 and 12 years, which also recorded the internalising scale used in this and in previous chapters. For conduct problems, the five items contained on the scale related to children's tendencies to lie, fight, steal, have a hot temper, and be obedient (reversed). Hyperactivity was assessed on five items exploring parent ratings on how distractible, persistent (reversed), restless, fidgety and reflective (reversed) they thought their children were.

6.2.2.4 Childhood psychosocial development variables

Parental negativity and parental discipline scales at ages 3 and 4 years were equivalent to those in Asbury et al. (2003) and Asbury, Dunn, and Plomin (2006). Parental discipline related to parents' approach to disciplining their child ranging from authoritarian and physically involved to withdrawn and lenient behaviours. Parental negativity assessed parents' feelings of happiness and closeness or alternatively anger with each twin. Peer problems and pro-social behaviour were assessed on the SDQ at ages 4, 7, 9 and 12 years. Peer problems assessed children's tendency to like and to be liked by peers, while pro-social behaviour was descriptive of children's willingness to

help. Scales contained five items each, scored on 3-point Likert scales (0-2). Example items are 'Picked on or bullied by other children' and 'Shares readily with other children' respectively.

Overall academic liking, and perceived academic ability by parent report at age 9 and self report at age 12 years corresponded to previously used scales to study NSE differences in TEDS (Greven, Harlaar, Kovas, Chamorro-Premuzic, & Plomin, 2009; Oliver et al., 2008). Academic liking contained nine items, of which three each referred to children's attitude to various tasks in school typical for English, Maths and Science classes. Perceived academic ability assessed the same nine items with respect to children's performance as judged by parents (at age 9 years) or children themselves (at age 12 years).

6.2.2.5 Pre/perinatal, neonatal problems and birth weight

These variables were equivalent to Ronald et al. (2010)'s previously used scales studying MZ differences and included birth weight, pre/perinatal problems and neonatal problems. The pre/perinatal problems questionnaire included 12 items about pregnancy, six about the labour. The neonatal problem scale included ten variables that were both continuous variables (degree of smoking and alcohol intake during pregnancy, the length of the labour, the length of gestation, the weight and length of the twins, time spent in hospital and in special care, and the number of days breastfed) and categorical variables such as whether the mother had amniocentesis during pregnancy (a detailed description is provided in Ronald et al., 2010).

6.3   Analysis

Data preparation was carried out using the strategies outlined in Chapter 2. Specifically, scales were created by summing items equally and converting to proportions of the total possible score given the number of items completed and using mean replacement (see section 4.3 for formula). Scale transformation was applied where appropriate. Data was coded as missing if half or less of the items were available.

6.3.1 MZ differences method: Creation of NSE difference variables

MZ twins provide a tool for the identification of specific nonshared environmental factors since they do not differ genetically (sharing close to 100% of their genes) and shared environment only further increases twin similarity. MZ twin differences are thus a direct index of the nonshared environment.

The aim of this design is to gain insight to what extent *twin differences* on an *environmental variable* A (e.g. birth weight) predict *twin differences* on an *outcome variable* X (e.g. adolescent autistic traits, adolescent internalising traits). A perfect correlation of 1.0 would mean that different experiences with this particular environment account for all of the twin differences on the outcome phenotype. Alternatively, the associations of differences in an *earlier psychiatric trait manifestation* B (early autistic traits, early internalising traits) and differences in outcomes in later life can also be studied.

In order to achieve this, relative difference scores are calculated subtracting MZ twin 2's score from twin 1's score on all measures (Pike et al., 1996b). Birth order was controlled for as per the selection procedures outlined in the methods in Chapter 2. NSE difference scores are then correlated with one another. Standardised scores are used to account for different ranges of scales across measures, and to simplify interpretation of twin scores with respect to the population mean during analyses.

$$Environmental\ variable\text{:} NSE_A \ = \ z(A_{twin1}) - \ z(A_{twin2})$$

$$Outcome\ variable\text{:} NSE_X \ = \ z(X_{twin1}) - z(X_{twin2})$$

$$Association\ of\ MZ\ differences\text{:} NSE_A * NSE_X$$

6.4 Results

Table 6.1 shows the descriptive statistics for the two adolescent variables – autistic traits at age 14 and internalising traits at age 12. These two variables are tested for their NSE association with all of the following childhood variables. The first mean in the descriptives tables displays the raw mean on all variables, while means for males and females are expressed with respect to their deviation from the overall sample mean.

Table 6.1 Adolescence variables (age 14 autistic traits, age 12 internalising traits): descriptives

Trait	Age (yrs)	All				Males				Females			
		N	Mean	Range	rMZ[a]	N	Mean	SD	rMZ[a]	N	Mean	SD	rMZ
Autistic traits	14	1225	20.69	0-72	.90	548	0.10	1.01	.91	677	-0.08	0.99	.89
Internalising traits	12	1091	1.62	0-10	.61	484	-0.08	0.92	.61	607	0.07	1.05	.60

[a] all p <.01

6.4.1 Childhood and Adolescent NSE Autistic and Internalising trait differences

Table 6.2 shows the descriptive statistics for earlier trait manifestations of internalising and autistic traits. All correlations between twins on the same variable are significant and all variables also show a degree of nonshared environment (rMZ = .46-.96).

Table 6.2 Childhood autistic traits, internalising traits: descriptives

Trait	Age (yrs)	All				Males				Females			
		N	Mean	Range	**rMZ[a]**	N	Mean	SD	**rMZ[a]**	N	Mean	SD	**rMZ**
Autistic traits													
	8	1034	4.7	0-31	**.81**	465	0.20	1.06	**.83**	569	-0.17	0.91	**.78**
	9	734	6.42	0-38	**.87**	317	0.14	0.99	**.86**	417	-0.11	0.99	**.88**
Internalising traits													
	2	777	3.26	0-12	**.54**	332	-0.03	0.95	**.52**	445	0.02	1.03	**.50**
	3	855	3.38	0-12	**.53**	383	-0.04	0.97	**.56**	472	0.03	1.03	**.52**
	4	1024	1.22	0-10	**.56**	457	-0.04	1.04	**.56**	567	0.03	0.97	**.56**
	7	1091	2.17	0-10	**.57**	483	-0.13	0.94	**.58**	608	0.10	1.03	**.56**
	9	733	1.68	0-10	**.61**	317	-0.13	0.91	**.65**	416	0.10	1.05	**.57**

[a] all p <.01. rMZ = phenotypic correlation between monozygotic twins. Significant values (p <.05) in bold.

Table 6.3 reports correlations between twins' childhood NSE differences on internalising and autistic traits with NSE differences in early adolescence on the same traits.

Correlations of childhood autistic trait NSE differences with adolescent autistic trait NSE differences are significant and moderate for both 8 and 9 years (r = .14-.20, all p <.01). The autistic trait longitudinal NSE differences in childhood correlated modestly with adolescent internalising NSE trait differences (r = .04-.12, p <ns-.01). Associations of NSE differences of childhood internalising trait differences with those on later internalising traits reached significance at age 3 years and increased across childhood (r = .02-.43, p <ns-.01). With exception of age 9, NSE differences associations of childhood internalising with later autistic traits were non-significant (r = -.04-.16, p <ns-.01).

Table 6.3 Childhood autistic and internalising traits: early NSE differences and association with adolescent NSE differences on age 14 autistic and age 12 internalising traits

| Childhood trait measures | Age (yrs) | Adolescent trait measures | | | | | |
| | | All | | Males | | Females | |
		Aut	Int	Aut	Int	Aut	Int
Autistic traits	8	**.15**[**]	.06	**.14**[**]	.05	**.15**[**]	.06
	9	**.17**[**]	**.08**[*]	**.20**[**]	**.12**[*]	**.15**[**]	.04
Internalising traits	2	.07	.05	.05	.02	.08	.07
	3	.04	**.08**[*]	**.10**[*]	.07	0	.08
	4	-.03	**.09**[**]	-.04	**.14**[**]	-.03	.05
	7	.05	**.03**[**]	.02	**.29**[**]	.07	**.25**[**]
	9	**.11**[**]	**.41**[**]	.03	**.38**[**]	**.16**[**]	**.43**[**]

* p <.05; ** p <.01. Aut = Autistic traits (age 14); Int = Internalising traits (age 12). Significant values (p <.05) in bold.

6.4.2 Childhood NSE externalising trait differences and Adolescent NSE Autistic and Internalising trait differences

Table 6.4 shows the descriptive statistics for hyperactivity and conduct problems. Table 6.5 shows the longitudinal correlations of NSE conduct and hyperactivity differences with adolescent NSE differences on autistic and internalising traits.

Table 6.4 Childhood externalising traits: descriptives

Trait	Age (yrs)	All				Males				Females			
		N	Mean	Range	rMZ[a]	N	Mean	SD	rMZ[a]	N	Mean	SD	rM...
Conduct problems													
	4	1023	1.91	0-10	**.64**	457	0.14	1.02	**.65**	566	-0.12	0.97	.6...
	7	1091	1.57	0-10	**.74**	483	0.11	1.03	**.76**	608	-0.09	0.97	.7...
	9	733	1.19	0-10	**.80**	317	0.19	1.09	**.82**	416	-0.14	0.90	.7...
	12	1090	1.119	0-10	**.78**	483	0.12	1.04	**.75**	607	-0.10	0.95	.7...
Hyperactivity													
	4	1022	3.84	0-10	**.50**	457	0.15	1.04	**.53**	565	-0.13	0.95	.4...
	7	1091	3.34	0-10	**.56**	483	0.16	1.03	**.59**	608	-0.13	0.96	.5...
	9	733	3.15	0-10	**.72**	317	0.21	1.07	**.71**	416	-0.16	0.91	.7...
	12	1091	2.7	0-10	**.76**	484	0.28	1.04	**.74**	607	-0.22	0.91	.7...

[a] all p <.01. rMZ = phenotypic correlation between monozygotic twins. Significant values (p <.05) in bold.

For conduct problems, NSE at age 4 years showed a weak negative correlation with later NSE differences on internalising traits (r = -.07, p <.05). At age 7, NSE differences on conduct problems showed a small positive correlation with NSE differences on adolescent autistic traits (r = .07, p <.05). No significant NSE differences associations were present at age 9, but weak significant correlations for females were found. By early adolescence, the association of NSE conduct problems differences with NSE internalising trait differences is positive (r = .11, p <.01).

For hyperactivity, NSE effects showed modest longitudinal associations with NSE adolescent trait differences on both autistic and internalising traits: NSE hyperactivity differences were significantly correlated with NSE autistic trait differences at age 4 and 12 (r = .07-.08, p <.05), and with NSE internalising trait differences at 7, 9 and 12 years (r = .06-.08, p <.05-.01). A clearer pattern emerges from the split sample, showing NSE hyperactivity differences to be associated with NSE autistic trait differences in females, and with NSE internalising differences in males.

Table 6.5 Childhood externalising traits: early NSE differences and association with adolescent NSE differences on age 14 autistic and age 12 internalising traits

Trait	Age (yrs)	All		Males		Females	
		Aut	Int	Aut	Int	Aut	Int
Conduct problems							
	4	-.02	**-.07**[*]	-.07	-.05	.03	-.08
	7	**.07**[*]	.05	.10[*]	.03	.06	.06
	9	-.01	.05	-.07	-.05	.03	**.12**[*]
	12	.06	**.11**[**]	**.09**[*]	**.14**[**]	.03	**.08**[*]
Hyperactivity							
	4	**.07**[*]	.06	.02	**.15**[**]	**.11**[*]	0
	7	.02	**.06**[*]	-.02	**.14**[**]	.05	.01
	9	.04	**.08**[*]	-.05	**.13**[*]	**.12**[*]	.03
	12	**.08**[*]	**.08**[**]	.02	**.15**[**]	**.13**[**]	.02

* p <.05; ** p <.01. Aut = Autistic traits; Int = Internalising traits. Significant values (p <.05) in bold.

6.4.3 Childhood NSE Psychosocial development differences and Adolescent NSE Autistic and Internalising trait differences

Table 6.6 presents the descriptives and Table 6.7 shows the correlations of NSE differences in twins' adolescent trait presentation on internalising and autistic traits with NSE differences on variables relevant to their psychosocial development.

There were no significant NSE differences associations for either parental discipline or parental negativity with adolescent measures of autistic and internalising traits. Peer problems NSE twin differences in childhood were the most highly correlated variable in this set of analyses (all but one NSE association with autistic and internalising traits significant) and showed increasing associations across development (autistic traits: $r = .07\text{-}.20$, internalising traits: $r = .05\text{-}.22$). NSE differences on prosocial behaviour were also assessed and showed significant negative correlations with NSE autistic trait differences in males from 7 years onwards ($r = -.10\text{--}-.13$). Academic liking showed no significant NSE associations in the overall sample. In early adolescence, NSE differences on perceived academic ability showed significant negative associations with NSE differences on internalising traits ($r = -.09$, $p < .01$), though not with NSE differences on autistic traits.

Table 6.6 Childhood psychosocial development variables: descriptives

Trait	Age (yrs)	All				Males				Females			
		N	Mean	Range	rMZ[a]	N	Mean	SD	rMZ[a]	N	Mean	SD	rMZ[a]
Parental discipline	3	857	0.08	-9-7	**.79**	386	-0.03	1.00	**.79**	471	0.02	1.00	**.80**
	4	1036	0.09	-9-7	**.83**	463	0.09	1.00	**.82**	573	-0.08	0.99	**.83**
Parental negativity	3	852	0.07	-9-7	**.73**	385	0.00	0.96	**.71**	467	0.00	1.03	**.74**
	4	1039	-0.05	-9-7	**.71**	465	0.04	0.97	**.73**	574	-0.03	1.03	**.70**
Peer problems	4	1023	1.24	0-10	**.69**	457	0.09	1.04	**.74**	566	-0.07	0.96	**.64**
	7	1091	0.82	0-10	**.61**	483	0.07	1.03	**.59**	608	-0.06	0.97	**.62**
	9	733	0.9	0-10	**.71**	317	0.07	1.00	**.72**	416	-0.05	1.00	**.70**
	12	1091	1	0-10	**.76**	484	0.09	1.06	**.76**	607	-0.07	0.94	**.76**
Prosocial behaviour	4	1024	7.35	0-10	**.58**	457	-0.15	1.00	**.57**	567	0.12	0.98	**.56**
	7	1091	8.22	0-10	**.65**	483	-0.17	1.01	**.67**	608	0.14	0.97	**.61**
	9	735	8.32	0-10	**.82**	318	-0.23	1.11	**.80**	417	0.17	0.87	**.83**
	12	1090	8.61	0-10	**.72**	483	-0.14	1.05	**.79**	607	0.12	0.94	**.63**
Academic liking	9	729	3.97	1-5	**.85**	317	-0.06	1.04	**.83**	412	0.05	0.97	**.86**
	12[b]	1087	3.51	1-5	**.51**	486	-0.03	1.00	**.49**	601	0.03	1.00	**.54**
Perceived academic ability	9	730	4.09	1-5	**.90**	316	0.00	1.03	**.89**	414	0.00	0.98	**.91**
	12[b]	1087	3.91	1-5	**.58**	487	0.08	0.97	**.57**	600	-0.06	1.02	**.58**

[a] all p <.01. [b] children's self report. rMZ = phenotypic correlation between monozygotic twins. Significant values (p <.05) in bold.

Table 6.7 Childhood psychosocial development: early NSE differences and association with adolescent NSE differences on age 14 autistic and age 12 internalising traits

Trait	Age (yrs)	All		Males		Females	
		Aut	Int	Aut	Int	Aut	Int
Parental discipline							
	3	-.04	.01	-.01	.01	-.06	0
	4	0	-.02	-.08	-.07	.06	.01
Parental negativity							
	3	.01	-.04	.03	-.01	-.02	-.08
	4	.03	-.02	.07	.01	.01	-.04
Peer problems							
	4	**.07***	.05	.03	.04	**.10***	.05
	7	**.10****	**.07***	.08	**.12***	**.12****	.02
	9	**.12****	**.13****	.08	**.17****	**.14****	**.11***
	12	**.20****	**.22****	**.23****	**.26****	**.18****	**.19****
Prosocial behaviour							
	4	-.02	.03	.05	.02	-.08	.03
	7	-.04	.03	**-.11***	-.04	.01	.08
	9	-.02	-.01	**-.13***	-.04	.09	.04
	12	-.06	.05	**-.10***	.02	-.04	.08
Academic liking							
	9	-.02	-.05	.09	**-.12***	**-.13****	.01
	12^b	0	-.03	.01	-.09	-.02	.02
Perceived academic ability							
	9	-.05	-.05	.03	**-.12***	**-.11***	.01
	12^b	-.03	**-.09****	.01	**-.15****	-.06	-.04

* p <.05; ** p <.01. Aut = Autistic traits; Int = Internalising traits. ^{b} children's self report. Significant values (p <.05) in bold.

6.4.4 Pre/perinatal, neonatal and birth weight NSE differences and Adolescent NSE Autistic and Internalising trait differences

Descriptives and correlations of twin differences on autistic, internalising, and co-occurrence traits with birth weight, pre/perinatal and neonatal problems are shown in Table 6.8 and Table 6.9 respectively. Of the first year variables, only birth weight was significantly negatively correlated (autistic traits: r = -.09, internalising traits: r = -.10, p <.01), and the split sample indicates that the females largely drove this association.

Table 6.8 Pre/perinatal, neonatal problems and birth weight variables: descriptives

Trait	All				Males				Females			
	N	Mean	Range	**rMZ[a]**	N	Mean	SD	**rMZ[a]**	N	Mean	SD	**rMZ**
Birth weight (in grams)	1194	2434	596-4560	**.47**	529	0.06	1.05	**.50**	665	-0.05	0.95	**.43**
Neonatal problems	1223	1.71	0-10	**.72**	547	0.13	1.03	**.72**	676	-0.10	0.97	**.70**
Pre/perinatal problems	1221	4.69	0-17	**.91**	546	0.08	1.02	**.91**	675	-0.06	0.98	**.91**

[a] all p <.01. rMZ = phenotypic correlation between monozygotic twins. Significant values (p <.05) in bold.

Table 6.9 Pre/perinatal, neonatal problems and birth weight variables: early NSE differences and association with adolescent NSE differences on age 14 autistic and age 12 internalising traits

Trait	All		Males		Females	
	Aut	Int	Aut	Int	Aut	Int
Birth weight	**-.09**[**]	**-.10**[**]	.01	-.06	**-.16**[**]	**-.13**[**]
Neonatal problems	-.03	.01	-.04	.01	-.01	.02
Pre/perinatal problems	-.02	0	-.04	0	-.01	.01

* p <.05; ** p <.01. Aut = Autistic traits; Int = Internalising traits. Significant values (p <.05) in bold.

6.5 Discussion

The present study examined NSE effects in early adolescence, studying the association of internalising and autistic trait differences at age 12/14 years first with earlier within- and cross-trait level NSE differences, second with NSE externalising trait differences and third with NSE differences on a range of specific environmental variables.

6.5.1 A general note on the patterns observed

The measures that permitted investigation of NSE effects on adolescent trait differences at multiple time points showed association patterns some of which increased in their magnitude with increasing proximity of the first measure with the adolescent measure (e.g. early with later internalising). This was expected. As discussed in the introduction, both autistic traits and internalising traits show an extent of phenotypic trait stability. The current study shows that this replicates at the aetiological level for NSE as more recently arisen twin differences were more predictive of dissimilarities in early adolescence. However, the magnitude of some associations was also very consistent across time (e.g. male hyperactivity with later internalising). Next, some childhood variables consistently failed to show significant NSE associations (parental discipline, parental negativity, prosocial behaviour, academic liking, neonatal problems and pre/ perinatal problems), while others reached significance on single occasions (perceived academic ability). This highlights the importance of including longitudinal data across child development in order to have guidance on how individual NSE effects should be interpreted.

6.5.2 NSE effects of differences in earlier trait manifestations on later internalising, autistic trait differences

Associations of earlier trait manifestations with later differences on the same trait revealed modest correlations for autistic traits in mid-childhood (age 8/9 years: r = .15-.17) and moderate correlations at the same age for internalising traits (age 9 years: r = .41). At first, this appears to suggest large differences in NSE effects for the two traits. However, it should be borne in mind that the correlation relating to autistic traits was performed on age 9/14 years using different measures, while for internalising this was age 9/12 years on the same instrument. Second, a more useful statistic is, to compare the percentage of variance in nonshared environment this association accounts for at their respective ages (this is the squared correlation divided by the nonshared environmental estimate e^2 from Chapter 4 at age 12 and age 14 respectively). This approach reveals that although the amount of adolescent trait variance accounted for by earlier

differences does indeed differ (2-4% and 14-18%), this gap narrows when viewed as a function of the relative importance of nonshared environmental aspects on the respective trait (i.e. $\frac{r^2_{(NSEchildhood \times NSEadolescence)}}{e^2_{(adolescent\ trait)}}$; autistic traits: males $= \frac{.20^2}{.07} = 57\%$, females $= \frac{.15^2}{.09} = 25\%$; internalising: males $= \frac{.38^2}{.39} = 37\%$, females $= \frac{.43^2}{.40} = 46\%$).

Beyond such NSE contributions of early towards later trait differences on the same trait, small cross-trait NSE difference effects were also observed in mid-childhood, such that the twin with greater mid-childhood autistic traits was also the twin with greater adolescent internalising traits and vice versa. These cross-trait NSE were of the magnitude of 1-2% on total variance.

6.5.3 NSE effects of externalising trait differences on internalising, autistic trait differences in early adolescence

No clear pattern was found for conduct problems. Childhood hyperactivity was associated via NSE with adolescent internalising behaviours in males, and autistic traits in females (both predicted 2% of total variance). The associations with internalising trait differences are in line with previous findings (Kessler et al., 2006) that in the general adult population, 10-13% of individuals with internalising disorders also meet diagnostic criteria for ADHD, and even more (38-47%) of those with a primary diagnosis of ADHD go on to develop an internalising disorder. In addition, the frequent symptom overlap between autistic and ADHD traits has been reported (Gargaro, Rinehart, Bradshaw, Tonge, & Sheppard, 2011; Taurines et al., 2012).

With respect to the sex differences found, at the phenotypic level it has previously been suggested (Dworzynski et al., 2012) that in order for girls to meet ASD diagnostic criteria they often show a range of additional problems, in the absence of which autistic traits do not translate into clinically relevant symptomatology. Thus, the NSE association of earlier hyperactivity and adolescent autistic trait differences may indicate the effects of such added 'stress' in females. Importantly, the current analyses also go beyond previous phenotypic descriptions and indicate that nonshared environment acts as a significant contributing factor.

6.5.4 NSE effects of differences in psychosocial development variables on internalising, autistic trait differences in early adolescence

While one previous study reported NSE effects of ~4% of differential parental feelings (positive and negative discipline, positivity and negativity) on NSE internalising trait

differences at age 4 years (Asbury et al., 2003), such early parenting differences were no longer significantly associated with either autistic or internalising trait differences in early adolescence. For autistic traits, the lack of a significant association is perhaps not surprising given that social theories (e.g. 'refrigerator mother') have been unsuccessful in fully explaining outcomes on this phenotype. Parental aloofness may thus reflect wider characteristics of the broader autism phenotype that are unlikely to result in NSE effects via *differential* parental treatment. For internalising traits, a possible reason is that as adolescents become increasingly independent, they rely less on their parents' high regard and approval, and therefore protective effects of differential positive parental behaviour, and negative effects of detrimental parental behaviour play a lesser role for how different twins are from each other on adolescent internalising traits. Instead, peer relations may replace the normative role of parents. Indeed, in the current study childhood peer problems was one of the variables most consistently showing significant NSE effects across childhood in relation to adolescent trait differences accounting for 2% of total variance on autistic traits in females, and 3% on internalising traits in males. In this context it is important to note that the undertaken set of analyses focused on the investigation of effects of early peer problems on later autistic-like and internalising behaviour but the reverse direction of causation, specifically the extent to which early psychiatric traits cause peer problems was not studied. However, reciprocal effects are likely and it has been shown that it is possible to discriminate between autistic and typically developing children in toddlerhood based on their behaviour toward peers (Clifford, Young, & Williamson, 2007). Beyond the associations with problem scales, the prosocial behaviour scale also significantly predicted lowered autistic-like behaviour (2%), but only in males.

Against the initial hypothesis of school environment as an important predictor of psychological outcomes, the associations found were only modest. No clear pattern emerged for academic liking. For males, NSE differences in perceived academic ability were negatively associated with NSE differences in adolescent internalising traits (2%). Tentatively, this association relates to males' self-confidence where greater levels could be expected to result in relatively lower internalising traits. However, the failure to detect greater effects may be owed to timing effects. Specifically, at the phenotypic level, one study (Masten et al., 2005) found that in childhood, it was externalising problems that undermined academic competence by adolescence. This in turn showed a negative effect on internalising traits in young adulthood. This cascade of effects suggests that results may not have been very clear as the window investigated precedes

that of relevance for the traits studied, and the direction of effects may be reversed. However, the just mentioned phenotypic results do not permit any direct inferences on NSE differences.

6.5.5 NSE effects of differences in pre/perinatal problems, neonatal problems and birth weight on internalising, autistic trait differences in early adolescence

A previous MZ difference study by Ronald et al. (2010) reported small negative significant NSE difference effects of prenatal, neonatal problems and birth weight (<1%) on mid-childhood NSE autistic trait differences at ages 7/8 years. When examined for their role in early adolescence NSE autistic trait differences, only NSE birth weight differences remained a significant predictor; in addition, it also correlated with adolescent internalising trait differences. Both NSE effects were driven by females (2% and 3% respectively) and were nonsignificant in males only. Interestingly, birth weight differences were also found to have a significant NSE effect on academic achievement in mid-childhood, as well as on a range of psychological traits in toddlerhood (Asbury et al., 2006), and the effect size increased when selecting the most discordant MZ pairs. These findings confirm that low birth weight is an important NSE general predictor in child development not only of medical outcomes (e.g. (Royer, 2009) and is not specific to differences in outcomes on specific phenotypes such as internalising and autistic traits.

6.5.6 Limitations

The environments, behaviours, attitudes and traits investigated in this study were collected using a variety of measures at different ages and drawing on parent and self report. Therefore, the magnitudes of significant NSE difference associations found should not be used to draw inferences about which environments are more or less influential when different raters/ measures have been used, as associations across raters and measures would have been more difficult to detect than those within rater and measure.

Next, the ability to detect NSE effects across traits for pairing with adolescent autistic trait differences was somewhat limited by the high similarity of TEDS twins on this trait in early adolescence (rMZ = .90). As discussed previously in Chapter 4 and Chapter 5, this represents an increased parent rating of twin similarity compared to age 12 – and as the next Chapter will show – twins' self ratings do however suggest a greater role for nonshared environment. Some of the MZ differences correlation may also be

attributable to correlated measurement error. Correlated measurement error has received little attention but has been suggested by some to have small significant effects on some medical phenotypes (Rifkin, 1995). Further, as previously discussed (Chapter 3) although twinness does not have an effect on overall psychological trait *levels*, environmental variables such as birth weight are known to be different on average in twins compared to singletons.

Nevertheless, the nonshared environmental influences found at age 14 (e^2 = .07-.11) would have set upper expected NSE differences at maximally a moderate level (r = .33). With nonshared environmental influences of ~40% on internalising traits, the scope for discovery of relevant NSE effects was much greater. The contributions the reported NSE differences make to individual traits may thus be best viewed as effects in their own right rather than in comparison with the effect they have on the respective other outcome measure.

In summary, this study has identified a number of significant NSE effects on adolescent internalising and autistic traits of which NSE birth weight differences was an early predictor. Specifically, relatively lower birth weight may put twins at a greater developmental disadvantage, potentially contributing to delay and/or altered trajectories. Previous studies on toddlerhood had implicated parenting variables as producing some of the most frequently reported NSE effects on psychosocial outcome differences, however this was not replicated in adolescence. Instead, peer problems throughout childhood were showing the clearest NSE associations with both adolescent autistic and internalising traits in both males and females. Therefore, greater peer problems should be acknowledged as a warning sign for future diverging development on autistic and internalising traits via NSE. In conclusion, the study shows that in addition to studying the aetiology of twin similarities, twin *differences* are important and specific contributing factors on such NSE differences on adolescent autistic and internalising traits can be identified. The following Chapter will turn to late adolescence, teasing apart univariate influences on depression and anxiety traits individually and relating them to autistic traits at age 16.

Chapter 7 Co-occurrence with autistic traits in late adolescence: Associations with depression traits and anxiety sensitivity

Chapter 4 presented data on the co-occurrence of autistic and internalising traits in early adolescence. The first aim of the current chapter is to investigate this co-occurrence in later adolescence, thereby closing the gap between previous child and adult studies on these traits. Chapter 5 strived to address how specific autistic-like traits relate to internalising behaviour; in the following, the second aim is to further elucidate anxiety and depression as specific internalising disorders and their association with autistic traits.

7.1 Background

As outlined in the introductory chapter, the fractionable autism triad hypothesis suggests that autistic features may show differently strong associations with one another at individual levels of explanation, such as only moderate correlations between the three diagnostic domains at the phenotypic level and a varying extent of aetiological overlap (e.g. (Happé & Ronald, 2008; Happé et al., 2006; Robinson et al., 2012; Ronald et al., 2011). The co-occurrence of depression and anxiety has previously been introduced and described in section 1.7.3. Chapter 5 showed that autistic trait subdomains differed in their univariate aetiology and in their aetiological associations with internalising traits in early adolescence.

7.1.1 Using anxiety sensitivity as an anxiety trait measure

Previously in chapter 4, the SDQ was introduced and utilised as a measure of omnibus internalising traits in early adolescence. As discussed, items on this scale relate to symptoms associated with anxiety and depression. At age 16, questionnaires continued to take this approach for the depression measure. However, data collection on autistic, anxiety and depression traits in late adolescence included both self and parent report measures and allowed for two complimentary conceptual approaches to be taken with respect to anxiety traits. First, the TEDS-created Anxiety Related Behaviours Questionnaire (ARBQ, description below in section 7.2.2.2.2) was employed as a parent measure in the current study. Conceptually similar to the SDQ, it records anxiety-related *symptoms*.

A different approach using a pre-existing instrument, the Childhood Anxiety Sensitivity Index (CASI, Silverman, Fleisig, Rabian, & Peterson, 1991), has been taken for twins' self report; this will be introduced in the following.

Anxiety sensitivity refers to sensitivity to the physical and emotional symptoms of anxiety and the belief that these are harmful (Reiss, Peterson, Gursky, & McNally, 1986). The two constructs trait anxiety and anxiety sensitivity are distinguishable and use different relevant aspects – past anxiety experiences and cognition biases respectively – to predict future anxiety and fear (Reiss, 1997; Sandin, Chorot, & McNally, 2001).

It has been argued that anxiety sensitivity is superior to measures of trait anxiety due to evidence of respondents' tendencies to report their *state* anxiety in relation to anticipation of threatening situations, in the prediction of fear and panic (McNally, 1996; Taylor, 1996). Anxiety sensitivity emerges in middle childhood (around age 7 years) simultaneously with children's ability to consider anxious physical symptoms (Muris, Vermeer, & Horselenberg, 2008; Reiss, Silverman, & Weems, 2001). A comparable proxy for depression has been suggested in the concept of attributional style (Seligman, Abramson, Semmel, & Baeyer, 1979; Sweeney, Anderson, & Bailey, 1986). Initially conceptualised as relevant to internalising generally, anxiety sensitivity has since been shown to relate more strongly to anxiety than depression traits and symptoms (Joiner et al., 2002; Rabian, Embry, & MacIntyre, 1999; Smari, Erlendsdottir, Bjorgvinsdottir, & Agustsdottir, 2003; Weems, Hammond-Laurence, Silverman, & Ginsburg, 1998). It is also predictive of panic and anxiety in both clinical and nonclinical samples (Benitez et al., 2009; Maller & Reiss, 1992; Plehn & Peterson, 2002; Schmidt et al., 2010; Schmidt, Lerew, & Jackson, 1997). In summary, anxiety sensitivity is now considered a vulnerability factor for many anxiety subtypes.

A number of recent quantitative genetic studies have investigated anxiety sensitivity in adolescence and early adulthood using the CASI (Brown et al., 2012; Waszczuk, Zavos, & Eley, 2013; Zavos, Gregory, & Eley, 2012a; Zavos, Rijsdijk, & Eley, 2012b; Zavos et al., 2012c). In the sample of initially 12-19 year-olds assessed at three subsequent time points, moderate phenotypic correlations between time points and moderate degrees of both continuity and innovation of genetic influences were found. In contrast, nonshared environmental influences were largely time-specific. Briefly, with respect to possible expected findings in the current study, this research also suggests (McAdams et al., 2013) that anxiety sensitivity is moderately heritable throughout adolescence with

substantial nonshared environmental contributions. Given the similarity of autistic and internalising trait aetiologies reported in mid-childhood and early adolescence, hypothesised findings for later adolescence are to find a phenotypic and aetiological twin similarity that is not much changed compared to these earlier time points.

7.1.2 Findings on co-occurring anxiety and depression traits in adolescence

The introductory chapter has illustrated the high comorbidity of disorders within the internalising category, and particularly anxiety and (major) depressive disorders. However, a wealth of research has also suggested a multitude of genetic and environmental factors specific to anxious and depressive behaviours. A review of these is beyond the scope of this thesis, though a behaviour genetic overview is available (Rice, Fowler, Scourfield, & Thapar, 2003; Rice & Thapar, 2010). Instead, hereafter a recent study will be summarised which most relevantly demonstrates the phenotypic and aetiological patterns of anxious and depressive traits throughout adolescence, as it presents longitudinally the aetiological trait compositions and trait overlaps within a twin sample using the same twin modelling strategies as in the current sample.

In a sample of twins recruited from the Netherlands Twin Register (NTR), Lamb et al. (2010) analysed data provided at the ages of 12, 14 and 16 years (N age subsets = 1,400-2,000). Both anxiety and depression traits were measured on the Youth Self Report instrument (YSR, Achenbach, 2009) using the anxious/depressive and withdrawn/depressive scales respectively. Girls tended to score higher than boys, and this difference was more pronounced on anxious traits than on depression traits. Mean scores also increased with age. Phenotypic correlations between traits were high in both sexes at all ages (r = .59-.69).

The aetiological composition of univariate anxiety and depression traits at age 12 included shared environmental effects, which were not included in the best-fitting models at ages 14 and 16 years. No sex-specific aetiology on either trait was found. At age 12, twin similarity on anxiety traits was due to moderate genetic (a^2 = .35) and shared environmental (c^2 = .21) effects, with the remainder of the trait variance accounted for by nonshared environmental influences (e^2 = .45). At 14, twin similarity was comparatively greater and fully accounted for by genetic effects (a^2 = .67), while nonshared environmental influences became less pronounced (e^2 = .33). At 16, twin similarity resembled that of age 12, but in common with age 14, this was due to genetic influences only (a^2 = .55, e^2 = .45).

Depression traits at age 12 showed very little genetic influences ($a^2 = .03$), and moderate shared environmental influences ($c^2 = .38$). The strongest aetiological component were nonshared environmental influences ($e^2 = .60$), and remained so at all ages. At age 14, twin *similarity* had shifted to an entirely genetic aetiology ($a^2 = .37$, $e^2 = .63$). A similar aetiological trait composition was observed at age 16 ($a^2 = .45$, $e^2 = .55$).

At the bivariate level between anxiety and depression traits, both genetic and shared environmental correlations at age 12 showed complete overlap ($r_g = 1$, $r_c = 1$), though confidence intervals were wide at this time point; the nonshared environmental correlation was substantial ($r_e = .50$). At ages 14 and 16, the genetic overlap had reduced but remained high (both $r_g = .85$), with nonshared environmental overlaps (age 14: $r_e = .42$, age 16: $r_e = .51$) similar to age 12.

In summary, overall levels of heritability remained the same during the investigated adolescent years. Shared environmental influences and shared environmental correlations were only detected at age 12. With respect to potentially increasing shared environmental effects across development on autistic traits, it is of interest to investigate if this pattern is a distinguishing factor between autistic and internalising traits, or alternatively owed to the fact that shared environmental effects are less frequently detected on self report measures. The bivariate statistics suggest that to the extent that twins are similar across anxiety and depression traits, there is complete genetic and shared environmental overlap at age 12. Of note, both later time points suggest genetic aetiological factors on anxiety and depression traits are no longer wholly shared and the covariation of anxious and depressive traits was partially influenced by nonshared factors. This poses an added incentive to address the co-occurrence of these traits separately beyond early adolescence, to uncover aetiological overlap with other psychiatric traits e.g. autistic traits as investigated hereafter.

7.1.3 Findings on co-occurring autistic and specific internalising traits in childhood

Of the previously discussed twin studies on co-occurring autistic and internalising traits, two studies used an overall internalising trait measure (Hallett et al., 2009a; Hallett et al., 2010) and one study sample was tested for aetiological associations with anxiety only (Lundström et al., 2011). Just two studies (Hallett, 2010; Lundström et al., 2011) investigated associations of autistic traits with both anxiety and depression separately

(as was reviewed in detail in Chapter 4). The first investigated anxiety and depression traits in ASD probands, the second focused on an adult sample.

In addition, one recent twin study (Hallett et al., 2012) has contributed data on mid-childhood relating specific internalising traits to specific autistic traits, using a more bottom-up data-driven approach to internalising behaviours. Drawing on parent report on ~7,000 TEDS twin pairs aged 7 and 8 years, autistic traits were assessed on the CAST (description above in section 4.2.2.2) and internalising traits on the ARBQ. Three autistic trait domains were investigated as suggested by the three subscales of the CAST, namely social difficulties, communication impairments and RRBIs.

Specific internalising trait domains had been constructed previously (Hallett, Ronald, Rijsdijk, & Eley, 2009b) using factor analysis on the ARBQ. In this fashion, five factor scales were obtained, descriptive of generalised anxiety/ negative cognitions, negative affect, fear, social anxiety and obsessive-compulsive behaviours (OCB) respectively. Since the OCB scale only contained two items, it was deemed not sufficiently comprehensive to be included in multivariate analysis. Accordingly, three CAST subscales were analysed with respect to their phenotypic and aetiological associations with four ARBQ subscales.

All but two phenotypic correlations between variable pairs were significant and ranged from low to moderate (r = .04-.36). Associations of social difficulties with fears and with generalised anxiety were nonsignificant. The highest phenotypic overlap across autistic-like and internalising trait categories in both males and females was for communication problems with negative affect (r = .33-.36).

At the aetiological level, the three autistic trait domains social difficulties, communication problems and RRBIs were all substantially heritable and more so in males (a^2 = .61-.70) than females (a^2 = .57-.65). Shared environmental influences were low to modest (males: c^2 = .04-.18, females: c^2 = .04-.26) and nonshared environmental influences were modest to moderate (males: e^2 = .19-.25, females: e^2 = .16-.31). Being based on the same sample, age group and measure as described in Chapter 4 when discussing the TEDS mid-childhood studies, these findings were expected, but add to prior knowledge by further differentiating by sex and CAST subdomain.

Univariate parameter estimates of the four internalising traits social anxiety, fears, generalised anxiety and negative affect also showed significant heritability for both males (a^2 = .52-.60) and females (a^2 = .49-.59). Like autistic traits, there were low to

moderate shared environmental and moderate nonshared environmental influences (males: c^2 = .06-.19, e^2 = .24-.42; females: c^2 = .07-.23, e^2 = .23-.39). These findings using the ARBQ demonstrate similar levels of aetiological environmental contributions on phenotypic internalising behaviours compared to the results using the SDQ as an omnibus internalising measure. The genetic influences on the factor-derived trait scales tended to be more substantial than all other previously described estimates on childhood internalising in the general population using the SDQ (i.e. with exception of the clinical ASD study, where heritability of internalising traits was over 80%).

Bivariate analysis produced a complex matrix of aetiological associations between specific anxiety traits and autistic trait subdomains that differed both between trait pairs and by sex. Genetic correlations for males were significant and modest for social difficulties with all four internalising trait domains (r_g = .08-.19). Equally, all male genetic correlations of communication problems were significant, being modest for social anxiety and fears (both r_g = .08), and moderate for generalised anxiety and negative affect (r_g = .29-.30). Male genetic correlations of RRBIs were significant with all but the social anxiety domain, the other three correlations ranging from modest to moderate (r_g = .18-.29). Female genetic correlations were lower throughout, and for social difficulties only generalised anxiety showed significant low genetic overlap (r_g = .05). Genetic correlations of female communication problems with social anxiety and fears were nonsignificant and moderate with generalised anxiety and negative affect (r_g = .23-.24). An exception to the relatively lower genetic female overlap was for RRBIs and social anxiety, which (unlike the correlation in males) was modest and significant (r_g = .10); correlations between RRBIs in females with fears, generalised anxiety and negative affect were modest to moderate (r_g = .12-.23).

For shared environmental overlap, correlations described as nonsignificant all had non-zero point estimates but also wide confidence intervals attached, which overlapped with zero. One male shared environmental correlation of social difficulties was significant and showed substantial overlap with generalised anxiety (r_c = .64). For male communication problems their shared environmental influences all significantly overlapped with shared environment on the four internalising traits, ranging from substantial to almost full correlations (r_c = .46-.96). Shared environment on male RRBIs showed significant substantial correlations with shared environmental influences on social anxiety and negative affect (r_c = .60-.69). Of the female shared environmental correlations on social difficulties, only those with negative affect were significant (r_c =

.48). Female shared environment in communication problems correlated significantly with all internalising domains (r_c = .54-.76). Female RRBIs showed significant moderate shared environmental associations with fears and negative affect (r_c = .29-.33). However, with all these shared environmental correlations it should be borne in mind that C accounted for relatively little of the trait variance on either trait.

Nonshared environmental correlations were all low to modest, which is in line with previous studies generally finding relatively lower nonshared environmental overlap compared to the trait overlap for genetic reasons reported on autistic and internalising traits. Specifically, nonshared environment on male social difficulties correlated modestly with nonshared environment on social anxiety (only) (r_e = .14). Nonshared environmental correlations of male communication problems with internalising traits were significant for all four trait pairs (r_e = .10-.18), as were those of specific internalising traits with RRBIs (r_e = .06-.16). Females did not show significant nonshared environmental overlap on internalising-social difficulties pairings, but all internalising domains overlapped with communication problems (r_e = .06-.17). Female nonshared environmental correlations of RRBIs were significant with generalised anxiety and negative affect (r_e = .01-.04).

An important consideration, which has not yet been addressed in presenting the Hallett et al. (2012) results is to what extent the ARBQ represents anxiety traits. The four internalising domains social anxiety, fears, generalised anxiety and negative affect are somewhat descriptive of both anxious and depressive behaviours. With respect to the items composing the factor-derived scales in Hallett et al. (2012), social anxiety relates to anxious and withdrawn behaviour in social situations (see Appendix VII). Fears relates mostly to phobic reactions and childhood fears. A greater range of concepts is contained in generalised anxiety (originally named negative cognitions, Hallett et al., 2009b), comprising items relating to self confidence, locus of control and general anxiety and worries. Negative affect was composed of anhedonia and items relating to physiological reactions to anxiety. Of note, the factor analysis was based on the ARBQ childhood measure, first developed for toddlers with additional items added to ensure relevance in mid-childhood. As has been suggested by the authors of the factor analysis (Hallett et al., 2009b), the factors of social anxiety and fears may reflect clinical subtypes of anxiety disorder, while generalised anxiety and negative affect may represent aspects of temperament. Indeed, on comparing the items used in toddlerhood

(Eley et al., 2003), most have been allocated to the generalised anxiety and negative affect scales.

In summary, Hallett et al. (2012) have provided important information on specific associations between autistic and anxious traits associated with some specific anxiety disorders, highlighting that phenotypic levels of trait presentation are based on the composition of aetiological factors whose contributions differ in complex ways. However, in this study, none of the internalising subdomains focused on depression traits separately. In the child sample, Lundström et al. (2011) reported co-occurrences of autistic traits with anxiety traits only. Thus to date, no population-based twin study has investigated the association of autistic traits with depression traits in either childhood or adolescence.

7.1.4 Objectives

The present study investigates associations of late adolescent autistic traits with depression and anxiety traits individually, also testing for quantitative sex differences on aetiological factors. In consideration of potential differences between introspective self ratings and parent observed behaviours, both data from both raters has been included.

7.2   Methods

7.2.1 Current Sample

Complete questionnaire data on autistic traits, depression traits and anxiety traits was obtained from 4807 parents and 4792 twin pairs at the age of 16 years (mean = 16.32, SD = 0.68). The same exclusion criteria as previously described applied (see section 4.2.1.3). Bivariate analyses using parent data were based on 715 MZM, 670 DZM, 999 MZF, 882 DZF and 1530 DZOS pairs. Self report bivariate analysis included 705 MZM, 662 DZM, 990 MZF, 885 DZF and 1506 DZOS pairs.

Table 7.1 TEDS study data returns at age 16 years

Cohort	1	2	3 & 4	Total
Pairs included at birth	3,746	5,564	7,400	16,810
Age 16: Contacted	2,340	3,864	4,670	10,874
Age 16: Valid Parent data returns	1,142	1,607	2,374	5,123
(Percentage)	49%	42%	51%	47%
Age 16: Valid Twin data returns	1,121	1,586	2,365	5,072
(Percentage)	48%	41%	51%	47%

7.2.2 Measures

7.2.2.1 Autistic traits

Autistic traits at age 16 years were assessed on the shortened AQ, the same measure as used in the previous chapters (described in 4.2.2.2). At 16 years, self report relating to autistic traits was collected on 28 items, while parent report was based on 13 items. Items were scored on a 4-point (0-3) Likert scale out of a maximum of 84 and 39, respectively. Both the AQ parent (Cronbach's α = .84) and AQ self report (Cronbach's α = .77) showed good internal consistency.

7.2.2.2 Anxiety traits

7.2.2.2.1 Self report

Anxiety traits as reported by twins themselves were measured using the Childhood Anxiety Sensitivity Index (CASI, items shown in Appendix VIII; Silverman et al., 1991). The CASI shows good test-retest reliability in non-clinical (r = .76, within 2 weeks) and clinical samples (r = .79, within 1 week) and it has been validated in relation to fear, anxiety and panic (Hayward et al., 1997; Lau, Calamari, & Waraczynski, 1996). A study (Kearney, Albano, Eisen, Allan, & Barlow, 1997) comparing the psychometric properties of other anxiety trait measures found the CASI to classify individuals similarly and to correlate well with the Revised Children's Manifest Anxiety Scale (RCMAS, Reynolds & Richmond, 1985), the State-Trait Anxiety Inventory for Children (STAIC, Spielberger, 1973) and the Fear Survey Schedule for Children (FSSC-R,

Ollendick, 1983). CASI scores of individuals identified as having a clinical diagnosis of panic disorder on the Anxiety Disorders Interview Schedule (ADIS-C, Silverman & Nelles, 1988) were also significantly higher than those of controls. More recently, a meta-analytic review of child anxiety sensitivity in child anxiety including 15 studies (N = 6,579) suggested a positive correlational relationship between anxiety sensitivity and anxiety for children ($r = .26$) and adolescents ($r = .36$) and higher levels of AS for anxiety disordered youth than non-clinical youth ($d = .64$; Noel & Francis, 2011).

The CASI is a self report tool designed to measure anxiety sensitivity across childhood between the ages of 7 and 17 years. Consisting of 18 items, the CASI is scored on a 3-point Likert scale (0-2), to a possible total of 36. Items relate to fearful thoughts about symptoms of anxiety (e.g. 'When I am afraid, I worry that I might be crazy'). Responses at age 16 to the items showed good internal consistency (Cronbach's $\alpha = .86$), matching that reported by the test authors (.87), and means and variances were similar to those previously reported in a large community sample of children and adolescents (Walsh, Stewart, McLaughlin, & Comeau, 2004).

7.2.2.2.2 Parent report

Parent reported anxiety traits were recorded using the Anxiety Related Behaviour Questionnaire (ARBQ, Eley et al., 2003). The ARBQ is a collection of items designed to investigate anxiety-related behaviours commonly assessed in children, combining items from existing reliable and valid measures of temperament and psychopathology (Achenbach, 1991; Behar & Stringfield, 1974; Berg, Whitaker, Davies, Flament, & Rapoport, 1988; Elander & Rutter, 1996; Goodman & Scott, 1999). The ARBQ has been used previously in the TEDS sample at age 4 using 13 items (Eley et al., 2003). At ages 7 and 9 years, 9 additional items were included in order to assess cognitive traits that are more age-appropriate in middle childhood (Hallett et al., 2009b). The addition of these items ensured continued similarities with other screening questionnaires (Birmaher et al., 1999; Spence, Barrett, & Turner, 2003) beyond toddlerhood (Hallett, 2010). At age 9, parent report on the ARBQ and the SDQ emotional symptoms subscale correlated highly ($r = .86$). While there is no second parent reported anxiety measure available for comparison, at age 16 across raters, parent report on the ARBQ showed moderate agreement with self rating on the SDQ emotional symptoms subscale at the same age ($r = .33$).

At age 16, the ARBQ contains 19 items assessing five dimensions of anxious behaviours: Generalised Anxiety, Negative Cognitions, Fears, Social Anxiety, and

Obsessive-Compulsive Behaviours (items are listed in Appendix VII). Scored on a 3-point Likert scale (0-2), it has a range of 0-38. Internal consistency was good (Cronbach's α = .84) and equivalent to that reported in the same sample at age 9 years.

7.2.2.3 Depression traits

Depression traits were assessed using the Moods and Feelings Questionnaire (MFQ, Angold et al., 1995; Angold et al., 1987). This screening instrument can be completed by children and adolescents themselves or by their parents. The longform MFQ consists of 32 items and has been shown to have good clinical validity (Costello & Angold, 1988; Wood, Kroll, Moore, & Harrington, 1995). Test-retest reliability in a sample of children with major depressive disorder was good (r = .78, within 18 days) and there was moderate parent-child agreement on the total MFQ score (r = .51) as well as individual symptoms (**κ** = .31). A shortform of 13 items, the Short Mood and Feelings Questionnaire (SMFQ) has been validated for use within community samples of typical children (Thapar & McGuffin, 1998) and more recently, data on the TEDS sample at age 12 has become available, using a version of the SMFQ containing 11 items of the MFQ and two additional items from the SDQ that were contextually identical (Wilkinson, Trzaskowski, Haworth, & Eley, 2013).

At age 16, the MFQ self consisted of 13 items (identical to the just cited previous TEDS study) and the MFQ parent consisted of 11 items (i.e. did not include the additional SDQ items), scored on 3-point Likert scales (0-2) with maximum scores of 26 and 22. Internal consistency was highest on this measure (Cronbach's α self = .88, parent = .86).

7.2.3 Analysis

7.2.3.1 Data preparation and phenotypic analysis

All measures showed varying degrees of skew and appropriate transformations were applied. However, the parent reported depression trait measure remained highly skewed (skew >2). In line with standard behaviour genetic procedures, age of the twins at testing and sex were regressed out of all scores, and the residual scores were used in all ensuing model fitting. The associations between autistic traits and anxiety/ depression trait measures were assessed using Pearson's correlations. Independence among cases was maintained by randomly selecting one twin per pair.

7.2.3.2 The twin design and model-fitting

The methodology of the twin design has been described in Chapter 2. The steps involved in the analysis of the twin data were identical to the rationale outlined

previously in section 4.3. Briefly, first phenotypic univariate and CTCT twin correlations were obtained to ensure sufficient degrees of covariance to continue with model-fitting and to obtain an initial overview of the trait aetiology. Second, univariate and bivariate twin model-fitting proceeded from full models, modelling autistic traits, anxiety traits and depression traits individually, and bivariate pairs between same-rater autistic with anxiety traits and autistic with depression traits. Incrementally, parameters were dropped for comparison, in order to select the best-fitting models according to the produced model fit parameters. This procedure was applied to self report and parent report data separately, quantitative sex differences were modelled.

7.3 Results

7.3.1 Descriptives

The descriptive statistics for the self report and parent report measures are presented in Table 7.2. The results showed significant sex effects on all measures ($p < .001$), with males scoring higher on autistic traits (self report: $F_{1, 4787} = 16.52$; parent report: $F_{1, 4804} = 138.96$, $p < .001$), and females scoring higher on the anxiety (self report: $F_{1, 4790} = 436.97$; parent report: $F_{1, 4806} = 170.046$, both $p < .001$) and depression trait measures (self report: $F_{1, 4791} = 191.01$; parent report: $F_{1, 4803} = 33.03$, both $p < .001$). Significant zygosity effects on mean scores were only found on one scale (depression traits self report: $F_{1, 4791} = 3.45$, $p < .05$). Significant sex-by-zygosity interactions were found for autistic traits using both self report ($F_{2, 4787} = 4.77$, $p < .01$) and parent report ($F_{2, 4804} = 9.95$, $p < .001$), and for parent reported anxiety traits ($F_{2, 4806} = 5.31$, $p < .01$) and parent reported depression traits ($F_{2, 4803} = 3.79$, $p < .05$).

Table 7.2 Descriptive statistics: age 16 self and parent report on autistic traits, anxiety traits and depression traits

Trait Measure (range/ rater)	No. of items	Cronbach's alpha	Mean Score (SD)							ANOVA				
			Whole Sample	MZM	MZF	DZM	DZF	DZOM	DZOF	Sex	Zyg	Sex-Zyg		R^2
Autistic traits (0-39/ **S**)	13	.77	11.91 (5.80)	12.22 (6.06)	11.70 (5.93)	11.96 (5.70)	11.79 (5.67)	12.62 (5.56)	11.28 (5.82)	<.001***	.93	<.01**		.004
Autistic traits (0-84/ **P**)	28	.84	24.21 (10.93)	25.50 (10.92)	22.73 (10.50)	25.56 (10.91)	22.85 (10.00)	27.71 (12.20)	21.75 (9.99)	<.001***	.42	<.001***		.03
Anxiety traits (0-36/ **S**)	18	.86	7.93 (5.85)	6.02 (4.88)	9.26 (6.01)	5.95 (4.75)	9.37 (5.99)	6.23 (4.82)	9.79 (6.60)	<.001***	.14	.93		.08
Anxiety traits (0-38/ **P**)	19	.84	3.59 (4.27)	2.68 (3.69)	4.53 (4.93)	2.64 (3.36)	4.28 (4.47)	3.00 (3.84)	3.88 (4.29)	<.001***	.92	<.01**		.04

Trait Measure (range/ rater)	No. of items	Cronbach's alpha	Mean Score (SD)							ANOVA			
			Whole Sample	MZM	MZF	DZM	DZF	DZOM	DZOF	Sex	Zyg	Sex-Zyg	R^2
Depression traits (0-26/ **S**)	13	.88	3.60 (4.14)	2.45 (3.46)	4.29 (4.84)	2.76 (3.56)	4.18 (4.71)	2.84 (3.65)	4.63 (5.11)	<.001***	<.05*	.16	.04
Depression traits (0-22/ **P**)	11	.86	.99 (2.31)	.62 (1.78)	1.21 (2.68)	.82 (2.01)	1.09 (2.35)	.88 (1.96)	1.18 (2.69)	<.001***	.15	<.05*	.008

Note: P = Parent rated, S = Self rated.

7.3.2 Phenotypic correlations

Table 7.3 presents the phenotypic correlations between all measures. All phenotypic correlations were significant at p <.001. Associations of autistic traits with same rater anxiety and depression traits were moderate-to-high (r= .26-.53). Phenotypic overlap within raters by sex between autistic traits and anxiety and depression measures is shown in Figure 7.1. The amount of variance explained by these associations was lowest for males on parent reported autistic traits with depression traits (7%) and highest for females using parent reported autistic traits with anxiety traits (28%).

Table 7.3 Phenotypic correlations: age 16 self and parent report on autistic traits, anxiety traits and depression traits

	Pearson Correlation				
	Autistic Traits (**S**)	Autistic Traits (**P**)	Anxiety Traits (**S**)	Anxiety Traits (**P**)	Depression Traits (**S**)
Autistic Traits (**P**)	.38				
Anxiety Traits (**S**)	**.32**	.13			
Anxiety Traits (**P**)	.25	**.52**	.20		
Depression Traits (**S**)	**.29**	.15	**.46**	.24	
Depression Traits (**P**)	.11	**.30**	.18	**.49**	.35

Note: all correlations significant at p <.01. P = Parent rated, S = Self rated. Correlations in bold are using the same rater.
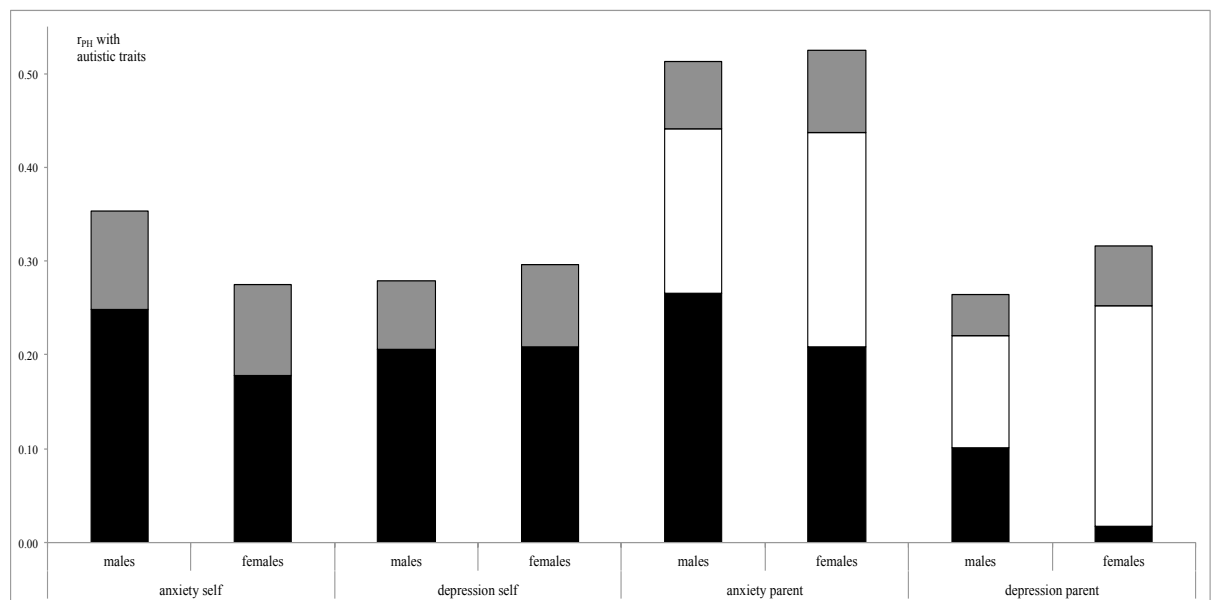
Figure 7.1 Phenotypic correlations of age 16 same rater autistic traits, with anxiety and depression traits and the proportions of correlations accounted for by genetic, shared and nonshared environmental effects

Table 7.4 Legend for Figure 7.1

	Anxiety Self		Depression Self		Anxiety Parent		Depression Parent	
	males	females	males	females	males	females	males	females
r_{PH}	.36	.28	.28	.30	.49[x]	.53	.26	.32
Genetic factors	.25 (69%)	.18 (64%)	.21 (75%)	.21 (70%)	.27 (55%)	.21 (40%)	.10 (38%)	.02 (6%)
Shared environment	-	-	-	-	.17 (35%)	.23 (43%)	.12 (46%)	.24 (75%)
Nonshared environment	.11 (31%)	.10 (36%)	.07 (25%)	.09 (30%)	.07 (10%)	.09 (17%)	.04 (16%)	.06 (19%)

Note: Aetiological factors in the table summatively show the phenotypic trait covariance with autisti
traits. *E.g. Anxiety Parent females r_{ph} = .21 + .23 + .09 = .53.* [x] Aetiological components add to .51 due t
small differences in sample composition for phenotypic analysis vs. twin modelling.

7.3.3 Twin correlations

Table 7.5 presents the intraclass and CTCT correlations. Univariate twin correlations on autistic traits were all higher as a rule than for anxiety and depression traits. In addition, correlations obtained using the parent data were higher than those from the twins themselves.

The univariate twin correlations of both parent and self report data show that MZ twins were more similar on traits than DZ twins, such that genetic influences on all individual traits are apparent. No perfect MZ correlation of 1.0 was found, indicating that all traits are also subject to nonshared environmental effects. At the bivariate level, CTCT correlations were low to moderate, and higher for MZ than DZ twins, indicating that some genetic and nonshared environmental effects would also be found on the associations across traits.

Differences in aetiology are however observable from the shown MZ correlations in respect to DZ correlations. All parent rated traits suggest the presence of shared environmental influences, since $r_{DZ} > .5\ r_{MZ}$. Overall, the correlation matrix thus implicates ACE models for all univariate and bivariate parent rated models of autistic traits with anxiety traits and depression traits.

For the self rated scales, twin correlations did not clearly favour either ACE or ADE models, and both were tested during model-fitting. Specifically, twin correlations suggested dominance effects due to low DZ correlations ($<.5\ r_{MZ}$) on self reported autistic traits, for male self reported anxiety traits, and for females on the bivariate model between self reported autistic traits and anxiety traits. For all bivariate models, female DZ CTCT correlations were more similar to their MZ counterparts than for males, indicating relatively less overlap for genetic reasons between the investigated traits for females.

Table 7.5 Intraclass and CTCT correlations of age 16 same rater autistic traits, anxiety traits and depression traits

	Self report					Parent report			
	Autistic Traits	N				**Autistic Traits**	N		
MZM	.57 (.52-.62)	704			MZM	.92 (.91-.93	715		
DZM	.22 (.15-.30)	662			DZM	.57 (.52-.62)	670		
MZF	.50 (.46-.55)	991			MZF	.88 (.87-.90)	999		
DZF	.24 (.18-.30)	884			DZF	.66 (.63-.70)	882		
DZOS	.20 (.15-.25)	1503			DZOS	.55 (.51-.58)	1532		
			CTCT with					CTCT with	
	Anxiety Traits	N	Autistic Traits			**Anxiety Traits**	N	Autistic Traits	
MZM	.40 (.33-.46)	705	.26 (.19-.32)		MZM	.67 (.63-.71)	715	.47 (.41-.53)	
DZM	.13 (.05-.20)	662	.13 (.05-.20)		DZM	.49 (.43-.54)	670	.33 (.26-.40)	
MZF	.43 (.38-.48)	990	.21 (.15-.27)		MZF	.75 (.72-.77)	999	.46 (.41-.50)	
DZF	.23 (.17-.29)	885	.03 (-.04-.10)		DZF	.52 (.47-.57)	882	.33 (.27-.39)	
DZOS	.24 (.19-.29)	1506	.11 (.06-.16)		DZOS	.47 (.43-.51)	1530	.29 (.25-.34)	

	Self report				Parent report		
			CTCT with				CTCT with
	Depression Traits	N	Autistic Traits		**Depression Traits**	N	Autistic Traits
MZM	.37 (.31-.43)	706	.24 (.17-.31)	MZM	.58 (.53-.62)	713	.27 (.20-.34)
DZM	.23 (.16-.30)	662	.15 (.08-.23)	DZM	.39 (.32-.45)	670	.17 (.10-.25)
MZF	.45 (.39-.49)	992	.27 (.21-.32)	MZF	.61 (.57-.65)	997	.29 (.23-.35)
DZF	.36 (.30-.42)	884	.14 (.08-.21)	DZF	.45 (.40-.50)	882	.25 (.18-.31)
DZOS	.23 (.18-.28)	1503	.13 (.08-.18)	DZOS	.38 (.33-.42)	1531	.19 (.14-.23)

Note: 95% confidence intervals shown in parenthesis. CTCT = Cross-twin cross-trait correlations. DZF = DZ females; DZM = DZ males; DZOS = DZ opposite-sex twin pairs; MZF = MZ females; MZM = MZ males.

7.3.4 Model-fitting results for Self ratings

Bivariate fit statistics of autistic traits with anxiety traits are shown in Table 7.6, and Table 7.7 presents results of autistic traits with depression traits. (Fit statistics on univariate models are presented in Appendix IX). Sex limitation models for all investigated bivariate models provided the best fit, however overall bivariate models fits are also shown for information, to give an indication of the model fits for the whole sample.

Proceeding from univariate ADE models, the best fitting univariate model of self reported autistic traits was without dominance effects (AE). A univariate AE model after dropping dominance effects was also fitted for self reported anxiety traits. Proceeding from an initial univariate ACE model on self reported depression traits, it was found that it was not possible to drop shared environmental influences (p <.001).

For the bivariate sex limitation model of self reported autistic traits and depression traits, an AE model provided the best fit. For consistency it was thus decided to proceed showing the results of the AE model rather than the full ACE model for the overall bivariate model of the two measures. Figure 7.2 shows the parameter estimates for the two self rated final models.

Genetic influences on self rated autistic traits and non-shared environmental influences accounted for half of the variability each (males: $a^2 = .55$, $e^2 = .45$; females: $a^2 = .50$, $e^2 = .50$). In comparison, the variability in anxiety traits (males: $a^2 = .37$, $e^2 = .63$; females: $a^2 = .42$, $e^2 = .58$) and depression traits (males: $a^2 = .38$, $e^2 = .62$; females: $a^2 = .47$, $e^2 = .53$) involved moderate heritability overall. The genetic correlations between autistic traits and anxiety traits (males: $r_g = .55$, females: $r_g = .39$), and between autistic traits and depression traits (males: $r_g = .45$, females: $r_g = .43$) showed that some of the genetic influences on autistic traits are shared with anxiety and depression traits, respectively. In contrast, the non-shared environmental correlations of both models were modest (self reported autistic traits – anxiety traits males: $r_e = .18$, females: $r_e = .21$; self reported autistic traits – depression traits males: $r_e = .14$, females: $r_e = .17$).

As shown in Figure 7.1 above, for self reported autistic traits and anxiety traits, the genetic contribution to the observed correlation was 70% in males and 64% in females. Nonshared environment explained one third of the phenotypic correlation between self reported autistic and anxiety traits (males: 31%, females: 36%). Similarly, for self reported autistic traits and depression traits, most of the phenotypic overlap was explained by genetic factors (males: 75%, females: 70%), while nonshared environment explained about a quarter of the covariance (males: 25%, females: 30%).

Table 7.6 Fit statistics of bivariate models of age 16 self rated autistic traits and anxiety traits

	Overall Fit of Model					relative Fit of Model			
	−2LL	χ²	df	p	AIC	χ²	df	p	AIC
Saturated	35426.421								
ACE	35459.246	32.825	17	.05	-1.175				
ADE	35457.738	31.317	17	.05	-2.683				
AE	35459.246	32.825	20	.04	-7.175	0	3	1	-6.000
CE	35607.719	181.298	20	.001	141.298	148.473	3	.001	142.473
E	36409.239	982.818	23	.001	936.818	949.993	6	.001	937.993
Saturated (Sex lim)	35365.064								
ACE	35419.506	54.442	34	.05	-13.558				
ADE	35416.130	51.066	34	.05	-16.934				
AE	**35420.684**	**55.62**	**40**	**.06**	**-24.380**	**4.554**	**6**	**.60**	**-7.446**
E	36382.745	1017.681	46	.001	925.681	963.239	12	.001	939.239

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ² = likelihood ratio χ² test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences. Best-fitting model shown in bold.

Table 7.7 Fit statistics of bivariate models of age 16 self rated autistic traits and depression traits

	Overall Fit of Model					relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	35373.002								
ACE	35414.591	41.589	17	.001	7.589				
ADE	35430.956	57.954	17	.001	23.954				
AE	35434.393	61.391	20	.001	21.391	19.802	3	.001	13.802
CE	35531.795	158.793	20	.001	118.793	117.204	3	.001	111.204
E	36471.510	1098.508	23	.001	1052.508	1056.919	6	.001	1044.919
Saturated (Sex lim)	35319.000								
ACE	35377.419	58.419	34	.01	-9.581				
ADE	35396.429	77.429	34	.001	9.429				
AE	**35400.339**	**81.339**	**40**	**.001**	**1.339**	**3.910**	**6**	**.69**	**-8.090**
CE	35497.871	178.871	40	.001	98.871	120.452	6	.001	108.452
E	36437.504	1118.504	46	.001	1026.504	1060.085	12	.001	1036.085

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences. Best-fitting model shown in bold.

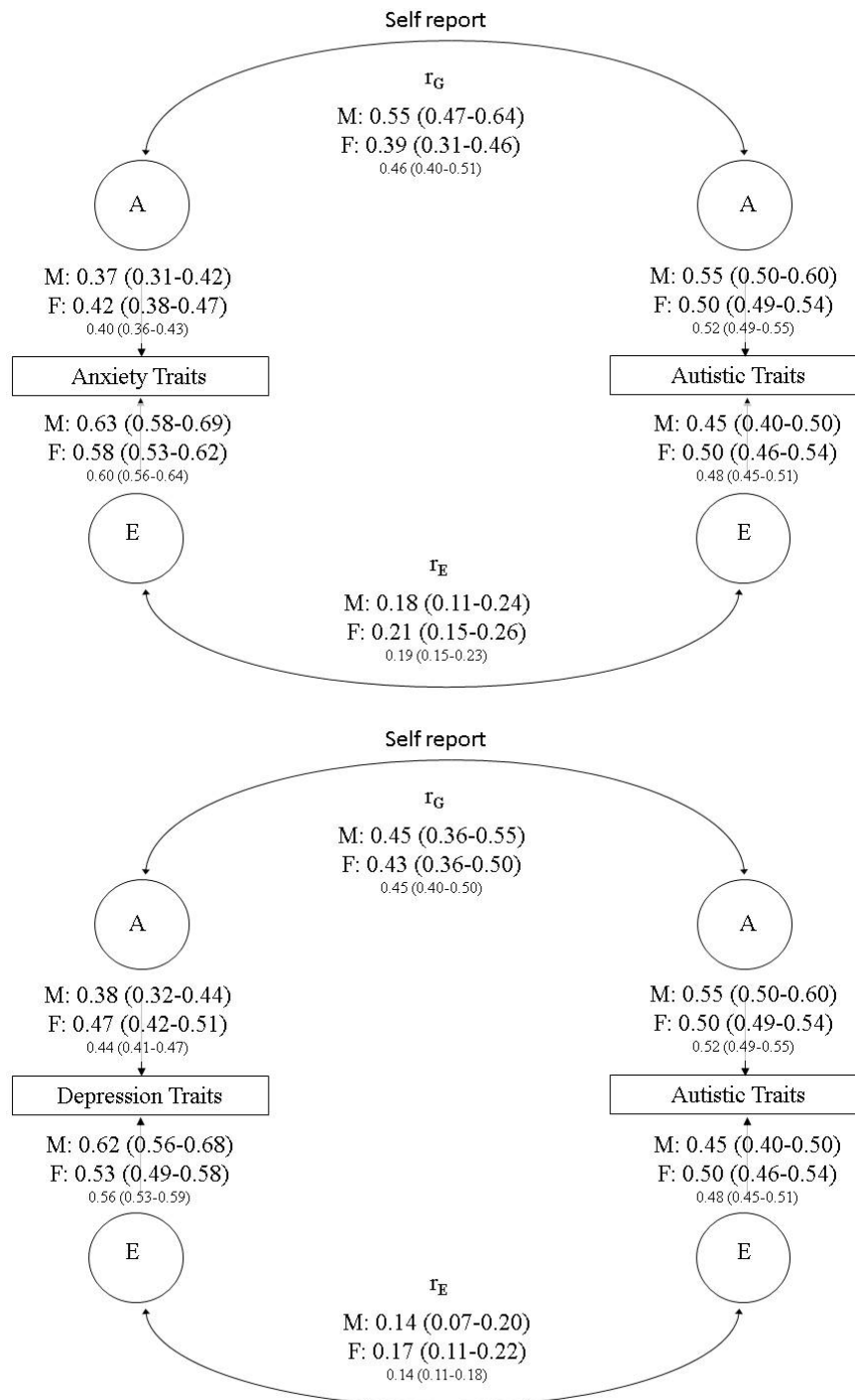**Figure 7.2 Path diagram showing results from the correlated factors solution of age 16 self report bivariate models of autistic traits with anxiety traits and depression traits**

Note: r_G = genetic correlation; r_C = shared environmental correlation; r_E = nonshared environmental correlation; *nonsignificant path (confidence intervals overlapping with 0). Parameters for overall sample shown in smaller font.

7.3.5 Model-fitting results for Parent ratings

Fit statistics for parent models on autistic and anxiety traits are shown in Table 7.8 and for autistic traits – depression traits in Table 7.9. (Fit statistics on univariate models are presented in Appendix IX). Bivariate models showed full ACE Cholesky models with quantitative sex differences to provide the best fit as per the lowest AIC value compared to the nested models (Table 7.8 and Table 7.9). Parameter estimates are presented in Figure 7.3.

Parent ratings on autistic traits suggest a substantial male heritability and moderate female heritability (Figure 2; males: $a^2 = .70$, females: $a^2 = .43$). Being at twice the level of males, female shared environmental influences account for as much of the variability as do their genetic influences (males: $c^2 = .22$, females: $c^2 = .45$). Low levels of nonshared environmental influences were observed in both males and females (males: $e^2 = .08$, females: $e^2 = .12$).

For anxiety traits, there was an even distribution of variability accounted for by genetic, shared and nonshared environmental influences (males: $a^2 = .36$, $c^2 = .31$, $e^2 = .33$; females: $a^2 = .44$, $c^2 = .30$, $e^2 = .26$).

Depression traits showed moderate male heritability and moderate female heritability, while shared environmental influences were low for males and moderate in females. Moderate levels of nonshared environmental influences were observed (males: $a^2 = .50$, $c^2 = .12$, $e^2 = .38$; females: $a^2 = .29$, $c^2 = .31$, $e^2 = .58$).

The genetic correlations between parent rated autistic traits and anxiety traits were substantial (males: $r_g = .53$, females: $r_g = .48$). A similar level of overlap was observed for their nonshared environmental correlations (males: $r_e = .44$, females: $r_e = .50$). Considerable overlap between parent rated autistic traits and anxiety traits was observed on their shared environmental influences (males: $r_c = .67$, females: $r_c = .62$).

The shared environmental correlations of parent rated autistic traits and depression traits were similar for both sexes to those shown for autistic traits and parent rated anxiety (males: $r_c = .74$, females: $r_c = .63$). However the genetic and nonshared environmental correlations were low to moderate for parent rated autistic traits and depression traits (males: $r_g = .17$, $r_e = .25$; females: $r_g = .05$, $r_e = .29$).

The genetic contribution to the observed phenotypic correlation of parent rated autistic traits and anxiety traits were 55% and 39% for males and females respectively. Shared

environment accounted for 35% and 43%, and nonshared environment explained 14% and 17% of the shared variance between parent reported autistic and anxiety traits.

For parent rated autistic traits and depression traits in males, the contributing factors were 38% genetic, 46% shared environment, and 16% nonshared environment. In females the genetic contribution was very low with aetiological contributions of 6%, 75%, and 19%, for genetic, shared environment and nonshared environment, respectively.

Table 7.8 Fit statistics of bivariate models of age 16 parent rated autistic traits and anxiety traits

	Overall Fit of Model					relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	29796.220								
ACE	29810.266	14.046	17	.66	-19.954				
ADE	29951.608	155.388	17	.001	121.388				
AE	29951.608	155.388	20	.001	115.388	141.342	3	.001	135.342
CE	30647.646	851.426	20	.001	811.426	837.38	3	.001	831.380
E	34936.114	5139.894	23	.001	5093.894	5125.848	6	.001	5113.848
Saturated (Sex lim)	29698.875								
ACE	**29728.616**	**29.741**	**34**	**.68**	**-38.259**				
ADE	29888.388	189.513	34	.001	121.513				
AE	29888.388	189.513	40	.001	109.513	159.772	6	.001	147.772
CE	30610.684	911.809	40	.001	831.809	882.068	6	.001	870.068
E	34914.819	5215.944	46	.001	5123.944	5186.203	12	.001	5162.203

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences. Best-fitting model shown in bold.

Table 7.9 Fit statistics of bivariate models of age 16 parent rated autistic traits and depression traits

	Overall Fit of Model					relative Fit of Model			
	−2LL	χ^2	df	p	AIC	χ^2	df	p	AIC
Saturated	31626.455								
ACE	31645.211	18.756	17	.34	-15.244				
ADE	31775.913	149.458	17	.001	115.458				
AE	31775.913	149.458	20	.001	109.458	130.702	3	.001	124.702
CE	32432.713	806.258	20	.001	766.258	787.502	3	.001	781.502
E	36244.132	4617.677	23	.001	4571.677	4598.921	6	.001	4586.921
Saturated (Sex lim)	31426.797								
ACE	**31476.571**	**49.774**	**34**	**.04**	**-18.226**				
ADE	31621.100	194.303	34	.001	126.303				
AE	31984.734	557.937	40	.001	477.937	363.634	6	.001	351.634
CE	32308.066	881.269	40	.001	801.269	831.495	6	.001	819.495
E	36102.317	4675.52	46	.001	4583.52	4625.746	12	.001	4601.746

Note: Values on the left of the table show the difference between the bivariate models and the saturated model. Values on the right of the table show the difference between the Cholesky and the best fitting submodel (if applicable). -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences. Best-fitting model shown in bold.

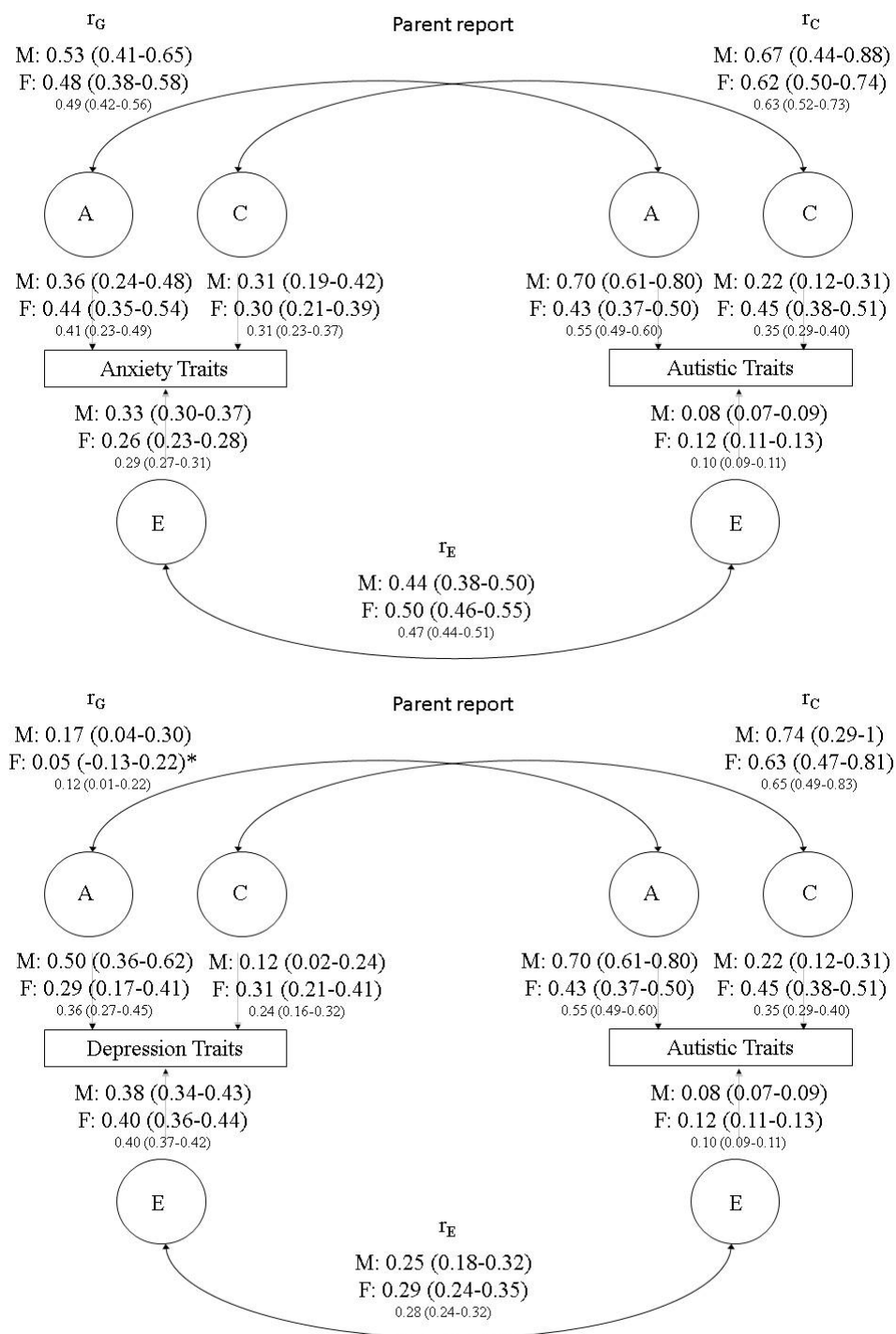**Figure 7.3 Path diagram showing results from the correlated factors solution of age 16 parent report bivariate models of autistic traits with anxiety traits and depression traits**

Note: r_G = genetic correlation; r_C = shared environmental correlation; r_E = nonshared environmental correlation; *nonsignificant path (confidence intervals overlapping with 0). Parameters for overall sample shown in smaller font.

7.4   Discussion

Both twins and their parents provided ratings in the current study on 16-year-olds' co-occurrence of autistic traits, first with anxiety traits and second with depression traits. This adds novel data on their respective trait overlaps during the time of late adolescence, as well as being the first study to address aetiological autistic and depression trait overlap in any underage twin sample.

7.4.1 Anxiety traits at age 16

7.4.1.1 Self report

Self report data of anxiety traits showed reduced heritability and doubled nonshared environment compared to the omnibus parent report internalising measure in TEDS at 12 (Chapter 4 and Chapter 5). Phenotypic correlations of anxiety symptoms (similar to the parent questionnaire) and anxiety sensitivity (self report) have previously been reported as of the magnitude of ~.70 (Zavos, Rijsdijk, Gregory, & Eley, 2010), which indicates good overlap but may also go some way to explain the differences between rater outcomes. Further, as has been discussed previously (Chapter 4), there is some debate whether observers can ever accurately report on *internalising* symptoms, and in combination with waning access to the increasingly autonomous adolescents, some may argue that self report should be given a priori preference.

Therefore, a more appropriate comparison of the self report results (all following comparisons in this discussion will be on same raters) might be one with the Lamb et al. (2010) study discussed in the introduction, presenting self report data across adolescence on anxiety traits (on the YSR): in relation to their 16-year results of heritability around 50%, the TEDS results suggest significantly lower genetic effects. In other words, unlike this Dutch sample, self report in TEDS did not suggest increasing trait similarity for genetic reasons across adolescence.

Taking into account the fact that self rated univariate twin correlations are comparing responses from two individual twins, twin similarities on anxiety traits of up to .76 (MZF) in their sample are remarkably high. Indeed, current results more closely resembled their 12-year estimates. A difficulty in making any statements about different developmental patterns observed by the two studies is however that some participants in Lamb et al. (2010) only took part in the study at a single time point, and others in any two out of three, the implications of which are unclear. Of note, in the previously discussed adult twin study including anxiety traits (Lundström et al., 2011), genetic

influences were reported as only modest and lower than the two just discussed adolescent studies.

More specifically with respect to anxiety sensitivity, previous studies have estimated heritability to be around 35% in children (Eley, Gregory, Clark, & Ehlers, 2007) and 50% (Stein, Jang, & Livesley, 1999) in adults. Similarly to the results just presented, heritability in adolescence has been shown to fall within that range (.34-.43; Zavos et al., 2010). However, the authors of the latter study also report that despite significant differences in levels of phenotypic association of anxiety sensitivity with depression (~.50) and anxiety (~.70), it shows a strong genetic correlation with both these conditions. If, as is further suggested (Zavos et al., 2010), anxiety sensitivity acts as a more general (mostly genetically driven) risk factor for internalising phenotypes, it seems overstretching the data to make any inferences about the clinical implications for anxiety specifically based on current results.

7.4.1.2 Parent report

With respect to the aetiological components found, the ACE model of parent report is consistent with the results on parent reported internalising traits at age 12 (also including the shared environmental aetiological component) using a different measure (SDQ, see Chapter 4 and Chapter 5), and with parent report at age 12 using four factor-derived subscales on the same measure (ARBQ; study discussed in detail in this chapter's introduction, Hallett et al., 2012).

The finding of three equally important contributing factors (A, C and E) can be interpreted in several ways. First, it would suggest that interventions aiming to reduce anxious cognitions at age 16 should address shared factors such as family environment as much as twin-specific issues. However, with age, therapeutic approaches tend to move away from family-based settings to treat adolescents individually. The previous chapter found no significant *NSE* effects of (early) parenting on early adolescent internalising NSE differences. Given the finding of no *differential* effects of parenting in adolescence, this indicates that if parenting is having an effect on anxiety at age 16, it is likely to play a shared environmental role on the phenotype instead. Therefore, the continued inclusion of parents as part of treatment plans could be beneficial – in a seminal review on the nature and effect of parent-child relationships in adolescence, Steinberg (2001) similarly concludes that authoritative parents who are warm, firm, and accepting of their offspring's needs for psychological autonomy are creating conditions most conducive to positive development. The previous chapter also reported some

significant NSE effects, which could be informative of how to tackle twin-specific anxiety problems. For instance, childhood peer problems and hyperactivity in males were both identified as significant contributors to greater adolescent internalising traits via nonshared environment. A possible mechanism by which these variables may affect outcomes are suggested by a recent study by Fite, Rubens, Preddy, Raine, and Pardini (2013), who report an association of reactive aggression (the disposition to easily react or be provoked into impulsive aggressive behaviour) with elevated internalising problems, but only in those 16 year-olds who also had high levels of peer rejection and/ or poor communication with their parent.

7.4.2 Depression traits at age 16

7.4.2.1 Self report

For self rated depression traits, phenotypic outcomes were mostly due to nonshared environment, with genetic influences of less than half (males: 38/ 62%, females: 47/ 53%). The aetiological findings using self ratings of depression resembled both those reported in a different sample at the same age (Lamb et al., 2010) and in an adult sample (Lundström et al., 2011). However, somewhat different results have been reported by Gregory et al. (2011), based on a mixed cohort adolescent and young adult sample. Due to the preferred fit of an ADE model over the ACE alternative, the authors report point estimates for additive genetic effects of only 10%, supplemented by 34% of genetic dominance; nonshared environmental influences were similar to the current study at 56%.

A recent review (Uher, 2011) summarises the importance of knowing the relative contributions of aetiological factors to clinical depression: In the treatment of depression, evidence-based medicine today does not account for the vast individual variability in therapeutic response within each diagnostic group and clinical characteristics are relatively weak predictors of who will improve. The study of depression has produced affective, biological, and cognitive models of depression (Hyde, Mezulis, & Abramson, 2008).

In a next step, it will therefore be important to research the intersection of genes and environment. As is currently true of psychiatric traits in general (including autistic traits, as discussed in Chapter 1), a growing body of literature reports on significant GxE effects on the depressive phenotype (Jasinska, Lowry, & Burmeister, 2012) and the potential of specific polymorphisms in pharmacogenetics and using antidepressants

(Uher, 2011). The environmental side of the story, which at age 16 appears to be the most influential contributing factor, has also been explored. First, early adversity and maltreatment including physical, emotional abuse and neglect and sexual abuse have been highly consistently associated with higher risk, earlier onset, more chronic course and more comorbidity, and this association extends throughout the life course into old age (Nanni, Uher, & Danese, 2012). Second, stressful and negative life events including natural disasters, serious illness, death of a close other, relationship breakdowns, job loss and assault are known to be relevant factors typically within weeks or months prior to the onset of depressive disorders (La Greca, Lai, Joormann, Auslander, & Short, 2013).

7.4.2.2 Parent report

Depression traits as rated by parents showed the same extent of overall twin similarity as compared to twins' self ratings, however at the aetiological level in males this was attributable to mostly genetic influences and low shared environmental influences (50% and 12%). In females, genetic and shared environmental influences accounted for trait variance in equal parts (29% and 31%). Nonshared environment at age 16 was equally important for male and female trait presentation (38-40%). Reasons for taking a cautionary approach to interpreting the results of this specific measure will be addressed below when discussing limitations.

7.4.3 Autistic traits at age 16

Twin correlations of all self reported measures were lower than those from parent report, therefore the moderate heritability from self ratings was an expected effect. Nevertheless, heritability of around half of phenotypic outcomes points to substantial involvement of genetic factors. Indeed, a study (Hoekstra et al., 2007a) analysing responses of participants from the Netherlands Twin Register (NTR) on the same measure (AQ) found a closely similar aetiological composition ($a^2 = .53$, $e^2 = .47$) in 18-year-olds. Only moderate heritability (32%) was reported in the Swedish adult twin sample, though this may be owed to the fact that MZ twin correlations on most of their collected psychiatric trait measures were <.50. A potential reason for greater self rated twin similarity up until the age of 18 could be that twins with a large proportion of shared genes live in largely the same environments, resulting in the expression of likely more of the same autistic-like traits. In contrast, participants of the Swedish adult sample ranged from 20 to 47 years of age, by which time most of them would have left

their parents' home and created more twin-specific environments for themselves that may have influenced their expressed autistic trait levels.

Parent report reproduces the patterns of greater male heritability and relatively more important shared environment in females which has previously been found for twins' autistic traits at ages 8, 12 (Hallett et al., 2010) and age 14 (Chapter 4). Male genetic influences showed a tendency for increased heritability compared to ages 12 and 14, though similar levels had been found in mid-childhood at age 8. Similarly, the Swedish mixed cohort sample has reported equivalent heritability at ages 9/12 (Lundström et al., 2011).

Some of the difference in heritability in late adolescence in the current study found between parent and self report may have resulted from a different number of items (28 and 13 respectively) being used. As discussed in Chapter 5, some reduction of the original 50 items on the AQ has been shown to improve scale reliability while maintaining face validity. However, limiting the twin measure to 5 non-social, and 4 social and communication items each increased the relative contribution of individual item scores and may have affected twin correlations.

7.4.4 Patterns of overlap between autistic and anxiety traits, and autistic and depression traits in late adolescence

Aetiological trait overlap of autistic traits and anxiety traits as rated by parents was significant and substantial for all correlations (r_g = .48-.53, r_c = .62-.67, r_e = .44-.50). The genetic correlation on self reported overlap was equivalent, but the nonshared environmental correlation found was lower (r_g = .43-.45, r_e = .14-.17). The magnitude of parental overlap estimates were similar to those reported using the mixed-age mid-childhood sample, though the latter did not include shared environmental overlap. In relation to earlier TEDS data, all aetiological correlations had increased again (as previously from age 8) on age 12/14. The correlations obtained from self report resemble those found in Swedish adult twins (Lundström et al., 2011).

As discussed above, anxiety sensitivity has been proposed as a vulnerability factor in the genesis initially only of panic disorders has now been broadened to include other internalising disorders. Individuals with ASD are known to experience both somatisation and alexithymia (inability to describe emotions in the self, Taylor, 1984), and anxiety sensitivity relates primarily to a cognitive bias to interpret physiological changes as threatening. In addition, it has also been argued that anxiety sensitivity itself

may arise as a separate trait-like construct or could be acquired as learned experience. The significant bivariate associations with autistic traits found at the genetic and the environmental level may be interpreted in this light. Both suggestions are consistent with examples such as the familiality of alexithymia (Szatmari et al., 2008) on the one hand, and the occurrence of hyper- and hyposensitivities (Kern et al., 2006) on the other hand, which are stable in some, changing in others in individuals with ASD.

Parent rated aetiological trait overlap of autistic traits and depression showed non-significant genetic correlations for females and a low estimate for males (r_g = *ns*-.17). Environmental correlations were high on shared and moderate on nonshared influences for both sexes (r_c = .63-.74, r_e = .25-.29). Of note, as with the univariate parent depression ratings, the bivariate parent depression-autistic trait model should be interpreted with caution.

Adolescent self ratings demonstrated substantial genetic and modest nonshared environmental correlations between autistic and depression traits (r_g = .43-.45, r_e = .14-.17). As stated previously, there are no population based twin studies in childhood available to compare this specific trait overlap to. The pattern found in self ratings appears to replicate that found in adulthood (Lundström et al., 2011). Furthermore, the Hoekstra et al. (2007a) study previously mentioned in the discussion on autistic traits above studied the AQ in relation to withdrawn behaviour on the YSR in young adults, finding estimates of 64% bivariate heritability and 36% bivariate nonshared environment.

7.4.4.1 Generalist genes hypothesis

As shown in Figure 7.1, shared factors (genetic in the self rated models and genetic + shared environmental in the parent rated models) across autistic and anxiety/ depression traits accounted for 64-90% of the phenotypic correlations. Bivariate heritability ranged from just under half to fully three quarters of the trait covariances (ignoring parent rated depression, see limitations below). This finding warrants further discussion.

High genetic correlations and bivariate heritability suggests the involvement of largely the same genetic factors on bivariate trait pairs. In the 'generalist genes' hypothesis, such mechanisms have been suggested with respect to cognitive abilities and disabilities (Kovas & Plomin, 2006). Similarly, Kendler, Prescott, Myers, and Neale (2003) investigated the structure of genetic and environmental risk factors for common psychiatric and substance use disorders finding strong relationships between genetic

factors affecting broader categories (internalising/ externalising) and the specific disorders contained therein. On the micro end of the spectrum, general genes have also been proposed for anxiety sensitivity and anxiety symptoms (Eley, 1997; Waszczuk et al., 2013). Given this pattern of common and unique genetic effects at each level of analysis, it appears probable that there could exist general genes relevant to psychopathology in general, and for the overlap of autistic-like and internalising behaviours in particular. Indeed, recently evidence has been put forward for a 'generalist genes, specialist environments model' (Lahey et al., 2012; Lahey, Van Hulle, Singh, Waldman, & Rathouz, 2011) consistent with the lower overlap of nonshared environmental influences reported here.

As previously discussed, a recent study reports that age 16 anxiety and depression have high but less than full correlation on genetic factors (Lamb et al., 2010). Though with the information given it was not possible to calculate the exact bivariate heritability, the range of phenotypic correlations between anxiety and depression traits given (r = .59-.69) corresponds to a proportionate effect of their bivariate heritability of 60% to 71%. Remarkably then, in late adolescence in the current study, autistic traits share as much of their genetic aetiology with depression and anxiety traits as do the two internalising traits within the category of internalising.

Chapter 3 has discussed the familiality of psychiatric disorders in general, and the increased risk of homotopic and heterotopic transmission of ASD and internalising disorders. Another emerging pattern of importance in the literature is also that genetic factors are generally a source of continuity over time to a higher degree than environmental factors, which tend to bring innovation and are time-specific (e.g. (Zavos et al., 2012a). In adolescence, to the extent that individuals are predisposed to experience negative outcomes on single traits for genetic reasons (i.e. ~40% for internalising, 50-70% for autistic traits), almost three quarters of genetic factors can also act as 'troublemakers' for the respective other trait.

7.4.5 Limitations

At repeated points in the discussion, caution was issued in interpreting the results of the parent reported depression traits. As reported in the results section, mean depression symptoms were very low, indicating that parents did not see a great number of depressed behaviours in youths. Particularly with respect to the sharply rising incidence of depression after puberty in the general population, the low parent reported symptoms are of concern and could point to parents missing crucial signs in their offspring. This

resulted in high skew of the MFQ measure. Of note, the MFQ has been recognised as as a scale that produces particularly highly skewed distributions with most respondents endorsing zero scores (Sharp, Goodyer, & Croudace, 2006). Of the three studied parent reported traits, this measure also showed the lowest degrees of twin similarity, reducing the covariance to be partitioned during twin modelling. Upon investigating the potential reasons for such low observed depression symptoms, it was initially reasoned that factors such as adolescents' increased independence resulting in less time spent with parents, and perceptions of the 'moody teenager' may have affected ratings leading to systematic under-reporting. In addition, it has also been suggested that parents' ratings of their own depression may affect the perceptions of depression in their children (Moretti, Fine, Haley, & Marriage, 1985). Significantly higher self identified behavioural problems in adolescence have been demonstrated on other measures (CBCL, YSR; Sourander, Helstela, & Helenius, 1999; Verhulst & Vanderende, 1992), providing an important reason for including self report in TEDS at this developmental stage.

Alternatively, low ratings may point to issues with parent report on the instrument (in TEDS) more generally, as suggested by a similarly skewed scale obtained at age 12 (Wilkinson et al., 2013). Data on the criterion validity of the MFQ and SMFQ found contradicting results with respect to who (parents or children themselves) more conservatively or freely observed and labelled behaviour as 'depressed' as compared to clinical judgement (Daviss et al., 2006; Thapar & McGuffin, 1998); – somewhat contradicting to what would be supposed from the TEDS data here, the authors of the respective studies recommended a higher clinical cut-off score for self ratings on the longform MFQ, but on the short MFQ, the cut-off for parent ratings was higher.

Another limitation as mentioned above is that the moderate shared environmental effects that were found using parent report but not self report could be partially due to rater effects. It should be noted that when the same informant reports on the behaviour of both twins, rater bias may lead to an overestimation of shared environmental effects due to correlated rater bias across the twin pair (Bartels, Boomsma, Hudziak, van Beijsterveldt, & van den Oord, 2007). Rater bias has not been tested in the current study. Indeed, it has been suggested that behaviour genetic research thus far has found shared environmental influences on only a few disorders (Plomin, 2011; Plomin, DeFries, McClearn, & McGuffin, 2008). Thus, the question of whether twin similarity

is subject to apparently increasing genetic and/ or shared environmental influences across development will require further future exploration.

Finally, in comparison to any previous child data on these trait co-occurrences, bivariate heritabilities found in this study represent a notable increase. Comparing self report measures, current outcomes are also 10-20% higher than those reported across adulthood. In arguing for the involvement of generalist genes, it is unclear whether they are particularly relevant in late childhood and once again abate in adulthood, such that genetic overlap on many psychiatric traits is especially high during adolescence. Another possibility is that higher bivariate heritability in adolescence in TEDS compared to the adult STAGE sample (Lundström et al., 2011) could reflect differences in sample characteristics.

To summarise, the results provided in this chapter have added to data in late adolescence, which is lacking for patterns of co-occurrence of autistic with anxiety traits and particularly with depression. Self ratings suggested a similar aetiological composition of anxiety and depression traits with less of variances explained by genetic factors than nonshared environment. Self rated anxiety and depression trait overlap with autistic traits showed genetic correlations of ~.50 and more modest nonshared environmental correlations. Parent ratings exhibited greater sex differences and greater male heritability on autistic and depression traits, while shared environment was a component of equal importance on all traits in females. Twin similarity on traits also showed substantial overlap. These findings demonstrate evidence for the involvement of generalist genes. The following, final chapter will summarise the main findings of this thesis and provide a general discussion.

Chapter 8 General Discussion

8.1 Summary of Background

ASD are disorders with important ramifications for individuals' ability to function in society and on their life quality. Internalising disorders including anxiety and depression often pose frequent additional challenges for people with ASD. Internalising disorders become more common across childhood and particularly during adolescence. ASD and anxiety disorders commonly co-occur. Less is known about to what extent the same or different factors contribute to the co-occurrence of these two phenotypes and the aim of the thesis was to elucidate their individual and shared aetiologies during adolescence using a trait approach, which quantitatively measures the behavioural characteristics across the full dimension of expression.

As previously discussed in Chapter 3, family studies have demonstrated that characteristics of both the autism and internalising phenotypes are often found in parents and siblings of people with ASD, suggesting familiality. Previous research suggests some specificity in the transgenerational transmission of disorders with signs and symptoms in parents frequently also presenting in filial generations. Beyond this, there also appears to be more general transgenerational vulnerability factors demonstrated by the fact that relatives of individuals with clinical status for one disorder are also at greater risk of displaying clinically relevant behaviour for another psychiatric disorder (references provided in Chapter 3). However, family studies cannot address to what extent family resemblance for psychopathology stems from genetic and shared environmental influences respectively.

Twin studies provide a powerful tool for the consideration of relative contributions of aetiological factors, though some assumptions are being made. Greater statistical power can be garnered by studying psychological phenotypes as quantitative traits within the general population (again with some limitations as to how directly this informs us of associations between the clinical disorders). The aetiology of co-occurring autistic and internalising traits specifically in adolescence was the theme of this thesis.

8.2   Summary of Findings

8.2.1 What are the phenotypic and aetiological associations between autistic and internalising traits in early adolescence?

For the first time in this age group, 14-year-old twins' autistic traits were investigated in relation to their internalising traits (at age 12 years) in a twin sample. Beyond a moderate phenotypic association, results showed substantial genetic influences on autistic traits and moderate genetic influences on internalising traits. With respect to environmental influences, shared environment further increased twin similarity on autistic traits, while nonshared environment acted on internalising traits. Nonshared environmental influences were low for autistic traits. Overlap in the trait aetiology in early adolescence between autistic and internalising traits was demonstrated in significant modest to moderate genetic and modest nonshared environmental correlations.

Studying the co-occurrence of autistic and internalising traits in mid-childhood, Hallett (2010) previously noted that in the general population (as assessed via the same TEDS twin sample at a younger age), these traits did *not* show the strong shared phenotypic and genetic influences that might have been expected from clinical reports emphasising the heightened levels of internalising difficulties in children with ASDs. Together with previous phenotypic studies on the trait overlap between autistic and internalising traits (Constantino & Todd, 2003; Hoekstra et al., 2007a) and more recent aetiological work on mid-childhood and adulthood (Lundström et al., 2011), this observation of modest phenotypic and genetic overlap has now been shown to hold across multiple samples and age groups, and was also replicated in this thesis.

8.2.2 Are there distinguishable patterns of overlap between specific autistic subdomains and internalising traits?

Given the breadth of behaviours contained within the autistic spectrum, and the previously reported fractionation of the autistic triad contingent on level of analysis (Happé & Ronald, 2008), another aim within this thesis was to test specific autistic-like subdomains for their association with internalising traits. These analyses confirmed that specific autistic behaviours each have their unique individual aetiology and overlap with internalising behaviours. An autistic trait social unease factor appeared to show most phenotypic and genetic overlap with internalising behaviours. The other four derived

specific autistic trait subdomains showed low phenotypic, genetic and environmental overlap with internalising traits.

Mid-childhood data from TEDS (Hallett, 2010) suggests that of the autistic triad of impairments, communication problems showed the strongest association with internalising traits in the general population. However, in a proband-ascertained sample (a subsample of TEDS twins who were suspected or confirmed ASD cases, and their co-twins) no such differences between individual autistic symptom subdomains in their association with internalising traits were found (Hallett, 2010). Difficulties in communication in mid-childhood and autistic-like social unease in early adolescence are therefore associated with internalising traits at the trait level. The co-occurrence with non-social difficulties plays a lesser role in relation to internalising traits. This provides further support to the assertion laid out in the fractionable autism triad hypothesis (Happé & Ronald, 2008) that phenotypic presentation is affected by a complex aetiology that varies at different levels of analysis and whose relevant additional contributing factors are subject to high individual variability, once again emphasising the value of investigating causal questions separately for the different autistic subdomains (Sucksmith et al., 2011).

8.2.3 Which specific nonshared environmental factors contribute to autistic and internalising trait twin differences in adolescence?

Nonshared environment consistently plays a small aetiological role in phenotypic autistic trait levels, and a greater role in internalising traits. Specific environmental effects on internalising traits have been suggested mainly for early childhood, but the same NSE differences have not been tested systematically for their effect in adolescence. Analyses were able to identify nonshared environmental factors important for both internalising and autistic trait differences. Findings replicate the previously reported significant NSE effect of lower birth weight but not pre/peri- and neonatal problems on later NSE autistic trait differences and newly showed an important role of peer problems in mid-childhood on differential outcomes in twin pairs with respect to autistic and internalising traits in early adolescence. The effects demonstrated were small and this was expected based on the findings of previous MZ differences studies.

To further illustrate this, two publications deserve mention here, one summarising twin studies describing the effect of twin birth weight differences on mental, motor and physical development (Datar & Jackowitz, 2009) and the other studying (non-twin)

perinatal and neonatal risk factors for autism, (Gardener, Spiegelman, & Buka, 2011). Gardener et al. (2011) identified over 60 significantly associated risk factors including low but not high birth weight. However, controlling for the influence of maternal, environmental and genetic factors, Datar and Jackowitz (2009) showed only small effects on twin *differences* in developmental outcomes. With respect to the current findings, these studies suggest that there are a range of potential environmental contributors to autistic trait outcomes, but that effects on twin differences tend to be small.

Peer problems are known predictors of later internalising behaviours (Hymel, Rubin, Rowden, & LeMare, 1990; Troop-Gordon & Ladd, 2005). With respect to autistic traits, less impairment in social interaction skills are known to predict friendships, peer relationships and the participation in social and recreational activities (Gardener et al., 2011), though it has been suggested that intra-familial relationships and conflict are more predictive of ASD symptomatology than peer relationships (Kelly, Garnett, Attwood, & Peterson, 2008). An alternative explanation may however be that family-factors are important because familial accumulation of autistic-like characteristics affects outcomes. The findings presented in this thesis show that NSE twin *differences* in how well twins relate to their peers in mid-childhood are predictive of both internalising and autistic NSE trait differences in early adolescence. As such, children with more frequent and more severe peer conflict throughout childhood are likely to display relatively greater autistic and internalising difficulties in early adolescence in part via nonshared environmental mechanisms.

8.2.4 What are the phenotypic and aetiological associations between autistic and specific internalising traits in late adolescence?

The aetiology of co-occurring autistic traits with anxiety and depression traits in twins has never been specifically investigated at age 16 years, though their association may conceivably be highly relevant in the domains of progress and well-being in a school environment, the formation of romantic relationships and independent living. The individual aetiologies of the three traits studied were similar to those found in early adolescence (earlier autistic traits similar to later autistic trait aetiology, earlier internalising traits similar to later anxiety/ depression aetiological findings) and quantitative sex differences continued to play a role. Some differences also were found between results derived from self report and parent report measures.

A novel finding at age 16 years was that the phenotypic trait overlap between autistic and anxiety traits, and between autistic and depression traits could mostly be accounted for by genetic factors. Genetic correlations between self rated autistic-anxiety and autistic-depression trait pairs, and for the parent rated autistic-anxiety trait pair were substantial, and environmental correlations for these pairings were modest. In the parent rated models, similarity across traits was also indicated by substantial shared environmental correlations. These correlations translated into bivariate heritabilities of 50-75% (with the exception of the model including parent rated depression traits, whose limitations have been discussed previously).

Given the differences in typical age of onset between anxiety and depressive disorders, with anxious behaviours observed throughout childhood and depression becoming more prevalent from adolescence, it was important to establish separately the associations of anxiety and depression traits with autistic traits. Results showed small differences in their aetiology and aetiological association that lent support to the rational of studying these traits as conceptually coherent under the umbrella term of internalising traits, but also to study their individual co-occurrences with autistic traits.

The finding of large bivariate heritability of autistic with anxiety and autistic with depression traits was suggested to point to the involvement of 'generalist' genes, which could contribute to outcomes on multiple traits. The idea of generalist genes has been applied by others (Scharf & Mathews, 2010) to broadly related phenotypes, noting that the same large (>100kb) deletions/ duplications at specific loci throughout the genome appear to be present in patients with a wide range of neurodevelopmental phenotypes including autism, developmental disability, schizophrenia, ADHD, Tourette's and seizures. Generalist genes have also been suggested to be responsible for the performance on several cognitive processes (Kovas & Plomin, 2006). The shared genetic aetiology of the studied traits at age 16 years could indicate that generalist genes may also play a role across disorders in the sense of a general psychiatric vulnerability (as suggested by transgenerational heterotopic transmission of disorders in family studies, and pleiotropic effects in psychiatric disorders; Smoller et al., 2013; Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013).

8.3   Implications

In light of the high number of individuals with ASD who are affected by additional comorbid conditions, and the observed increase of internalising conditions during

adolescence in the general population, the present thesis had as its aim to quantify how closely autistic and internalising traits are associated in adolescence both at the phenotypic and at the aetiological level.

First, the most unexpected finding of the analyses presented was from Chapter 7, which showed a substantial bivariate heritability between autistic and anxiety traits, and autistic and depression traits, i.e. *across* psychiatric phenotypes. Similar levels have been found *within* diagnostic categories among internalising conditions (Lamb et al., 2010). Given significant genetic overlap in late adolescence, it may make some sense to test genes associated with internalising traits for their role in autistic traits. In comparison, the findings from early adolescence suggest only modest to moderate genetic overlap. Studies on gene-environment interactions of serotonin, genotype and maternal anxiety (Kumsta et al., 2010; Zavos et al., 2012c) may give a first indication of some candidate gene systems that could be involved. However, with respect to generalist genes it may also be hypothesised that potential genes involved are likely to relate to biological functions that are not exclusive to ASD, such as the recently demonstrated role of some calcium-channel subunits in a range of structural and functional brain phenotypes, including circuitry involved in emotion processing, executive function, attention and memory (Smoller et al., 2013).

Second, the study of nonshared environmental factors gave an insight into specific variables contributing via nonshared environmental to autistic traits and internalising traits in early adolescence. Previous studies have emphasised the NSE role of parental personality and home characteristics on a range of psychological trait outcomes. Therefore, it is of particular note that no significant NSE effects of parental discipline and negativity at ages 3 and 4 years on adolescent outcomes on internalising and autistic trait differences were found, however NSE effects of peer problems on autistic traits and internalising traits were significant as early as age 4 and effects further strengthened across the ages 7, 9 and 12 years. Child development is often portrayed as a journey towards full self-sufficiency and independence, during the early years of which a child is largely dependent on their parents for guidance and confirmation, while a greater role of peer evaluation is usually allowed for during adolescence (Fergusson & Horwood, 1996). Yet, at least with respect to nonshared environment, relationships with peers appear to be formative, or at least informative, of later adolescent autistic trait outcomes from a very early age in this study. An implication of this is that facilitation of

successful peer relationships is likely to have small significant effects on later adjustment independent of genetic and familial influences.

Third, the development and subsequent use of the AQ autistic trait subdomains showed that all autistic-like behaviours are not equal in their association with internalising traits. As expected from the rotation and extraction strategy used, individual factors accounted for different proportions of trait variance in the sample. The magnitude of phenotypic associations with internalising traits showed a pattern that of higher correlations for the social/communication autistic trait domains over the non-social ones. A somewhat contrasting hypothesis to the findings of this thesis suggests that rigid thinking and behaviour might be associated with anxiety (Rodgers, Riby, Janes, Connolly, & McConachie, 2012), e.g. when events occur that violate individuals' expectations and rituals. However, in TEDS, the association of autistic trait domains and internalising traits has been consistently greater for the social/communication domains compared to the non-social domain (Hallett, 2010). Specifically, the findings presented in this thesis show that the phenotypic correlation of social unease and internalising traits was more than twice as high as any other subdomain association, highlighting the potentially special role of social unease in relation to internalising.

8.4 Limitations

Limitations specific to the individual chapters have been previously discussed and will not be repeated here. The focus will instead be on general and recurring themes that apply to the thesis as a whole.

8.4.1 Twin design

All studies in this thesis utilised twin data and thus rely on the twin modelling assumptions outlined in section 2.2.1. Among the basic premises are that of the equal environments assumption and absence of gene-environment interaction, which cannot be tested in a twin-only design and has been reviewed in the methodology chapter. In addition, with regard to generalisability, the twin design assumes that twins and singletons are comparable in terms of their autistic and internalising trait levels. While Curran et al. (2011) found no major effect of twinning on autistic traits, it is important to bear in mind that others (Greenberg et al., 2001) found that twins are at greater risk of ASD. Chapter 6 found a NSE birth weight differences to be associated with early adolescent trait differences on autistic and internalising traits. However, in interpreting this result a limiting factor is that twins, competing for intrauterine space, are on

average of lower birth weight compared to singletons. Another rare case causing MZ twin birth weight differences which is not generalizable to singletons is twin-twin transfusion syndrome, occurring in 1 in 58 twin gestations, though presently a significant proportion of these will not result in live births (Gandhi, Teach, Papanna, Johnson, & Moise, 2011).

8.4.2 Sample characteristics

With TEDS being a longitudinal study, the sample is subject to a low level of attrition bias, which may impact the current work. Response rates and representativeness of the sample in childhood have been presented by Oliver and Plomin (2007), and an extension into adolescence is provided in Haworth et al. (2013). With respect to response rates, a special case is the age 14 year datapoint. As noted earlier in Chapter 4, several factors (e.g. changes in data collection, constraints on funding) resulted in the lowest to date TEDS data returns, meaning relatively less statistical power during twin modelling compared to other ages. That said, the respective data analyses nevertheless included >3,000 twin pairs.

In addition, in its goal to be a comprehensive study of learning abilities and disabilities, the autistic trait and internalising trait questionnaire measures included in TEDS were sometimes limited. For instance, no dedicated internalising trait measure was available at age 14 years. For the study of early adolescence, the co-occurrence of autistic traits at age 14 years with internalising traits was investigated looking at cross-age correlations with age 12 years internalising traits.

Next, in comparing the current findings from adolescence to the results obtained from the TEDS sample in mid-childhood, it is of note that children with ASD were not included in all stages of the data collection. Specifically, known ASD cases were included at age 8 years but excluded at age 9 years. A comparison of 8-year results first including then excluding these individuals did however not produce significant differences in findings (Hallett, 2010).

8.4.3 Questionnaire measures

A range of measures was used throughout the thesis to record autistic traits, internalising traits and anxiety and depression traits separately. Results consistently produced modest to moderate phenotypic associations, suggesting limited covariation between measures. While the multitude of measures is a limiting factor in directly comparing the findings in TEDS at different ages, the consistency of the reported levels

of trait associations across those measures, across raters and across development suggests that the extent of trait independence is unlikely to be solely accounted for by measurement error. As presented in their respective chapters, all measures used have been previously demonstrated to have good construct validity and test-retest reliability.

8.4.3.1 Autistic trait measures

Autistic traits in TEDS throughout adolescence have been collected using parent report (age 14, 16 years) and self report (age 16 years) on the AQ. The AQ is one of the most commonly used autistic trait measures and as such has been used in a variety of contexts. However, of the 50 items included in the original AQ, only 38 items were included in TEDS at age 14 years, and 28 items at age 16 years using parent report, and self reported autistic traits at age 16 relied on 13 items. Chapter 5 has discussed evidence that test accuracy may indeed be improved by reducing item numbers of the original AQ, and this has resulted in the publication of the AQ-short (Hoekstra et al., 2011), on which the TEDS parent questionnaire at age 16 was based. The 13 items used in self report were owed to the need for a very brief autistic trait measure and items were selected to represent the social (4 items), communication (4 items) and non-social domain (5 items). Reducing item numbers this much may have affected results, though internal consistency of the scale remained good (alpha of .77 in comparison to .84 from the parent data). It would have been of interest to explore self rated subdomains but the self report scale, containing less than half of the items of the original AQ, was judged too different from the original instrument to be able to construct meaningful AQ-subdomains from.

A question that this thesis hasn't specifically addressed is the continuity of trait measures at the 10%, 5%, 2% and 1% extremes. This question has however been addressed in TEDS at an earlier age using data from age 12 (Robinson et al., 2011a). Moderate to high heritability was found for autistic traits in the general population (52% for females; 76% for males). High heritability was found in extreme scoring groups. There were no differences in heritability among extreme groups or between the extreme groups and the general population. A continuous liability shift towards autistic trait affectedness was seen in the cotwins of individuals scoring in the top 1%, suggesting shared aetiology between extreme scores and normal variation. Since many of the high scorers on quantitative measures will meet diagnostic criteria for an ASD during clinical interview, establishing the aetiology of traits within individuals falling on the

quantitative extreme of the spectrum can give an approximation of the genetic and environmental influences within clinical ASD.

The trait measures employed in the current thesis are a convenient proxy for measuring characteristics typical of individuals with ASD but clearly cannot replace clinical assessment of suspected cases. An indication how trait levels at the high end relate to reported symptoms, the broader autism phenotype and clinical status can nevertheless be seen in the findings by the same group (Robinson et al., 2011b) reporting on the Avon Longitudinal Study of Parents and Children (ALSPAC) sample. These findings suggest that autistic traits are highly stable in the general population, even in individuals with the highest concentrations of autism-like behaviours. Phenotypic stability is consistent with expectations for individuals with autism spectrum disorders, providing further support for a phenomenologic continuum across the clinical threshold.

8.4.3.2 Internalising trait measures

The emotional symptoms scale of the SDQ – the internalising trait measure at age 12 years in TEDS – has been used with the sample throughout childhood (age-appropriate Behar questionnaire versions at age 2 and 3 years, and identically at ages 4, 7 and 9 years). The brevity of the measure (5 items) has already been discussed as a limitation. In addition, it is worth noting that the focus of the measure is symptoms of anxiety and depression that are closely aligned to the DSM diagnostic criteria for internalising disorders. This represents difference to the autistic trait measure used, which emphasises biases, likes and dislikes over symptoms. In this respect, the approach taken in late adolescence, using anxiety sensitivity (on the CASI) as the anxiety trait measure may more closely match the approach taken with autistic traits. However, it would have been interesting to be able to draw on a measure of attributional style for depression traits, which has been suggested as the conceptual trait equivalent, rather than continuing to use symptom reporting on the MFQ.

8.4.3.3 Informants

The analyses presented on early adolescence have relied on parent report, while TEDS data on autistic, anxiety and depression traits from both parents and twins themselves was available in late adolescence. Studying the specific trait co-occurrence that this thesis focused on may have presented a particular challenge: As discussed in chapter 4, it has been suggested that internalising traits, since they represent biases and feelings that are largely internal to the person in question, are difficult for an observer to report

on. Conversely, a common characteristic in ASD is the lack of introspection, such that someone external may be better placed to report on observed autistic-like behaviours. One simple solution might have been to combine parent rated autistic traits and self rated internalising traits in one model, though in early adolescence this would have meant a cross-rater cross-age (age 12 internalising, age 14 autistic traits) solution. At age 16 one of the aims of the analyses undertaken was precisely to study differences in parent and self ratings which would have been lost by creating such a combined model. However, one alternative, which would have made use of all available data that has not been explored in this thesis, is constructing a rater bias model. Yet, given the very low parent reported depression symptoms on the MFQ and the resulting skew on the measure, and the conceptual differences between the self report anxiety trait measure CASI (anxiety sensitivity) and the parent reported ARBQ (anxiety symptoms), a decision was made not to pursue this model.

8.5   Future research directions

With regard to future research directions, some questions remain unanswered, both on the co-occurrence of autistic traits and internalising traits, and on their association in adolescence more specifically.

8.5.1 ASD+Anxiety vs. Anxiety in ASD

Given the challenges observed in individuals with ASD, an extension to the point made above about being able to accurately report on autistic trait levels, individuals may also find it difficult to report on their emotions. Alternatively, higher autistic traits may represent a bias against experiencing *cognitions* of anxiety, but anxiety could still be represented as a state of high arousal. Distinguishing displays of non-social autistic-like behaviours that are integral to ASD (i.e. RRBIs) and may indeed serve self-soothing purposes from the nervous energy owed to feelings of anxiety and distress therefore poses an interesting future avenue of research. Some (Wood & Gadow, 2010) have also been debating whether co-occurring anxious symptoms in individuals with ASD meet the criteria of a true co-occurrence. The findings of this thesis suggest that in adolescence there exists a noteworthy role of shared genetic aetiology between autistic traits and internalising traits but also an extent of aetiological independence. A further question has been whether standard clinical and trait measures of anxiety are appropriate to detect possibly differently expressed anxiety symptoms in ASD. Recently, attempts have been made to develop such specific measures (Rieske et al.,

2013) and it would be interesting both at the phenotypic and at the aetiological level to see this measure applied to a large twin sample.

8.5.2 Cognitive ability as a moderator

As noted initially in the general introduction, the autistic spectrum is very heterogeneous and autistic trait measures have been designed to collect data on behaviours characteristic of ASD largely irrespective of cognitive ability (although items may not be applicable to the very low IQ (intellectual disability) end of the spectrum). It has been suggested that children with higher IQ and greater social impairment experience the most severe anxiety (Sukhodolsky et al., 2008), although no significant difference in IQ was found in another study between children with PDD-NOS who had comorbid internalising disorders and those who did not (De Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007). Somewhat related to the previous section, cognitive ability may moderate the way that anxiety is experienced and there is emerging evidence for associations between anxiety, sensory hypersensitivity and degree of social impairment (Bellini, 2006; Pfeiffer, Kinnealey, Reed, & Herzberg, 2005). Therefore future work could include specific cognitive abilities as moderator variables, which have previously been influential in ASD explanatory frameworks, e.g. theory of mind (ToM), executive functioning (EF), or have direct bearing on emotion processing, e.g. alexithymia.

8.5.3 Clinically minded studies on autistic and internalising traits in adolescence

As was laid out repeatedly, utilising the twin design and using a trait based approach with participants recruited from the general population gives both the methodological and statistical power to study the trait co-occurrence of autistic and internalising traits. Despite challenges in finding an appropriate trade-off in power vs clinical relevance of findings, given the paucity in available aetiological data on the trait overlap in late adolescence, it would be desirable to replicate their bivariate aetiology (i.e. of a high proportion of genetic aetiology accounting for their co-occurrence) with an adolescent clinical sample, or alternatively using a proband-ascertained or broader autism phenotype (BAP) sample.

Another finding that lends itself to a follow-up is that of the social unease autistic trait subdomain showing the greatest phenotypic and genetic overlap with internalising traits. A possible future study could select on high social unease scores (e.g. the 10% most

socially uneasy individuals) and investigate their outcomes with the prediction that they would show elevated internalising and related difficulties. The study of nonshared environmental effects also demonstrated effects of peer problems on autistic traits. Therefore, it would be of relevance to explore whether peer problems also lead to greater social unease specifically. Finally, again in the chapter focusing on nonshared environment, differences in NSE hyperactivity had significant effects on later outcomes. Work on co-occurring autistic and externalising traits in TEDS in early adolescence is currently being carried out (Taylor et al., 2013) and a review contrasting findings on co-occurring autistic and internalising against externalising traits is beyond the scope of this thesis but would be beneficial.

8.6 Concluding remarks

Proceding from the knowledge that ASD is highly comorbid with a wide range of psychiatric disorders, of which internalising disorders are a common and increasing across development co-occurrence, this thesis was interested in establishing the aetiological factors that are unique to the autistic and internalising phenotypes, and those that are shared across the two. Using quantitative genetic techniques and a large population twin sample, it focused on adolescence, which has been a relatively understudied developmental period. Findings demonstrated that while individual trait aetiologies remained relatively stable across adolescence, increasingly across these years, the moderate phenotypic similarity on autistic and internalising traits (anxiety/ depression traits) was driven by common genetic factors shared across traits. This observation of a greater phenotypic association translating into greater genetic commonality was also true of the autistic trait subdomain 'social unease', which had been identified as particularly associated with adolescent internalising traits. A complimentary approach was taken studying nonshared environmental twin differences that was able to identify small significant effects on differences in adolescent autistic/ internalising trait outcomes resulting from twin-specific childhood peer problems and relatively lower birth weight. This suggested an additional role of specific environments altering twins' developmental trajectories on the studied traits.

Overall, results of this thesis point to a complex bivariate trait aetiology of autistic and internalising traits. It would be beneficial for future work to explore the effects of IQ and potential differences of primary internalising conditions to their presentation within ASD. It was demonstrated that there is a multitude of aspects to consider even within a single document including differences across time (early/ late adolescence), rater

(parent/ self), by modelling approach (twin modelling/ factor analysis/ MZ differences) and level of analysis (phenotypic/ aetiological). Crucially, with respect to the repeatedly cited fractionation of the triadic autism features, it is important to continue to explore the causes and mechanisms underlying these difficulties using the breadth of quantitative genetic, molecular, and clinical work to inform both clinical diagnosis and effective intervention practices.

Appendices

Appendix I Position of disorders on the autism spectrum in DSM-5
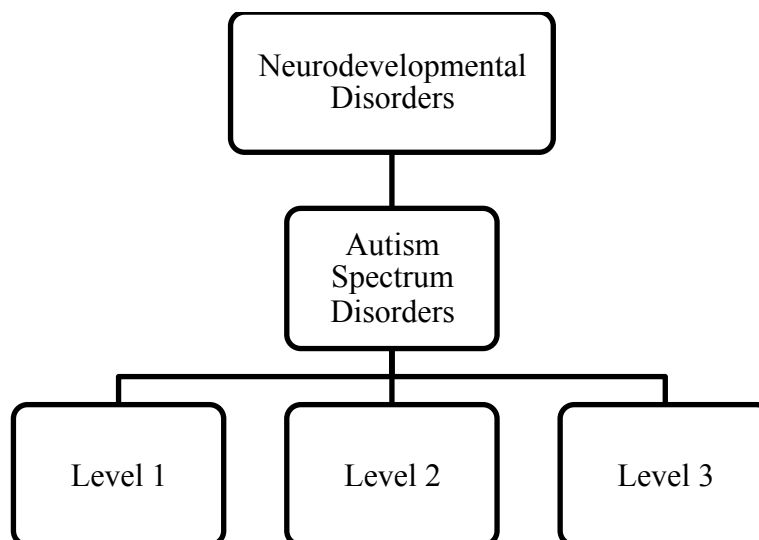
Appendix II DSM-5 Proposed levels of severity for autism spectrum disorders

Severity Level for ASD	Social Communication Impairments	Restricted Interests & Repetitive Behaviours
Level 3 'Requiring very substantial support'	• Severe deficits in verbal and nonverbal social communication skills cause severe impairments in functioning; • Very limited initiation of social interactions and minimal response to social overtures from others.	• Preoccupations, fixated rituals and/ or repetitive behaviours markedly interfere with functioning in all spheres. • Marked distress when rituals or routines are interrupted; • Very difficult to redirect from fixated interest or returns to it quickly.
Level 2 'Requiring substantial support'	• Marked deficits in verbal and nonverbal social communication skills; • Social impairments apparent even with supports in place; • Limited initiation of social interactions and reduced or abnormal response to social overtures from others.	• RRBIs and/ or preoccupations or fixated interests appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. • Distress or frustration is apparent when RRBIs are interrupted; • Difficult to redirect from fixated interest.
Level 1 'Requiring support'	• Without supports in place, deficits in social communication cause noticeable impairments. • Has difficulty initiating social interactions and demonstrates clear examples of atypical or unsuccessful responses to social overtures of others. • May appear to have decreased interest in social interactions.	• Rituals and repetitive behaviours (RRBIs) cause significant interference with functioning in one or more contexts. • Resists attempts by others to interrupt RRBIs or to be redirected from fixated interest.

(APA, 2013)

Appendix III Phenotypic correlations of overall autistic traits, autistic trait subdomains and CAST

	Pearson Correlation					
	Autistic traits^	Attention to Details/ Special Interests	Social Ease	Poor Mentalising	Solitariness	Poor Imagination
CAST total	.54	.28	.39	.36	.23	.33
CAST Communication	.46	.17	.32	.36	.10	.28
CAST Non-Social	.39	.35	.25	.12	.19	.13
CAST Social	.33	.05	.13	.16	.07	.14

Note: CAST = Childhood Asperger Syndrome Test – parent report taken at age 12 in same sample; all significant at p <.001.

^ 38-item AQ as used in Chapter 4 (Autistic Traits)

Appendix IV MZ, DZSS and DZOS: intraclass and CTCT correlations of age 12 internalising traits and age 14 autistic trait subdomains

	Internalising Traits	N		Attention to Details/ Special Interests	N	CTCT	Social Unease	N	CTCT
MZ	.76 (.73-.78)	1093		.97 (.96-.97)	1093	.14 (.03-.23)	.92 (.91-.93)	1093	.48 (.42-.54)
DZSS	.51 (.44-.57)	938		.86 (.84-.88)	937	.15 (.03-.25)	.61 (.56-.66)	938	.36 (.27-.43)
DZOS	.54 (.47-.60)	836		.79 (.76-.82)	835	.18 (.05-.28)	.58 (.52-.63)	836	.34 (.24-.42)
	Poor Mentalising	N	CTCT	Solitariness	N	CTCT	Poor Imagination	N	CTCT
MZ	.96 (.95-.96)	1088	.32 (.24-.40)	.89 (.87-.90)	1088	.17 (.06-.26)	.94 (.94-.95)	1087	.26 (.17-.35)
DZSS	.70 (.67-.74)	936	.29 (.19-.37)	.54 (.48-.59)	935	.18 (.07-.28)	.77 (.74-.80)	937	.24 (.13-.33)
DZOS	.67 (.62-.71)	831	.24 (.13-.34)	.44 (.36-.50)	832	.15 (.03-.26)	.68 (.63-.71)	835	.13 (.01-.24)

Note: 95% confidence intervals shown in parenthesis. CTCT = Cross-twin cross-trait correlations with internalising traits. DZOS = DZ opposite-sex twin pairs; DZSS = DZ same-sex twin pairs MZ = MZ twin pairs.

Appendix V Fit statistics and parameter estimates from the best fitting univariate models of age 12 internalising traits and age 14 autistic trait subdomains

	Model Fitted	χ^2	*df*	*p*	AIC	A	C/ D	E
Internalising traits	Saturated							
	ACE	0.873	6	.99	-11.127	.52 (.40-.60)	.09 (.02-.19)	.39 (.36-.43)
Attention to Details/ Special Interests	Saturated	-	-	-	-			
	ACE	10.34	6	.11	-1.66	.33 (.29-.38)	.61 (.56-.66)	.06 (.05-.06)
Social Unease	Saturated	-	-	-	-			
	ACE	26.19	6	.001	14.19	.52 (.45-.61)	.37 (.29-.45)	.11 (.09-.12)
Poor Mentalising	Saturated	-	-	-	-			
	ACE	8.36	6	.21	-3.64	.71 (.62-.80)	.20 (.11-.29)	.09 (.08-.10)
Solitariness	Saturated	-	-	-	-			
	AE	8.99	7	.25	-5.01	.84 (.82-.85)	D^2 dropped	.16 (.15-.18)

	Model Fitted	χ^2	*df*	*p*	AIC	A	C/ D	E
Poor Imagination	Saturated	-	-	-	-			
	ACE	17.80	6	.007	5.80	.42 (.35-.49)	.47 (.40-.54)	.11 (.10-.13)

Note: Values of the best fitting model are compared with the saturated model. Estimates of A, C/ D, and E are provided on the right of the table, taken from the best fitting univariate model. Confidence intervals are given in parentheses. -2LL = log likelihood fit statistic; χ^2 = likelihood ratio χ^2 test with df comparing model to saturated model; AIC = Akaike Information Criterion (lower values reflect a better model fit); A = additive genetic influences; C = shared environmental influences; D = genetic dominance effects; E = nonshared environmental influences.

Appendix VI Properties of social unease autistic trait subdomain after exclusion of potential confounding items

	Phenotypic association with internalising traits (r_{PH})
Autistic traits (38 items) as used in Chapter 4	.29 (males)/ .30 (females)
Total autistic trait scale (24 items) including all items used in factor analysis in Chapter 5	.32
Social Unease (7 items) as used in Chapter 5	.38
Social Unease excluding 2 potential confounding items:	**.32**
item No. 11 'finds social situations easy'	
item No. 46 'new situations make him/her anxious'	

	Intraclass twin correlations
MZM	.94
DZM	.67
MZF	.92
DZF	.69
DZOS	.63

	Parameter estimates in univariate ACE model
A	53%
C	40%
E	7%

Appendix VII Items of the anxiety-related cehaviours questionnaire (ARBQ) at age 16

Generalised Anxiety:	*Negative Cognition:*	*Social Anxiety:*
Asks for reassurance that he/she is OK	Is afraid of small enclosed spaces, heights, water, or the dark	Takes a long time to warm to strangers
Has low self confidence	Insists on doing something over and over, to the extent that it interferes with day to day life	Is afraid in social situations
Tends to blame him/herself	Doesn't tend to enjoy him/herself	Tends to be shy and timid
Is anxious that bad things will happen	Often makes comments critical of him/herself	*New items at age 16 (all Obsessive-Compulsive Behaviours):*
	Complains or whines a lot	
Fears:	Often seems worked up, on edge or tense	Tends to check things are done exactly right
Is afraid of medical procedures such as going to see the doctor/dentist	Is afraid of animals or insects (like dogs, spiders, or snakes)	Has twitches, mannerisms, or tics of the face and body
Is often extremely upset or distressed when parent leaves wound up or stressed		Is fussy or over-particular

Note: Items listed under domain headings derived from factor analysis on the ARBQ in (Hallett et al., 2009b)

Appendix VIII The childhood anxiety sensitivity Index (CASI)

Items as used for twins' self report in TEDS at age 16

I don't want other people to know when I feel afraid

When I cannot keep my mind on my schoolwork, I worry that I might be going crazy

It scares me when I feel "shaky"

It scares me when I feel like I am going to faint

It is important for me to stay in control of my feelings

It scares me when my heart beats fast

I feel embarrassed when my stomach rumbles or makes noise

It scares me when I feel like I am going to throw up

When I notice that my heart is beating fast, I worry that there might be something wrong with me

It scares me when I have trouble getting my breath

When my stomach hurts, I worry that I might be really ill

It scares me when I cannot concentrate on my schoolwork

Others my age can tell when I feel shaky

Unusual feelings in my body scare me

When I am afraid, I worry that I might be crazy

I get scared when I feel nervous

I don't like to let my feelings show

Funny feelings in my body scare me

(Silverman et al., 1991)

Appendix IX Fit statistics of the best fitting univariate models of age 16 self report

Autistic Traits	Overall Fit of Model					relative Fit of Model			
	−2LL	AIC	χ^2	df	p	AIC	χ^2	df	p
Saturated	17914.254								
ADE	17926.642	0.388	12.388	6	.06				
AE	**17928.058**	**-0.196**	**13.804**	**7**	**.06**	**-0.584**	**1.416**	**1**	**.23**
E	18574.393	644.139	660.139	8	<.001	643.751	647.751	2	<.001

Anxiety Traits	Overall Fit of Model					relative Fit of Model			
	−2LL	AIC	χ^2	df	p	AIC	χ^2	df	p
Saturated	18125.367								
ADE	18136.113	-1.254	10.746	6	.10				
AE	**18136.176**	**-3.191**	**10.809**	**7**	**.15**	**-1.937**	**0.063**	**1**	**.80**
E	18503.046	361.679	377.679	8	<.001	362.933	366.933	2	<.001

Depression Traits	Overall Fit of Model					relative Fit of Model			
	−2LL	AIC	χ^2	df	p	AIC	χ^2	df	p
Saturated	17983.961								
ACE	**17992.363**	**-3.598**	**8.402**	**6**	**.40**				
AE	18010.731	12.77	26.77	7	<.001	16.368	18.368	1	<.001
CE	18004.474	6.513	20.513	7	<.001	10.111	12.111	1	<.001
E	18475.709	475.748	491.748	8	<.001	479.346	483.346	2	<.001

Appendix X Fit statistics of the best fitting univariate models of age 16 parent report

Autistic Traits	Overall Fit of Model					relative Fit of Model			
	−2LL	AIC	χ^2	df	p	AIC	χ^2	df	p
Saturated	14857.444								
ACE	**14859.731**	**-9.713**	**2.287**	**6**	**.32**				
AE	14963.432	91.988	105.988	7	<.001	101.701	103.701	1	<.001
CE	15531.153	659.709	673.709	7	<.001	669.422	671.422	1	<.001
E	18447.757	3574.313	3590.313	8	<.001	3584.026	3588.026	2	<.001

Anxiety Traits	Overall Fit of Model					relative Fit of Model			
	−2LL	AIC	χ^2	df	p	AIC	χ^2	df	p
Saturated	16856.911								
ACE	**16861.25**	**-7.661**	**4.339**	**6**	**.63**				
AE	16918.249	47.338	61.338	7	<.001	54.999	56.999	1	<.001
CE	16985.635	114.724	128.724	7	<.001	122.385	124.385	1	<.001
E	18556.68	1683.769	1699.769	8	<.001	1691.43	1695.43	2	<.001

Depression Traits	Overall Fit of Model					relative Fit of Model			
	−2LL	AIC	χ^2	df	p	AIC	χ^2	df	p
Saturated	25945.82								
ACE	**25954.783**	**-3.037**	**8.963**	**6**	**.18**				
AE	25986.054	26.234	40.234	7	<.001	29.271	31.271	1	<.001
CE	26066.888	107.068	121.068	7	<.001	110.105	112.105	1	<.001
E	27263.159	1301.339	1317.339	8	<.001	1304.376	1308.376	2	<.001

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